Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)

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Thesis Abstract

Background

With 1.4 million deaths in 2019, tuberculosis remains a leading global infectious cause of death, second only to COVID-19 in 2020. Antimicrobial resistant infections are projected to cause over 10 million deaths annually by 2050, in part reflecting non-pathogen directed prescription secondary to limited point-of-care diagnostics and laboratory infrastructure. These two major global public health threats intersect through diagnostic algorithms that have for decades encouraged broad-spectrum antibiotic prescriptions ("trial-of-antibiotics") during the diagnostic work-up leading to ~5 million people being treated annually for mycobacteriology-negative tuberculosis. However, the underlying assumption that post-treatment symptom improvement "rules out" tuberculosis, had no clear evidence-base.

Aims

To use systematic review and a randomised controlled trial to evaluate and address evidence gaps in three key areas: 1) diagnostic performance, 2) safety of withheld prescription, and 3) impact on antimicrobial resistance of trial-of-antibiotics.

Methods and results

For the systematic review and meta-analysis (CRD42017083915), I searched MEDLINE, Embase and Global Health databases for studies addressing the diagnostic performance of trial-of-antibiotics against mycobacteriology tests in adults with tuberculosis symptoms. Pooled values for sensitivity and specificity of trial-of-antibiotics (index test) versus mycobacteriology tests (reference) were estimated using random-effects bivariate modelling and I^2 statistic.

Only 8/9410 screened studies were eligible, with no randomised trials. Treatment duration, antibiotics used, and definition of response to treatment varied substantially. Pooled sensitivity (67%, 95% CI 42, 85) and specificity (73%, 95% CI 58, 85) of trial-of-antibiotics versus mycobacteriology were below internationally-defined minimum performance profiles for tuberculosis diagnostics with substantial heterogeneity (I^2 96% for sensitivity, 99% for specificity) and low QUADAS-2 quality assessments.

My trial (NCT03545373) aimed to strengthen this weak evidence-base. I randomised (1:1:1) Malawian adults attending primary care for illness ≥2 weeks including cough with no immediate indication for hospitalisation, no recent antibiotic or tuberculosis treatment or prevention, to: azithromycin (500mg daily, 3 days)

amoxicillin (1g three times/day, 5 days); or standard-of-care (SOC) with no immediate antibiotic. Sputum at enrolment and day 8 was tested using microscopy, Xpert MTB/RIF, and culture. Primary outcomes were day 8 specificity (percentage with symptom improvement among mycobacteriology-negative), and day 29 clinical outcomes (composite: death, hospitalisation or missed tuberculosis diagnosis). The secondary outcome was day 29 risk of resistant *Streptococcus pneumoniae* identified by culture of nasopharyngeal swabs.

After screening 5825 adults, 1583 were randomised of whom 6.3% (100/1583) had positive baseline mycobacteriology. Compared to SOC (79.1% of 530), trial-of-antibiotics improved tuberculosis specificity: azithromycin (527 patients) vs. SOC difference +8.6% (95% confidence interval 3.9%, 13.3%); amoxicillin (526 patients) vs. SOC difference +8.8% (4.0%, 13.6%), but with extremely low sensitivity (10.7% azithromycin, 23.3% amoxicillin). Day 29 composite clinical outcomes were similar (SOC 1.1%, azithromycin 1.1%, amoxicillin 2.1%). Compared to SOC (5.3%), proportions with day 29 resistant *Streptococcus pneumoniae* were higher in azithromycin +2.5% (-0.5, 5.5), but similar in amoxicillin +0.2% (-2.9, 2.5) arms.

Using standard decision-analysis tools I compared antibiotic prescription and Xpert MTB/RIF requirements of 3 algorithms combining Xpert MTB/RIF and trial-of-antibiotics against Xpert alone in 100,000 hypothetical patients with tuberculosis prevalence of 5%. Antibiotic prescriptions (trial-of-antibiotics plus out-of-protocol) needed to identify one tuberculosis patient varied from 22.7 to 220.4 in combination algorithms, with minimal benefit over Xpert alone (NNS =22.5 Xpert tests, plus 4.0 antibiotic prescriptions). Trial-of-antibiotics exposed patients to considerable misclassification risks.

Conclusion

Harms of trial-of-antibiotics likely outweigh benefits, with the poor diagnostic performance, lack of additional clinical impact, high cost, and likely impact on antimicrobial resistance arguing strongly against routine prescription. National tuberculosis and antimicrobial stewardship programs should limit outpatient prescription of empirical broad-spectrum antibiotics to patients with strong clinical or microbiological indications. Future research should focus on strengthening diagnostics for tuberculosis and other respiratory pathogens, evaluating antibiotic-sparing tuberculosis diagnostic algorithms in less clinically stable patients, and antimicrobial stewardship programs targeting tuberculosis-related prescribing.

Involvement in the response to COVID-19

My COVID-19 work started with joining the Malawi-Liverpool Wellcome Trust (MLW) COVID-19 Committee in the role of inter-Institutional Coordination for Blantyre. The Blantyre COVID-19 Response is hosted by Blantyre District Health Office and includes, MLW, University of Malawi College of Medicine, Queen Elizabeth Central Hospital, Kamuzu College of Nursing, and the Blantyre City Council.

I view my main contribution, however, as being providing public health advice to the public via mainstream and social media, and as an Epidemiology advisor to the government of Malawi national response through participation in various committees described below. I got motivated and started reaching out to government officials with advice after observing that Malawi would experience high numbers of morbidity and mortality. I worked with Prof Liz Corbett to develop a community shielding intervention for older adults that is now being implemented by MLW and College of Medicine ("Kuteteza") in their community-based malaria trial sites.

My public engagement activities have involved explaining the epidemic curve, the various experiences and expectations at any given stage in the pandemic, and providing advice on what individual Malawians and families should do. I essentially made it my personal duty to make COVID-19 the top subject amidst all the political activity and misinformation that was going on at the time. My initial main form of communication was social media (Facebook page, personal Facebook profile, Twitter and LinkedIn), which lead onto invitations for mainstream media interviews. I was constantly in national newspapers, television, and radio. I have been on all top media interviews and front pages of all daily papers.

The government then invited me to formally support the national response first as a member of the Malawi National Surveillance Committee as an epidemiology advisor. When the Ministry of Health Expert Advisory Group on COVID-19 was established, I was also invited to join. Apart from these two committees I was also part of the Infection Prevention Committee, and COVID-19 Research Subcommittee. Outside government, I remained a member of the MLW Committee, and was later invited to the University of Malawi-wide COVID-19 Committee where I supported development of school reopening criteria and guidelines for campus infection prevention and shielding.

As part of the Surveillance Committee, I prepared two National Survey Protocols. I prepared funding applications and defended proposals to Norwegian Institute of Public Health and the Centre for Disease Control and Prevention, and led implementation, and analysis and dissemination of results, which we hope to publish in a peer-reviewed journals. One of my tasks on the Expert Advisory Group was to investigate why there was a disconnect between the public and the national response in several parts of the country. My work on this led to a set of policy recommendations to the Directorate of Prevention to improve delivery of community engagement.

Towards the end of the first wave (Sep 2020), I conducted an in-depth analysis of the epidemic including assessment of the implementation efficiency of the national response plan. I also developed proposals for national recovery and co-existence with COVID-19. After input from the Expert Advisory Group, I was invited to present the work at the Ministry of Health Senior Management Meeting. Our community shielding intervention (Kuteteza) was taken up by the Social Protection cluster, and I was invited to give a presentation, which led to several meetings and incorporation into national policy.

The demand for information was so high that May to Sep 2020 were intensely busy. In June 2020 I interrupted my studies to cope with my workload from the COVID-19 response. I am thankful to the London School for allowing the 3 month long interruption of studies to allow me to serve my nation.

The second COVID-19 wave that started in Dec 2020 caused a number of high profile deaths in Malawi. I have maintained the above roles but with a reduced meeting attendance and provision of advice as needed, to allow completion of this thesis. However, I still faced a challenge when the numbers of severe disease and deaths started rising rapidly. The demand for information (from media houses) and requests from various committees started piling up. I was also invited by the Vice President of Malawi to join his team of special advisors (COVID-19 Think Tank for the Vice President) to support the restructuring of the national response and increase capacity to address a rapidly rising epidemic.

In summary, volunteering for the Malawi National response was an extremely busy but very fulfilling task which delayed my doctoral training by a couple of months. I am thankful to the support and understanding of my supervisors and the London School as I took on the various roles of the national response.

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Abbreviations and acronyms

ACASI	Audio-Computer Assisted Self-Interview
ACT-TB	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis
ART	antiretroviral therapy
CAD	Computer-aided detection software
CDC	Centers for Disease Control and Prevention
COVID-19	Coronavirus Disease 2019
DNA	Deoxyribonucleic acid
DOTS	Directly observed treatment-short-course
DSMB	Data and Safety Monitoring Board
GLASS	Global Antimicrobial Resistance Surveillance System
HIV	human immunodeficiency virus
IQR	interquartile range
LAM	lateral flow lipoarabinomannan
LAMP	loop-mediated isothermal amplification
LED	Fluorescent light-emitting diode
LSHTM	London School of Hygiene and Tropical Medicine
MTB	Mycobacterium tuberculosis
NNS	Number needed to screen
NTP	National Tuberculosis program
PROSPERO	The International Prospective Register of Systematic Reviews
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SDG	Sustainable Development goals
TSC	Trial Steering Committee
UKAPTB	UK Academics & Professionals to End TB
UN HLM TB	UN high-level meeting on Tuberculosis
WHO	World Health Organisation

1 Introduction

1.1 Background

Tuberculosis and antimicrobial resistance independently threaten the attainment of at least half of the 17 Sustainable Development Goals by 2030, through ill health and severe disruption of productivity at individual, national and global levels. Despite declining incidence, tuberculosis remains a major global health problem, and the leading infectious killer after COVID-19. Antimicrobial resistance is a global health and economic emergency that is projected to be both the leading cause of death and healthcare expenditure in a few decades. The suboptimal nature of pulmonary tuberculosis diagnostics drives the use of “trial-of-antibiotics”, a course of broad-spectrum antibiotics without activity against *Mycobacterium tuberculosis* given to distinguish pulmonary tuberculosis from bacterial lower respiratory tract infection in patients with suggestive symptoms such as prolonged cough. Trial-of-antibiotics may be prescribed more than once in the course of a given illness, before or after investigations such as sputum diagnostics or chest radiography. Millions of trial-of-antibiotics doses are prescribed each year but the underlying assumption --- that patients with lower respiratory tract infection will improve, while those with pulmonary tuberculosis will not --- has an unclear evidence-base.

1.2 Aim

The aim of this thesis is to investigate the clinical utility, benefits, and antimicrobial resistance risks of including trial-of antibiotics in tuberculosis diagnostic algorithms for symptomatic adults without signs of severe illness.

1.3 Objectives

The research included in this thesis had the following three objectives:

A. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis in adults presenting to primary care with prolonged cough.
B. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.
C. To evaluate the effect of trial-of-antibiotics on antimicrobial resistance.

1.4 Structure of thesis

This thesis follows the "*research paper style*" approach which involves including a set of related manuscripts that are either published or prepared for publication. Three chapters of this thesis are published articles, and another chapter includes a manuscript undergoing peer review at the time of this writing. Each of these four manuscripts is introduced by a cover page and some background text linking it to the previous section of the thesis. Where additional material is necessary to complete the discussion those are marked accordingly and included after the manuscript.

I complete this first chapter by, summarising the structure of the thesis, describing my role in the work presented in the thesis, ethical considerations and the funding that supported the work. In the second chapter, I present the thesis background. Chapter three is a manuscript of a systematic review and meta-analysis investigating the diagnostic performance of trial of antibiotics against mycobacteriology tests. The goal of this work was to summarise available evidence on routine use of trial of antibiotics. The protocol for the systematic review was registered with PROSPERO and published in BMC Systematic Review [1] before data analysis. This protocol manuscript is included in the appendix of this thesis. I presented results at the 49th Union World Conference on Lung Health in October 2018 in The Hague. The results manuscript is published in The Lancet Infectious Diseases.[2]

Upon establishing through the systematic review that the use of trial of antibiotics lacked an evidence base, I designed and conducted an individually randomised controlled trial investigating diagnostic accuracy, clinical impact and antimicrobial resistance impact of trial-of-antibiotics. I present the protocol manuscript and statistical analysis plan in chapter four. I presented an abstract of the protocol manuscript at the 2018 London School of Hygiene & Tropical Medicine (LSHTM) Research Degree Poster Day and I secured the best poster presenter award. I registered the protocol with clinicaltrials.gov (NCT03545373), then prepared and published the manuscript describing the protocol in BMJ Open.[3] Chapter four is complemented by the trial's statistical analysis plan which I prepared and secured endorsements from Trial Steering Committee (TSC) and Data and Safety Monitoring Board (DSMB), before completing the trial data collection.

In chapter five, I present a manuscript of the results for the randomised controlled trial. These results were first presented to the Malawi National TB Program and the Blantyre District health Office and other local stakeholders at a dissemination meeting I organised. I have been invited by the National TB Program to support the translation of the work into policy. The trial results were accepted for oral presentation in The Union/CDC late-breaker session on TB of the 51st Union World Conference on Lung Health in October 2020. I have prepared and submitted the results manuscript for publication.

Chapter six presents my platform for potential postdoctoral work (if funded), extending the success of the tuberculosis diagnosis-related antimicrobial stewardship work of the randomised controlled trial to prescribing approaches in the broader respiratory clinical setting. I have presented in this chapter, a manuscript of a protocol for systematic review and network meta-analysis of randomised controlled trials investigating the relationship between the duration of antimicrobial exposure and development of, or selection for, resistance. Published in BMC Systematic Reviews,[4] this proposed review facilitated my successful bid to join the Medical Research Foundation National PhD Training Programme for Antimicrobial Resistance Research which selected only 150 out of all UK PhD students. In addition, my abstract was among the 50 selected for presentation at the program's annual conference in Bristol. Data collection for this work has just commenced and publication is expected in 2021.

I have used chapter seven to highlight major findings, strengths, and limitations of the thesis. I have also provided an interpretation of the work in the context of previous knowledge, and have prepared a set of recommendations for policy and future research.

1.5 Contribution of the author

I conceived the research question from my experience as a clinician managing patients with tuberculosis symptoms in Malawi. With the help of my supervisors and advisors I was able to better frame and develop the question over time. The detailed research approach was improved as I led the preparation of my research funding applications. I secured a scholarship from the Commonwealth Scholarship Commission in the UK, and research funding from the Helse Nord RHF Norway.

After designing the systematic review that I describe in chapter three, my supervisors helped me assemble a team from their collaborations to support with various roles including second screening and data extraction. I am thankful to the LSHTM Transferable Skills Programme and the LSHTM library for taking me through the process of how to conduct systematic reviews, one of the core skills I have obtained from my PhD. I framed my systematic review question, developed a protocol, prepared statistical analysis plan and analysis program in Stata, conducted the analysis, and interpreted results.

I designed and led the implementation of the trial described in chapters four and five as the chief investigator and received support from my supervisors, advisors, and co-authors. I also led local stakeholder consultations that improved the design and aligned it with clinical guidelines. I secured trial sponsorship from the LSHTM. Prior to study implementation, I obtained ethics review and approval from committees at LSHTM, University of Malawi College of Medicine, and Norwegian University of Science and Technology; and regulatory approval from the Malawi Pharmacy and Medicines Regulatory Authority. I then hired and trained study staff, developed data collection forms, and designed and led a pilot study. With the support of my supervisors, I organised the trial DSMB, TSC, and trial monitoring and coordinated their meetings and site visits throughout the study. I prepared a data management plan before trial implementation. I prepared a statistical analysis plan and analysis program code (Stata do-files) before trial completion. I performed the statistical analysis and led the write up and dissemination.

I conceived the research question in chapter 6 with the help of my supervisors and co-authors. I then developed the review protocol, registered with PROSPERO, and submitted for publication. My co-author, Dr Helen Stagg (at the time at University College London), was very instrumental in providing guidance on the design of the network meta-analysis approach, and with her guidance, I attended a formal training from the University of Bristol.

1.6 Ethical considerations

The trial was reviewed and approved by University of Malawi College of Medicine Research and Ethics Committee, LSHTM Research Ethics Committee, and Regional Committee for Health and Research Ethics –Norway, and Malawi Pharmacy and Medicines Regulatory Authority.

1.7 Funding

I am grateful to the Helse Nord RHF and the people of Norway for providing research funding for the trial. I am grateful for the PhD scholarship I received from the Commonwealth Scholarship Commission. The funders of the study did not influence the design, data collection, data analysis, data interpretation, or writing of any of the studies.

1.8 References

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2 Background

2.1 The two epidemics

My thesis covers an important but poorly described intersection between two major global public health, economic, and scientific challenges: 1) tuberculosis, and 2) antimicrobial resistance. Tuberculosis is the leading cause of mortality of all infectious diseases[1] (second to COVID-19 for 2020) in part because available diagnostics are suboptimal leading to misdiagnosis, underdiagnosis, and a protracted evaluation period requiring multiple clinic visits. Antimicrobial resistance, with an estimated 700,000 deaths in 2016, and projected to increase burden to 10 million deaths per year by 2050, is a public health emergency.[2,3] In this background chapter, I will introduce these distinct topics and discuss the nature of their overlap.

2.1.1 Tuberculosis: a treatable disease with high mortality

Tuberculosis is a multisystemic disease caused by *Mycobacterium tuberculosis*, a non–spore-forming, nonmotile, aerobic bacillus that is an obligate pathogen and has been causing disease in humans for thousands of years.[4] Tuberculosis has a myriad presentations and manifestations, but most commonly affects the lungs (pulmonary tuberculosis), reflecting the airborne nature of transmission. In 2019, 85% of registered tuberculosis patients had pulmonary disease.[1]

Tuberculosis is characterised by an extremely high case-fatality rate and has for some years been the global top cause of mortality out of all infectious agents, and the sixth out of all causes according to WHO.[5] In 2020 however, mortality due to SARS-CoV-2 exceeded that of tuberculosis. The estimated global burden of tuberculosis in 2019 was an incidence of 130 cases per 100,000 population, a total of 10 million people with active disease, and 1.4 million deaths.[1] Most people with tuberculosis were from WHO regions of South-East Asia (44%), Africa (25%) and the Western Pacific (18%). Tuberculosis deaths follow a similar global distribution to incidence with the bulk of the burden in South-East Asia and Africa.[6] The epidemiology of tuberculosis in Africa is mainly influenced by HIV as described under 2.4.1.

2.1.2 Antimicrobial resistance: a rapidly escalating global health burden

Antimicrobial resistance is a term used to describe protective mechanisms that microbes develop against antimicrobials, rendering treatments ineffective and

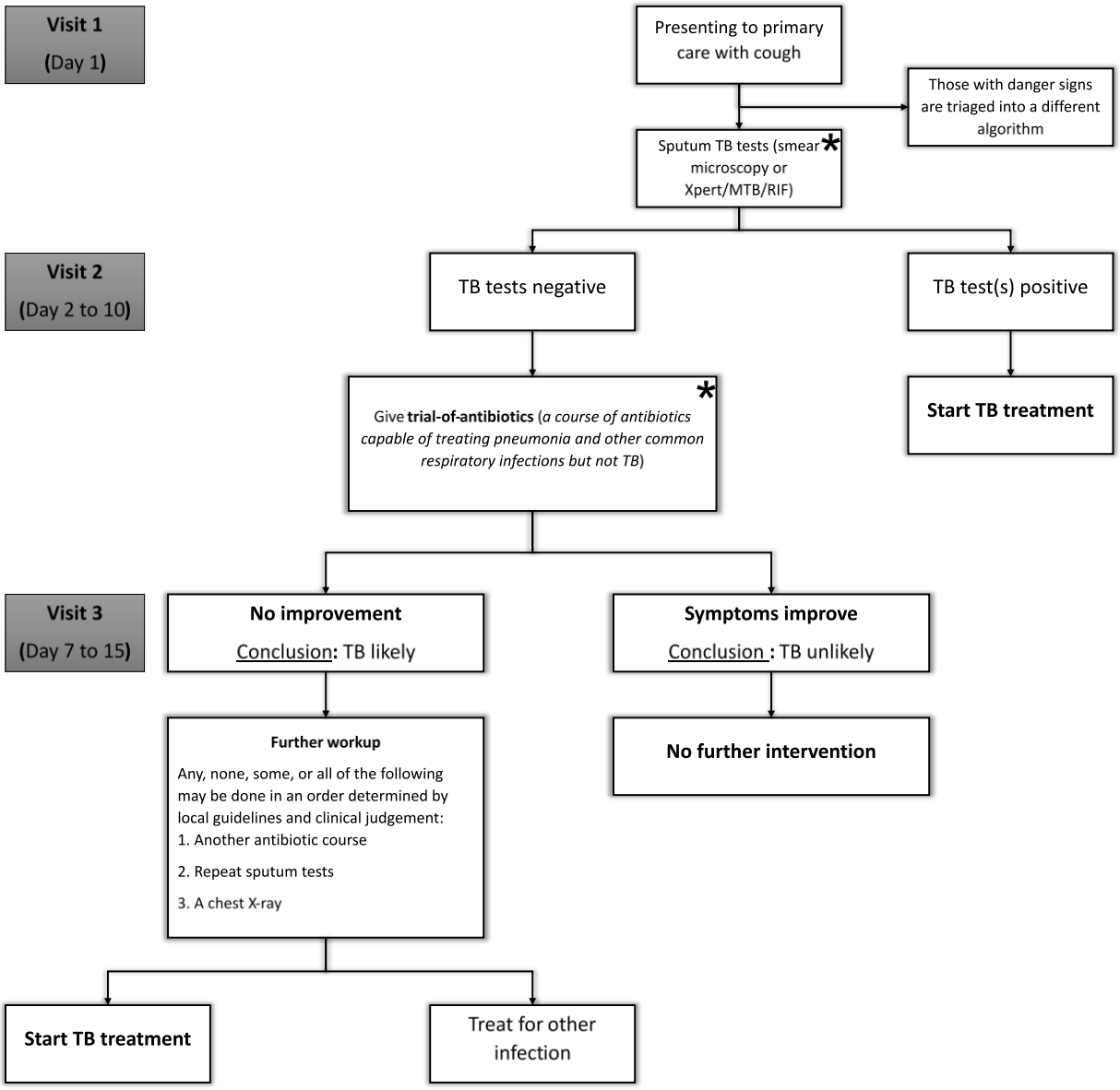
increasing the disease management costs and risk of death.[7] The discovery of penicillin and most of the lifesaving antimicrobials still in use today were among the most transformative events of the 20th century.[8] Disturbingly, antimicrobial resistance threatens the achievement[7] in what has become a rapidly escalating global challenge that may lead to a loss of 3.5% of Gross Domestic Product or US$100 trillion by 2050.[3]

The global distribution of the current and projected burden of antimicrobial resistance follows the pattern of major antimicrobial-dependent infections. Modelling work based on current burden and inaction on antimicrobial resistance of *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli (E. coli),* HIV, tuberculosis and Malaria, projects a disproportionately higher mortality burden in Asia (4.7 million/10 million global deaths) and Africa (4.1 million/10 million global deaths) by 2050.[3] The disproportionately high projected burden for Africa mainly reflects the much higher burden of treatment resistant strains of HIV, tuberculosis and malaria in Africa compared to other global regions as well as an increased risk of lower respiratory infections.[9]

2.2 Under-resourced tuberculosis epidemic drives antimicrobial resistance

2.2.1 Trial-of-antibiotics to "rule-out" tuberculosis: a course of broad-spectrum antibiotics bridging a key diagnostic gap

Despite being over a thousand years old and after over 100 years of human knowledge and investigations, tuberculosis is yet to have an accurate point-of-care diagnostic test, and all commonly used laboratory diagnostics have poor sensitivity.[10,11] The uncertainty that a negative mycobacteriology test result presents in the context of low diagnostic test accuracy triggers a common clinical question: "is this test-negative tuberculosis or bacterial chest infection?"[12,13] To address this question, tuberculosis diagnostic algorithms (since early 90s) provide for "rule-out" clinical decision-making that includes prescribing a trial-of-antibiotics, a course of broad-spectrum antibiotics with negligible *Mycobacterium tuberculosis* activity.[14,15]. If patients with negative sputum mycobacteriology respond to the antibiotic treatment, they are considered tuberculosis "negative" while those who remain symptomatic are deemed likely to have tuberculosis and undergo further evaluations leading on to receiving tuberculosis treatment see figure 2.1 below.[12,16]



*The common clinical practice is that outpatients start antibiotics at the time of submitting sputum, to avoid the need for a third clinic visit to complete the algorithm.

Figure 2.1 Implementation of trial-of-antibiotics in Malawi TB diagnostic algorithm, National TB control program (NTP Manual 2012).

2.2.2 Why empirical broad-spectrum antibiotics?

In settings and circumstances where diagnostic options are limited, uncertainties in diagnosis are followed by empirical treatment of the most likely aetiology, most commonly using a positive response to treatment to “rule-in” the diagnosis. For tuberculosis, this approach is still occasionally used as part of investigation of fever of unknown origin and other cryptic presentations in which tuberculosis cannot be confirmed or excluded by other means.[17] However, this requires withholding rifampicin (which has broad-spectrum antibiotic activity but is also the most potent bactericidal antituberculosis drug) and using response to 2 weeks of the narrow-spectrum anti-tuberculosis drugs isoniazid, pyrazinamide, and ethambutol with response to fever used to determine whether or not to continue to the full tuberculosis treatment course. This approach has been discouraged since the 1990s because of unknown effectiveness and the context of increasing prevalence of anti-tuberculous drug resistance.[18]

Tuberculosis treatment is long and toxic.[19,20] The current internationally recommended first-line therapy for drug-susceptible tuberculosis is a 6-month regimen of isoniazid, rifampicin, ethambutol and pyrazinamide,[19,20] although an effective 4 month regimen has very recently been identified.[21,22] Longer and more toxic drug combinations are needed for drug resistant tuberculosis.[19,23] Prolonged treatment regimens are needed even for fully susceptible *Mycobacterium tuberculosis* to avoid an unacceptably high risk of recurrence. This reflects the ability of *Mycobacterium tuberculosis* bacilli to enter a nonreplicating state that carries a degree of phenotypic resistance through interaction with host factors,[24] including the environment within tuberculous granulomas that are also poorly penetrated by most antituberculosis drugs.[25]

Therefore, the main value of attempting to rule out alternative bacterial causes of symptoms is the ease of treatment and rapidity of the expected clinical response. Unlike tuberculosis, uncomplicated bacterial pneumonia responds to a three-day course of azithromycin, or amoxicillin, both which are safe and better tolerated than tuberculosis chemotherapy.[26]

The antibiotic class for use as a “rule-out” trial-of-antibiotics (referred to hereafter as “trial-of-antibiotics“) is chosen on the basis of having negligible Mycobacterium tuberculosis activity.[16] Trial-of-antibiotics therefore complements the suboptimal mycobacteriology diagnostics by ruling out bacterial differential diagnoses and leaving tuberculosis as the most likely aetiology of symptoms because it cannot respond to a course of broad-spectrum antibiotics.[14-16,27]. My systematic review described in Chapter 3 provides a list of common choices of antibiotics, timing of prescription, duration, and how response to treatment is measured.

2.2.3 Trial-of-antibiotics and global antimicrobial resistance

Accurate estimates of global antibiotic use for trial-of-antibiotics are not available. The 2.6 million patients (43% of total global notifications) with pulmonary tuberculosis diagnosed without bacteriological confirmation[1] are each likely to have received trial-of-antibiotics. To establish a rough estimate of the overall antibiotic use, we start by assuming that each of the 2.6 million patients received on average two courses of broad-spectrum antibiotics before commencing tuberculosis treatment,[16] and that for each registered bacteriology-negative case there are at least three other patients whose symptoms resolve after trial-of-antibiotics,[27] then trial-of-antibiotic prescriptions will outnumber bacteriology-negative TB patients by five-fold, which for 2019 translated to13 million courses. This is likely to be an underestimate of the global picture because it does not include courses of antibiotics used before diagnosis of microbiologically confirmed pulmonary tuberculosis,[28] or the 3 million missed cases, or the 1.6 million notified extrapulmonary cases.[1] This widespread use of antibiotics without a microbiologically confirmed indication will inevitably be contributing to antimicrobial resistance[29-31].

2.3 Antimicrobial resistance in primary care setting

2.3.1 Mechanism of antimicrobial resistance

Development of resistance genes predates formal use of antimicrobials.[32,33] Under drug pressure, natural selection rapidly amplifies resistance-causing mutations that can also be transferred or acquired by horizontal genetic transfer machanisms.[34,35] The five best described expressions of resistance-conferring mutations in bacteria

include 1) production of enzymes that inactivate antibiotics, 2) mutations that make the cell wall less permeable to antibiotics, 3) mutations that utilise efflux pumps to remove antibiotics from the cell, 4) mutations that modify target antibiotic binding sites in ways that prevent binding or reduce affinity, and 5) creating new metabolic pathways bypassing those utilised by antibiotics.[34] These mechanisms are often organism specific, but it is not uncommon for one type of bacteria to host multiple mutations affecting different pathways.

2.3.2 Drivers of selection pressure

Selection for resistance is primarily secondary to drug pressure from antimicrobial exposure in the agriculture industry, environment, and health care.[34] The agriculture industry is under constant pressure to provide large amounts of animal protein for the growing global population.[36] The rising demands lead to intensive farming and veterinary approaches that involve use of large quantities of antibiotics.[37,38]

The environment hosts naturally produced antibiotics,[39,40] and is a recipient of more antimicrobials from human activity including agriculture and waste disposal.[41-43] Resistance-encoding genes are therefore ubiquitous in environmental bacteria.[40,44] Limitations in available culture and molecular investigation technologies prevent establishment of the full diversity of bacteria and resistance genes [45] necessary for quantitative documentation of the associations between environmental resistance genes and human pathogens.[44,46,47]

It is estimated that in humans as many as 50% of the clinical encounters that result in prescription of antimicrobials are unnecessary.[34] Unnecessary prescriptions are usually secondary to clinical, patient and clinician factors.[34] Presence of fever, purulent sputum, abnormal respiratory exam, and tonsillar exudate are some of the clinical-presentation factors associated with prescription of a course of antibiotics to patients with acute respiratory symptoms.[48] Presenting a direct request, being a regular patient, having more symptoms, and being an older adult, are some of the patient factors associated with antibiotic prescribing.[49,50] Clinician related factors include being older, fear of adverse outcome, tolerance of risk and uncertainty, relationship with patient; [49,51] and the underlying driver of inappropriate prescribing is absence of diagnostic assays to inform treatment decisions at the point of care.[34,52-54] Human antimicrobial use outside healthcare is also very common and another source of selection pressure.[55]

To sum up, addressing sources of drug pressure requires a careful, multisectoral and multipronged approach involving determinants from the agriculture industry, environment, and human health care.[2,35,56] Attempts at addressing such a wide span of action areas has led to the development of the one health approach,[57,58] defined as “designing and implementing programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes.”[59]

2.3.3 Transmission of resistance in health care

While exposure to antimicrobials leads to selection for resistance, onward transmission in health care settings is propagated by poor infection control practices, poor access to quality-assured antimicrobials, and lack of diagnostics.[60,61] The spread of nosocomial infections is facilitated by poor hand hygiene by patients and staff, lack of water supply and poor sanitation; and poor or poorly implemented infection prevention protocols.[61,62] Health facilities in low-income countries experience a higher burden of nosocomial infections compared to those in high income countries in part because of weak water, sanitation and hygiene infrastructure.[60,63]

Effective treatment is a critical measure for preventing onward transmission of pathogens including antimicrobial resistant organisms. Unfortunately, the current era is characterised by sharply declining investment from the pharmaceutical industry in the development of effective new antimicrobials; far fewer new compounds are developed annually now than during the 1990s.[64] In addition to the dry antibiotic research and development pipeline, low income countries, hosts of the largest proportion of the global infectious disease burden, have two related problems: 1) they cannot afford effective antimicrobials necessary to manage drug resistant infections, 2) do not have effective regulation and enforcement capacity, and 3) as a consequence, are on the receiving end of counterfeit or other substandard medications which lead to undertreatment and further worsening of the antimicrobial resistance burden.[65]

The limitations to access by low-income countries also extend to essential vaccines aimed at reducing infectious disease burden, and to infectious disease diagnostics, which are key in ensuring correct and prompt treatment of infections. Reducing

global transmission of resistant organisms will therefore not be possible without addressing infection prevention practices, and access challenges for effective antimicrobials, vaccines and diagnostics faced by low-income countries.

2.3.4 Diagnostic limitations and empirical antimicrobial treatment in low-income countries

What can be categorised as unnecessary antimicrobial prescriptions (in antimicrobial stewardship terms) in medical settings[66] are in part due to lack of accurate point-of-care diagnostics.[52,67,68] The diagnostics limitations in resource-limited settings lead to “syndromic management” of suspected infections which involve prescribing antimicrobials without microbiological confirmation but based on the most likely aetiology.[69-71] Clinical improvement following empirical antibiotics is the desired outcome, failure to respond leads to prescription of antibiotics of a different class or additional diagnostic efforts or referral to higher level care.

The relationship between lack of point-of-care diagnostics and antimicrobial resistance is well documented.[72,73] Similarly, the clinical benefits of empirical antimicrobial treatment in reducing morbidity and mortality, have been previously described.[70,74] Striking a balance between these two needs (preventing antimicrobial resistance, and preventing mortality) for infections with high case fatality rates like pneumonia and tuberculosis often favours empirical treatment for mortality prevention.[31,68,75]

In summary, until accurate, rapid, and point-of-care diagnostics for most infectious diseases, are identified, unnecessary antimicrobial prescribing is likely to continue especially for life threatening infections. Balancing the diagnostic gap against the mortality threat may be one of the main reasons for the continued existence of trial-of-antibiotics despite threat of the antimicrobial resistance.

2.4 Tuberculosis in adult primary care setting of Africa

The aim of this section is to contextualize trial-of-antibiotics by describing the epidemiology of tuberculosis in a primary care setting, the pathway to diagnosis, and the modern options for tuberculosis diagnosis.

2.4.1 Epidemiology of tuberculosis in primary care attending adults

Tuberculosis commonly presents with of cough, weight loss, fever, night sweats, dyspnoea, chest pain and haemoptysis.[76] These symptoms inform tuberculosis screening algorithms.[10] The symptoms are so non-specific that nearly 20% of adults presenting to primary care meet criteria for tuberculosis screening.[77] The differential diagnosis of the symptoms is also diverse but malaria, bacterial infections and tuberculosis are the critical life threatening aetiologies requiring rapid identification and treatment.[52,78] While there are effective point of care diagnostics for malaria, challenges remain for most tuberculosis and non-tuberculosis bacterial infections.[52]

In Malawi, respiratory infections contributed approximately half (46%) of all causes of non-malarial fevers in an outpatient survey,[79] and bacterial aetiologies were identified in 53% of 1065 articles included in a review of African and Asian literature on causes of fever.[78] In a 2014 study, tuberculosis accounted for 19% of adult primary care presentations in Malawi.[80] However, a community cohort study conducted at the same time identified high 12-month risk of mycobacteriologically confirmed tuberculosis in adults with (8.9%) and without (3.7%) chronic cough.[81]

The key determinants of tuberculosis in Africa are HIV[82] and poverty.[83] Incidence of tuberculosis and mortality have decreased substantially with the wide availability of antiretroviral therapy and integration of HIV and tuberculosis services.[84,85] In section 2.8.3, I demonstrate the synchronised change in the epidemiology of HIV, tuberculosis and mortality in Malawi over a 20 year period.

2.4.2 Screening and diagnosis of tuberculosis

Prompt identification and treatment of those with active pulmonary disease eliminates *Mycobacterium tuberculosis* from respiratory secretions[86] and prevents both onward community transmission and mortality.[87,88] This “case-finding and treatment” strategy remains the cornerstone of global TB control efforts.[89,90] However, an estimated 3 million of the 10 million people who developed active tuberculosis in 2019, were not identified and their disease outcomes remain unknown.[1] Similar numbers were reported in previous global tuberculosis reports.[91] This large case-notification gap, termed “missed cases,” comprises of both patients with active but undiagnosed tuberculosis[92,93] and some who are diagnosed but unreported especially in countries with large private healthcare sector.[94]

Underdiagnosis is most common in low-income settings where, in addition to geographical and financial barriers,[88,95] poor diagnostic tests are a major source of delay in the pathway to effective treatment.[86] The diagnostic inefficiencies threaten timely attainment of the 2035 global target for ending tuberculosis by enhancing transmission, morbidity and mortality. One of the clearest indicators of how suboptimal tuberculosis diagnostics are is that nearly half (43%) of global notifications reported to World Health Organization (WHO) are diagnosed clinically, without mycobacteriological confirmation.[1]

In recognition of diagnostic delays, high burden of undiagnosed tuberculosis, poor care seeking and access barriers, in 2013 the WHO introduced systematic screening for active disease, which is defined as the systematic identification of people with suspected active tuberculosis, in a predetermined target group, using tests, examinations or other procedures that can be applied rapidly.[96,97] A key principle of tuberculosis screening is that it must be directed towards populations with higher prevalence of disease where individual benefits are likely to outweigh risks, and delivered with patient convenience as a key priority.[96,98]

At facility level, systematic screening targets people living with HIV, previously treated for tuberculosis, the elderly, and all with any form of immunosuppression risk factor.[96] Outside health facility settings, target populations for systematic screening programmes depend on local epidemiology, but the WHO proposed several groups: 1) underserved communities in high prevalence areas, 2) residential institutions such as prisons, shelters and military, 3) immigrants and refugee camp settings, 4) key workplaces including healthcare and mines.[96]

2.4.3 Primary care diagnosis and care pathway

The primary care diagnosis and care pathway for adult presumptive tuberculosis patients starts with presentation to healthcare services, followed by the need for healthcare workers to elicit symptoms, initiate and complete tuberculosis diagnostic investigations by interpreting results and communicating to patients, before commencing and supporting completion of effective anti-tuberculosis treatment.[99] National programs and researchers monitor progress along this pathway using a tuberculosis “cascade of care” model (Figure 2.2).

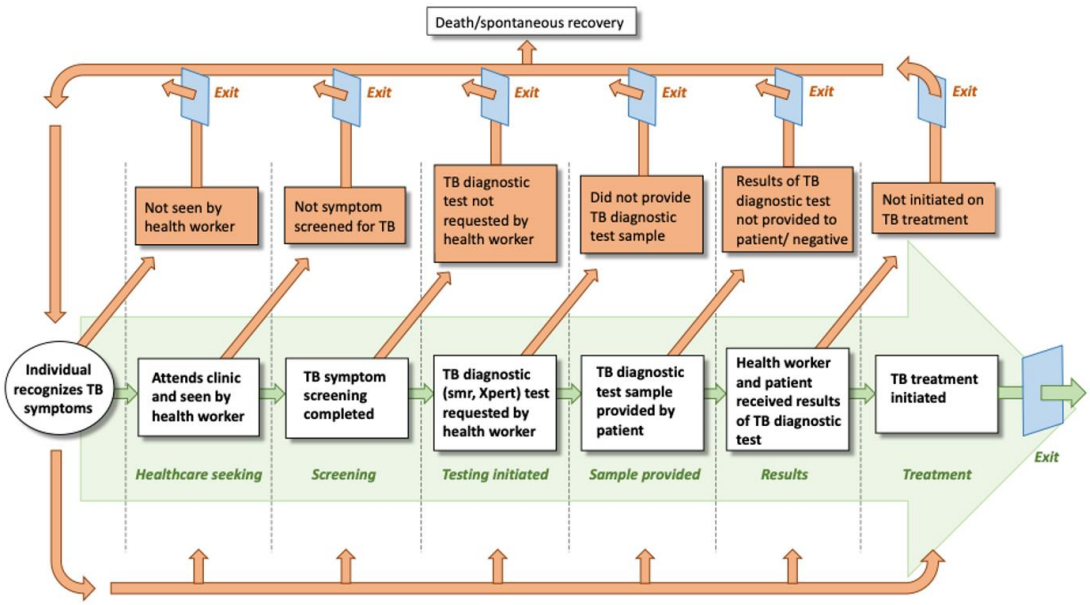


Figure 2.2 The diagnostic and care pathway for tuberculosis at health facility level, outlining opportunities for tuberculosis diagnosis and treatment in a symptomatic individual[100]

Key indicators of cascade progress include: percentage of facility attenders in whom tuberculosis symptoms are elicited; percentage of tuberculosis symptomatic individuals who are offered and complete tuberculosis diagnostic testing; percentage of patients with tuberculosis disease (identified either by diagnostic test or clinical diagnosis) who initiate tuberculosis treatment; and percentage of patients who start treatment, are retained to treatment completion and achieve recurrence-free survival for at least a year. [86,99]

2.4.4 Effectiveness of symptom screening: the entry point for all tuberculosis care

Symptom screening is currently the main triage test towards almost all of tuberculosis diagnostic and treatment efforts. The International Standards for Tuberculosis Care recommend that all patients attending a health facility with unexplained cough of two to three weeks or more should be investigated for tuberculosis.[101] However, symptoms of tuberculosis are often missed by healthcare workers,[102] leading to diagnostic and care delay.[103] The scale of missed tuberculosis symptoms is poorly defined, but thought to make a considerable contribution to tuberculosis underdiagnosis at the global level.

To establish how effectively tuberculosis symptoms are recognized and acted upon under routine programmatic conditions, I conducted a systematic review[100] collating evidence from the 48 countries that appear in the three lists of WHO-defined high-tuberculosis burden countries (HBCs) for general tuberculosis, tuberculosis/HIV and multidrug-resistant tuberculosis. Specifically, I investigated proportions of patients who successfully progress to the next stage of the pathway of care from the time they present with tuberculosis symptoms through to treatment initiation (Figure 2.2 above).

The systematic review found that tuberculosis symptom-screening, the critical entry point for diagnosis of tuberculosis, had not been done for 40% (1474/3604),[104] 50% (633/1255),[105] and 96% (407/423)[102] of symptomatic participants in the three studies that reported this outcome. This is very worrying but not surprising because it is consistent with long-standing concerns about the quality of tuberculosis care provided at primary care level facilities, with high levels of missed identification of symptoms and suboptimal management once symptoms are identified, and contributing to inefficiency in the tuberculosis diagnostic pathway.[106]

Optimising facility-based management of self-presenting patients with tuberculosis symptoms should be a priority for national tuberculosis programmes because it addresses the targeting of the “missing millions,” infection control, and complements community-based active case finding.[87] Failure to promptly identify symptomatic patients will also reduce the likely patient and public health impact of new tuberculosis diagnostics because most of the target population would simply not be offered the testing they should receive.[107]

2.4.5 Tuberculosis diagnosis in primary care settings

Following symptom screen, identified patients are offered a diagnostic test. I have described the range of available diagnostic technologies in section 2.5.3 but the most widely used assay in Africa is smear microscopy followed by Xpert MTB/RIF which is being rolled out. The WHO now recommends making a molecular assay (any of Xpert MTB/RIF, Truenat MTB or Truenat MTB Plus) as the initial diagnostic initial diagnostic tests in adults with signs and symptoms of pulmonary TB.[108]

My systematic review[100] established that the proportion of patients with tuberculosis symptoms offered a mycobacteriology test in 13 studies across high-tuberculosis

burden countries[104,109-118] achieved a study level median of only 38% (IQR: 22% to 45%, range 5% to 84%). I identified three studies that assessed proportion of participants that successfully underwent TB investigation after being offered, and they reported the following proportions: 0.50 (2/4),[102] 0.46 (61/134),[119] and 0.24 (230/932).[120]

The successive losses along the care pathway were also recently investigated in our care cascade study of a Malawian primary care setting, in which out of 256 symptomatic patients who were asked for presence of cough by their attending clinician, only 36 were asked to submit sputum, 21 submitted sputum, and only 1 received same day results (the desired outcome).[77] These findings demonstrate weaknesses and some of the missed opportunities for successful tuberculosis screening and management in primary care settings.

Apart from the broader health system and human resource capacity weaknesses, a possible explanation for the failure to offer or conduct a mycobacteriology test, may be the common practice of providing empirical antibiotics (implementing trial-of-antibiotics) to patients with respiratory symptoms before or after conducting mycobacteriology tests.[12,13,121] I have described the role of trial-of-antibiotics in section 2.2.1 and figure 2.1.

2.5 Containing the tuberculosis epidemic

Nearly 30 years ago, the global community declared tuberculosis an emergency and hoped to have ended it by now.[122] Unfortunately, global targets towards ending the tuberculosis burden have persistently been missed not because of being overly ambitious, but because of insufficient progress on the development of new diagnostics, treatment and vaccinations. Revisiting the global targets that have driven the response to the tuberculosis epidemic in the past two decades is illuminating in this respect.

2.5.1 Global targets for ending tuberculosis are dependent on timely diagnosis

After recognising neglect and slow progress, WHO member countries renewed their commitment to ending tuberculosis burden during the 44th World Health Assembly (1991) by agreeing to achieve a global case detection rate (proportion of estimated

cases that are identified) of at least 70%, and a treatment success rate of no less than 85% by 2000. The main tool for achieving the year 2000 targets was a pragmatic approach to improving “passive case-finding and treatment” exemplified by the 1993 WHO DOTS strategy,[123] which stands for Directly observed treatment-short-course. The DOTS strategy has five components, namely 1) government commitment, 2) case detection, 3) standardized treatment regimen observed by a healthcare worker for at least the first two months, 4) drug supply, and 5) standardized recording and reporting system that allows assessment of treatment results. In 1993 tuberculosis was declared a global health emergency,[124] and by 2000 most countries had adopted the DOTS strategy, which continues being the cornerstone for national programs.[125] Neither case detection nor treatment success targets were met, being 60% for case detection rate (60%) and 84% for treatment success rate [125], with the 2000 targets deferred to 2005.

The commitment to ending tuberculosis carried forward with WHO member countries adopting in 2005 the millennium declaration’s goal 6 (signed in 2000) to halt and reverse the incidence of TB by 2015 [126] through signing the “Stop TB Strategy”. The Stop TB Strategy included two targets: 1) by 2015, reduce prevalence and deaths due to tuberculosis by 50% compared with a baseline of 1990, and 2) eliminate TB as a public health problem by 2050.[127] Most countries achieved the 2015 targets giving confidence to the 67th World Health Assembly in May 2014 to launch a successor program aimed at ending the global tuberculosis epidemic by 2035.[128] The “End TB Strategy” target is to reduce incidence to less than ten new cases per 100 000 population worldwide (a 90% reduction), and tuberculosis deaths by 95%, using 2015 as baseline. Similar to the Stop TB Strategy, the End TB Strategy absorbs broader United Nations targets (Sustainable Development goals [SDGs] target 3.3) but differs from predecessor programs in that it also sets interim milestones for 2020, 2025, and 2030.

In 2018, for the first time in history, tuberculosis was the subject of the United Nations General Assembly in a meeting termed the UN high-level meeting on Tuberculosis (UN HLM TB).[129] I was privileged, as part of the UK Academics & Professionals to End TB (UKAPTB), to have actively participated in the preparation of the UN HLM TB resolution. The September 2018 resolution complemented the SDGs and End TB Strategy by establishing new targets and funding commitments for the period 2018 to 2022, although progress towards these targets has been much slower than expected.[130]

Central to achieving all the global targets for tuberculosis is timely diagnosis and prompt treatment both of which are persistently underequipped, with poor diagnostic accuracy and long treatment regimens, respectively. To date tuberculosis screening still relies largely on symptom screening as the first step towards laboratory-based tests (smear microscopy or Xpert /MTB/RIF) and trial-of-antibiotics.

2.5.2 The key elements for ending tuberculosis

Tuberculosis incidence is only declining by 2% per annum, which is far below the 10% annual decline necessary to end the epidemic by 2035.[128,131] There are several major barriers against rapid progress towards achieving a tuberculosis-free world.[131,132] First is the widespread nature of limitations to accessing prevention, diagnosis and treatment services. Addressing these access limitations will be key to achieving the commitments of the 2018 UN High level meeting which included ensuring provision of preventive therapy and treatment to 30 million and 40 million people respectively, between 2018 to 2022.[129]

The second well-recognised gap is the lack of new tools that can rapidly facilitate wider access to both prevention and treatment.[132] The key enablers include point-of-care tuberculosis diagnostics, effective vaccines and shorter treatment regimens. Table 2.1 summarises Stop TB partnership's targets for these three elements and progress so far.[132]

Table 2.1 Requirements for the accelerated development of essential new tools to end tuberculosis and current status

Required tool	Deadline	Current progress (Global TB Report 2020)
Tuberculosis vaccines ready to enter the registration process for global use	2025	14 vaccine candidates in clinical trials. Most promising is M72/AS01E which demonstrated 50% (90% CI: 12–71%) active disease prevention efficacy in people with TB infection after 3 years of follow-up.[133]
Affordable point-of-care tuberculosis diagnostics for new infections and drug resistance	2025	8 molecular rapid detection tests for tuberculosis are under WHO review. The most promising is the GeneXpert® Omni® (Omni) which includes remote functionality using an auxiliary battery for power and a tablet for data transfer.[134]
A 2-month or less oral cure for tuberculosis and its drug resistant forms	2028	22 drugs in Phase II or III trials. The most promising is a 4 month regimen from TBTC Study 31/A5349 study, a combination of high-dose rifapentine, isoniazid, pyrazinamide and moxifloxacin, has achieved non-inferiority against currently standard six-month regimen in a phase III study.[22]

2.5.3 The status of diagnostic tools and place for trial-of-antibiotics

Original use of trial-of-antibiotics was designed based on the diagnostic performance of light microscopy. The past two decades have seen improvements in microscopy and development of new technologies for detecting tuberculosis. Notable tuberculosis diagnosis advances include Fluorescent light-emitting diode (LED) microscopy,[135] Xpert MTB/RIF[136,137] and Xpert MTB/RIF Ultra[138] by Cepheid, loop-mediated isothermal amplification (TB-LAMP),[139] lateral flow lipoarabinomannan (LAM),[140] Truenat (MTB, MTB Plus and MTB-RIF) by MolBio Diagnostics,[141] and Computer-aided detection software (CAD) for automated interpretation of chest radiography.[142]

Despite these improvements, there has been no breakthrough equivalent of the highly sensitivity and specific device-free point of care lateral flow assays that exist for HIV diagnosis and are already available for SARS-CoV-2.[143] In consequence, barriers accessing tuberculosis diagnosis remain substantial and trial-of-antibiotics remains part of tuberculosis screening in outpatient settings in low- and middle-income countries. While trial-of-antibiotics does not fulfil the described definition of point of care diagnostic, the ease with which prescription fit into the high throughput of consulting rooms, may make it more attractive to clinicians for an initial or follow up diagnostic. Research and development efforts for high throughput true point-of-care diagnostics for tuberculosis are urgently needed.[144]

2.6 Containing the antimicrobial resistance epidemic

The goal of this section is to contextualise measures of containing antimicrobial resistance at primary care level with a historical and global perspective. The global nature of antimicrobial resistance is well recognised, and so is the need for urgent and effective national and global action.[145]

2.6.1 Global frameworks for containing antimicrobial resistance

In recognition of the escalating threat and impending global impact, WHO member countries passed resolution WHA51.I7 on containing antimicrobial resistance during the 51st World Health Assembly (1998).[146] Specifically, the seven point plan encouraged member states to develop sustainable surveillance systems, educational programs, infection prevention platforms, prescription controls, and legislative measures.[146] This was the first World Health Assembly resolution on antimicrobial resistance and was followed by several others with global policy efforts peaking in 2015 with the endorsement of the Global Action Plan on Antimicrobial Resistance.[2]

The five strategic objectives which the global action plan put forward are similar to resolution WHA51.I7 of 1998, but this time member states were asked to develop national action plans by 2017.[2] The World Health Organization, the Food and Agriculture Organization, and the inter-governmental World Organisation for Animal Health have over the years intensified coordination of efforts signifying the connectedness of the problem and solutions as advocated in the one health

approach.[147] In 2016, the global action plan was endorsed by the United Nations General Assembly at the first high level meeting on antimicrobial resistance.[148]

To sum up, the global response to antimicrobial resistance depends on successful implementation of national action plans and global collaboration grounded in the following principles:[149] 1) raising awareness and achieving behaviour change, 2) strengthening knowledge and evidence through surveillance, 3) responsible use of antimicrobials, 4) infection prevention and control measures, 5) strengthening regulatory frameworks, 6) financial resources and the economic case for investments in combating antimicrobial resistance, 7) strengthening public-private partnerships to promote research and development.

2.6.2 Containing antimicrobial resistance in primary care settings

Elements of the global framework that directly speak to health care settings and clinicians can be summarised from the 2019 report of the United Nations Secretary-General[149] as awareness, professional education, surveillance, infection prevention, immunisation, and optimised antimicrobial use. The WHO has developed a competency framework for health workers' education and training on antimicrobial resistance to ensure that health workers acquire the necessary prevention knowledge and skills.[150] The value of a range of behaviour change interventions for health workers, care givers and the public, has been demonstrated in previous observational and experimental studies.[151-155]

All global frameworks for combatting antimicrobial resistance mention surveillance as a critical element, yet it remains either absent or severely under-resourced in most low-income countries.[156-158] Investment in infection prevention is also mostly suboptimal in low income countries, and is compounded by the broader water, sanitation, and hygiene challenges.[159]

Immunisations reduce incidence of infection and the demand for antimicrobials.[160,161]. Although immunisation uptake is generally on the increase, coverage varies among and within countries.[162,163] Primary care centres and community health workers play a vital role in ensuring that their catchment population accesses available immunisations

Reducing inappropriate antibiotic use in primary care requires a detailed understanding of the determinants: diagnostic uncertainty, clinician, and patient behaviour are the key factors. Clinician, and patient behaviour have been addressed under education and behavioural change interventions. Eliminating diagnostic uncertainty has been shown to reduce unnecessary antimicrobial prescribing in primary care settings.[52,72]

In circumstances where a diagnostic tool is unavailable, common for primary care centres in low income settings and for diseases such as tuberculosis, alternatives are urgently needed. Scoring systems such as the Centor score,[164,165] CURB 65[166] and FeverPAIN score[167] have been used but their predictive potential is far from perfect.[168] An ongoing cluster randomised trial in Switzerland is evaluating utility of a computerised decision support system linked to medical records, as an intervention to minimise antimicrobial prescribing.[169] Another antimicrobial stewardship strategy that has been used is delaying antimicrobial prescriptions, but applicability beyond mild illnesses would be challenging.[170,171]

2.7 Appropriateness of trial-of-antibiotics: outstanding questions

Two decades after introduction as a placeholder for better diagnostics, now may be the right time to re-examine the continued role of trial-of-antibiotics. Having described trial-of-antibiotics in the context of tuberculosis diagnosis and the potential for widespread antimicrobial resistance consequences, three key questions arise: 1) whether it offers the intended diagnostic value to tuberculosis screening algorithms, 2) whether it contributes to antimicrobial resistance, and 3) whether there are other clinical benefits that should be considered. In this thesis, I use a systematic review and a randomised controlled trial to investigate these three key questions.

2.7.1 How accurate is trial-of-antibiotics as a diagnostic?

As an approach that is being used on a large scale, trial-of-antibiotics should ideally have a strong evidence-base of how much diagnostic value it brings to the tuberculosis diagnostic algorithm.[172,173] This should be among the most important considerations when deciding whether it is worth the trade-off with potential for antimicrobial resistance. Such evidence could come from either a randomised controlled trial or several well-designed prospective studies.[172-174] Other related

outstanding questions relate to 1) the choice of antibiotics, apart from the recommendation to avoid those with anti-tuberculosis activity (like fluoroquinolones), and 2) the definition for clinical resolution when determining the outcome of trial-of-antibiotics.

2.7.2 Is trial-of-antibiotics a significant driver of antimicrobial resistance?

Antimicrobial resistance relating to antibiotic use during evaluation for presumed tuberculosis has not been investigated before. However, previous work has shown that empirical antibiotics can drive rapid emergence of antimicrobial resistance.[175,176] Co-trimoxazole prophylaxis for HIV-positive patients, introduced in 2005, was followed by near-universal resistance in bloodstream infections by 2010.[177] Mass drug administration of azithromycin for trachoma control initially reduces nasopharyngeal carriage of *Streptococcus pneumoniae*, but with increased macrolide-resistance 6 months later.[178,179]

2.7.3 Are there benefits of antibiotics beyond diagnostic role?

Empirical antibiotics during tuberculosis investigations could be life-saving especially in areas of high HIV prevalence where mortality immediately before and after tuberculosis diagnosis is high, [81,180] and is often secondary to severe bacterial infections.[180-182] The leading aetiologies of infection and death on tuberculosis treatment as well as among outpatients with tuberculosis-like symptoms are *Streptococcus pneumoniae* and non-typhoidal salmonellae: both can present with cough (primary cause) or as co-morbidities (super-infections) in patients presenting with active *Mycobacterium tuberculosis* disease.[180-182] If effective treatment of this type of life-threatening primary/super-infection reduces mortality during the diagnostic work-up of suspected TB in people living with HIV (PLHIV), then empirical use of broad-spectrum antibiotics would be indicated for this purpose alone, irrespective of any diagnostic contribution to TB treatment decisions.

2.8 The study setting: Blantyre, Malawi

2.8.1 Organisation of the health system

The Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB) randomised trial was conducted at Limbe and Ndirande health centres in Blantyre city, Malawi. Blantyre city has a population of 800,264 and is located in the southern part of Malawi (population 17,563,749, median age 17 years).[183] Malawi has a three-tier health system with the bulk of primary care provided at either health centres or rural hospitals, secondary care provided by 28 district hospitals, and tertiary care offered at four referral hospitals.[184] Most of the health care is provided by public facilities. The private health sector is very small and mostly present in cities.[184] Most of the tuberculosis diagnosis, treatment and follow up is provided by the public health sector.[184] Screening and treatment guidelines are developed by the Malawi National Tuberculosis Program and are usually in line with the most recent WHO guidance.[14]

2.8.2 Tuberculosis screening and treatment

The estimated national tuberculosis prevalence from a 2015 survey was 452 per 100,000 (95% CI: 312 to 593).[185] National guidelines recommend that all patients attending a health facility with cough of any duration be investigated for tuberculosis.[186] The initial diagnostic options are Xpert /MTB/RIF and smear microscopy with 24 hours as the target turnaround time, [186] but this is often ≥5 days. Most primary care facilities have smear microscopy but Xpert MTB/RIF is becoming more widely available. Targeted urban community screening uses mobile chest radiography read by Computer-Aided Detection for TB (CAD4TB) software and confirmed by Xpert /MTB/RIF. The recommended first-line treatment for drug-susceptible tuberculosis is a 6-month regimen of isoniazid, rifampicin, ethambutol and pyrazinamide and is provided at a primary care level.[186] Second-line tuberculosis treatment is initiated at secondary care level, with post-hospital follow up conducted by primary healthcare teams.[186]

2.8.3 The reciprocal decline of HIV and tuberculosis incidence

Similar to most of sub-Saharan Africa, HIV is the main risk factor for tuberculosis in Malawi. The 2019 national HIV prevalence in adults aged 15 to 49 years was

estimated to be 8.9% (95% CI: 7.6%,9.6%).[187] Malawi has been performing well towards the UNAIDS Fast Track Strategy targets, with 2019 statistics showing that 90% of all people living with HIV knew their status, 88% of all people diagnosed with HIV infection were on antiretroviral therapy (ART), and 92% of all people receiving ART had achieved viral suppression.[187,188] The progress on the HIV epidemic is also reflected in the consistent decline of incidence from 4.25 per 1000 person years (95% CI: 3.94, 4.62) in 2010, 2.77 (95% CI: 2.5, 3.05) in 2015, to 1.94 (95% CI: 1.62, 2.25) in 2019 (Figure 2.3).[187]

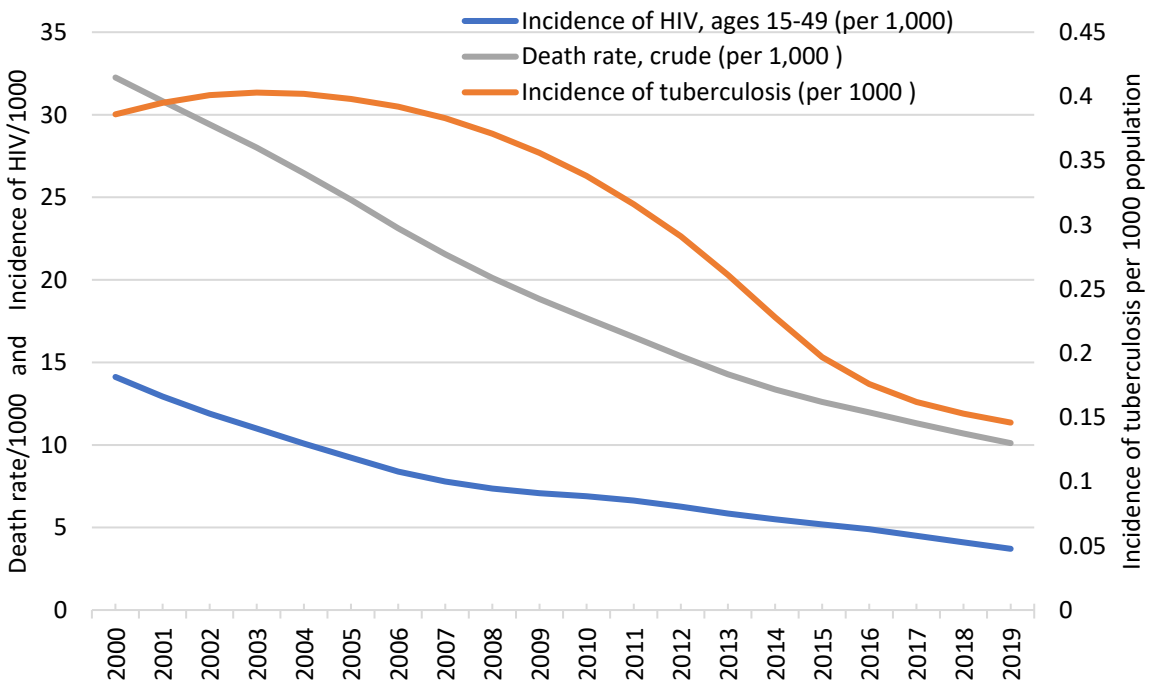


Figure 2.3 Epidemiology of HIV, tuberculosis, and mortality in Malawi

The successes in the HIV epidemic are thought of as the main contributor to proportional declines in general death rate and tuberculosis incidence in Malawi (figure 2.3).[189,190] The decline in mortality is also thought to be secondary to the strong integration of HIV and tuberculosis care, which improves coverage of both services.[191] Systematic screening and linkage for HIV in tuberculosis patients and of tuberculosis in people living with HIV, is part of routine care for both diseases. Longitudinal evaluation of routine data between 2008 and 2017 showed 1) improving proportion of people with HIV/TB co-infection already on ART by the time they start tuberculosis treatment (21% in 2008 to 81% in 2017), 2) decrease in tuberculosis incidence among people on ART (6.0 per 100 person-years in 2008, to 1.1 per 100 person-years in 2013).[192]

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3 A systematic review and meta-analysis of the use of broad-spectrum antibiotic treatment to exclude pulmonary tuberculosis in adults

3.1 Introduction

I conducted a systematic literature review to collate available evidence on the performance of trial-of-antibiotics as a diagnostic test and to explore the timing, interpretation, and decision-making process. The primary objective was to determine the sensitivity and specificity of using a trial-of-antibiotics compared to sputum mycobacteriology for diagnosis of pulmonary tuberculosis. I registered the systematic review protocol with PROSPERO (CRD42017083915) and published it with *Systematic Reviews,* prior to implementation. I have included the published systematic review protocol as an appendix to this thesis. The results manuscript was submitted to *The Lancet Infectious Diseases* in March 2019 and published in May 2020 as described below.

3.2 Manuscript of the systematic review and meta-analysis

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SECTION A – Student Details

Student ID Number	1700548	**Title**	Dr
First Name(s)	Titus, Henry		
Surname/Family Name	Divala		
Thesis Title	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)		
Primary Supervisor	Professor Katherine Fielding		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	THE LANCET Infectious Diseases		
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SECTION E

Student Signature	
Date	29 December 2020

Supervisor Signature	
Date	23Feb2021

Utility of broad-spectrum antibiotics for diagnosing pulmonary tuberculosis in adults: a systematic review and meta-analysis

Titus H Divala, Katherine L Fielding, Chikondi Kandulu, Marriott Nliwasa, Derek J Sloan, Ankur Gupta-Wright, Elizabeth L Corbett

Summary

Background Suboptimal diagnostics for pulmonary tuberculosis drive the use of the so-called trial of antibiotics, a course of broad-spectrum antibiotics without activity against *Mycobacterium tuberculosis* that is given to patients who are mycobacteriology negative but symptomatic, with the aim of distinguishing pulmonary tuberculosis from bacterial lower respiratory tract infection. The underlying assumption—that patients with lower respiratory tract infection will improve, whereas those with pulmonary tuberculosis will not—has an unclear evidence base for such a widely used intervention (at least 26·5 million courses are prescribed per year). We aimed to collate available evidence on the diagnostic performance of the trial of antibiotics.

Methods In this systematic review and meta-analysis we searched the MEDLINE, Embase, and Global Health databases for studies published up to March 15, 2019, that investigated the sensitivity and specificity of the trial of antibiotics against mycobacteriology tests in adults (≥15 years) with tuberculosis symptoms. We used the QUADAS-2 tool to assess the risk of bias. We estimated pooled values for sensitivity and specificity of trial of antibiotics (as the index text) versus mycobacteriology tests (as the reference standard) using random-effects bivariate modelling, and we used the I^2 statistic to assess heterogeneity between studies contributing to these estimates. This study is registered with PROSPERO, number CRD42017083915.

Findings Of the 9410 articles identified by our search, eight studies were eligible for inclusion. The studies were from seven countries in Africa, South America, and Asia, and involved 2786 participants. Six studies used mycobacterial culture as the reference standard, and six used penicillins for the trial of antibiotics. The treatment duration, number of antimicrobial courses, and definition of what constituted response to treatment varied substantially between studies. The pooled sensitivity (67%, 95% CI 42–85) and specificity (73%, 58–85) of the trial of antibiotics versus mycobacteriology tests were below internationally defined minimum performance profiles for tuberculosis diagnostics and had substantial heterogeneity (I^2 was 96% for sensitivity and 99% for specificity). Each included study failed on one or more domain of the QUADAS-2 tool.

Interpretation Current policy and practice regarding the trial of antibiotics appear inappropriate, given the weak evidence base, poor diagnostic performance, potential contribution to the global antimicrobial resistance crisis, and adverse individual and public health consequences from the misclassification of tuberculosis status. Antibiotic strategies during tuberculosis investigations should instead optimise clinical outcomes, ideally guided by clinical trials in both inpatient and outpatient groups.

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Introduction

Tuberculosis is the leading cause of adult mortality due to infectious disease, with 10 million new cases and 1·6 million deaths annually,[1] but it is curable when correctly diagnosed in a timely manner. However, current diagnostics are suboptimal, missing many cases.[2] Recognising the limitations of current diagnostic tests, the standard diagnostic algorithms that are endorsed by WHO[3,4] and that have been routinely promoted by national tuberculosis programmes[5] include the level of response to a course of broad-spectrum antibiotics as a means of excluding (or including) tuberculosis as a cause of symptoms. The course of broad-spectrum antibiotics, commonly referred to as a trial of antibiotics, has negligible activity against *Mycobacterium tuberculosis* (MTB) and is given to symptomatic patients with negative sputum mycobacteriology (panel, appendix pp 3–4).[6] Patients with negative sputum mycobacteriology whose symptoms respond to the antibiotic treatment are considered tuberculosis negative, whereas those who remain symptomatic are deemed in need of further evaluations, potentially leading to tuberculosis treatment.[6,7]

See Online for appendix

We estimated conservatively that at least 26·5 million courses of antibiotics are prescribed in the course of diagnosing 5·3 million smear-negative tuberculosis registrations per year, which raises concerns about the

Research in context

Evidence before this study

Antimicrobial resistance and tuberculosis are both serious threats that together cause 2·5 million deaths each year, are part of the 2030 agenda for sustainable development, and are two of only five health issues to ever secure a dedicated United Nations High Level Meeting. Apart from drug-resistant tuberculosis, a less discussed but key overlap between these two threats is that tens of millions of doses of broad-spectrum antibiotics are used in the diagnostic work-up for tuberculosis, with the so-called trial of antibiotics probably being the most used tuberculosis diagnostic globally. The trial of antibiotics reflects the suboptimal nature of current tuberculosis diagnostics, which miss a substantial fraction of tuberculosis cases. The underlying assumptions are that symptoms that respond to antibiotics are attributable to other respiratory infections (assumed to be sensitive to the broad-spectrum antibiotic used), whereas non-responsive symptoms are likely to be due to tuberculosis.

Two previous systematic reviews documented the role of broad-spectrum antibiotics in the diagnosis of tuberculosis, although neither addressed their specific diagnostic value. The scarcity of evidence in this area was first highlighted in the 2007 WHO guidelines on tuberculosis diagnosis in HIV-prevelant and low-resource settings, which recommended the use of antibiotics in patients with HIV to treat presumptive bacterial infections, but not for diagnostic purposes. The 2018 WHO recommendations, however, retain response to antibiotic treatment as a key part of clinical evaluation of patients both with and without HIV following a negative Xpert MTB/Rif test.

Added value of this study

To our knowledge, this is the first systematic review and meta-analysis, and the most comprehensive assessment, of the performance of the trial of antibiotics in tuberculosis diagnostic algorithms. Our study shows little evidence to support the continued implementation of the trial of antibiotics.

The available studies are few in number, of poor quality, and do not use standardised methodologies, leading to high interstudy heterogeneity. The pooled sensitivity (67%, 95% CI 42–85; I^2=96%) and specificity (73%, 58–85; I^2=99%) of the trial of antibiotics versus sputum mycobacteriology were both below internationally defined minimum performance profiles for tuberculosis diagnostics.

Implications of all the available evidence

The trial of antibiotics, despite being part of global recommendations for over three decades, has yet to be supported by evidence. The poor diagnostic performance, potential to increase antimicrobial resistance, and public health consequences of the misclassification of tuberculosis status warrant urgent and well designed prospective trials.

contributions of this practice to antimicrobial resistance.[8] This estimate assumes an average of five antibiotic courses per treatment initiation for a sputum-negative patient, including two courses given to the patient before tuberculosis treatment and three more given when tuberculosis is ruled out by the patient's response to antibiotics.[5,7] Despite the widespread use of the trial of antibiotics, no systematic review has focused on its diagnostic performance.

Other important evidence gaps concern the choice of antibiotics for the trial of antibiotics (except for the advice to avoid those with known anti-tuberculosis activity), the duration of treatment, the number of antibiotic trials, and the definition of treatment response. The inadequate consolidation of evidence in these areas is reflected in pronounced variations in how the trial of antibiotics is implemented across national programmes.[5]

The poor evidence on the use of the trial of antibiotics is also reflected in WHO recommendations, which evolved from bold recommendation of a routine trial of antibiotics in 1997[3] to more cautious language in 2018.[4] The 1997 WHO guidelines[3] included the absence of a clinical response after 1 week of broad-spectrum antibiotics as part of the case definition for smear-negative tuberculosis. 10 years later, in 2007, the guidelines for people living with HIV or AIDS called for more research into the diagnostic benefit of the trial of antibiotics and recommended that the primary role of antibiotics should not be as a diagnostic aid but as treatment for concomitant bacterial infection.[9] After another decade, and in the context of growing concern about antimicrobial resistance, the 2018 WHO model algorithms still support the trial of antibiotics (appendix p 3).[4] In practice, national guidelines and routine clinical practice in low-income settings still follow the 1997 approach to the trial of antibiotics (appendix p 4).

The objective of this systematic review was to assess existing evidence for the diagnostic accuracy (sensitivity and specificity) of the trial of antibiotics compared with sputum mycobacteriology tests for the diagnosis of tuberculosis. We also describe the choice of antibiotic, duration of treatment, and definition of post-treatment improvement.

Methods

Search strategy and selection criteria

For this systematic review and meta-analysis, we searched MEDLINE, Embase, and Global Health using the Ovid platform for studies published up to March 15, 2019, when the search was run. The search strategies are described in the appendix (pp 1–2). We included all studies published in any language that included adults (≥15 years) who were being investigated for pulmonary tuberculosis, which reported outcomes of both a trial of antibiotics and mycobacteriology investigations as part of a standardised diagnostic work-up.

Acceptable study designs were cross-sectional, cohort, or randomised controlled trials. To be eligible, studies had to recruit adults on the basis of symptoms suggestive of tuberculosis (with or without a preceding chest radiograph), include a trial of antibiotics as the index test and any sputum-based mycobacteriology test as the reference test, and report the proportions of participants whose mycobacteriology tests were positive or negative who were correctly or incorrectly identified through a trial of antibiotics (ie, both sensitivity and specificity).

The protocol for this systematic review, including detailed methods, is published elsewhere.[10] This study is registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42017083915. We prepared our study protocol, performed the systematic review, and prepared the report according to recommendations by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[11]

Data extraction

Two reviewers independently screened the titles and abstracts of the articles identified through the electronic searches against the eligibility criteria: THD and MN assessed articles published from Jan 1, 1993, to March 15, 2019; and on Aug 6, 2019, following the advice of a peer reviewer, THD and CK assessed all articles indexed by the selected databases up to Dec 31, 1992. THD, MN, and CK independently assessed the full texts of the included papers, documented the reasons for non-inclusion, and identified additional articles from reference lists. KLF resolved disagreements in eligibility. Huan Zhang and Mengyun Liu (London School of Hygiene & Tropical Medicine, London, UK) independently assessed the full texts of Chinese-language articles. THD, MN, and CK extracted data from the eligible articles into an Excel database and resolved discrepancies by consensus.

The following data were extracted from eligible papers: first author, year of publication, country of data collection, antibiotics used for the trial of antibiotics, duration of antibiotic treatment, method of assessing response to antibiotic treatment, reference mycobacteriology tests, and number of patients given both a trial of antibiotics and a mycobacteriology reference test. Articles were defined as eligible for meta-analysis estimation of sensitivity and specificity if they provided data on numbers of patients that were true positives, false positives, false negatives, and true negatives. For studies with missing or incomplete information for the meta-analysis, we contacted the authors for data. In cases where data were unavailable, we included in narrative synthesis as much information as the study could provide.

Assessment of study bias

We assessed risk of bias at the level of the study using QUADAS-2 (University of Bristol, Bristol, UK), the recommended tool for evaluating primary studies for inclusion in systematic reviews involving assessment of diagnostic accuracy.[12] We assessed the risk of bias and applicability concerns using four domains: patient selection, index test, reference standard, and patient flow and timing of tests. The level of risk or concern was reported as either high, low, or unclear.

Panel: Antibiotics as diagnostics for tuberculosis

Tuberculosis should be investigated in all patients presenting with respiratory symptoms using sputum-based tuberculosis diagnostic tests (smear microscopy or Xpert MTB/Rif). However, negative results on these tests do not rule out tuberculosis. The 2018 WHO model diagnostic algorithm (appendix p 3) advises clinical re-evaluation of patients with negative sputum results, with suggestions of "chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Xpert MTB/RIF testing, or culture".[4] Of these options, clinical response to broad-spectrum antimicrobial agents, the so-called trial of antibiotics, has long been the priority for national programmes in resource-limited settings (appendix p 4).

The trial of antibiotics serves two distinct goals: first, to empirically treat bacterial respiratory tract infections using one or more antibiotics with minimal or no anti-mycobacteriological activity; and second, to use the response to treatment to determine the need for further tuberculosis investigations, assuming that illness due to active tuberculosis will not respond. The focus of this systematic review is on the second diagnostic goal, whereby a trial of antibiotics is used to distinguish tuberculosis from other infectious causes of respiratory illness.

Meta-analysis

We included in the meta-analysis all studies that provided data that allowed us to calculate sensitivity and specificity of a trial of antibiotics against a reference standard of mycobacteriology tests. The meta-analysis was done using MIDAS (version 15.0),[13] which uses joint modelling of sensitivity and specificity. We estimated point estimates and 95% CIs for sensitivity and specificity for each study and for pooled data using bivariate random effects modelling.

To provide an inference of diagnostic quality, we plotted a summary receiver operating characteristic curve, in which the diagnostic accuracy of the trial of antibiotics was estimated by the area under the curve and the summary operating point.

We assessed heterogeneity across studies using the I^2 statistic, and we used a bagplot to examine the spread of the observed data and identify outliers. We examined clinical utility of trial of antibiotics using a Fagan plot, and we used the Deeks funnel plot to identify evidence of publication bias in studies of diagnostic performance.

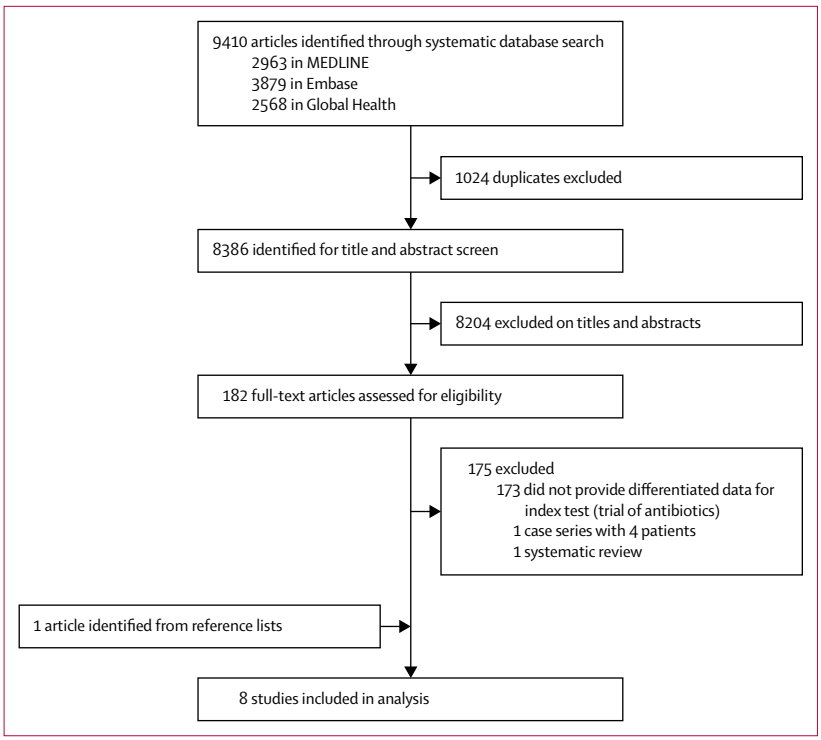

Figure 1: **Study selection**

We did subgroup and sensitivity analyses. For the subgroup analysis, we used univariate meta-regression. Our a-priori subgroups were study setting (whether a study was done in sub-Saharan Africa) and reference test (whether the study used MTB culture as the reference standard). In a post-hoc analysis, we stratified the data by use of chest radiography (in addition to tuberculosis symptoms) for pre-screening. For the sensitivity analyses, we restricted the meta-analysis to high-quality studies (showing high risk of bias in no more than one domain of QUADAS-2).

Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or in the writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We identified 9410 articles from the electronic searches, which reduced to 8386 after removing duplicates and to 182 after screening of the title and abstract (figure 1). After a full-text review, seven articles were included in the systematic review, which increased to eight following review of reference lists (figure 1).

The eight eligible studies were published between 1997 and 2016 and included 2786 participants from seven countries in Africa,[7,14–17] South America,[18] and Asia[19,20] (table). Seven studies evaluated participants in hospital settings or in clinics specialised in care of patients with HIV and tuberculosis. Two studies recruited only participants who were HIV-positive and one was restricted to participants who were HIV-negative. In all studies, the trial of antibiotics was used in a pre-screened population who tested tuberculosis-negative by smear microscopy. In addition to microscopy, three studies required a chest radiograph, but each of these excluded patients on the basis of a different radiographical finding: either features that were consistent with acute pneumonia,[17] suggestive of respiratory diseases other than tuberculosis or other pathologies such as cardiac disorders,[7] or suggestive of tuberculosis.[14] Six studies used MTB culture as their reference diagnostic test, with samples collected from smear-negative participants at baseline, before antibiotics were prescribed. The remaining two studies[15,20] first prescribed antibiotics to smear-negative participants at baseline and then collected sputum for a combination of MTB culture and smear microscopy (the reference standard) on the same day as evaluation for treatment response (index test outcome).

The choice of antibiotics for the trial of antibiotics varied across the studies, and four studies used more than one type of antibiotic. The most common class in the eight studies was penicillin, reported in six of the eight studies (table). Other antibiotic classes included macrolides in three studies, tetracyclines in two, and cephalosporins in one. The duration of treatment was also variable, ranging from 5 days to 14 days. Participants were assessed for their response to antibiotic treatment between 5 days and 14 days from the start of treatment. Although most studies implemented a single course of antibiotic treatment, two of them used two courses. One of these studies involved assessing the response to treatment before prescribing the second course,[7] whereas the other study asked participants to return for assessment only after completing both courses.[19]

There was no consistent definition of the response to treatment, and approaches ranged from using self-reported improvement to using a combination of clinical and radiological assessments (table). The approaches for measuring the response to treatment were largely subjective in all studies. One study included in their definition for the outcome "a negative smear on day 14".[19] The treatment response evaluation approaches were more rigorous in studies involving hospitalised participants. For example, Wilkinson and colleagues[7] used changes in cough, the amount of sputum produced, and body temperature as reported by a nurse. One study did not report how response to treatment was assessed.[17]

All eight studies had disaggregated data, which allowed estimation of the pooled sensitivity and specificity of the trial of antibiotics compared with a mycobacteriology reference (MTB culture, smear microscopy, or both). The unadjusted individual study estimates for both specificity and sensitivity were not consistent across the studies

	Setting	Study design	Proportion HIV positive	Screened population*	Pre-screening assessments*	Reference diagnostic test	Antibiotic, dose, and duration	Follow-up (days from baseline)	Definition of clinical response	Contribution to review population (n=2786)
Wilkinson et al (1997)[17]	South Africa; hospital inpatients	Cohort	58% of target population, but study-specific proportion not reported	≥3 weeks' cough and sputum production, weight loss, night sweats, or chest pain	Three negative smears and chest x-ray; patient excluded if clinical and radiological features of acute pneumonia were present	MTB culture (Lowenstein–Jensen and Middlebrook 7H11 agar)	Ampicillin 500 mg four times daily for 7–10 days	Not reported	Not reported	237 (9%)
Wilkinson et al (2000)[7]	South Africa; hospital inpatients	Cohort	70%	≥3 weeks' respiratory symptoms (cough, chest pain, sputum production, shortness of breath, tachypnoea, or haemoptysis) and an abnormal chest x-ray compatible with tuberculosis; or community-acquired pneumonia (acute cough, fever, and sputum production) that did not respond to antibiotic treatment taken as an outpatient	Three negative smears and chest x-ray; patient excluded if clinical and radiological features consistent with other respiratory infections or cardiac pathologies were present	MTB culture (Lowenstein–Jensen and Middlebrook 7Hll agar)	Amoxycillin 500 mg three times daily for 5 days (erythromycin 500 mg four times daily given if no improvement from amoxycillin)	5	Patients met all four criteria: (1) cough ceased or substantially decreased (reported by both nurse and patient); (2) sputum production ceased or substantially decreased (measured in sputum container); (3) apyrexial for 48 h (measured on temperature chart); and (4) judgment by attending clinician, including above and change in pulse and respiratory rates	120 (4%)
Kudjawu et al (2006)[15]	Guinea; primary care clinic	Cohort	15%	≥3 weeks' cough; patient excluded if they were previously diagnosed with chronic lung disease, had received more than 72 h treatment for the acute condition that prompted consultation,or had a history of tuberculosis	Three negative smears	Smear microscopy (Ziehl–Neelsen and phenolauramine) or MTB culture (culture type not reported)	Amoxicillin 1500 mg daily for 10 days	14	Clinical definition: diminished cough, defervescence, and improved wellbeing; radiographical definition: appreciable clearing on day 14 film of densities noted on day 1 film	359 (13%)
Siddiqi at al (2006)[20]	Pakistan; tuberculosis clinic at referral hospital	Cohort	Not reported	≥3 weeks' cough; patient excluded if they had a history of tuberculosis or were on anti-tuberculosis therapy	Three negative smears	Smear microscopy or MTB culture (culture type not reported)	Penicillin or macrolide (dose not reported) for 7–10 days	7 to 10	Clinical judgment of a study-trained physician (no specific definition provided)	1000 (36%)
Soto et al (2011)[18]	Peru; referral hospital (26% inpatients; 74% referred from peripheral centres)	Cohort	0%	≥2 weeks' cough plus at least one of dyspnoea, thoracic pain, fever, night sweating, or weight loss	Three negative smears	MTB culture (Ogawa, Middlebrook 7H9 media, and mycobacteria growth indicator tube)	Doxycycline 100 mg twice daily for 10 days	14	Reduction or resolution of constitutional and respiratory symptoms plus resolution of signs at clinical examination	264 (9%)

(Table continues on next page)

	Setting	Study design	Proportion HIV positive	Screened population*	Pre-screening assessments*	Reference diagnostic test	Antibiotic, dose, and duration	Follow-up (days from baseline)	Definition of clinical response	Contribution to review population (n=2786)
(Continued from previous page)										
Huerga et al (2012)[14]	Kenya, tuberculosis clinic at referral hospital	Cohort	68%	≥2 weeks' cough; patient excluded if they had taken fluoroquinolones or anti-tuberculosis drugs in the past month	Two negative smears and chest x-ray; patient excluded if chest x-ray suggested tuberculosis or if patient was in severe clinical condition	MTB culture (Lowenstein–Jensen and thin layer agar)	Amoxicillin 1 g three times daily for 5 days	5	Resolution judged as either complete resolution (resolution of all clinical symptoms with a normal physical examination), partial resolution (improvement with persistence of clinical symptoms or signs), or no resolution (absence of improvement or clinical worsening)	285 (10%)
Padmapriyadarsini et al (2013)[19]	India; network of HIV clinics	Cohort	100%	≥2 weeks' cough or fever in the past ≥2 weeks, or both	Three negative smears	MTB culture (Lowenstein–Jensen)	Amoxicillin 500 mg every 6 h for 7 days, followed by doxycycline 100 mg twice daily for 7 days	14	Patients considered not to have tuberculosis if they met all three criteria: (1) none or improved symptoms (cough or fever), (2) normal chest x-ray, and (3) negative sputum smears after 14 days	440 (16%)
Walusimbi et al (2016)[16]	Uganda; HIV clinic	Cohort	100%	≥2 weeks' cough or fever, or noticeable weight loss or excessive night sweats; patient excluded if they were on quinolone medication	Two negative fluorescent tuberculosis microscopy tests, and negative GeneXpert	MTB culture (mycobacteria growth indicator tube)	Macrolides and cephalosporins (dose and duration not reported)	14	Self-reported absence of symptoms to clinical staff	81 (3%)

MTB=*Mycobacterium tuberculosis*. *Screened population refers to the eligibility criteria for the part of the study in which the index test was evaluated; pre-screening tests were done for eligible patients before the index test.

***Table:* Characteristics of included studies**

(figure 2). Point estimates for sensitivity in the eight studies ranged from 15% to 97% (sample size range three to 235; median 56) and for specificity ranged from 41% to 96% (sample size range 66 to 905; median 188). Compared with mycobacteriology tests, the pooled sensitivity of the trial of antibiotics was 67% (95% CI 42–85; I^2=96%) and the pooled specificity was 73% (58–85; I^2=99%). The area under the summary receiver operating characteristic curve was 0·77 (95% CI 0·73–0·80; figure 3).

In subgroup analyses, pooled estimates of sensitivity and specificity by study setting and reference standard definition still showed substantial heterogeneity (appendix p 12), although these analyses should be interpreted with caution because of the small number of included studies. Sensitivity was lower and specificity higher in studies that used MTB culture alone for the reference standard, although again these need to be interpreted with caution considering the small numbers. Fagan's nomogram showed that if the prevalence of pulmonary tuberculosis is 20%, the trial of antibiotics would increase the probability of correctly detecting mycobacteriology-positive pulmonary tuberculosis in the study population by an absolute value of 19% (from a pre-test probability of 20% to a post-test probability of 39%). When participants reported resolution of symptoms after a course of antibiotics (ie, testing tuberculosis-negative for the trial of antibiotics), the probability that they could nonetheless have mycobacteriology-positive pulmonary tuberculosis was 10% (appendix p 5). The Deeks' funnel plot for the eight studies included in our meta-analysis indicated that there was no evidence of publication bias (p=0·84 for Deeks' funnel plot asymmetry test; appendix p 11).

Studies within the 95% confidence bounds of the median distribution in the bagplot were not clustered together (appendix p 6). There were two outliers (the 2006 study in Guinea[15] and the 2012 study in Kenya),[14] but excluding these studies from the meta-analysis in a post-hoc sensitivity analysis did not account for the substantial heterogeneity of the full model (appendix p 12). Of note, the 2012 Kenya study categorised outcomes of antibiotic treatment as either complete resolution, partial resolution, or no resolution (table) and considered only complete (not partial) resolution as improvement. However, to be consistent with the definitions used in the other eligible studies, we re-categorised the data from the 2012 Kenya study such that clinical improvement referred to any improvement (either partial or complete resolution) and no improvement referred to no resolution. Using the authors' definitions did not significantly change the pooled estimates for sensitivity and specificity.

Evaluating our main question (of the diagnostic accuracy of the trial of antibiotics compared with sputum mycobacteriology tests for the diagnosis of tuberculosis) against the eight studies, we established that each study had a potential risk of bias in at least one of the four

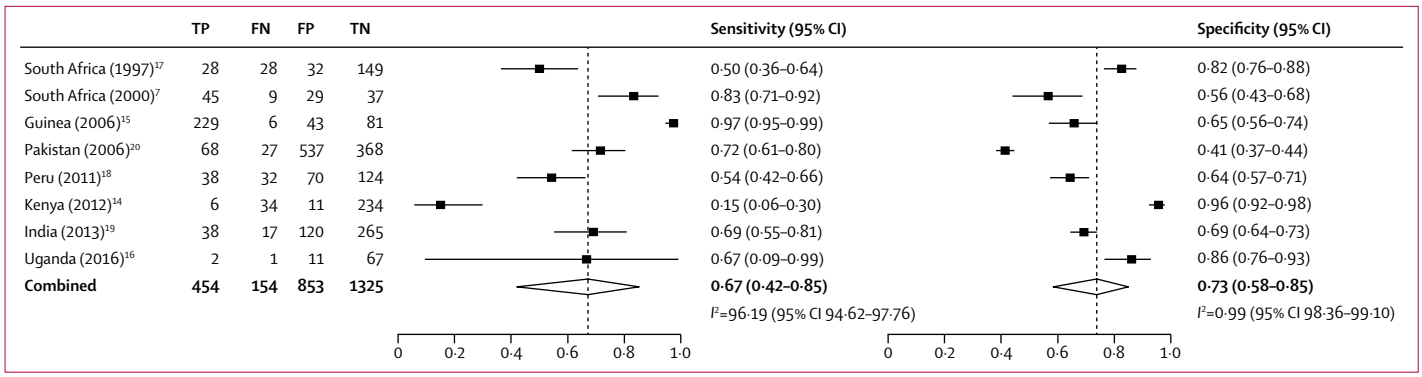

Figure 2: **Diagnostic sensitivity and specificity of the trial of antibiotics versus mycobacteriology tests**
Meta-analysis of the diagnosis of pulmonary tuberculosis in the eight studies included. Mycobacteriology tests included culture only or culture plus smear microscopy. Dashed vertical lines show the pooled estimates. TP=true positive. FN=false negative. FP=false positive. TN=true negative.

domains of the QUADAS-2 tool (appendix pp 7–10). A sensitivity analysis that involved doing the meta-analysis without the one study that showed a high risk of bias in at least three QUADAS-2 domains yielded sensitivity, specificity, and I^2 estimates that were similar to the full analysis (appendix p 12). In all studies, the patient selection process and conduct of both index and reference tests matched the expectation of our main question.

Discussion

We report, to our knowledge, the first systematic review to assess rigorously the diagnostic performance of the trial of antibiotics against mycobacteriology for sputum-negative tuberculosis. Our main findings are that the available evidence base is insufficient and limited by incomplete geographical coverage and inconsistencies on the choice of antibiotics, duration of treatment, and case definition for post-treatment clinical improvement. However, the pooled sensitivity (67%) and specificity (73%) estimates fall well below minimum recommendations for new tuberculosis triage and diagnostic tests for adults.[21] As the medical community moves towards meeting End TB goals,[22] clinicians and those designing public health programmes need to be aware of how substantial the misclassification by trial of antibiotics can be.

Our results call for reconsideration of the appropriateness of retaining routine trial of antibiotics in any international guidelines and national tuberculosis diagnostic algorithms. Algorithms that instead promote mycobacteriology and early chest radiography, repeated as needed, are likely to have better diagnostic accuracy.[23] Broad-spectrum antibiotics will still be needed to treat clinically suspected bacterial infection, with the crucial evidence gap then being how different antibiotic strategies affect clinical outcomes[24] and antimicrobial resistance[25] during tuberculosis investigation, including among key subgroups such as inpatients, people living with HIV, children, and participants identified through tuberculosis screening initiatives.

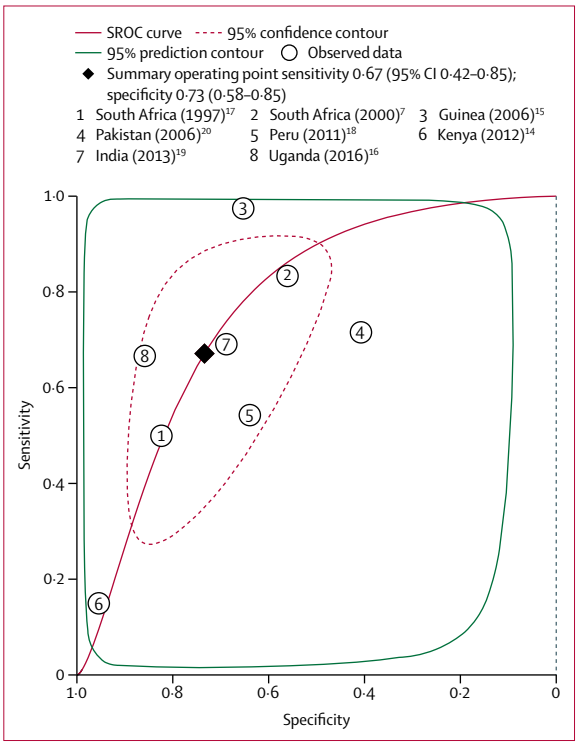

Figure 3: **SROC meta-analysis of the diagnostic performance of the trial of antibiotics against reference mycobacteriology tests for diagnosing pulmonary tuberculosis in eight studies**
Area under the SROC is 0·77 (95% CI 0·73–0·80). Mycobacteriology tests included culture only or culture plus smear microscopy. The confidence contour shows the range that is likely to contain the population summary operating point and the prediction interval is the range that is likely to contain where study data that are not yet observed would fall. SROC=summary receiver operating characteristic curve.

We identified only eight published studies investigating the diagnostic performance of the trial of antibiotics for tuberculosis, which is well below the number needed for making informed health-care choices. This number is especially striking given that tuberculosis is a life-threatening illness and that the trial of antibiotics

might be the most commonly used tuberculosis diagnostic test globally,[26] resulting in non-pathogen-directed prescription of tens of millions of doses of antibiotics each year.[27] Consistent data from well performed randomised controlled trials are required for high-quality evidence,[28] but our review did not identify any randomised controlled trials, and most of the observational studies that we identified were not optimally designed or sized. Instead, four of the eight studies included in this Article assessed the diagnostic performance of the trial of antibiotics as a secondary or exploratory outcome using a small subset of the original study population, reducing power and increasing the risk of selection bias. Methodological concerns are highlighted by the suboptimal scores for each included study on the QUADAS-2 tool for assessing risk of bias. The thin evidence and poor methodological quality that we have observed with the trial of antibiotics does not match the past 10 years' rapid accumulation of high-quality trial data informing the rational use of antibiotics for the treatment of presumed chest infections when tuberculosis is not under consideration.[29]

The poor diagnostic performance reported here is unsurprising given the wide differential of tuberculosis symptoms, including viral and non-infectious causes.[30] Misleading responses could also arise from partial response to antibiotics in patients with tuberculosis with concurrent bacterial infections. This situation is best described in (but not limited to) patients with HIV, which led to the 2007 WHO recommendation to separately investigate and manage tuberculosis and bacterial infections in people living with HIV.[9] Misclassifying tuberculosis is costly to both the patient and the health system. False-positive tuberculosis diagnoses expose patients to unnecessary tuberculosis chemotherapy and its associated toxicity, stigma, hospital visits, lost schooling or employment, and any consequences from delayed diagnosis of the true cause of illness. False-negative tuberculosis diagnoses are associated with the individual and public health consequences of delayed diagnosis and ongoing transmission.[31]

A framework for evaluating the diagnostic performance of the trial of antibiotics is provided by comparing our estimates against target product profiles for new non-sputum tuberculosis triage tests (minimum sensitivity of 90% and specificity of 70%) and sputum-based replacements for smear microscopy at the primary care level (minimum sensitivity of 60% for smear-negative tuberculosis and specificity of 98%).[21] Additional attributes of diagnostic tests that are important to patients and are not met by the trial of antibiotics include timely diagnosis[5,7] and low cost (the recommendation from WHO[21] of <US$6 for a new diagnostic will be exceeded with the trial of antibiotics once expenses incurred by patients,[32] costs of drugs, and staff time[33] are included). The main attributes that are likely to drive the continued use of the trial of antibiotics globally are, therefore, the ease with which prescription fits into the high throughput of consulting rooms, as well as patients' expectations and clinicians' habitual prescription of antibiotics for respiratory consultations—considerations that should be discouraged and not encouraged in an era of rising threat from antimicrobial resistance.[34–36]

For more on the **WHO End TB Strategy** see https://www.who.int/tb/strategy/end-tb/en/

Our meta-analysis showed substantial heterogeneity, which is consistent with the non-standardised nature of choice and duration of antibiotics and the definition of response to treatment. Other variables potentially affecting heterogeneity include site-specific factors, such as antibiotic resistance patterns and exposure to tobacco smoke and air pollution, level of health care, pre-study investigations (eg, whether chest radiography was done), and HIV prevalence. The small number of eligible studies limited our power to explore these variables. Altogether, the heterogeneity in and underlying differences between studies highlight the variations that exist in the interpretation of WHO guidelines in different settings.

The main limitations of this systematic review and meta-analysis are the small number of studies identified, the suboptimal number of participants per study, the pronounced variation in the definitions and methods used, and the suboptimal reference standard. Suboptimal reference standards are a concern for studies in tuberculosis diagnostics.[37] The studies included in our review used either one or a combination of MTB culture and smear microscopy, each of which can misclassify patients' tuberculosis disease status, thereby misinterpreting the true sensitivity and specificity of the trial of antibiotics. We were unable to explore the probable causes of heterogeneity given the data limitations. We restricted our search strategy to peer-reviewed articles and will therefore have omitted eligible studies published in conference proceedings or in programme reports. We might also have missed some articles, including peer-reviewed papers, because data on trials of antibiotics are often reported as secondary or exploratory outcomes to the main study objective. In addition, the result of the Deeks' funnel plot should be interpreted with the understanding that the model works best if it has at least ten studies. In the absence of a better tool, we thought that Deeks' funnel plot could still give a reasonable estimate for publication bias.

The End TB Strategy calls for major expansion of tuberculosis testing to find the missing millions of undiagnosed tuberculosis cases and to save lives; making treatment available to the target of 40 million tuberculosis cases by 2022 will involve testing up to 1 billion people. The ethical obligation to minimise individual harms is especially pertinent in the context of systematic screening strategies, in which patients have not initiated the diagnostic process.[38] Studies investigating the role, if any, of the trial of antibiotics in patients identified through systematic screening are missing from this meta-analysis but are urgently needed, both to minimise individual harms and from the equally important perspective of antibiotic stewardship.

In conclusion, despite more than 30 years of international guidelines and national algorithms promoting the trial of antibiotics for tuberculosis diagnosis, the small amount of data presented here on its diagnostic utility do not support the underlying rationale. Antibiotics might still be indicated for the treatment of suspected bacterial infections, but in line with strategies for addressing antimicrobial resistance, their use during tuberculosis investigations should otherwise be minimised. More data are needed to guide the minimisation of antibiotic use as we scale up tuberculosis testing globally. We urge donors to prioritise support for well conducted implementation research studies and randomised controlled trials that aim to evaluate rigorously the effect of different antibiotic strategies on outcomes such as short-term mortality, need for hospitalisation or so-called rescue antibiotics, and antimicrobial resistance. These studies should include trials of the safety of antibiotic minimisation protocols[29] to support the rapid generation of sufficient data to guide evidence-based, patient-centred management of presumptive tuberculosis patients, including key subgroups and populations for whom the relative benefits and harms of antibiotics are likely to vary from routine clinic adults—notably, young children, people with HIV,[39] people with diabetes, and tuberculosis screening participants.

Contributors

All authors contributed to the conception and design of the study and reviewed all documents and materials. THD collected data, performed data analysis, interpreted results, and wrote the first draft of the manuscript. MN and CK reviewed the protocol, screened articles, extracted data, and reviewed the results and manuscript. KLF and ELC contributed to the systematic review protocol and critically reviewed the results and manuscript. MN, DJS, and AG-W contributed to the protocol development and reviewed the manuscript. All authors read and approved the final manuscript. THD is the guarantor for this work.

Declaration of interests

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THE LANCET Infectious Diseases

Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Divala TH, Fielding KL, Kandulu C, et al. Utility of broad-spectrum antibiotics for diagnosing pulmonary tuberculosis in adults: a systematic review and meta-analysis. *Lancet Infect Dis* 2020; published online May 18. https://doi.org/10.1016/S1473-3099(20)30143-2.

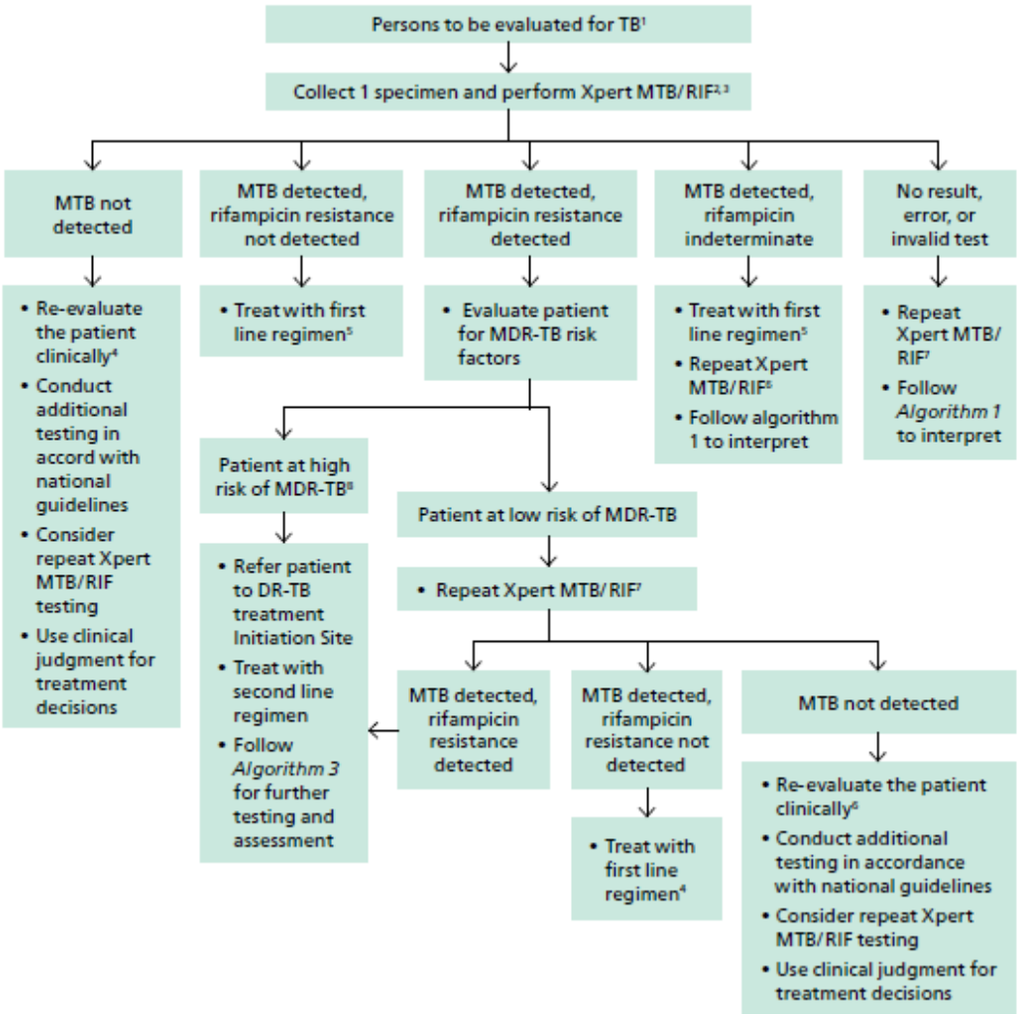
Utility of broad-spectrum antibiotics for diagnosing pulmonary tuberculosis in adults: a systematic review and meta-analysis

Appendix 1: Additional figures and tables

Appendix Table 1: Search strategy for MEDLINE using Ovid platform

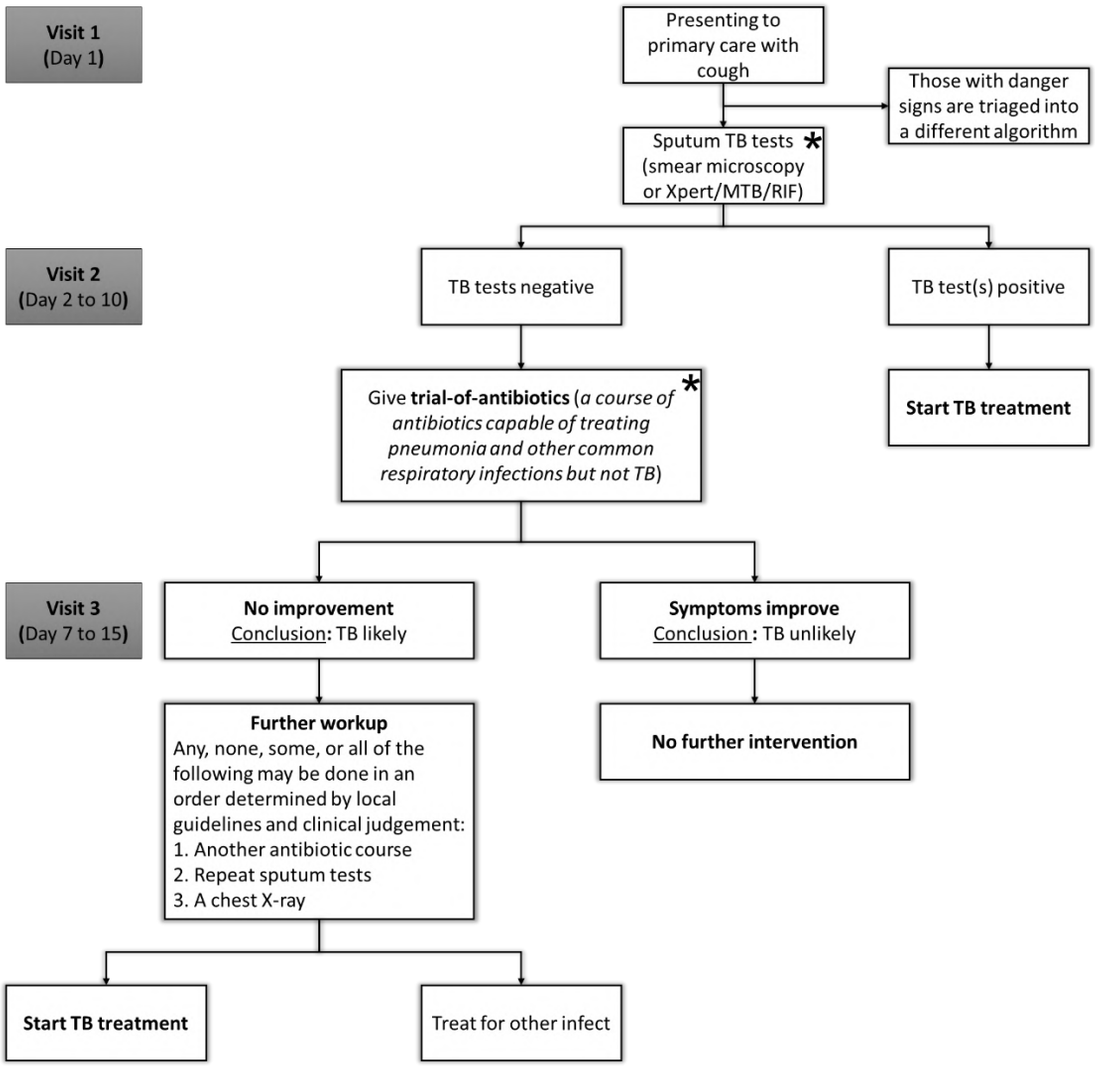
Search line	Search terms
Part 1	*Defining study population:*
1.	exp Tuberculosis/
2.	tuberculosis.mp.
3.	(suspect* adj3 (TB or Tuberculosis)).mp.
4.	(presumpt* adj3 (TB or Tuberculosis)).mp.
5.	(probabl* adj3 (TB or Tuberculosis)).mp.
6.	exp Cough/
7.	tb.mp.
8.	(suspect* adj3 (TB or Tuberculosis)).mp.
9.	or/1-8
Part 2	*Defining study intervention*
10.	(Antibiotic* adj3 trial).mp.
11.	antibiotic*.mp.
12.	Anti-Bacterial Agents/
13.	(oral* adj3 antibiotic*).mp.
14.	(amox?cillin or erythromycin or azithromycin or doxycyclin* or Vibramycin or clavulanic acid or co-amoxiclav).mp.
15.	or/10-14
Part 3	*Defining study outcome*
16.	exp "Sensitivity and Specificity"/
17.	sensitivity.mp.
18.	specificity.mp.
19.	accuracy.mp.
20.	exp "Predictive Value of Tests"/
21.	((positive or negative) adj2 predictive value).mp.
22.	(ppv or npv).mp.
23.	or/16-22

Part 4	*Subject combinations*
24.	9 and 15 (*population and intervention*)
25.	23 and 24 (*Population and intervention and outcome*)
Part 5	*Applying pre-defined limits*
26.	limit 25 to yr="1993 -Current"



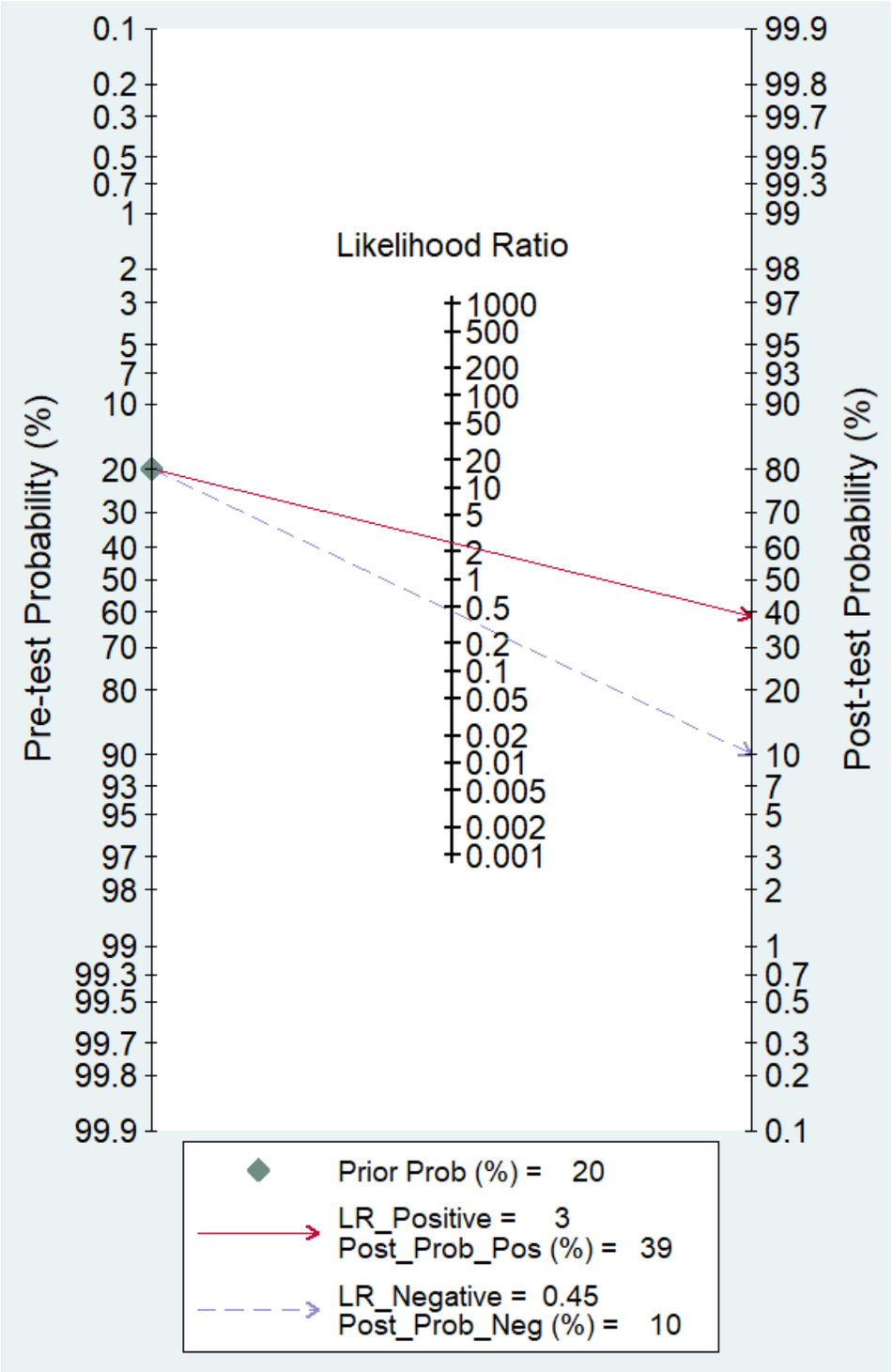
1. Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node and other tissue specimen from persons being evaluated for extrapulmonary TB. For persons being evaluated for TB who are HIV positive and have CD4 counts ≤100 cells/µl or are seriously ill, see Algorithm 4.
2. The new generation Xpert MTB/RIF Ultra assay (Ultra) uses the same semi-quantitative categories used in the Xpert MTB/RIF assay, with an additional semi-quantitative category "trace call" that corresponds to the lowest bacillary burden for Mycobacterium tuberculosis (MTB) complex detection. If MTB is detected with a "trace call", then no interpretation can be made regarding rifampicin resistance and results should be reported as MTB detected, trace, RIF indeterminate (Follow section on "MTB detected, rifampicin indeterminate" under Algorithm 1). The "trace call" positive result is sufficient to initiate therapy in those with known or suspected HIV infection, children and for patients with extrapulmonary samples. For other categories of patients repeating test may be considered with use of second Ultra test for clinical decisions and patients follow-up. (See GLI Planning for country transition to Xpert MTB/RIF Ultra Cartridges).
3. Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the Xpert MTB/RIF test. The second specimen may be used for the additional testing described in this algorithm. For persons being evaluated for pulmonary TB, sputum is the preferred specimen.
4. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Xpert MTB/RIF testing, or culture.
5. Patients should be initiated on a first-line regimen according to national guidelines. A sample may be sent for molecular or phenotypic DST for isoniazid , particularly if the patient has been previously treated with isoniazid or if there is a high prevalence of isoniazid resistance not associated with rifampicin resistance (i.e., isoniazid mono- or poly-resistance) in this setting or for DST for rifampicin if rifampicin resistance is still suspected.
6. Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Use the rifampicin result of the second Xpert MTB/RIF test in this algorithm for a decision(s) regarding choice of regimen (first line or second line regimen).
7. Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second Xpert MTB/RIF test for clinical decisions.
8. Patients at high risk for multidrug-resistant TB (MDR-TB) include previously treated patients including those who had been lost to follow-up, relapsed, and failed a treatment regimen; non-converters (smear positive at end of intensive phase); MDR-TB contacts; and any other MDR-TB risk groups identified in the country.

Appendix Figure 1a: WHO/GLI Model Algorithm 1; Preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance (June 2018)

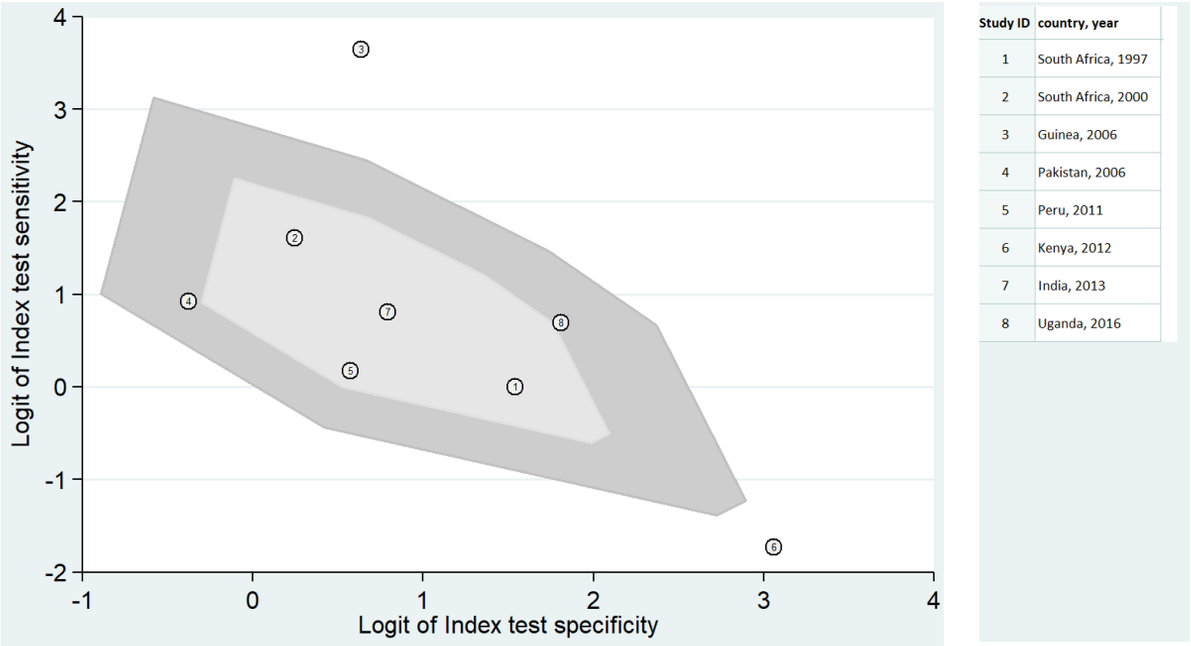


*The common clinical practice is that outpatients start antibiotics at the time of submitting sputum, to avoid the need for a third clinic visit to complete the algorithm. In some guidelines, trial-of-antibiotics is implemented after chest X-ray.

Appendix Figure 1b: The position of trial-of-antibiotics in most national tuberculosis diagnostic algorithms showing how countries interpret the WHO GLI model guidelines (based on national guidelines from Ghana, Malawi and South Africa).



Appendix figure 2: Fagan's nomogram demonstrating clinical utility of trial-of-antibiotics by plotting post-test probabilities of detecting mycobacteriology positive PTB. In this analysis, the pre-test probability, fixed at 20%, is investigators suggestion of TB prevalence based on reference standard diagnosis. The interpretation of the post-test probabilities is as follows: with an estimated TB prevalence of 20%, if a patient tests positive using trial-of-antibiotics, the probability that they truly have TB is 39% (solid line in red); if patient tests negative, the probability that they have TB is 10% (blue dotted line).



Appendix figure 3: The Bagplot demonstrating the level of heterogeneity using the spread of the 8 studies included in meta-analysis

Appendix table 3: Assessment of the quality of included studies against the review question using QUADAS 2 tool (University of Bristol)

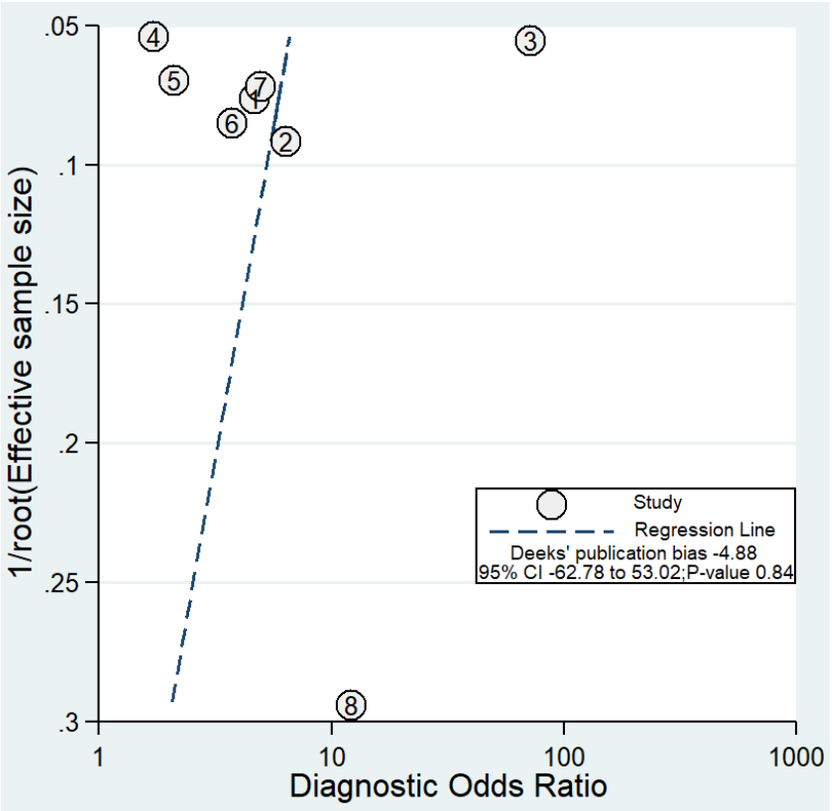
Author	**South Africa**	**South Africa**	**Guinea**	**Pakistan**	**Peru**	**Kenya**	**India**	**Uganda**
Year	**1997**	**2000**	**2006**	**2006**	**2011**	**2012**	**2013**	**2016**
Domain 1: Patient selection								
Was a consecutive or random sample of patients enrolled?	yes	yes	yes	yes	yes	yes	yes	yes
Was a case-control design avoided?	yes	yes	yes	yes	yes	yes	yes	yes
Did the study avoid inappropriate exclusions?	No, one of their exclusion criteria was clinical picture consistent with pneumonia	No, they excluded patients based on clinical and radiological features consistent with pneumonia. Inclusion was also based on CXR consistent with TB.	yes	No, 64% of the 2794 patients treated with antibiotics did not have their outcome evaluated (loss to follow up)	yes	No, 66 of 380 patients were excluded from receiving antibiotics and put on presumptive TB treatment either based on CXR or other clinical TB diagnosis	No, started 17 patients on TB treatment based on clinical judgement and excluded them from receiving antibiotics	No, study started with 162 patients, 157 received antibiotics and reported outcome; but only 110 patients had culture done, of which only 81 had valid results
Could the selection of patients have introduced bias? (Low if YES to all above; High if any NO)	**high risk**	**high risk**	**low risk**	**high risk**	**low risk**	**high risk**	**high risk**	**high risk**

Author	South Africa	South Africa	Guinea	Pakistan	Peru	Kenya	India	Uganda
Year	**1997**	**2000**	**2006**	**2006**	**2011**	**2012**	**2013**	**2016**
Is there concern that the included patients do not match the review question?	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**
Domain 2: Index test								
Were the results of trial of non-TB antibiotics interpreted without knowledge of the results of the reference standard?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the interpretation approach of trial-of-antibiotics outcome pre-specified?	No	Yes	Yes	No	Yes	Yes	No	Yes
Could the conduct or interpretation of trial-of-antibiotics as a diagnostic test have introduced bias? (Low if YES to all above; High if any NO)	**high risk**	**low risk**	**low risk**	**high risk**	**low risk**	**low risk**	**high risk**	**low risk**
Is there concern that the trial of antibiotics, its conduct, or interpretation differ from the review question?	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**
Domain 3: Reference test								
Is the reference TB microbiology test likely to correctly detect TB?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Author	**South Africa**	**South Africa**	**Guinea**	**Pakistan**	**Peru**	**Kenya**	**India**	**Uganda**
Year	**1997**	**2000**	**2006**	**2006**	**2011**	**2012**	**2013**	**2016**
Were the TB microbiology test results interpreted without knowledge of the outcome of the trial of non-TB antibiotics?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Could the TB microbiology test, its conduct, or its interpretation have introduced bias? (Low if YES to all above; High if any NO)	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**
Is there concern that the target condition as defined by the reference standard in the paper does not match the review question?	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**	**low risk**
Domain 4: Patient flow								
Was there an appropriate interval between antibiotics and reference TB microbiology test?	Yes	Yes	No, sample for reference standard was taken while index test outcome was known	No, sample for reference standard was taken while index test outcome was known	Yes	Yes	Yes	Yes
Did all the included patients have a TB microbiology test?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

Author	**South Africa**	**South Africa**	**Guinea**	**Pakistan**	**Peru**	**Kenya**	**India**	**Uganda**
Year	**1997**	**2000**	**2006**	**2006**	**2011**	**2012**	**2013**	**2016**
Did patients receive the same TB microbiology test?	Yes	Yes	No, used smear for reference in some patients, in those who were smear negative, used culture	Yes	Yes	Yes	Yes	Yes
Were all patients who received index test included in the analysis?	Yes	Yes	yes	No, 64% of patients were lost to follow up	No, 21 of 285 were lost to follow up of	No, final sample missing 32 patients due to inconclusive culture	yes	No, 76 of 167 patients with available index test outcome had no valid reference test results
Could the patient flow have introduced bias? (Low if YES to all above; High if any NO)	**low risk**	**low risk**	**high risk**	**high risk**	**high risk**	**high risk**	**low risk**	**high risk**

Number of high-risk domains out of four	**2**	**1**	**2**	**3**	**1**	**2**	**2**	**2**



Study ID	country, year
1	South Africa, 1997
2	South Africa, 2000
3	Guinea, 2006
4	Pakistan, 2006
5	Peru, 2011
6	Kenya, 2012
7	India, 2013
8	Uganda, 2016

Appendix figure 4: Deeks' funnel plot to evaluate publication bias in the 8 studies included in the meta-analysis

Appendix table 2a: Subgroup analysis

Covariate (refer to Table 1)	category	Number of studies	Sensitivity (95% CI)	p-value for difference in sensitivity	Specificity (95% CI)	p-value for difference in specificity	Joint model I^2 (%)
Sub-Saharan Africa	Yes	5	0.69 (0.41, 0.97)	0.83	0.81 (0.70, 0.92)	0.35	72
	No	3	0.65 (0.29, 1.00)		0.58 (0.36, 0.80)		
Culture only for reference standard	Yes	6	0.55 (0.31, 0.79)	0.03	0.79 (0.68, 0.90)	0.24	52
	No	2	0.90 (0.76, 1.00)		0.53 (0.24, 0.81)		
These analyses are exploratory and should be interpreted with caution considering the small number of included studies.							

Appendix table 2b: Sensitivity analyses attempting to explain high heterogeneity

Description	Included studies	Sensitivity (95% CI)	Sensitivity I^2 (95% CI)	Specificity (95% CI)	Specificity I^2 (95% CI)
All studies	1, 2, 3, 4, 5, 6,7, and 8	67 (42, 85)	96 (95, 98)	73 (58, 85)	99 (98, 99)
Excluding studies (1/8) based on quality (with high risk of bias in at least three domains of Quadas 2 tool).	1,2, 3, 4, 5, and 7	66 (37, 87)	97 (95, 98)	77 (64, 87)	95 (92, 97)
Excluding studies (2/8) outside the 95% CI of the median distribution of the bagplot.	1, 2, 4, 5, 7, and 8	64 (53, 74)	82 (69, 96)	67 (53, 79)	98 (98, 99)
1. South Africa, 1997 **2.** South Africa, 2000 **3.** Guinea, 2006 **4.** Pakistan, 2006		**5.** Peru, 2011 **6.** Kenya, 2012 **7.** India, 2013 **8.** Uganda, 2016			
These analyses are exploratory and should be interpreted with caution considering the small number of included studies.					

Appendix 2: Stata Code for meta-analysis

A. Preliminary steps:
   i. Start stata as administrator
   ii. Install (if not installed) midas
   iii. Install (if not installed) metan
   iv. Install (if not installed) mylabels
   v. Install (if not installed) gllamm

B. Load the following data

Studyid	*author*	*year*	*sampsize*	*tp*	*fn*	*fp*	*tn*	*reference*	*ref*	*country*	*region-ssa*
1	South Africa	1997	237	28	28	32	149	culture	1	South Africa	1
2	South Africa	2000	120	45	9	29	37	culture	1	South Africa	1
3	Guinea	2006	359	229	6	43	81	Smear+culture	0	Guinea	1
4	Pakistan	2006	1000	68	27	537	368	Smear+culture	0	Pakistan	0
5	Peru	2011	264	38	32	70	124	culture	1	Peru	0
6	Kenya	2012	285	6	34	11	234	culture	1	Kenya	1
7	India	2013	440	38	17	120	265	culture	1	India	0
8	Uganda	2016	81	2	1	11	67	culture	1	Uganda	1

C. Perform the following analyses

```
*Summary Statistics

    midas tp fp fn tn, res(all)

*Forest plot to demonstrate study-specific on right y-axis

    midas tp fp fn tn, id(author year) ms(0.75) ford fors bfor(dss)

*Summary ROC Curve with prediction and confidence Contours

    midas tp fp fn tn, plot sroc(both)

*Linear regression test of funnel plot asymmetry

    midas tp fp fn tn, pubbias

*Fagan's plot

    midas tp fp fn tn, fagan(0.20)

*Bagplot

    midas tp fp fn tn, bivbox scheme(s2color)
```

4 Design of a randomised controlled trial

4.1 Introduction

The systematic review established a dearth of evidence to support continued use of trial-of-antibiotics tuberculosis diagnosis algorithms. In this chapter I describe a randomised controlled trial I designed to address diagnostic accuracy, clinical impact and antimicrobial resistance impact of trial-of-antibiotics. I registered the randomised controlled trial with clinicaltrials.gov and published the following manuscript with BMJ Open. While the manuscript describes the work to detail, I have included the full protocol in the appendix. Immediately after the manuscript, I have presented the trial statistical analysis plan, which I prepared and shared before completing data collection.

4.2 Protocol manuscript for the randomised controlled trial

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1700548	**Title**	Dr
First Name(s)	Titus, Henry		
Surname/Family Name	Divala		
Thesis Title	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)		
Primary Supervisor	Professor Katherine Fielding		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMJ Open		
When was the work published?	25 March 2020		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study described in the protocol manuscript, wrote the study protocol, led the writing of the manuscript, and submitted it for publication

SECTION E

Student Signature	[redacted]
Date	29 December 2020

Supervisor Signature	[redacted]
Date	23Feb2021

BMJ Open

Accuracy and consequences of using *trial-of-antibiotics* for TB diagnosis (ACT-TB study): protocol for a randomised controlled clinical trial

Titus Henry Divala ,[1,2,3] Katherine L Fielding,[1,4] Derek J Sloan,[5] Neil French,[6] Marriott Nliwasa,[1,2] Peter MacPherson,[3,7] Chikondi Charity Kandulu,[2,3] Lingstone Chiume,[2,3] Sanderson Chilanga,[2,3] Masiye John Ndaferankhande,[3] Elizabeth L Corbett[1,2,3]

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ABSTRACT

Introduction Over 40% of global tuberculosis case notifications are diagnosed clinically without mycobacteriological confirmation. Standard diagnostic algorithms include 'trial-of-antibiotics'—empirical antibiotic treatment given to mycobacteriology-negative individuals to treat infectious causes of symptoms other than tuberculosis, as a 'rule-out' diagnostic test for tuberculosis. Potentially 26.5 million such antibiotic courses/year are prescribed globally for the 5.3 million/year mycobacteriology-negative patients, making trial-of-antibiotics the most common tuberculosis diagnostic, and a global-scale risk for antimicrobial resistance (AMR). Our systematic review found no randomised controlled trial (RCT) to support use of trial-of-antibiotic. The RCT aims to determine the diagnostic and clinical value and AMR consequences of trial-of-antibiotics.

Methods and analysis A three-arm, open-label, RCT randomising (1:1:1) Malawian adults (≥18 years) seeking primary care for cough into: (a) azithromycin 500 mg one time per day for 3 days or (b) amoxicillin 1 g three times per day for 5 days or (c) standard-of-care (no immediate antibiotic). We will perform mycobacteriology tests (microscopy, Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampicin) and *Mycobacterium tuberculosis* culture) at baseline. We will use audiocomputer-assisted self-interview to assess clinical improvement at day 8. First primary outcome will be proportion of patients reporting day 8 improvement out of those with negative mycobacteriology (specificity). Second primary outcome will be day 29 incidence of a composite endpoint of either death or hospitalisation or missed tuberculosis diagnosis. To determine AMR impact we compare proportion of resistant nasopharyngeal *Streptococcus pneumoniae* isolates on day 29. 400 mycobacteriology-negative participants/arm will be required to detect a ≥10% absolute difference in diagnostic specificity with 80% power. We will estimate measures of effect by comparing outcomes in antibiotic arms (combined and individually) to standard-of-care.

Ethics and dissemination The study has been reviewed and approved by Malawi College of Medicine Research and Ethics Committee, London School of Hygiene & Tropical Medicine (LSHTM) Research Ethics Committee and Regional Committee for Health and Research Ethics – Norway, and Malawi Pharmacy, Medicines and Poisons Board. We will present abstracts at relevant conferences, and prepare a manuscript for publication in a peer-reviewed journal.

Trial registration number The clinical trial is registered with ClinicalTrials.gov, NCT03545373

Strengths and limitations of this study

- ► To our knowledge this is the first randomised controlled trial to address benefits and consequences of using antibiotics as an exclusion diagnostic for tuberculosis, a widely used practice that results in millions of antibiotic prescriptions/year.
- ► We will also contribute evidence on antimicrobial resistance affecting common antimicrobials used for managing respiratory infections.
- ► The use of audio computer-assisted self-interview for assessing clinical response and adherence to antibiotic treatment which can be used in future studies.
- ► Acknowledged weaknesses include limited power to evaluate safety of deferred antibiotic treatment, conduct subgroup analysis by HIV status and the possibility that participants randomised to the standard-of-care arm may find alternative access to antibiotics therefore misclassifying exposure/intervention status.

INTRODUCTION

The high case-fatality rate for tuberculosis, the leading global infectious cause of death in adults[1] with approximately 10 million cases and 1.6 million deaths in 2017,[2] in part reflects suboptimal diagnostics.[3–6] To complement this diagnostic gap, standard algorithms throughout the world include a 'trial-of-antibiotics' (figure 1). This is a course of broad-spectrum antibiotics, with negligible

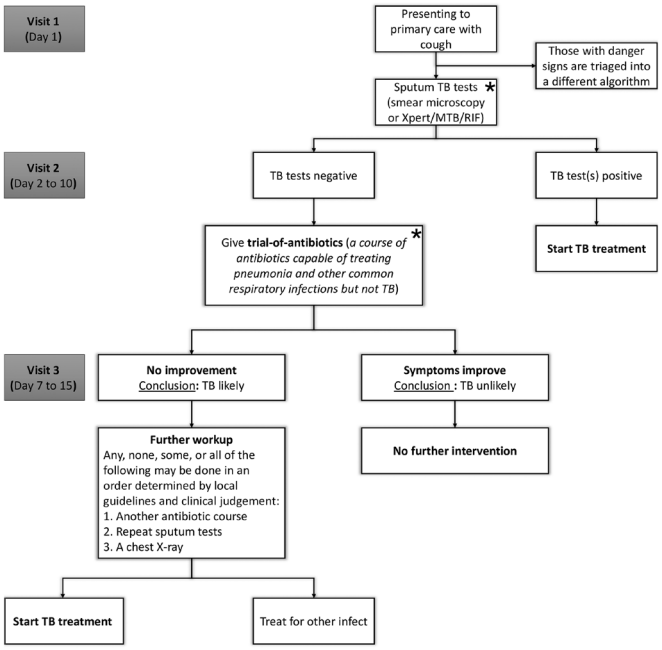

Figure 1 The position of trial-of-antibiotics in standard algorithms for diagnosis of tuberculosis in low- and middle-income countries (based on the 2018 WHO GLI model guidelines and as implemented in national guidelines, for example, Ghana, Malawi and South Africa). *The common clinical practice is that outpatients start antibiotics at the time of submitting sputum, to avoid the need for a third clinic visit to complete the algorithm. GLI,Global Laboratory Initiative; MTB, *Mycobacterium tuberculosis;* RIF, rifampicin; TB, tuberculosis.

Mycobacterium tuberculosis activity, given to patients with symptoms such as cough in order to 'rule-out' or 'rule in' tuberculosis.[7–9] In clinical practice and most national guidelines (summarised in figure 1), patients who have negative sputum mycobacteriology and have responded to antibiotic treatment are considered tuberculosis-negative while those who remain symptomatic are deemed likely to have tuberculosis and undergo further evaluations potentially leading on to receiving tuberculosis treatment.[7–9]

We estimate that 26.5 million courses of antibiotics are prescribed in the diagnosis of the 5.3 million smear-negative tuberculosis registrations recorded annually,[10] making antibiotics the most common diagnostic for tuberculosis.[11] Our 26.5 million estimate assumes that for every one smear-negative tuberculosis case detected, five antibiotics courses are used: the first two courses being given to patients are ultimately registered as smear-negative tuberculosis, while the other three courses represent patients whose symptoms resolved without starting anti-tuberculosis treatment.[4 12] This high frequency of prescription of important broad-spectrum antibiotics raises a global-scale risk for antimicrobial resistance (AMR) which like tuberculosis, is a major crisis, becoming in 2016 one of only four health topics ever to be discussed at the United Nations General Assembly.[13–16]

We performed a systematic literature review[17] which demonstrated that, despite being in global and national guidelines for decades, trial-of-antibiotics has a limited supporting evidence base but with the available evidence suggesting poor diagnostic performance.[18] None of the identified studies was an randomised controlled trial (RCT) and most of the observational studies were very small and not primarily designed to assess the benefits and consequences of trial-of-antibiotics. Pooled sensitivity and specificity of trial-of-antibiotics versus mycobacteriology tests were below internationally defined minimum performance profiles for tuberculosis diagnostics.[19]

We hypothesise that use of antibiotics in the course of evaluating patients for tuberculosis has both benefits and risks that need to be weighed carefully to optimise patient and public health outcomes. We will address evidence gaps related to (a) accuracy, (b) antimicrobial resistance and (c) impact on clinical outcomes of trial-of-antibiotics by conducting an RCT (ACT-TB study) recruiting adult patients with cough presenting to health centres in Blantyre, Malawi. To our knowledge this is the first randomised controlled trial to rigorously address these questions.

METHODS AND ANALYSIS

Study design

This is a three-arm individually randomised (1:1:1), open-label controlled clinical trial (RCT) investigating accuracy and broader clinical, and antimicrobial resistance impact of using trial-of-antibiotics to rule-out tuberculosis among adults presenting with cough at primary care centres in Malawi (figure 2). The trial is registered with ClinicalTrials.gov (online supplementary appendix 2). The full trial protocol is provided as online supplementary appendix 3.

Study setting

We will screen adults aged at least 18 years presenting to Limbe and Ndirande health centres in Blantyre, Malawi. Blantyre has an estimated tuberculosis prevalence of 1014 per 100 000 (95% CI: 486 to 1542), and an estimated adult HIV prevalence of 12.7% (95% CI: 11.9 to 13.6).[20]

Eligibility criteria

We will offer enrolment to patients who satisfy the following inclusion and exclusion criteria.

Inclusion criteria

- Ambulatory clinic attendees presenting with cough.
- Unwell for at least 14 days.
- Aged at least 18 years.
- Reside in Blantyre and willing to return to the same clinic for follow-up visits over the entire study period.

Exclusion criteria

- Self-reported allergy to study medications.
- WHO/Malawi National Tuberculosis Programme danger signs: respiratory rate >30/min, temperature >39°C, heart rate >120/minute, confused/agitated, respiratory distress, systolic blood pressure <90 mm Hg, inability to walk unassisted.

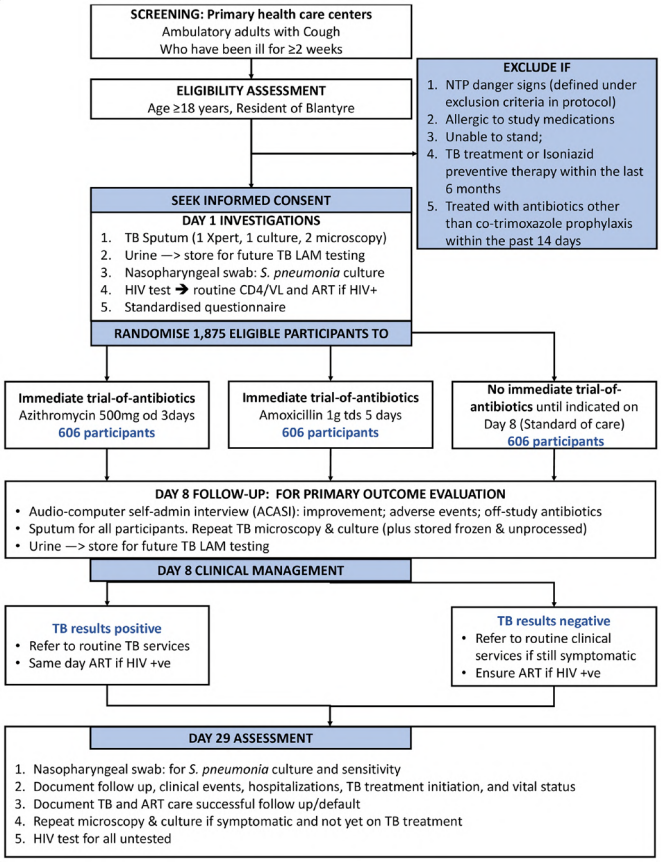

Figure 2 Flow diagram for the clinical trial in Blantyre, Malawi. ART;antiretroviral therapy; LAM, lipoarabinomannan; NTP, Malawi National Tuberculosis Programme; TB, tuberculosis; VL, viral load.

- Treated with antibiotics other than co-trimoxazole prophylaxis within the past 14 days.
- Tuberculosis treatment or isoniazid preventive therapy within the last 6 months.

Interventions

We will randomise participants, in a ratio of 1:1:1, to the following arms:

- **Arm 1** (azithromycin): Azithromycin 500 mg taken one time per day for 3 days from enrolment day.
- **Arm 2** (amoxicillin): Amoxicillin 1 g taken three times per day for 5 days from enrolment day.
- **Arm 3** (standard of care): No study antibiotic prescription.

Rationale for interventions

Amoxicillin was chosen because it is the standard antibiotic used as first-line treatment and for trial-of-antibiotics in Malawi. However, amoxicillin may not demonstrate the best performance for trial-of-antibiotics because of increasing resistance, and a narrow coverage for aetiology of community acquired pneumonia and 'atypical' organisms. We chose azithromycin to represent the optimal biological specificity of an oral regimen due to more complete coverage of atypical organisms that cause community acquired pneumonia (eg, mycoplasma and chlamydia), and also the low resistance rates in Malawi where macrolides are rarely used. The dose for azithromycin is as recommended in the British National Formulary (BNF) as treatment for community-acquired pneumonia.[21] The dose for amoxicillin is the BNF recommendation for severe infections but it is the recommended first-line established by the Department of Medicine at Queen Elizabeth Central Hospital (Blantyre, Malawi) based on local microbiology.

Timing of interventions

The standard of care in Malawi defined by National Tuberculosis Programme guidelines for primary care patients presenting with cough who are otherwise well (no danger signs) is to take two sputum specimens for smear microscopy or Xpert and ask patients to return for results, typically 3 days to 1 week later (figure 1). The Malawi tuberculosis diagnostic algorithm recommends use of broad-spectrum antibiotics as trial-of-antibiotics after negative sputum tests are provided to patients who remain symptomatic. Therefore, the ideal population for randomisation for this study are patients on who already have negative results for smear microscopy or Xpert. However, that may have ethical challenges considering the implications of withholding treatment (if randomised to reference arm) from a symptomatic patient who, according to guidelines, should be given antibiotics. The first visit therefore was the most ideal time for randomisation and is in line with recommendations for test interval in investigations evaluating diagnostic tests with respect to the time interval between the index test (trial-of-antibiotics) and the reference test (mycobacteriology sputum sample collection). The timing also conforms to common clinical practice of prescribing trial-of-antibiotics at the same time as sputum collection to reduce diagnostic delay. The design was discussed with the District Health Office and the National Tuberculosis Programme ahead of ethics submission.

Known drug reactions

Azithromycin and amoxicillin have a long registration history, have been widely used globally and are well tolerated. Rare side effects for azithromycin include nervousness, dermatological reactions including Stevens-Johnson syndrome, anaphylaxis and prolonged QT interval. Rare side effects for amoxicillin are mental state changes, lightheadedness, photosensitivity and severe allergic reactions.

Concomitant medication and interaction with other therapies

We do not have any restrictions with respect to concomitant medications apart from those listed in the exclusion criteria. We expect some participants to be on HIV antiretroviral drugs and some to subsequently start tuberculosis therapy. Important interactions therefore would be those between the product and HIV antiretroviral drugs. There is no moderate or major interaction between either azithromycin or amoxicillin with the classes of HIV antiretroviral drugs currently used in Malawi.

Trial restrictions

We do not require participants to have any dietary restrictions. We will also accept co-administration with contraception. Azithromycin and amoxicillin are both considered safe in pregnancy, so we will include pregnant women should they be eligible.

Assessment of compliance

On Day 8, we will document self-reported compliance adherence of study products.

Withdraw of interventions

The investigator may also terminate a participant from study product if indicated by an adverse reaction. If a participant stops taking study product either voluntarily or by investigator decision, they will be encouraged to remain in follow-up and their data will form part of intention-to-treat analyses.

Study outcomes

The clinical trial has two separately powered, and distinctly assessed primary outcomes, one for diagnostic evaluation (Primary outcome 1: Day 8) and the other for clinical impact (Primary outcome 2: Day 29) of the intervention. The following are descriptions of all study outcomes:

Primary outcome 1: specificity of day 8 symptom change versus mycobacteriology

The first primary outcome is the proportion of patients without tuberculosis (by sputum tests) who report improvement of their baseline illness when asked 7 days after randomisation (Day 8 study visit). This outcome can be thought of as diagnostic specificity if you take sputum test results as a reference standard and *change in symptoms at Day 8* as the investigational test (figure 3). In this case the possible results of the investigational test are improvement and no improvemet (no change or worsened) in response to the question: *on Day 1, you reported that you were unwell; compared with that day, has your illness worsened, remained the same or improved?*

As with all self-rated outcomes, social desirability bias (tendency of participants to answer questions in a manner that will be viewed favourably by healthcare worker), and interviewer bias (interviewers' subconscious or conscious influencing subject response) may affect the outcome. To minimise these biasses in evaluation of improvement of baseline symptoms the interview will be conducted using audio computer-assisted self-interview (ACASI), a platform that allows patients to report their health state in private and directly into a database via an audio questionnaire administered by a tablet. The lack of human-to-human interaction will minimise interviewer, ascertainment and social desirability biasses. Another concern with open-label design is placebo-effect favouring those randomised to antibiotics over the standard of care arm that is however not addressed in our design.

We developed, piloted and optimised the ACASI questionnaire in the study target population and arrived at the question: *on Day 1, you reported that you were unwell; compared with that day, has your illness worsened, remained the same or improved?* Before proceeding to the self-interview, participants will be oriented using test questions until study staff are sure that they will be able to go through the interview on their own. We will term ACASI interview outcome as ACASI-test-negative if the participant reports improvement or ACASI-test-positive if the participant reports no change or worsening (figure 3).

The mycobacteriology reference standard will be defined in participants with at least one valid sputum test result on Days 1 and 8 as sputum-test-positive if there is at least one positive of smear microscopy, Xpert MTB/RIF or MTB culture; and as sputum-test-negative if none of the tests is positive. To minimise bias, the sputum tests will be performed by a high-quality research laboratory in the University of Malawi College of Medicine by staff with no access to participant treatment allocation information or symptom results.

The specificity of Day 8 symptom change (the index test measured using ACASI) against mycobacteriology tests (reference test) is defined as: proportion of sputum-test-negative who are ACASI-test-negative.

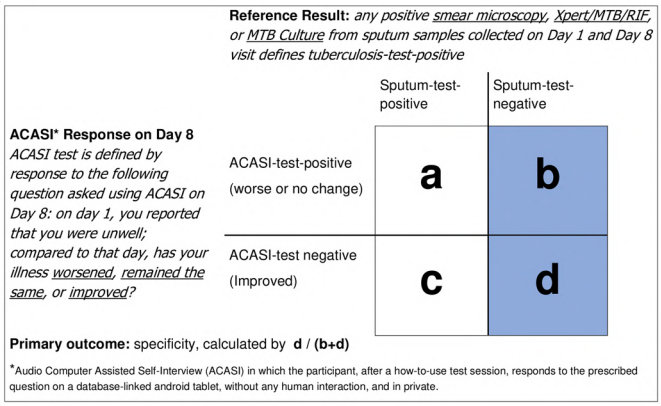

Figure 3 Assessing the diagnostic value of a change in symptoms from baseline to day 8. MTB,*Mycobacterium tuberculosis;* RIF, rifampicin.

Primary outcome 2: clinical impact of trial-of-antibiotics

We will investigate the overall clinical impact of trial-of-antibiotics by comparing the Day 29 risk of any of death, hospitalisation and 'missed tuberculosis' (untreated mycobacteriological or radiological tuberculosis). All these events can lead to mortality and are potential consequences of trial-of-antibiotics; therefore, grouping them as a composite endpoint appropriately represents the effect of the intervention because: (1) there are similarities in the importance of each of the components, (2) the components occur with similar frequencies in the patient population and (3) the direction of effect is anticipated to be the same for all.[22]

The connection between trial-of-antibiotics and risk of hospitalisation and death assumes a protective effect of antibiotics. In patients presenting with chronic cough at primary care in high HIV prevalence settings, frequencies

of mortality and hospitalisation over a 2 months period are similar, ranging from 2% to 6%.[23]

We have included missed tuberculosis diagnosis in our composite clinical outcome because this too can lead to death. We are defining 'missed tuberculosis' as participants who meet standard mycobacteriological and radiological tuberculosis definitions but are incorrectly classified as tuberculosis-negative and not yet on tuberculosis treatment by Day 29. Clinical, radiological and microbiological evaluation for tuberculosis will be done at Day 8, Day 29, as well as day between these two for patients who report worsening symptoms.

Secondary outcome 1: impact of trial-of-antibiotics on antimicrobial resistance

We will use *Streptococcus pneumoniae* isolated from swabs of the nasopharynx as the indicator pathogen for AMR evaluation. An ecological niche for many bacterial species, the upper respiratory tract also presents a convenient window for investigating antimicrobial resistance. *S. pneumoniae* is the organism of choice not only for being an important cause of respiratory tract infections but also because it often colonises the upper respiratory tract, acquires resistance readily and has well documented laboratory investigation procedures in place.[24]

We will define AMR positive as having nasopharyngeal isolates of *S. pneumoniae* that are resistant to any of the following commonly used antibiotics: ceftriaxone, amoxycillin, cefoxitin, azithromycin and erythromycin as determined using disc diffusion technique; and AMR negative as either (1) not isolating any *S. pneumoniae* or (2) isolating any *S. pneumoniae* that is not resistant to any of the assessed antibiotics. For each arm, and at both baseline and Day 29, we will report proportion of AMR positive participants. The study outcome will be the proportion of AMR positive participants at Day 29.

Secondary outcome 2: diagnostic value of trial-of-antibiotics in all patients including those without a valid sputum result

In this analysis, all will remain as described for primary outcome 1 except for the denominator, which will now include those without a valid sputum test result. The mycobacteriology reference standard for secondary outcome 2 will be defined as sputum test positive if at least one positive of smear microscopy, Xpert MTB/RIF or MTB culture from samples collected on Days 1 and 8. The reference test will be sputum-test-negative if none of the tests is positive and where there is no valid sputum test result available. The most likely reason for not having a valid sputum result will be inability to produce sputum, but other explanations will be: lost sample before laboratory analysis, an invalid laboratory reading or contamination. We have opted to analyse this population because in symptomatic adults of the study setting, failure to produce sputum can be as high as 13%.[23]

Secondary outcome 3: economic evaluation

The objective of the economic evaluation is to undertake a cost-utility analysis to estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other. We will systematically compare costs and consequences associated with the interventions. We will perform a within trial comparison of the three treatment arms to estimate the incremental cost per quality-adjusted life year (QALY) gained for the azithromycin or amoxicillin arm in comparison to standard of care. Costs will be estimated from the Malawian Ministry of Health perspective. Health outcomes will be quantified in QALYs, estimated from participants' responses to the Chichewa version of the EQ-5D-3L, a health-related quality of life measure.[25 26] We will adopt a time horizon matching the length of participant follow-up to achieve the within trial evaluation.

Exploratory outcomes

Our exploratory analyses will be comparisons between the **azithromycin** and **amoxicillin** arms for all our primary and secondary outcomes.

Planned subgroup analyses

We will perform analysis of primary outcomes stratified by HIV status and by antiretroviral therapy (ART) status as documented on enrolment day. This is important because the study site has high prevalence of HIV and associated bacterial infections which may be amenable to antibiotics used for trial-of-antibiotics.

Study procedures

Figure 2 and table 1 presents the study time schedule including a summary of patient identification, baseline procedures and outcome ascertainment at Day 8 and Day 29 follow-up visits.

Screening

Study staff will approach patients with symptoms of pulmonary tuberculosis (including cough of any duration, fever, weight loss and night sweats) with information about the study and seek written informed consent (online supplementary appendix 4) from all patients who meet eligibility criteria. After consenting, a participant will be given a unique study identification number confirming enrolment.

Randomisation

Randomisation will be in the ratio 1:1:1 to the three arms of the trial, using block-randomisation with variable block sizes, and stratified by study site. An independent statistician will prepare the randomisation list using ralloc command in Stata software, then print each allocation alongside a randomisation number and seal in opaque envelopes. On confirming eligibility and consenting status a designated site staff will open the next available sequentially numbered randomisation envelopes and administer the allocated study arm.

Table 1 Key study procedures over the study period

	Study period		
	Enrolment	Follow-up	
Time point	Day 1	Day 8	Day 29
Enrolment			
Eligibility screen	x		
Informed consent	x		
Allocation	x		
Interventions			
Azithromycin	x		
Amoxicillin	x		
Standard of care	x		
Assessments			
Demographics	x		
History of antibiotic use	x	x	x
History and examination*	x	x	x
Sputum collection†	x	x	
Urine for TB LAM test‡	x	x	
Nasopharyngeal swab for AMR§	x		x
HIV test	x		
Linking to routine care	x	x	x
ACASI¶		x	
Clinical events**			x
Update contact & address		x	x

*For symptomatic participants, Day 8 sputum mycobacteriology should be fast-tracked to inform care before they leave the clinic.
†Give sputum bottles at the end of Day 1 visit for submission on Day 8. Also collect sputum and perform mycobacteriology at any time of the study when clinically indicated.
‡Urine lipoarabinomannan for tuberculosis diagnosis (TB LAM).
§Nasopharyngeal swab for *Streptococcus pneumoniae* culture and sensitivity as a way of determining risk of antimicrobial resistance (AMR).
¶Audio computer-assisted self-interview (ACASI) for documenting change of symptoms on Day 8 versus Day 1.
**Illnesses, clinic visits, radiological outcomes, new HIV diagnosis, new tuberculosis diagnosis, death, hospitalisation, missed tuberculosis diagnosis, HIV care loss to follow-up and tuberculosis care loss to follow-up.

Blinding

The study is not placebo controlled because of funding limitations, and so will not use blinding due to the nature of the study design. However, study team masking will be maintained with all study outcome assessment occurring without reference to randomisation arm.

Baseline procedures

At baseline, we will collect demographic data, clinical history, record vital signs, height and weight. Participants will be requested to provide two sputum samples for Xpert MTB/RIF and two more sputum samples the following morning for smear microscopy and MTB culture. We will also collect a urine sample for lipoarabamannan antigen detection (TB LAM); and a nasopharyngeal swab for pneumococcal culture and sensitivity testing. We will offer and perform HIV testing according to the national algorithm, and link all who test positive to care. To minimise loss to follow-up, we will collect contact phone numbers, a physical address and geolocation information.

Participant follow-up

On Day 8, the first activity (ahead of any other interaction with study staff) will be the ACASI. Other activities include providing results for Day 1 tuberculosis tests and linking those who test positive to care, collection of another sputum sample for smear microscopy and *Mycobacterium tuberculosis* (MTB) culture and management of ongoing symptoms and other illnesses. On visit Day 29, the final study visit, we will document participant vital status, hospitalisations and establish adherence to HIV and tuberculosis treatment. We will also collect nasopharyngeal swab samples from all participants, and sputum from those with tuberculosis symptoms.

Participant retention

To minimise loss to follow-up, we will record geolocation information of participants' place of residence using ePAL android application, a high-resolution mapping system validated in Blantyre. We will also record up to three contact phone numbers of the participant and their nominated friends and relatives. We will not replace participants who discontinue study participation or study treatment regardless of reason for withdrawal or discontinuation or the time either of these occurs.

Data management

We will collect data using TeleForm (paper based system that uses optical character recognition) and Open Data Kit systems (ODK, an electronic data capture system installed on android devices). Data will be committed to a secure database located at Malawi-Liverpool Wellcome Trust within 2 days for TeleForm, and 7 days for ODK.

Statistical approach

We will summarise the processes of recruitment including non-eligibility and reasons of exclusion in a CONSORT (Consolidated Standards of Reporting Trials) flow chart. We will describe the study participants by their baseline characteristics, by arm. We will perform analyses of all our outcomes based on an intention-to-treat analysis (using the arm patient was randomised to). Analysis for primary outcome 1 will be restricted to participants with a valid sputum test result. We will report measures of effect from the following comparisons: (i) azithromycin or amoxicillin (combined) versus standard of care, (ii) azithromycin versus standard of care and (iii) amoxicillin versus standard of care.

We will use a generalised linear model (GLM) with identity link to estimate risks differences and the GLM with log link to estimate risk ratios for the three comparisons, adjusting for study site. For each comparison, we will

report 95% CIs and p values from the likelihood test. If outcomes are rare, or the GLM model does not converge, we will use logistic regression to estimate the treatment effect using an OR. We will not perform adjustments for multiple comparisons but will report all effect sizes with their 95% CIs and p values to facilitate appropriate interpretation of our results.

We will perform data cleaning and analysis using Stata release 15 (StataCorp, College Station, Texas, USA). The statistical approach will be expanded in a detailed statistical analysis plan, which will be finalised before unblinding the study data.

Sample size and power

We performed power and sample size estimations for the diagnostic impact, clinical impact and AMR impact outcomes as described below. Our sample size estimations are based on planned analysis that will use χ^2 test for comparing two independent proportions.

Diagnostic impact outcome

We assume that at Day 8, change in well-being from baseline state in trial-of-antibiotics (azithromycin or amoxicillin) arms will correctly classify 60% of all mycobacteriology-negative participants (ie, 60% specificity of Day 8 symptom change in trial-of-antibiotics arms).[12] We wanted to estimate a sample size that would provide a discriminatory power of 80% at a two-sided significance level of 5%, to detect at least 10% difference in specificity (ie, ≤50% specificity of Day 8 symptom change in standard of care arm).

Sample size for a combination of two antibiotic arms against standard of care arm

The sample size estimates along with assumptions for this comparison are shown in the table 2A. To achieve the desired 80% discriminatory power, we will need to recruit at least 290 sputum-test-negative participants per arm. Accounting for TB prevalence, ability to produce and submit sputum, and loss to follow-up increases the sample to 453 per arm or 1359 for the whole study.

Sample size for one antibiotic arm against standard of care arm

The sample size estimates along with assumptions for this comparison are shown in the table 2B. To achieve the desired 80% discriminatory power, we will need to recruit at least 388 sputum-test-negative participants per arm. Accounting for TB prevalence, ability to produce and submit sputum, and loss to follow-up increases the sample to 606 per arm or 1819 for the whole study (The ethics approved protocol uses an older calculation that yields 625 per arm and 1875 for whole study).

Power for clinical impact outcome

For the clinical impact of trial-of-antibiotics outcome, we assume a 4% baseline risk of composite outcome, and a loss to follow-up of 10% by Day 29. Using the sample size of 625 participants per arm (obtained in table 2B), and a type I alpha of 5%, we will be able to detect the difference between arms with 80% power, if the risk in the intervention arm is twice that of the standard of care arm. This estimate is applicable to all comparisons shown in section 3.

Table 2A Sample size estimation for the *diagnostic impact outcome* comparing a combination of two antibiotic arms to standard of care arm (2:1 comparison)

Power (X2 difference between independent proportions)	Effect size (50% SoC vs 60% amoxycillin or azithromycin)	Effective sample per arm (sputum negative participants needed)
0.80	0.10	290
0.85	0.10	332
0.90	0.10	388

Highlighted entries indicates target power and respective sample size estimates based on knowledge of TB risk, ability to produce and submit sputum and loss to follow-up.
Stata code: power two proportions 0.5 and 0.6, test (χ^2), power (0.80), n ratio (2).
SoC, standard of care; TB, tuberculosis.

Table 2B Sample size estimation for the *diagnostic impact outcome* one antibiotic arm to standard of care arm (pairwise comparison)

Power (X2 difference between independent proportions)	Effect size (50% SoC vs 60% amoxycillin or azithromycin)	Effective sample per arm (sputum negative participants needed)
0.80	0.10	388
0.85	0.10	443
0.90	0.10	519

Highlighted entries indicates target power and respective sample size estimates based on knowledge of TB risk, ability to produce and submit sputum, and loss to follow-up.
Stata code: power two proportions 0.5 and 0.6, test (χ^2), power (0.80), n ratio (1).
SoC, standard of care; TB, tuberculosis.

Power for AMR outcome

Study arms will be compared based proportion of participants with resistant *S. pneumoniae* on Day 29. We assume that 45% of Day 29 nasopharyngeal swabs will successfully grow *S. pneumoniae*, and that 10% of the isolates will meet the definition of resistance (described earlier under outcomes), and that 10% will be lost to follow-up by Day 29. Therefore, on Day 29, the standard of care arm (of 625 participants) will have 253 *S. pneumoniae* isolates, 25 of which would meet the definition of resistance. This translates into a 4% (25/625) risk of AMR positive cases in the standard of care arm. To detect a two-fold change in odds of Day 29 AMR risk with at least 80% power, using Pearson's χ^2 test, at 0.05 alpha, we will need at least 431

and 553 participants per arm for the 2:1 and pairwise comparisons, respectively.

Monitoring and oversight

The trial will be monitored by the Research Support Centre Clinical Trials Unit of the University of Malawi College of Medicine. An independent Data and Safety Monitoring Board (DSMB), and a Trial Steering Committee have been set up and meet bi-annually.

Trial closure

We will consider the trial closed after completing follow-up of the last enrolled participant, and on recording all mycobacteriology laboratory reports. Antimicrobial resistance laboratory work will continue beyond trial closure. The trial may be terminated early by the Trial Steering Committee on recommendation of the DSMB. The halting rule for a trial arm is an unacceptable high level of deaths assessed using an alpha determined at the first DSMB meeting.

PATIENT AND PUBLIC INVOLVEMENT

Patients were involved in the design of the study especially the ACASI used for collecting primary outcome data. Health workers were involved in the design of study visits and patient flow.

DISCUSSION

The ACT-TB study will investigate the benefits and consequences of 'trial-of-antibiotics,' a widely promoted approach to many patients with suspected tuberculosis in low- and middle-income countries without solid evidence base. To our knowledge, ACT-TB study is the first RCT of this kind. Results of our trial will add to the evidence-base regarding routine diagnosis of tuberculosis in low- and middle-income countries and strengthen our fight against AMR. Both tuberculosis and AMR are diseases of major importance globally, with tuberculosis causing an estimated 1.6 million deaths in 2017 and AMR projected to cause 10 million deaths per year by 2050.[2 27]

Choice of study interventions

We have chosen amoxicillin because it is the first-line treatment for outpatient management of pneumonia in Malawi and is commonly used for trial-of-antibiotics. It also provides data of immediate programmatic relevance and a starting point to investigate exacerbation of pre-existing AMR pressure. However, amoxicillin may not demonstrate the full benefits for trial-of-antibiotics because of organisms with intrinsic ('atypicals') or acquired (common in gram-negative organisms, and *Staphylococcus aureus*) penicillin resistance.[28] Oral antibiotics that may provide the better diagnostic discrimination for bacterial versus mycobacterial causes of cough are macrolides, such as azithromycin, because of better intrinsic coverage of 'atypical' intracellular organisms such as *mycoplasma* species that cause community acquired pneumonia,[29–31] and low levels of acquired macrolide-resistance in bacterial isolates in Malawi.[28]

ACASI for post-treatment improvement assessment

Our systematic review[18] did not identify a consistent definition of tuberculosis or no tuberculosis based on trial-of-antibiotics. A definition of clinical change following antibiotic treatment is necessary for the trial-of-antibiotics as this determines who get categorised as well or tuberculosis-positive. Approaches that ranged from self-reported improvement to a combination of clinical and radiological assessments are likely to be highly subjective and prone to bias, as well as being a potentially avoidable source of heterogeneity between studies. In this study, we hope to address these biasses (particularly, inter-observer variability, and patient/interviewer reporting or ascertainment biasses) by using self-rated change of illness (on Day 8) recorded using a self-completed questionnaire, the ACASI (described under outcomes). The ACASI questionnaire, the delivery platform and the resulting data management can all be replicated in future studies, creating potential for more standardisation in assessment of clinical response to treatment.

Potential clinical impact of antibiotics

In areas with high HIV prevalence, empirical antibiotics during tuberculosis investigations could be life-saving: mortality immediately before and after tuberculosis diagnosis is high,[3 32] and is often secondary to severe bacterial infections.[32–34] The leading aetiologies of infection and death on tuberculosis treatment as well as among outpatients with tuberculosis-like symptoms are *S. pneumoniae* and non-typhoidal salmonellae: both can present with cough (primary cause) or as comorbidities (super-infections) in patients presenting with active *Mycobacterium tuberculosis* disease.[32–34] If effective treatment of this type of life-threatening primary/super-infections reduces mortality during the diagnostic workup of suspected tuberculosis in people living with HIV, then empirical use of broad-spectrum antibiotics would be indicated for this purpose alone, irrespective of any diagnostic contribution to tuberculosis treatment decisions. In this context, azithromycin may be the most effective arm, as salmonella infections are highly sensitive to azithromycin, but not to amoxicillin.[28]

AMR and trial-of-antibiotics

Antimicrobial resistance relating to antibiotic use during evaluation for suspected tuberculosis has not been investigated before. Previous work has shown that empirical antibiotics can drive rapid emergence of antimicrobial resistance.[35 36] Co-trimoxazole prophylaxis for HIV-positive patients, introduced in 2005, was followed by near-universal resistance in bloodstream infections by 2010.[37] Mass drug administration of azithromycin for trachoma control initially reduces nasopharyngeal carriage of *S. pneumoniae*, but with increased macrolide-resistance 6 months later.[38 39]

In this study we have the opportunity to assess the extent to which brief exposure drives antimicrobial resistance during diagnostic workup for tuberculosis. An ecological niche for many bacterial species, the upper respiratory tract also presents a convenient sampling opportunity for investigating antimicrobial resistance.[40] *S. pneumoniae* is the organism of choice not only for being an important cause of respiratory tract infections but also because it often colonises the upper respiratory tract, acquires resistance readily and has well documented laboratory investigation procedures in place.[24] As exploratory analyses, we will also assess nasopharygeal colonisation and antimicrobial resistance in relation to tuberculosis treatment and HIV status.

Important subgroups

Clinical response to trial-of-antibiotics is possible and indeed well-described in patients with bacteriologically confirmed tuberculosis (ie, false-negatives/low sensitivity from the perspective of tuberculosis diagnosis) may relate to multiple super-infections.[4 33] As such, this phenomenon may vary by HIV status, since multiple concurrent infections are a hallmark of advanced HIV immunosuppression, and are most commonly reported in patients with suspected tuberculosis in the pre-ART era. In 2015, in Malawi, 45% of adults who presented to primary care with prolonged cough (≥2 weeks) were HIV-positive, of whom only~20% started tuberculosis treatment on the basis of positive mycobacteriology.[23] As such, the benefits and consequences of trial-of-antibiotics may vary by HIV status and ART coverage, and by subsequent tuberculosis treatment decisions. We will, therefore, include a prespecified subanalysis of trial outcomes stratified by HIV and ART status.

Limitations

The study has several limitations. First, we did not use a placebo-control arm. Second, the study is not adequately powered to evaluate safety of deferred antibiotic treatment or conduct subgroup analyses of outcomes by HIV status, both of which are important evidence gaps. Other limitations include the possibility that participants randomised to the standard-of-care arm may find alternative access to antibiotics therefore misclassifying exposure/intervention status. There is also a possibility of misclassifying active tuberculosis status because of the suboptimal nature of the available tests.

ETHICS AND DISSEMINATION

The study has been reviewed and approved by the University of Malawi College of Medicine Research and Ethics Committee (COMREC; registration number P.04/18/2381), the London School of Hygiene & Tropical Medicine Research Ethics Committee (LSHTM EC; registration number 15232) and Regional Committee for Health and Research Ethics, NTNU-Midt, Norway (REK nord; registration number 208/1964). Regulatory approval has been granted by the Malawi Pharmacy, Medicines and Poisons Board (PMPB; registration number CTRC/III/14062018102). We will present any future protocol modifications to these bodies before implementing. We will submit results for publication in a peer-reviewed journal. We will submit abstracts to relevant national and international conferences. This work will also form part of a PhD thesis for THD, which he will submit to the LSHTM. This study will follow the standards set by CONSORT guidelines.

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Contributors THD, KF and ELC are the main contributors to the conception, and design of the study. DS, MN and PM contributed to the general study planning and clinical design. NF contributed to the general study planning and antimicrobial resistance design. CK, LC, SC, and MJN contributed to the design, piloting and refining of study and clinical procedures. THD developed the first draft of the manuscript. All authors carefully reviewed and substantially contributed to the development of the trial protocol and this manuscript. All authors read and approved the final manuscript. THD is the guarantor for this work.

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4.3 Randomised trial statistical analysis plan

Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)

Statistical Analysis Plan

Full Title	**Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)**		
Acronym	**A**ccuracy and **C**onsequences of using **T**rial-of-antibiotics for **TB** diagnosis (ACT-TB Study)		
Document History	**Version No.**	**Version Date**	**Description of Change**
	1.0	20 May 2020	Initial release
Protocol	Version 4.0, 27 Jan 2020		
Trial Registration	ClinicalTrials.gov (NCT03545373)		
Principal Investigators	Titus Divala		
SAP Authors	Titus Divala, Elizabeth Corbett and Katherine Fielding		

Table of Contents

1 Introduction

The present document comprises the Statistical Analysis Plan (SAP) for **A**ccuracy and **C**onsequences of using **T**rial-of-antibiotics for **TB** diagnosis (ACT-TB Study), a trial investigating benefits and risks of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients.

Scoping statement

The SAP contains:

- details of the planned statistical analyses associated with a clinical study so that the analyses are planned with the desired work product(s) in mind and can be conducted in a consistent, repeatable manner.
- detailed requirements and parameters for the reporting database, statistical programs/output reports, and any tests of the robustness and sensitivity of the analysis.
- example tables, figures and listings.

2 Study design summary

Title	Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)
Design	Three arm (625 per arm) individually randomised (1:1:1), open-label controlled clinical trial investigating standard care diagnostic approach for tuberculosis. The trial will not use any unlicensed products.
Objective	**Outcomes**
Primary	
1. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with cough (and have a valid sputum test result) at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among participants with a valid result from at least one sputum TB test
2. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.	Proportion of participants experiencing at least one of the following adverse outcomes by Day 29: 1) death 2) hospitalisation

	3) missed TB diagnosis
Secondary	
3 To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.	Proportion of day 29 nasopharyngeal *Streptococcus pneumoniae* isolates resistant to commonly used antimicrobials.
4. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in primary care presenting Malawian adults with cough including those unable to produce sputum.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among all randomised participants, with those who could not provide sputum classified as mycobacteriologically negative.
5. To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other. **The SAP does not cover outcomes under this objective.**	• Incremental cost per quality adjusted life year gained • Total direct medical costs per participant over 56 days • Eq-5D utility score
Exploratory	
Our exploratory analyses will be comparisons between the azithromycin and amoxicillin arms for all our primary and secondary outcomes.	
Population	Adults presenting to primary care centres in Malawi reporting cough.
	Inclusion criteria: • Ambulatory clinic attendees presenting with cough • Should have been ill for ≥ 14 days • Aged at least 18 years • Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period. Exclusion criteria: • Self-reported allergy to study medications • Acute danger signs defined in national TB program treatment guidelines • Tuberculosis treatment or isoniazid preventive therapy in the last 6 months • Treated with antibiotics, other than co-trimoxazole prophylaxis, for the current illness or within the past 14 days
Treatment	**Arm 1**: Azithromycin 500mg once daily for 3 days commencing on randomization day.

	Arm 2: Amoxicillin 1 g 3 times daily for 5 days commencing on randomization day. **Arm 3**: Standard of care in current national guidelines for patients presenting with cough and without danger signs (No treatment until re-evaluation with sputum TB test results)
Duration	Antibiotics will be prescribed on the randomisation day (Day 1) and follow up activities performed on days 8 and 29.

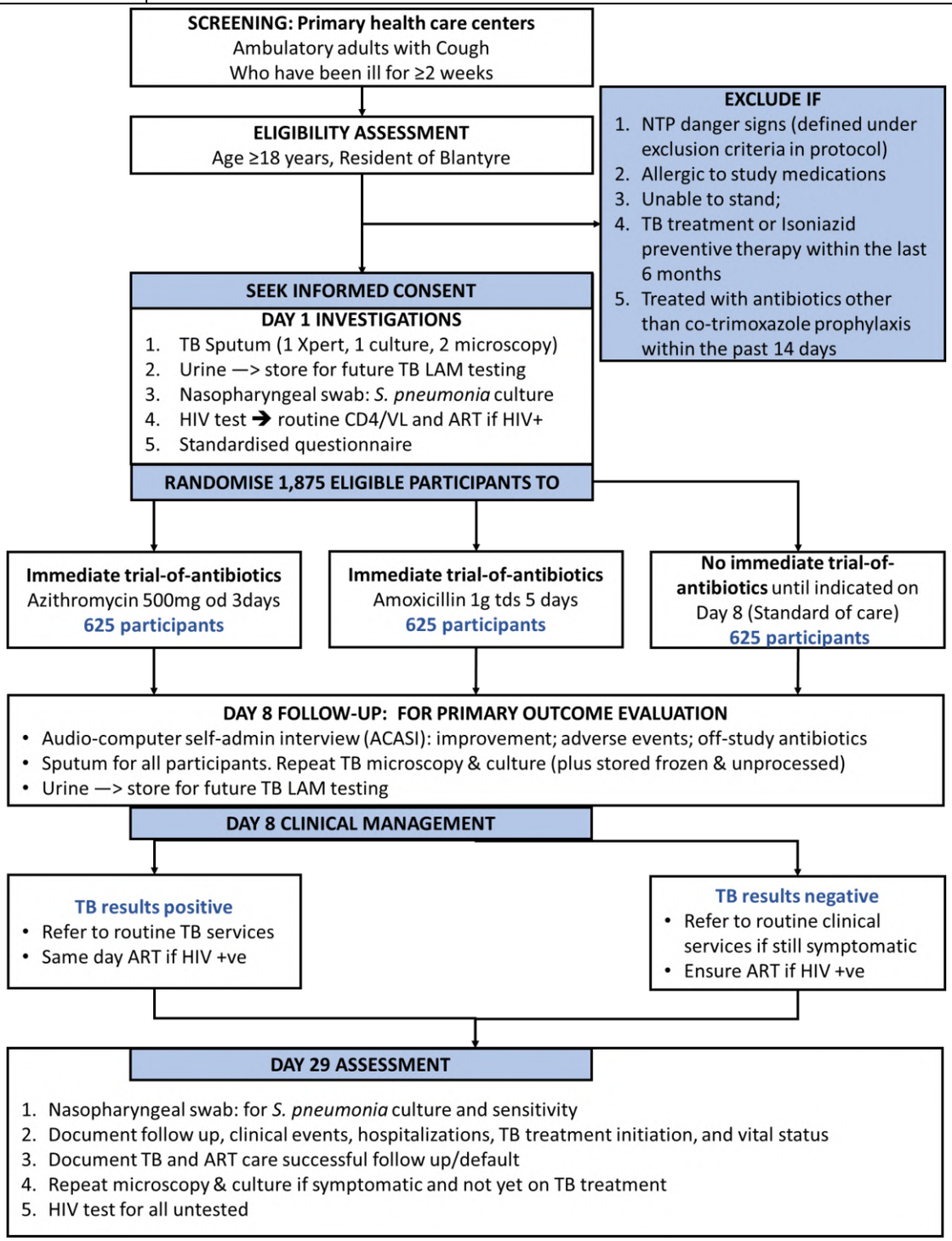


Figure 1: Flow diagram for the planned clinical trial in Blantyre, Malawi

3 General approach for outcome analysis

Study outcome definitions are expanded in sections 8, 9 and 10. For all outcomes, we will perform analyses and report measures of effect from the following comparisons:

a. azithromycin or amoxicillin (combined) versus standard of care

b. azithromycin versus standard of care

c. amoxicillin versus standard of care

d. Exploratory analysis: azithromycin vs amoxicillin

All analyses will be adjusted for randomisation strata of primary care centre (two strata). The main study results will be based on comparison a. Comparison d is exploratory. We will perform analyses b and c without accounting for multiple comparisons. Imbalances at baseline are unlikely, though will assessed without the use of hypothesis testing. Any major imbalances will be adjusted for.

4 Sample size justification

We performed power and sample size estimations for the diagnostic impact, clinical impact, and AMR impact outcomes as follows:

4.1 Diagnostic impact outcome

We assume that at Day 8, change in well-being from baseline state in trial-of-antibiotics (azithromycin or amoxicillin) arms will correctly classify 60% of all mycobacteriology negative participants (i.e 60%specificity in trial-of-antibiotics arms). [12] We wanted to estimate a sample size that would provide a discriminatory power of 80% at a two-sided significance level of 5%, to detect at least 10% difference in specificity (i.e ≤50% specificity in standard of care arm). The sample sizes will differ by the number of arms being compared, we have therefore provided two separate estimates in line with type of comparisons specified under section 7.

4.1.1 Sample size for a combination of 2 antibiotic arms against standard of care arm

The sample size estimates along with assumptions for this comparison are shown in the Table 1A. To achieve the desired 80% discriminatory power, we will need to recruit at least 305 sputum-test-negative participants per arm. Accounting for TB prevalence, ability to

produce and submit sputum, and loss-to-follow up increases the sample to 472 per arm or 1,416 for the whole study.

Table 1A: Sample size estimation for the *diagnostic impact outcome* comparing a combination of two antibiotic arms to standard of care arm

POWER (X2 difference between independent proportions**)**	**Effect size (**50% SoC vs 60% amoxycillin or azithromycin**)**	**Effective sample per arm (**Sputum negative**)**	**Include sputum positive (**20%**)**	**Include inability to submit sputum (**15%**) and LTFUP (**5%**)**	**Total sample size (**all three arms**)**
0.60	0.10	262	328	409	1,228
0.65	0.10	292	365	456	1,369
0.70	0.10	325	406	508	1,523
0.75	0.10	363	454	567	1,702
0.80	0.10	400	500	625	1,875
0.85	0.10	463	579	723	2,170
0.90	0.10	538	673	841	2,522
0.95	0.10	661	826	1,033	3,098
	Target power and respective sample size estimates based on knowledge of TB risk, ability to produce and submit sputum, and loss-to-follow up.				

4.1.2 Sample size for one antibiotic arm against standard of care arm

The sample size estimates along with assumptions for this comparison are shown in the Table 1B. To achieve the desired 80% discriminatory power, we will need to recruit at least 400 sputum-test-negative participants per arm. Accounting for TB prevalence, ability to produce and submit sputum, and loss-to-follow up increases the sample to 625 per arm or 1,875 for the whole study.

Table 1B: Sample size estimation for the *diagnostic impact outcome* one antibiotic arm to standard of care arm

POWER (X2 difference between independent proportions**)**	**Effect size (**50% SoC vs 60% amoxycillin or azithromycin**)**	**Effective sample per arm (**Sputum negative**)**	**Include sputum positive (**20%**)**	**Include inability to submit sputum (**15%**) and LTFUP (**5%**)**	**Total sample size (**all three arms**)**

0.60	0.10	196	245	306	919
0.65	0.10	218	273	341	1,022
0.70	0.10	243	304	380	1,139
0.75	0.10	271	339	423	1,270
0.80	0.10	305	381	477	1,430
0.85	0.10	347	434	542	1,627
0.90	0.10	403	504	630	1,889
0.95	0.10	495	619	773	2,320
	Target power and respective sample size estimates based on knowledge of TB risk, ability to produce and submit sputum, and loss-to-follow up.				

4.2 Power for clinical impact outcome

For the clinical impact of trial-of-antibiotics outcome, we assume a 4% baseline risk of composite outcome, and a loss to follow up of 10% by Day 29. Using the sample size of 625 participants per arm (obtained in Table 2B), and a type I alpha of 5%, we will be able to detect the difference between arms with 80% power, if the risk in the intervention arm is twice that of the standard of care arm. This estimate is applicable to all comparisons a, b and c shown in section 3.

4.3 Power for AMR outcome

Study arms will be compared based proportion of participants with resistant *Streptococcus pneumoniae* on day 29. We assume that 45% of Day-29 nasopharyngeal swabs will successfully grow *Streptococcus pneumoniae,* and that 10% of the isolates will meet the definition of resistance (described earlier under outcomes), and that 10% will be lost to follow up by Day 29. Therefore, on day 29, the standard of care arm (of 625 participants) will have 253 *Streptococcus pneumoniae* isolates, 25 of which would meet the definition of resistance. This translates into a 4% (25/625) risk of AMR positive cases in the standard of care arm. To detect a twofold change in odds of day 29 AMR risk with at least 80% power, using Pearson's Chi-squared test, at 0.05 alpha, we will need at least 431 and 553 participants per arm for the 2:1 and pairwise comparisons respectively.

5 General definitions for analysis

5.1 Study period and visit definitions

Recruitment of patients started on 25 March 2019 and will complete in or after March 2020. Following randomisation (day 1), each participant is expected to attend follow up visits at day 8 (+/-3 days) and day 29 (+3 weeks). Table 1 shows study activities planned for each study visit.

Table 1: Activities over the study period

	STUDY PERIOD			
	Enrolment	Follow up		
TIMEPOINT	Day 1	Day 8		Day 29
ENROLMENT:				
Eligibility screen	x			
Informed consent	x			
Randomisation	x			
INTERVENTIONS:				
Azithromycin	x			
Amoxicillin	x			
Standard of care	x			
ASSESSMENTS:				
Demographics	x			
History of antibiotic use	x	x		x
History & examination	x	x		x
TB symptoms	x	x		x
Sputum collection[1]	x	x		[x]
Urine for TB LAM test[2]	x	x		
Nasopharyngeal swab for AMR[3]	x			x
HIV test	x			
Linking to routine care	x	x		x
ACASI[4]		x		
Clinical events[5]				x

Update contact & address		x		x

1. On Day 29 collect sputum and perform mycobacteriology if the participant is symptomatic
2. Urine sample collected for mycobacterial lipoarabinomannan when Fujifilm SILVAMP TB LAMtest becomes available
3. Nasopharyngeal swab for Streptococcus pneumoniae culture and sensitivity as a way of determining risk of antimicrobial resistance (AMR)
4. Audio Computer Assisted Self-Interview (ACASI) for documenting change of symptoms on Day 8 versus Day 1
5. Illnesses, clinic visits, radiological outcomes, new HIV diagnosis, new tuberculosis diagnosis, death, hospitalisation, missed tuberculosis diagnosis

5.2 Study populations

Patients are considered randomized when they are assigned a randomization number. All analyses will follow the intention to treat principle. Each participant will be analysed based on the treatment arm they were allocated to regardless of adherence to study protocol. Analysis for the primary outcome 1 (proportion of sputum-test-negative participants who are ACASI-test-negative) will be restricted to participants who have at least one valid sputum-test-result.

5.3 Subgroup definitions

We will perform sub-group analyses for diagnostic accuracy and clinical benefit outcomes for the following variables; i) HIV status (positive, negative or unknown), ii) antiretroviral treatment (started/not started) as documented on enrolment day.

5.4 Treatment assignment and treatment arms

Randomization lists, stratified by study site (primary care centre), with variable block sizes and indicating a randomization number and which treatment is to be given, were produced prior to the start of the trial by a London based statistician not affiliated with the study. The code for each individual was provided in separate sealed envelopes and assigned to individuals in the order in which they are being enrolled in the study. Management of patients is not blinded due to the cost of arranging placebo. However, study team masking will be maintained with all study outcome assessment occurring without reference to randomisation arm.

6 Study patients

6.1 Patient flow

A clear accounting of all patients who entered the study, using figures and/or tables will be presented. Numbers of patients screened, randomized, and completed each phase of the study as well as reasons for withdrawals presented by treatment arm using a Consolidated Standards of Reporting Trials (CONSORT) flow chart (appendix 1). This is a generic flow chart and will be repeated for all scenarios for the primary outcome.

6.2 Inclusion and exclusion criteria

Inclusion and exclusion criteria are as follows:

6.2.1 Inclusion criteria:

To be eligible for inclusion in this trial, patients have to fulfil all of the following criteria:

- Ambulatory clinic attendees presenting with cough
- Unwell for at least 14 days
- Aged at least 18 years

Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period.

6.2.2 Exclusion criteria

Patients who meet any of the following criteria will not be eligible for the study.

- Self-reported allergy to study medications
- WHO/Malawi National tuberculosis Program (NTP) danger signs: respiratory rate > 30/min, temperature >39ºC, Heart rate >120/minute, confused/agitated, respiratory distress, systolic blood pressure <90 mmHg, inability to walk unassisted
- Treated with antibiotics other than co-trimoxazole prophylaxis within the past 14 days
- Tuberculosis treatment or isoniazid preventive therapy within the last 6 months

6.3 Incomplete follow up

Frequency of withdrawals and loss to follow up will be summarised by treatment arm along with respective reasons.

7 Demographics and baseline characteristics

Demographic and baseline clinical characteristics of the randomized patients will be summarised by treatment arm (see appendix 2 for table shells). The distribution of categorical variables will be summarised by percentages. Quantitative variables will be summarised using the mean and standard deviation (SD) or median and inter-quartile range (IQR), where appropriate, and the minimum and maximum and sample size of non-missing data. We will present demographic and baseline clinical characteristics for intention-to-treat and diagnostic assessment populations.

8 Diagnostic impact of trial of antibiotics

Primary and secondary diagnostic impact analyses will be based on the investigational and reference tests as defined in the following sections.

8.1 Definitions for investigational test

The investigational test is change in symptoms at Day 8 categorised as: improved or not improved (no change or worsened) in response to the following question delivered to patients using Audio Computer Assisted Self-Interview (ACASI): *on day 1, you reported that you were unwell; compared to that day, has your illness worsened, remained the same, or improved?*

We will term ACASI interview outcome as

- **ACASI-test-negative,** if the participant reports improvement (i.e TB ruled out)
- **ACASI-test-positive,** if the participant reports no change or worsening (i.e TB likely)

8.2 Definitions for reference standard test

The reference standard test will be defined based on sputum mycobacteriology test result on days 1 and 8. The interpretation of the reference standard will be as follows for each diagnostic impact outcome:

8.2.1 Reference standard definition for Primary outcome 1

- **Sputum-test-positive,** if there is at least one positive of smear microscopy, Xpert/MTB/RIF, or MTB culture;
- **Sputum-test-negative,** if none of the tests are positive AND at least one test is known to be negative.

8.2.2 Reference standard definition for Secondary Outcome 2

- **Sputum-test-positive,** if there is at least one positive of smear microscopy, Xpert/MTB/RIF, or MTB culture;
- **Sputum-test-negative,** if none of the tests are positive AND
    - at least one test is known to be negative
    - patient was unable to produce a sputum sample

8.3 Diagnostic assessment outcome

The diagnostic assessment outcome will be defined for each study arm as the proportion of sputum-test-negative who are ACASI-test-negative as illustrated in Figure 2.

		Reference Result: *any positive smear microscopy, Xpert/MTB/RIF, or MTB Culture from sputum samples collected on Day 1 and Day 8 visit defines tuberculosis-test-positive*	
		Sputum-test-positive	Sputum-test-negative
ACASI* Response on Day 8 *ACASI test is defined by response to the following question asked using ACASI on Day 8: on day 1, you reported that you were unwell; compared to that day, has your illness worsened, remained the same, or improved?*	ACASI-test-positive (worse or no change)	**a**	**b**
	ACASI-test negative (Improved)	**c**	**d**

Primary outcome: specificity, calculated by **d / (b+d)**

*Audio Computer Assisted Self-Interview (ACASI) in which the participant, after a how-to-use test session, responds to the prescribed question on a database-linked android tablet, without any human interaction, and in private.

-*+**Figure 2**: Assessing the diagnostic value of a change in symptoms from baseline to day 8

8.4 Analysis for assessing diagnostic impact of trial-of-antibiotics

We will perform comparisons (specified under section 7) of the proportion of sputum-test-negative participants who are also ACASI-test-negative (d/[b+d] in Figure 2) in trial-of-antibiotics (azithromycin and/or amoxicillin) arms to standard of care arm (Figure 2) (i.e a comparison of specificities with versus without trial-of-antibiotics using a generalised linear model (GLM) with binominal family, and (i) identity link to estimate risks differences and (ii) the GLM with log link to estimate risk ratios. If the outcome is rare or if GLM model with log link does not converge, we will use logistic regression to model odds and report odds ratios. All analyses will adjust for primary care centre based on where the participant was enrolled from (2 strata). For each comparison, we will report the point estimate, 95% CIs and p-values. Table shells for this analysis are shown in Appendix 3 for primary outcome, Appendix 4 for secondary outcome, and Appendix 5 for detailed diagnostic performance parameters.

9 Clinical impact of trial-of-antibiotics

Clinical impact of trial-of-antibiotics will be determined by comparing the proportion of participants experiencing at least one of the following adverse outcomes between arms: death, hospitalisation, TB misdiagnosis, HIV care loss to follow up, TB care loss to follow up.

9.1 Outcome definitions

The following are definitions of the components of this composite clinical outcome documented on day 29 (visit window: up to day 29+3 weeks):

Outcome component	Definition
death	death before or on Day 29
hospitalisation	being hospitalised for any reason at any point before or on Day 29
missed TB diagnosis	Participants not identified as TB positive at day 1 or day 8 but started on TB treatment based on either a day 29 sputum sample or routine care clinical decision made following study team referral on Day 29 visit

9.2 Analysis for assessing clinical impact of trial-of-antibiotics

For each component of the composite outcome, we will report cumulative incidence by study arm. We will perform comparisons (specified under section 7) of the proportion of participants experiencing the composite clinical outcome using a GLM with binomial family and, (i) identity link to estimate risks differences and (ii) log link to estimate risk ratios. If the outcome is rare or if GLM model with log link does not converge, we will use logistic regression to model odds and report odds ratios. All analyses will adjust for primary care centre based on where the participant was enrolled from (2 strata). For each comparison, we will report the point estimate, 95% CIs and p-values. Table shells for this analysis are shown in Appendix 3 for primary outcome, and Appendix 4 for secondary outcome.

10 Impact of trial-of-antibiotics on antimicrobial resistance (AMR)

Impact of trial-of-antibiotics on AMR will be determined by comparing, between arms, the proportion of participants meeting AMR positive definition at day 29.

10.1 Outcome definitions

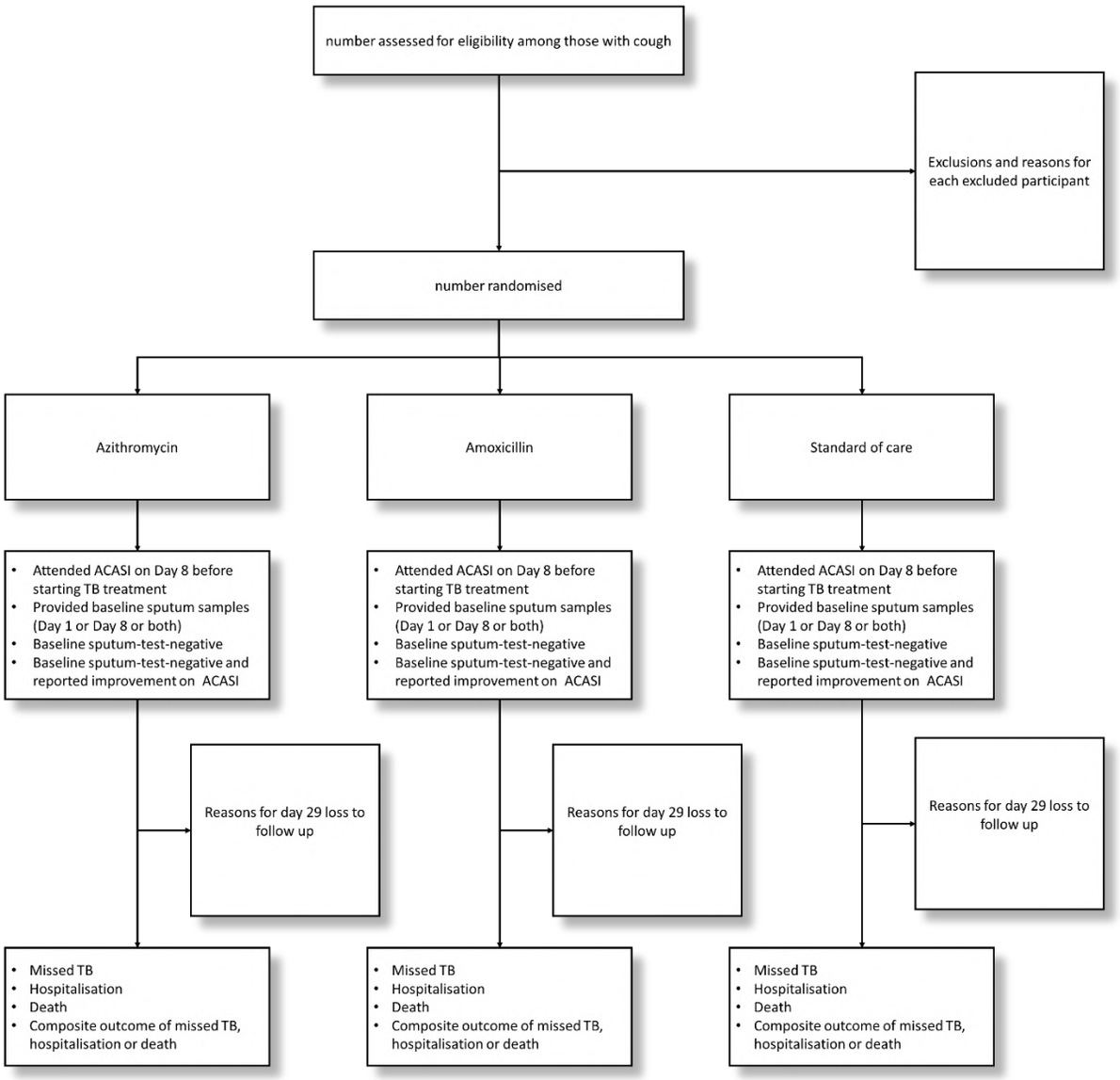
We will define **AMR positive** as having nasopharyngeal isolates of *Streptococcus pneumoniae* that are resistant to any of the following commonly used antibiotics: ceftriaxone, amoxycillin, cefoxitin, azithromycin, and erythromycin as determined using disc diffusion

technique; and **AMR negative** as either (1) not isolating any *Streptococcus pneumoniae* or (2) isolating any *Streptococcus pneumoniae* that is not resistant to any of the assessed antibiotics. For each arm, and at both baseline and day 29, we will report proportion of AMR positive participants. The study outcome will be the proportion of AMR positive participants at day 29.

10.2 Analysis for assessing clinical impact of trial-of-antibiotics

To determine impact of trial-of-antibiotics on AMR, we will perform comparisons (specified under section 7) of the Day 29 proportion of AMR positive participants using a GLM with binomial family and, (i) identity link to estimate risks differences and (ii) log link to estimate risk ratios. If the outcome is rare or if GLM model with log link does not converge, we will use logistic regression to model odds and report odds ratios. All analyses will adjust for primary care centre based on where the participant was enrolled from (2 strata). For each comparison, we will report the point estimate, 95% CIs and p-values. Table shells for this analysis are shown in Appendix 4.

11 APPENDIX 1: Study Consort Diagram



12 APPENDIX 2: Participant characteristics at baseline

		Amoxicillin and azithromycin			Azithromycin			Amoxicillin			Standard of care	
randomised (n, %)												
Site												
Limbe (n, %)												
Ndirande (n, %)												
Age in years (mean, SD)												
Gender												
Female (n, %)												
Male (n, %)												
Pregnant *(n, %)*												
Signs and symptoms												
Fever (n, %)												
Night sweats (n, %)												
Chest pain (n, %)												
Blood in sputum (n, %)												
Self-reported weight loss (n, %)												
BMI (mean, SD)												
Low BMI (defined as weight/height^2 of <19) (n, %)												
Previous TB *(n, %)*												
Months since last dose of TB treatment *(mean, sd)*												
HIV status												
HIV positive *(n, %)*												
HIV negative *(n, %)*												
HIV unknown *(n, %)*												
ART Status if HIV positive												
On ART *(n, %)*												
Not on ART *(n, %)*												
Years in education *(n, %)*												
AMR positive swab *(n, %)*												

13 APPENDIX 3: Results for primary outcomes

	Amoxicillin and azithromycin	Azithromycin	Amoxicillin	Standard of care
Day 8 Primary outcome: *proportion of sputum-TB-negative participants who report symptom improvement on Day 8 ACASI*				
Sputum-TB-positive (n, N)				
Sputum-TB-negative (n, N)				
Reported improvement on Day 8 ACASI (n, N)				
Reported improvement on Day 8 ACASI and were also Sputum-TB-negative				
n, N				
RD (95%CI), p-value*				*Reference*
RR (95%CI), p-value*				*Reference*
Day 29 Primary outcome: Proportion of participants experiencing missed TB, hospitalisation or death				
Missed TB (n, N)				
Hospitalisation (n, N)				
Death (n, N)				
Composite endpoint of missed TB, Hospitalisation and Death				
n, N				
RD (95%CI), p-value*				*Reference*
RR (95%CI), p-value*				*Reference*

RD risk difference RR risk ratio, CI confidence interval, ACASI Audio Computer Assisted Self-Interview

* adjusted for study site

Subgroup analyses for specificity

	Amoxicillin and azithromycin	Azithromycin	Amoxicillin	Standard of care
Day 8 Primary outcome: *proportion of sputum-TB-negative participants who report symptom improvement on Day 8 ACASI*				
OVERALL				
n, N				
RD (95%CI), p-value*				*Reference*
RR (95%CI), p-value*				*Reference*

		Amoxicillin and azithromycin		Azithromycin		Amoxicillin		Standard of care
Day 8 Primary outcome: *proportion of sputum-TB-negative participants who report symptom improvement on Day 8 ACASI*								
HIV POSITIVE (include previously diagnosed and newly diagnosed)								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*
HIV NEGATIVE								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*
HIV UNKNOWN								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*
HIV POSITIVE ON ART								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*
HIV POSITIVE NOT ON ART								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*

14 APPENDIX 4: Results for secondary outcomes

		Amoxicillin and azithromycin		Azithromycin		Amoxicillin		Standard of care
Day 8 Secondary outcome								
Either sputum-TB-negative or no valid sputum result available (n, N)								
Reported improvement on Day 8 ACASI and were also either sputum-TB-negative or could not produce sputum								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*
Day 29 Secondary outcome								
Antimicrobial resistance positive at Day 29 (n, %)								
n, N								
RD (95%CI), p-value*								*Reference*
RR (95%CI), p-value*								*Reference*

RD risk difference RR risk ratio, CI confidence interval, ACASI Audio Computer Assisted Self-Interview

* adjusted for study site

15 APPENDIX 5: Diagnostic performance of ACASI-reported change in symptoms against a sputum TB diagnostic reference

	Amoxicillin and azithromycin	Azithromycin	Amoxicillin	Standard of care
Part A: Participants with sputum test results				
Number of participants				
True positive (n, %)				
False negative (n, %)				
False positive (n, %)				
True negative (n, %)				
Prevalence (95% CI)				
Positive predictive value (95% CI)				
Negative predictive value (95% CI)				
Sensitivity (95% CI)				
Specificity (95% CI)				
ROC area (95% CI)				
Part B: All study participants included, treating those unable to produce sputum as reference test negative				
Number of participants				
True positive (n, %)				
False negative (n, %)				
False positive (n, %)				
True negative (n, %)				
Prevalence (95% CI)				
Positive predictive value (95% CI)				
Negative predictive value (95% CI)				
Sensitivity (95% CI)				
Specificity (95% CI)				
ROC area (95% CI)				

5 Results of the randomised controlled trial

5.1 Introduction

In this chapter, I present results of the randomised controlled trial (ACT TB study) as described in a manuscript I have submitted to The Lancet. The trial design and analysis approach is described in detail under Chapter 4 of this thesis.

5.2 Manuscript of the randomised controlled trial results

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1700548	**Title**	Dr
First Name(s)	Titus, Henry		
Surname/Family Name	Divala		
Thesis Title	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)		
Primary Supervisor	Professor Katherine Fielding		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	THE LANCET
Please list the paper's authors in the intended authorship order:	Titus H Divala MBBS, Prof Elizabeth L Corbett FMedSci, Chikondi Kandulu MBBS, Brewster Moyo MSc, Peter MacPherson PhD, Marriott Nliwasa PhD , Prof Neil French PhD, Derek Sloan PhD, Lingstone Chiume BSc, Masiye Ndaferankhande BPharm, Sanderson Chilanga BSc, Sabina

	Majiga-Kadzuwa RN, Prof Jon Øyvind Odland PhD, Prof Katherine L Fielding PhD
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study, wrote the study protocol, led the data collection and analysis, led the writing of the manuscript, and submitted it for publication

SECTION E

Student Signature	[redacted]
Date	29 December 2020

Supervisor Signature	[redacted]
Date	23 Feb 2021

Title

Diagnostic accuracy, clinical impact and antimicrobial resistance consequences of using trial-of-antibiotics for tuberculosis diagnosis: a randomised controlled trial (ACT-TB study)

Authors

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Summary

Background

In low-income countries diagnostic algorithms often assume that tuberculosis can be 'ruled-out' in mycobacteriology-negative individuals whose symptoms improve with a "trial-of-antibiotics". We conducted a randomised controlled trial to investigate diagnostic performance, and clinical and antimicrobial resistance consequences.

Methods

We randomised (1:1:1) Malawian adults attending primary care for illness ≥2 weeks including cough with no immediate indication for hospitalisation, no antibiotic use within 14 days, and no tuberculosis treatment or preventive therapy within 6 months to: azithromycin (500mg daily, 3 days) amoxicillin (1g three times/day, 5 days); or standard-of-care (SOC) with no immediate antibiotic. Sputum at enrolment and day 8 was tested for tuberculosis (microscopy, Xpert MTB/RIF, culture). Primary outcomes were day 8 specificity (percentage with symptom improvement among mycobacteriology-negative), and day 29 clinical outcomes (composite: death, hospitalisation or missed tuberculosis diagnosis). The secondary outcome was day 29 risk of resistant *Streptococcus pneumoniae* identified by culture of nasopharyngeal swabs. (NCT03545373).

Findings

Between Feb-2019 and March-2020, we screened 5825 adults and randomised 1583 (mean age 36 years, 14.9% HIV-positive) to SOC (530), azithromycin (527), or amoxicillin (526). Overall, 6.3% (100/1583) had positive baseline mycobacteriology. Compared to SOC (79.1%), trial-of-antibiotics improved tuberculosis specificity: azithromycin vs. SOC difference +8.6% (95% confidence interval 3.9%, 13.3%); amoxicillin vs. SOC difference +8.8% (4.0%, 13.6%), but with extremely low sensitivity (10.7% azithromycin, 23.3% amoxicillin). Proportions with day 29 composite clinical outcome were similar between arms (SOC 1.1%, azithromycin 1.1%, amoxicillin 2.1%). Compared to SOC (5.3%), proportions with *Streptococcus pneumoniae* antimicrobial resistance were higher in azithromycin +2.5% (-0.5, 5.5), but similar in amoxicillin +0.2% (-2.9, 2.5).

Interpretation

Routine outpatient trial-of-antibiotics led to modest improvement in tuberculosis diagnostic specificity, but had extremely low sensitivity, offered no additional clinical benefits, and may have increased risk of antimicrobial resistance. Broad-spectrum antibiotics should be reserved for patients with defined clinical or microbiological indications.

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Research in context

Evidence before this study

Tuberculosis and antimicrobial resistance independently threaten the attainment of at least half of the 17 Sustainable Development Goals through ill health and severe disruption of productivity at individual, national and global levels. Tuberculosis remains the leading global infectious cause of death after COVID-19, while antimicrobial resistant infections are the projected leading cause of death by 2050. Antimicrobial resistant infections are on the rise in part due to non-pathogen directed prescription secondary to limited point-of-care diagnostics and laboratory infrastructure. Diagnostic algorithms designed to augment suboptimal tuberculosis diagnostics have for decades encouraged millions of broad-spectrum antibiotic prescriptions—termed trial-of-antibiotics— and assumed, without evidence, that post-treatment symptom improvement rules out tuberculosis. As a diagnostic approach used on such a large scale, trial-of-antibiotics must have high quality evidence base.

We searched Medline, Embase and Global Health for randomised trials published in any language up to 15 February 2021. We combined terms for “tuberculosis”, “antibiotic treatment”, terms for diagnostic accuracy (“sensitivity”, “specificity” and “predictive value”), and a filter for randomised trials, and identified no articles. Our recent systematic review and meta-analysis which pooled available observational data (only 8 studies), concluded that trial-of-antibiotics was yet to be supported by evidence, and the meta-analysis suggested that diagnostic performance was very poor. None of the identified studies systematically assessed impact on other clinical outcomes and antimicrobial resistance.

Added value of this study

The Accuracy and consequences of using trial-of-antibiotics for TB diagnosis (ACT-TB) randomised controlled trial, is the first randomised, and the most comprehensive examination of the diagnostic, clinical and antimicrobial resistance impact of trial-of-antibiotics to rule out tuberculosis. Compared to standard of care (79.1%), trial-of-antibiotics modestly improved diagnostic specificity for mycobacteriologically-confirmed tuberculosis with either azithromycin (88.7%) or amoxicillin (89.4%), but with very low sensitivity (10.7% azithromycin, 23.3% amoxicillin), and weak evidence for increased risk of antimicrobial resistance (azithromycin arm +2.5% [-0.5, 5.5]). There was no apparent impact on clinical (co-primary) outcomes. These results confirm the lack of benefit of trial-of-antibiotics, presenting an opportunity for improved antimicrobial stewardship. Safety of discontinuing trial-of-antibiotics in favour of an antimicrobial-sparing approach is further supported by the currently decreasing tuberculosis and mortality risk in primary-care attending adults.

Implications of all the available evidence

The poor diagnostic performance, the lack of additional clinical impact, and the likely increased risk of antimicrobial resistance mean are strong arguments for discontinuing routine prescription of trial-of-antibiotics. National tuberculosis and antimicrobial stewardship programs should restrict prescription of empirical broad-spectrum antibiotics to cases where strong clinical or microbiological indication exists. Given the growing body of evidence relating to ease of acquisition of macrolide antibiotics resistance from brief exposure, clinicians should avoid empirical azithromycin if possible. New affordable and point of care diagnostics for tuberculosis and other respiratory pathogens are urgently needed to address the unmet clinical need.

Main body

Introduction

Despite over two decades of concerted investment, tuberculosis caused an estimated 1.4 million deaths in 2019,[1] and remains one of the leading causes of death among adults globally, second only to SARS-CoV-2 as an infectious cause of death in 2020.[2] Tuberculosis incidence and mortality are falling[3], but not at the rate needed to meet "End TB Strategy" targets.[4] Treatment access barriers remain pronounced. Despite advances, with new technologies such as molecular assays and digital chest radiography with computer-aided diagnosis now recommended, there is still no low cost highly accurate rapid diagnostic test that can provide instrument-free point-of-care diagnosis.[5,6] To exemplify the importance of diagnostic barriers, only 57% of pulmonary tuberculosis notifications to WHO in 2019 were mycobacteriologically confirmed, with the remainder diagnosed clinically.[1]

For decades, tuberculosis diagnostic algorithms have recommended use of broad-spectrum antibiotics ("trial-of-antibiotics") with negligible *Mycobacterium tuberculosis* activity for diagnostic purposes.[7,8] The goal is to treat alternative bacterial causes of respiratory symptoms, and use the clinical response to "rule-out" or "rule in" tuberculosis.[9-11] Tens of millions of such antibiotic courses are prescribed globally every year, making trial-of-antibiotics the most commonly used tuberculosis triage test[8,12,13] and a potentially important contributor to antimicrobial resistance, which, like tuberculosis, is a major global crisis.[14-16] Despite being part of internationally recommended diagnostic algorithms, our previous systematic review found no randomised controlled trial or other strong evidence base to support use of trial-of-antibiotics in tuberculosis diagnostic algorithms.[7]

Apart from diagnostic performance and antimicrobial resistance concerns, a key consideration before removing trial-of-antibiotics from diagnostic algorithms, is potential clinical benefit. In settings with high HIV prevalence, mortality during investigations for tuberculosis and immediately after diagnosis has been extremely high, [17,18] and often secondary to severe bacterial infections,[18-20] making empirical antibiotic treatment potentially lifesaving. Risks of death vary substantially by HIV status, extent of immunosuppression, and between in- and out-patients.[21] As such, the safety of moving away from recommending trial-of-antibiotics during tuberculosis diagnosis is an important consideration for patient management.

We therefore hypothesised that trial-of-antibiotics would be likely to have both benefits and risks that need to be weighed carefully to optimise patient and public health outcomes. We conducted the Accuracy and consequences of using trial-of-antibiotics for TB diagnosis (ACT-TB) randomised controlled trial to investigate diagnostic, clinical and antimicrobial resistance impact.[22]

Methods

Study design

The study design has been described in detail previously[22] and the protocol and statistical analysis plan are available in supplementary material (appendix 2 and 3). In brief, we conducted a three-arm individually randomised (1:1:1), open-label, controlled trial to investigate the accuracy and the broader clinical and antimicrobial resistance impacts of using trial-of-antibiotics to rule-out tuberculosis among adults presenting with cough at primary care centres in Malawi. The study was reviewed and approved by Malawi College

of Medicine Research and Ethics Committee, London School of Hygiene & Tropical Medicine Research Ethics Committee, Regional Committee for Health and Research Ethics –Norway, and Malawi Pharmacy, Medicines, and Poisons Board (supplementary material, appendix 4).

Study Participants

We introduced the study to adults presenting to either Limbe or Ndirande Health Centres in Blantyre, Malawi by first inviting all with cough to a brief talk, then conducted detailed eligibility screen for those who express interest. We included patients if they were aged ≥18 years, had cough, reported being unwell for at least 14 days, and did not have any danger signs (respiratory rate > 30/min, temperature >39°C, heart rate >120/minute, confused/agitated, respiratory distress, systolic blood pressure <90 mmHg). We excluded patients if they reported allergies to study medications, had taken antibiotics other than co-trimoxazole prophylaxis within the previous 14 days, or had taken tuberculosis drugs either for treatment or prevention within the previous 6 months. To participate, eligible patients provided written (or, if illiterate, witnessed thumbprint) informed consent.

Randomisation and masking

We used block-randomisation with variable block sizes, stratified by study site to allocate participants in 1:1:1 ratio, to either standard of care (no study antibiotic prescription), azithromycin (azithromycin 500mg taken one time per day for 3 days, from enrolment day, termed day 1), or amoxicillin (amoxicillin 1g taken three times per day for 5 days, from day 1). An independent statistician prepared a randomisation list using the ralloc command in Stata software Release 15 (Stata Corp. Texas, USA). Allocations were sealed in sequentially numbered opaque envelopes, opened and assigned by site staff upon confirming eligibility. Antibiotic arms dosage and self-administration was explained, and participants took their first dose in the presence of study staff at the clinic with the remainder self-administered at home. The study was not blinded, but mycobacteriology and antimicrobial resistance outcome assessment occurred without reference to arm.

The standard of care (SOC) arm of no antibiotics until clinically indicated was based on national and global guidelines.[23] We chose amoxicillin because it is the standard first line treatment used for trial-of-antibiotics in Malawi.[24] However, amoxicillin may not demonstrate the best performance for trial-of-antibiotics because of increasing resistance, and a narrow coverage for aetiology of community acquired pneumonia and “atypical” organisms. We therefore included azithromycin as a third arm to represent the optimal biological specificity of an oral regimen due to more complete coverage of atypical organisms that cause community acquired pneumonia (e.g. *Mycoplasma pneumoniae*. and *Chlamydia pneumoniae*), and the low resistance rates in Malawi.[24]

Procedures

On visit day 1 (baseline, randomisation day), questionnaires were used to collect demographic information, tuberculosis symptoms, and HIV testing and treatment history. HIV testing was offered to all not already known to be HIV-positive. Nasopharyngeal swabs were collected for pneumococcal culture and antimicrobial resistance testing, and two sputum specimens were collected at least one hour apart for mycobacteriology. To minimise loss to follow-up, we collected contact phone numbers, a physical address and geolocation information.

On visit day 8 (7 days from randomisation), the first activity conducted ahead of any other interaction with study staff was audio-computer-assisted interview (ACASI), during which participants reported the status of their symptoms compared to day 1 and whether they missed or had any remaining study medications. We used ACASI with the aim of minimising interviewer ascertainment and social desirability bias in evaluation of improvement of day 1 symptoms. The ACASI was developed and refined during the pilot study. Staff then communicated and acted on day 1 tuberculosis tests results (with referral for onsite treatment as required), collected one more sputum sample for mycobacteriological testing, and provided clinical management of ongoing symptoms and other illnesses.

On day 29, the final study visit (and home tracing where necessary), we documented participant vital status, hospitalisations, non-study medication use during follow up, collected a follow up nasopharyngeal swab sample from all participants, and sputum from those with tuberculosis symptoms. During the study period, participants were encouraged to present to the study clinic for all illnesses. At the study clinic, history, physical examination, and linkage to available study or routine care clinicians was done. Where bacterial infection was suspected, clinicians would prescribe non-study antibiotics according to national guidelines.

Laboratory procedures

Mycobacteriological testing of sputum specimens used Xpert/MTB/RIF (Cepheid, Sunnyvale, CA, USA) for one sample (day 1, run at the study clinic), and fluorescent microscopy and culture at a specialised tuberculosis research laboratory at the University of Malawi College of Medicine for all other specimens. Microscopy used auramine-O stain and Primo Star iLED™ microscopes (Carl Zeiss Microimaging, Oberkochen, Germany). Culture used BD BACTEC™ MGIT™ 960 Mycobacteria Growth Indicator Tube (MGIT) and Lowenstein Jensen (LJ) for mycobacterial culture with MPT 64 antigen test and microscopic cording for species identification.

Nasopharyngeal swabs used sterile nylon flocked swabs placed immediately into 1.5 ml skim milk tryptone-glucose-glycerol (STGG) medium for transport and storage at -80°C on the same day. We used sheep blood-gentamicin agar plates for culture, and examination of colony morphology plus optochin disc (Oxoid, Basingstoke, UK) susceptibility for identification. We determined susceptibility against ceftriaxone, amoxicillin, cefoxitin, azithromycin and erythromycin using the disc diffusion method (Oxoid, UK), in line with the British Society of Antimicrobial Chemotherapy's guidelines.[25]

Outcomes

We had two distinct co-primary outcomes; diagnostic impact, and clinical impact of the intervention. The diagnostic impact outcome was defined as the proportion of participants without tuberculosis (negative reference standard) who reported improvement of their day 1 symptoms at day 8; that is the specificity of self-reported symptom improvement (Supplementary figure 1). The index test was defined as positive (improvement) or negative (no improvement; no change, or worsened) in response to the ACASI question: *on day 1, you reported that you were unwell; compared to that day, has your illness worsened, remained the same, or improved?* The reference standard was defined as positive if at least one day 1 or 8 sputum was positive on smear microscopy, Xpert MTB/RIF or culture; and negative if none were positive and at least one test was known to be negative.

Our secondary pre-specified outcome for diagnostic accuracy included participants who could not produce sputum at day 1 and 8, who were categorised as mycobacteriology negative. We opted to analyse this population because in the study setting, as many as 13% of symptomatic adults fail to produce sputum.[26] In pre-specified analysis, we calculated test characteristics (sensitivity, predictive values, and area under the receiver operating characteristic curve) of trial-of-antibiotics versus reference standard and their respective 95% confidence intervals. Post hoc analyses were also conducted for sensitivity including (i) all mycobacteriology from day 1, day 8, and day 29, and (ii) in addition clinical diagnoses defined as initiation of tuberculosis treatment in mycobacteriology-negative patients based on clinical or radiological diagnosis, for the reference standard.

The clinical impact co-primary outcome was a composite measure defined as the risk of any of death, hospitalisation, or "missed tuberculosis" (tuberculosis not detected on day 1 or day 8 sputum, but documented based on day 29 mycobacteriology or radiological findings consistent with tuberculosis) by day 29.

We defined the antimicrobial resistance secondary outcome as the proportion of all randomised participants whose day 29 nasopharyngeal swabs grew *Streptococcus pneumoniae* resistant to any of the following commonly used antibiotics: ceftriaxone, amoxicillin, cefoxitin, azithromycin, and erythromycin as determined using disc diffusion technique. *Streptococcus pneumoniae* was chosen as a sentinel respiratory pathogen first because it can acquire resistance efficiently through DNA uptake.[27,28] Secondly because the treatment of choice for these *Streptococcus pneumoniae* are penicillins and macrolides, the same drugs used as study interventions. In a post-hoc analysis we considered only incident resistant isolates, excluding participants who had resistant isolates at baseline.

Statistical analysis

The statistical approach is described in the statistical analysis plan included in supplementary material (Appendix 3). For the diagnostic impact primary outcome, we assumed that day 8 symptom improvement in trial-of-antibiotics (azithromycin or amoxicillin) arms would correctly classify 60% of all mycobacteriology negative participants (i.e. 60% specificity).[29] We established that 388 (rounded to 400) reference standard negative participants per arm would provide 80% power at a two-sided type I error of 5%, to detect at least 10% difference in specificity. Accounting for tuberculosis prevalence (20%), inability to produce sputum (15%), and day 8 loss-to-follow up (5%) we increased the sample size to 625 per arm, and 1875 in total. For the clinical impact outcome, we assumed a 4% risk of composite outcome in SOC arm, and a loss to follow up of 10% by day 29. Assuming 625 participants per arm, a two-sided type I error of 5%, we would have 80% power, to detect a risk ratio of at least two in the intervention (single arm) versus standard of care arm.

We summarise screening and enrolment in a CONSORT (Consolidated Standards of Reporting Trials) flow chart and described baseline characteristics of participants by arm. All analyses were based on an intention to treat principle using the arm to which the participant was randomised. We report measures of effect for comparing azithromycin or amoxicillin arms separately and combined, with the standard of care.

We used a generalised linear model (GLM) with identity link to estimate risks differences and the log link to estimate risk ratios for the three study arm comparisons, adjusting for

study site. In cases where the GLM model did not converge, we used a modified Poisson model to estimate risk ratios.[30] Our priori design did not include adjusting for multiple comparisons, but reported all intervention effects with their 95% confidence intervals (CI) and p-values to facilitate appropriate interpretation. We used Stata release 16.1 (Stata Corp, College station, Texas, USA).

In our pre-specified subgroup analysis, we examined the diagnostic performance by HIV status. We did not conduct pre-specified subgroup analysis for the clinical impact primary outcome because of limited number of events. In post hoc per protocol analysis for diagnostic and antimicrobial resistance impact, we excluded participants who reported incomplete adherence to treatment (remaining with at least one study tablet by day 8) or taking non-study antibiotics by day 8.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

Results

Participants

We screened 5825 adults presenting with cough to Limbe and Ndirande Health Centres between 25 February 2019 and 14 March 2020, of whom 45.5% (2659/5825) expressed interest after a brief description of the study. Following detailed information and eligibility screening, 27.2% (1583/5825) met eligibility criteria, gave written consent to participate and were enrolled (Figure 1). A total of 1033 were ineligible, with most common reasons being recent antibiotic treatment (503; 48.7%), recent or current tuberculosis preventive treatment (198; 19.2%) and being aged ≤18 years (95; 9.2%). 43 adults were eligible but did not give consent. Participant demographics were similar between arms (Table 1). HIV prevalence was 14.9% (236/1583), with 97.7% (214/219) of the previously diagnosed patients already taking ART.

Overall, 1171 (73.9%) provided a sputum sample for Xpert/MTB/RIF (day1), while 1181, 818 and 65 contributed to smear microscopy and culture for days 1, 8 and clinically indicated day 29 specimens, respectively (Supplementary table 1). The prevalence of tuberculosis by day 8 (positive mycobacteriology at day 1 and/or 8) was 6.3% (100/1583). By day 29, an additional five mycobacteriologically-confirmed and 28 clinically-diagnosed cases were identified giving a prevalence 8.4% (133/1583). Four participants died, and another nine were hospitalised during the follow up. More participants missed day 8 visit (45, 2.8%) than day 29 visit (24, 1.5%) - Figure 1. Reasons for missing day 8 visit were not systematically recorded, but 24 missed day 29 visit due to either loss to follow up 70.8% (17/24) or withdrawal of consent29.2% (7/24) (figure 1).

Primary and secondary outcomes at day 8

A total of 1161 participants with negative mycobacteriology and known day 8 symptom status contributed to the diagnostic accuracy primary outcome (392, 383 and 387 in the standard of care, amoxicillin, and azithromycin arms, respectively). Compared to standard of care (79.1%; 310/392), trial-of-antibiotics improved specificity of tuberculosis diagnosis:

azithromycin vs. standard of care (difference +8.6% [95% CI 3.9%-13.3%]; p<0.001); amoxicillin vs. standard of care (difference +8.8% [4.0%-13.6%]; p=0.001) (table 2). Results were similar when the two antibiotic arms were combined and compared to the standard of care. In subgroup analysis, diagnostic impact did not vary by HIV status (table 2).

When participants who could not produce sputum were included in the denominator for diagnostic impact outcome (diagnostic accuracy secondary outcome), trial-of-antibiotics with either azithromycin (90.5%) or amoxicillin (91.1%) still demonstrated improvement in specificity compared to standard of care (82.6%), though the effect was smaller than in the primary outcome analysis (table 2).

The sensitivity of the three arms against the primary reference mycobacteriology, was: 25.6% for standard of care, 10.7% for azithromycin, and 23.3% for amoxicillin (table 2). In post hoc analyses the diagnostic sensitivity remained very low and similar to standard of care (standard of care 25.0%, azithromycin 10.7%, and amoxicillin 22.6%) when all mycobacteriology from day 1, day 8, and day 29 were included in the reference standard, and did not improve (standard of care 26.1%, azithromycin 15.8%, and amoxicillin 23.8%) after including clinical diagnoses (supplementary table 2).

Primary and secondary outcomes at day 29

Compared to standard of care 1.1% (6/530), the proportions of participants who experienced the day 29 composite clinical outcome (at least one of death, hospitalisation, or missed tuberculosis) did not differ by arm (azithromycin -0.2% [95% CI: -1.5, 1.1]; and amoxicillin 1.0% [95% CI: -0.6, 2.6]) (table 3). Results were similar when the two antibiotic arms were combined and compared to the standard of care (table 3).

A total of 1529 participants (96.6% of total recruitment) provided day 29 nasopharyngeal swab samples of which 10.9% (167/1529) grew *Streptococcus pneumoniae* (standard of care 55/506, azithromycin 57/512, amoxicillin 55/551) of which 57.5% (96/167) were resistant (table 3). Compared to standard of care, the proportions of participants whose day 29 nasopharyngeal swabs grew *Streptococcus pneumoniae* resistant to at least one commonly used antibiotic was 2.5 percentage points higher in the azithromycin (RD +2.5% [95% CI: -0.5, 5.5]; p =0.10) (secondary outcome, table 3). This increased to +3.1 (95% CI: 0.1, 6.1, p=0.04) in a post-hoc analysis when only incident resistant isolates were considered (excluding participants who had resistant isolates at baseline; n=139). Proportion of day 29 resistant isolates in the amoxicillin arm was similar (RD -0.2% [95% CI: -2.9, 2.5]; p =0.90) to that of standard of care (table 3).

Adherence to study interventions

More participants (118, 23.1%) in amoxicillin arm (dosage was12 tablets/day over 5 days) remained with at least one unused study medication tablet by day 8, compared to 11 (2%) in azithromycin arm (dosage 2 tablets/day over 3 days) (table 4). Participants from all three arms (standard of care 62 [11.7%] azithromycin 22 [4.2%], amoxicillin 16 [3.0%]) took non-study antibiotics before their day 8 visit. Post hoc per protocol analyses of the diagnostic and antimicrobial resistance outcomes excluding participants who took non-study antibiotics and those who missed any single study drug tablet, produced results similar to the main analyses (table 4).

Discussion

The main findings of this individually randomised trial to investigate the diagnostic, clinical and antimicrobial resistance impact of trial-of-antibiotics were that, compared to standard of care, trial-of-antibiotics with either azithromycin or amoxicillin modestly improved diagnostic specificity for mycobacteriologically-confirmed tuberculosis, but with very low sensitivity. Routine prescription of antibiotics did not improve day 29 clinical outcomes defined for this study (all-cause mortality, hospitalisation, missed diagnosis of tuberculosis), but may have generated antimicrobial resistance in the azithromycin arm.

Although the diagnostic specificity in azithromycin (88.7%) and amoxicillin (89.4%) arms were higher than for standard of care (79.1%), the practical benefit of this difference is limited by the extremely low sensitivity for both azithromycin (10.7%) and amoxicillin (23.3%). Neither antibiotic met WHO criteria (target product profiles) for tuberculosis diagnostic test performance for either a triage test (specificity >80%, sensitivity >90%) or smear microscopy-replacement test (specificity >98%, sensitivity >80%).[31] In our previous systematic review and meta-analysis, all 8 small studies (none randomised) reporting accuracy of trial-of-antibiotics performed poorly.[7] One study from Kenya had a similarly low sensitivity of 15% (specificity 96%) although our pooled sensitivity estimate (67%, 95% CI 42–85) was considerably higher, though with substantial heterogeneity.[7] We now provide high quality randomised trial evidence to support our systematic review conclusions, which were that response to trial-of-antibiotics should not be used for the purposes of establishing a diagnosis of tuberculosis.[7]

Apart from the poor diagnostic performance, safety is the other key consideration for national programs and clinicians before routine prescription of trial-of-antibiotics to outpatients without danger signs can be discontinued. Withholding a course of effective antibiotic treatment may affect patient safety because bacterial aetiologies in patients with respiratory symptoms[32] are common,[18-20] and are an important cause of hospitalisation and mortality.[33] The lack of difference in the risk of death, hospitalisations and missed tuberculosis between participants in standard of care arm and those taking trial of antibiotics (azithromycin or amoxicillin arms) is reassuring and strengthens the argument for discontinuation of routine prescription of broad-spectrum antibiotics to outpatients with respiratory symptoms. The lack of difference may also be explained by the fact that we registered very low morbidity and mortality, reducing study power below that anticipated for this outcome.

Our safety data results are consistent with those from a 2017 systematic review update comparing immediate prescription with antibiotic-sparing strategies for outpatients with uncomplicated acute respiratory infections.[34] This reported no difference between delayed, immediate and no prescribed antibiotics for patients with cough, but based on only 4 studies with limited geographical range.[34] Our study adds substantially to the available data on people living with HIV (PLHIV), a major factor affecting aetiology, management and prognosis of acute respiratory infection.[35] However, although 14.9% of participants in this trial were HIV positive, very few (17) were newly diagnosed and 97% of those previously diagnosed were already taking ART.

Current national estimates for Malawi are 90% PLHIV diagnosed, of whom 88% are on ART, of whom 92% are virally suppressed.[36] This reflects recent successful scale up of HIV testing and treatment programmes in Africa, leading to pronounced reductions in HIV-related opportunistic infections including tuberculosis, and to reduced rates of hospitalisation

and death. The Malawi National Tuberculosis programme has invested in improving diagnostic pathways and reducing undiagnosed tuberculosis in the community. The combined impact can be seen in the decline in the proportion of confirmed tuberculosis among adults attending Blantyre primary care clinics because of prolonged cough, which has fallen from 19.4% in 2014[26] to 6.3% in 2019 (this study)[37,38]. We were unable to conduct *a priori* subgroup analysis of clinical impact by HIV status due to low event rate. Therefore, our results on clinical outcomes may not be generalisable to high HIV prevalence settings with lower coverage of HIV diagnosis and antiretroviral therapy.

A further consideration is antimicrobial resistance. Here we show results consistent with previous reports of ready acquisition of resistance following brief exposure to azithromycin,[39,40] with the risk of resistant nasal pneumococcal isolates being +2.5% (95% CI: -0.5, 5.5) higher for patients randomised to azithromycin compared to standard of care. The difference was greater when patients with pre-existing (baseline) antimicrobial resistance were excluded from the analysis (+3.1 [95% CI: 0.1, 6.1]). These results add to the already existing strong body of evidence on the association between empirical antibiotic treatment and emergence of antimicrobial resistance.[41-43] We saw no similar suggestion of rapid emergence of resistance in the amoxicillin arm, despite higher pre-existing rates of resistance in Blantyre and lower treatment adherence for amoxicillin compared to azithromycin.[24] Unlike amoxicillin, azithromycin has a long half-life[44] due to extensive uptake in tissues.[44,45] The long half-life (azithromycin) has been postulated to lead to a wide mutant selection window (drug concentration range in which resistant mutants are selectively amplified) potentially allowing greater mutant amplification than is seen in amoxicillin.[39,46]

Antibiotic prescription for patients presenting with respiratory symptoms is common practice at the study sites, with nearly half (46.7% [503/1076]) of the potential participants excluded due to having taken antibiotics within 14 days, and self-reported use of non-study antibiotics by day 29 reported by 15.1% of standard of care arm participants and also by 6.3% azithromycin arm, and 5.3% amoxicillin arm participants. The most common non study antibiotic was amoxicillin, consistent with previous reports of wide availability and easy access in Malawi.[47] Taking non study antibiotics would tend to drive our AMR day 29 measures of effect towards the null by increasing risk of resistance (in the standard of care arm) or by clearing carriage (trial-of-antibiotic arms). However, post hoc analysis of antimicrobial resistance outcome excluding participants who reported missing at least one tablet of their study medication, and those who reported taking any non-study antibiotic by day 29, did not have any impact on effect measures.

Our main study limitations include the lack of blinding and consequential room for misclassifying exposure status for participants who may have accessed antibiotics outside the study beyond that reported, and possible misclassification of active tuberculosis status because of the suboptimal nature of the available tests. However, exploratory analysis of different tuberculosis diagnostic criteria, and exclusion of patients known to have taken antibiotics outside of the study prescriptions do not support a major impact on our key conclusions. We recruited participants from a single city, limiting generalisation to other settings. Finally, power to evaluate safety was limited by lower than anticipated event rates for adverse clinical outcomes, most likely due to the recent progress in HIV testing and treatment service scale-up.

In conclusion, our results do not support routine prescription of trial-of-antibiotics for the purposes of establishing a diagnosis of tuberculosis in ambulatory adult outpatients and add

to the data that suggest that prescription of antibiotics can be safely delayed in clinically stable outpatients. We cannot, however, comment on the safety of this approach in high HIV settings with less good progress towards ART scale up than in Malawi. The diagnostic performance of both azithromycin and amoxicillin was poor, with no additional clinical benefits, and some suggestion of increased risk of antimicrobial resistance with azithromycin, which has a relatively low threshold for acquired resistance. When trial-of-antibiotics were first introduced into national tuberculosis algorithms, the prevalence of tuberculosis in adults presenting with prolonged cough to primary care was high, and programmes for diagnosis and management of both tuberculosis and HIV were much weaker and less well funded in low- and middle-income countries than they are now. Our data show that this approach is no longer credible and should be replaced by greater emphasis on early and, if necessary, repeated microbiological diagnostic tests. At least for outpatients, antibiotics should be reserved for patients with distinct clinical or microbiological grounds to raise diagnostic concern about alternative or additional infections.

Contributors

THD, ELC and KF conceived the study. THD obtained the funding. All authors contributed towards the study design, protocol development and implementation. DS, MN, PM and JO contributed to the study planning and clinical design. NF contributed to the study planning and antimicrobial resistance design. THD, ELC, CK, BM, PM, MN and KF contributed to development of data collection forms. THD, LC and BM developed data management plan. THD, CK, LC, SC and SM developed and piloted clinical and laboratory protocols. TD and MJN developed pharmacy plan, and the flow and accountability of treatment allocations and study medications. THD, BM and LC conducted data management. THD developed the statistical analysis plan. THD, ELC, BM and KF were involved in the analysis. THD, ELC and KF were involved in interpreting the data. THD, ELC and KF wrote the first draft. All authors read and approved the final manuscript. THD is the guarantor for this work.

Declaration of interests

We have no competing interests.

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Supplementary Material

1. Additional tables and figures
2. Approved clinical trial protocol
3. Statistical analysis plan
4. Ethics and regulatory approvals

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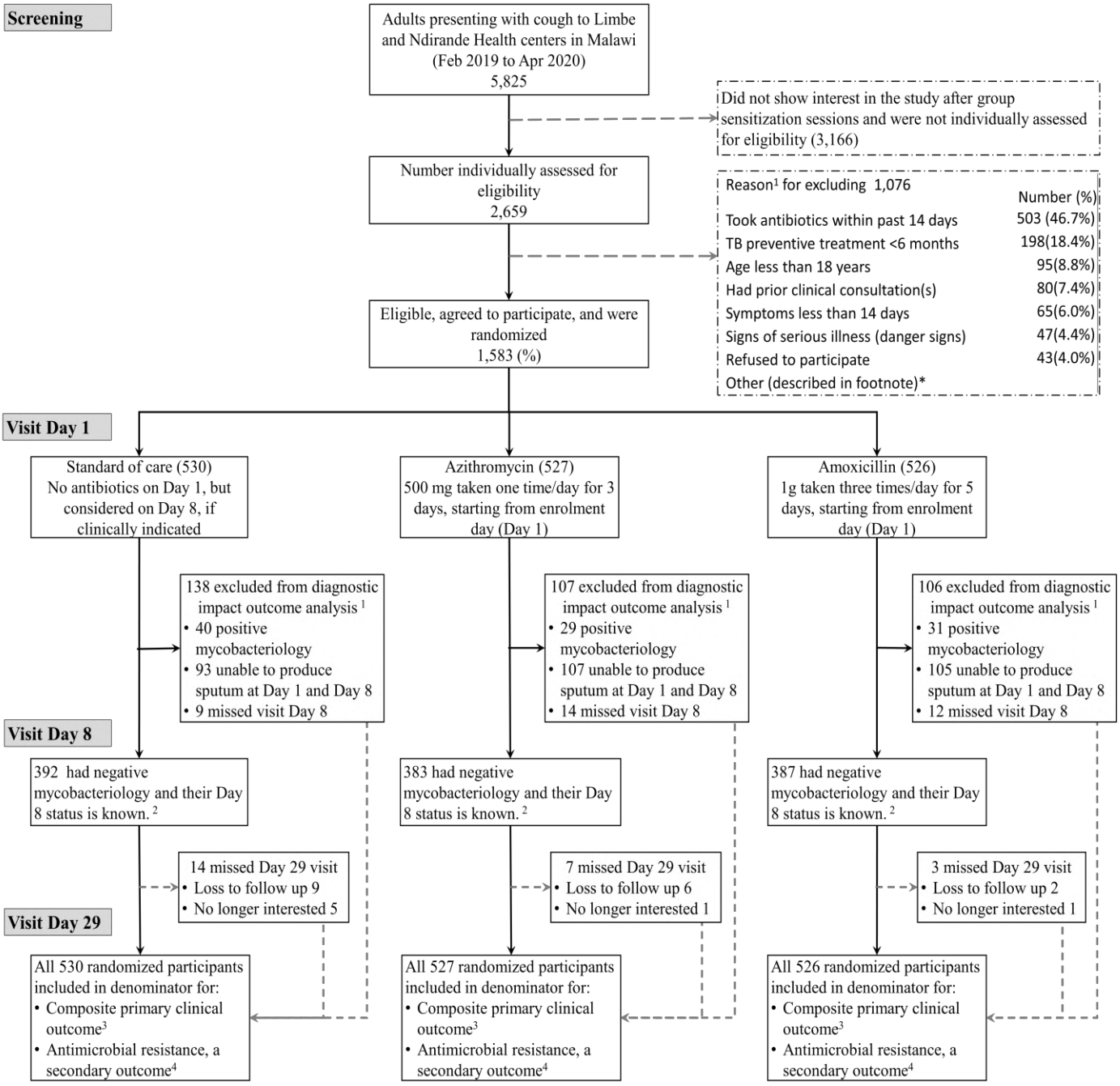
Tables and Figures

Figure 1. Study enrolment, randomization, and follow-up of the participant



*Other= Unable to return for follow up visits 42(3.9%), Lives outside study catchment area 25(2.3%), Unable to walk independently 13(1.2%), Took TB treatment in past 6 months 12(1.1%), Reported allergy to study medication 1(0.1%) 1=Reasons overlap, 2= denominator for diagnostic accuracy primary outcome, 3= experiencing either death, hospitalization or missed tuberculosis by day 29, 4= nasopharyngeal swab with *Streptococcus pneumoniae* resistant to commonly used antibiotics

Table 1. Baseline characteristics of the randomised population (n=1583)

Characteristic	**Standard of care (N= 530)**		**Azithromycin (N= 527)**		**Amoxicillin (N= 526)**		**Amoxicillin or azithromycin(N= 1053)**	
Limbe health centre (n, %)	169	31.9%	167	31.8%	168	32%	335	31.9%
Ndirande health centre (n, %)	361	68.1%	359	68.3%	357	68%	716	68.1%
Age in years (median, sd)	36.4	15.9	35.6	13.8	35.7	14.8	35.6	14.3
Female (n, %)	323	60.9%	302	57.4%	319	60.8%	621	59.1%
Male (n, %)	207	39.1%	224	42.6%	206	39.2%	430	40.9%
Pregnant (n, % of females)	20	6.2%	13	4.3%	13	4.1%	26	4.2%
Fever (n, %)	326	61.5%	343	65.2%	322	61.3%	665	63.3%
Night sweats (n, %)	241	45.5%	246	46.8%	228	43.4%	474	45.1%
Chest pain (n, %)	387	73%	386	73.4%	381	72.6%	767	73%
Blood in sputum (n, %)	34	8.2%	20	4.9%	24	5.9%	44	5.4%
Self-reported weight loss (n, %)	191	36.0%	183	34.8%	183	34.9%	366	34.8%
BMI, kg/m^2 (mean, SD)	24.1	5.0	24.1	5.4	23.8	4.7	24	5.1
BMI <19) (n, %)	52	9.8%	55	10.4%	47	8.9%	102	9.7%
Previous tuberculosis (n, %)	42	7.9%	26	4.9%	32	6.1%	58	5.5%
Months since last dose of TB treatment (median, IQR)	126.1	178.6	94.7	70.5	208.6	230.7	126.1	178.6
HIV positive*	83	15.7%	73	13.9%	80	15.2%	153	14.5%
On ART (n, % of HIV positive)**	75	90.4%	67	91.8%	72	90.0%	144	94.1%
AMR positive swab (n, %)	45	8.5%	42	8%	52	10%	94	8.8%

AMR = antimicrobial resistance. ART= antiretroviral therapy. BMI=Body Mass Index. IQR= interquartile rage. SD= standard deviation. *HIV status unknown in 55 participants (25= standard of care, 14= Azithromycin and 16= Amoxicillin), HIV newly diagnosed for 17 out of 122 tested (6/43= standard of care, 4/37= Azithromycin and 7/42= Amoxicillin). **96.8% (214/221) of the previously diagnosed HIV positive participants were already on ART.

Table 2. Diagnostic impact of trial-of-antibiotics (primary, secondary, and pre-specified outcomes)

Study arm (number randomised)	**standard of care (N= 530)**		**azithromycin (N= 527)**		**amoxicillin (N= 526)**		**amoxicillin or azithromycin (N= 1053)**	
Primary outcome								
Diagnostic specificity: n/N*, %	310/392	79.1%	340/383	88.7%	346/387	89.4%	686/770	89.1%
Difference in specificity between arms*** (95%CI), p-value	Ref		8.6% (3.9, 13.3)	<0.001	8.8% (4.0, 13.6)	0.001	8.9% (4.5, 13.3)	<0.001
Ratio of specificity between arms*** (95%CI), p-value	Ref		1.12 (1.06, 1.19)	<0.001	1.13 (1.07, 1.20)	<0.001	1.13 (1.07, 1.19)	<0.001
Secondary outcome								
Diagnostic specificity: *n/N**, %*	398/482	82.6%	439/485	90.5%	441/484	91.1%	880/969	90.8%
Difference in specificity between arms*** (95%CI), p-value	Ref		6.6% (2.7, 10.4)	0.001	6.8% (2.9, 10.7)	0.001	6.9% (3.3, 10.5)	<0.001
Ratio of specificity between arms*** (95%CI), p-value	Ref		1.10 (1.04, 1.15)	<0.001	1.11 (1.05, 1.16)	<0.001	1.10 (1.05, 1.15)	<0.001
Pre-planned subgroup analysis by HIV status								
a) HIV Positive								
Diagnostic specificity among HIV positive participants: n/N*, %	54/64	84.4%	52/54	96.3%	59/67	88.1%	111/121	91.7%
Difference in specificity between arms (95%CI), p-value	Ref		11.9% (1.7, 22.1)	0.02	3.7% (-8.1, 15.5)	0.61	7.4% (-2.8, 17.5)	0.16
b) *HIV Negative*								
Diagnostic specificity among HIV negative participants: n/N*, %	238/307	77.5%	278/315	88.3%	275/307	89.6%	553/622	88.9%
Difference in specificity between arms (95%CI), p-value	Ref		10.7% (4.9, 16.6)	<0.001	12.1% (6.2, 17.8)	<0.001	11.3% (6.1, 16.7)	<0.001
Interaction p value for Treatment arm and HIV status			0.85		0.21		0.49	
Pre-planned diagnostic performance panel****								
Sensitivity % (n/N, [95%CI])	25.6% (10/39 [13.0, 42.1])		10.7% (3/28 [2.3, 28.2])		23.3% (7/30 [9.9, 42.3])		17.2% (10/58 [8.6, 29.4)	
Positive predictive value % (n/N, [95%CI])	10.9% (10/92 [5.3, 19.1])		6.5% (3/46 [1.4, 17.9])		14.6% (7/48 [6.1, 27.8])		10.6% (10/94 [5.2, 18.7)	
Negative predictive value % (n/N, [95%CI])	91.4% (310/339 [87.9, 94.2])		93.1% (339/364 [90.0, 95.5])		93.8% (346/369 [90.8, 96.0])		93.5% (685/733 [91.4, 95.1])	
Area under the receiver operating characteristic curve (95%CI)	0.52 (0.45, 0.60		0.50 (0.44, 0.56)		0.56 (0.49, 0.64)		0.53 (0.48, 0.58)	

*Primary outcome denominator for specificity limited to mycobacteriology-negative participants. **Secondary outcome denominator for specificity includes participants with either negative mycobacteriology or failed to produce sputum. ***Effect measures are adjusted for study site. ****Diagnostic performance in participants with confirmed mycobacteriology status at day 1 and/or day 8. CI = Confidence Interval. Ref=reference arm. HIV= human immunodeficiency virus. All risk differences and ratios are adjusted for study site.

Table 3. Impact of trial-of-antibiotics on clinical (co-primary outcome) and antimicrobial resistance (secondary outcome) outcomes

Study arm (number randomised)	**standard of care (N= 530)**		**azithromycin (N= 527)**		**amoxicillin (N= 526)**		**amoxicillin or azithromycin (N= 1053)**	
Primary outcome								
Composite clinical endpoint of missed TB, hospitalisation and death by day 29 *n/N*, %*	7/530	1.1%	6/527	1.1%	12/526	2.3%	18/1053	1.7%
Risk difference (95%CI), p-value	Ref		-0.2% (-1.5, 1.1)	0.79	1.0% (-06., 2.6)	0.24	0.4% (-0.9, 1.6)	0.54
Risk ratio (95%CI), p-value	Ref		0.86 (0.29, 2.55)	0.79	1.73 (0.73, 4.35)	0.25	1.29 (0.54, 3.08)	0.56
Individual components of the clinical impact primary outcome								
Missed TB *n/N, %*	3/530	0.6%	3/527	0.6%	7/526	1.3%	10/1053	0.9%
Hospitalisation *n/N, %*	3/530	0.6%	3/527	0.6%	3/526	0.6%	6/1053	0.6%
Death *n/N, %*	2/530	0.4%	0/527	0.0%	2/526	0.4%	2/1053	0.2%
Secondary outcome								
Antimicrobial resistance positive at day 29 *n/N*, %*	28/530	5.3%	41/527	7.8%	27/526	5.1%	68/1053	6.5%
Risk difference*** (95%CI), p-value	Ref		2.5% (-0.5, 5.5)	0.10	-0.2% (-2.9, 2.5)	0.90	1.2% (-1.2, 3.6)	0.34
Risk ratio (95%CI), p-value	Ref		1.48 (0.93, 2.35)	0.10	0.97 (0.58, 1.66)	0.92	1.22 (0.79, 1.88)	0.35

*Number randomised. **Number who provided nasopharyngeal swab samples for *Streptococcus pneumoniae* culture. CI = Confidence Interval. Ref=reference arm. TB=Tuberculosis. All risk differences and ratios are adjusted for study site. *** In a post-hoc analysis excluding participants who had resistant isolates at baseline the percentages antimicrobial resistance positive at day 29 were 21/485, 36/485 and 18/474 in the standard of care, azithromycin and amoxicillin arms, respectively. Risk differences, adjusted for study site, were 3.1 (95% CI: 0.1, 6.1, p=0.04) and -0.6 (95% CI: -3.1, 1.9, p=0.66) for azithromycin and amoxicillin arms vs standard of care, respectively

Table 4. Diagnostic and antimicrobial resistance impact among participants who adhered* to study interventions

Study arm (number randomised)	**standard of care (N= 530)**	**azithromycin (N= 527)**	**amoxicillin (N= 526)**
Treatment adherence			
*Missed at least one tablet: n/N**, %	Not applicable	11/511 (2.2%)	118/511 (23.1%)
Took non-study antibiotics between day 1 and day 8 (n, %)	62/530 (11.7%)	22/527 (4.2%)	16/526 (3.0%)
Took at least one study or non-study antibiotic course between day 1 and day 29 (n, %)	80/530 (15.1%)	527/527 (100.0%)	526/526 (100.0%)
Took at least two antibiotic courses during study period (n, %)	9/530 (1.7%)	33/527 (6.3%)	28/526 (5.3%)
Took at least three antibiotic courses during study period (n, %)	4/530 (0.8)	6/527 (1.1)	8/526 (1.5)
Took at least four antibiotic courses during study period (n, %)	0	2/527 (0.4)	2/526 (0.4)
Number of occasions a course of antibiotics was taken	93	572	570
Number of occasions a non-study course of antibiotics was taken	93	41	38
Post hoc per-protocol analysis: including only participants who adhered to treatment arm			
Diagnostic specificity at day 8 n/N***, %	282/336 (83.9%)	322/357 (90.2%)	262/288 (91.0%)
Difference in specificity between arms (95%CI), p-value	Ref	6.8% (2.1, 11.5), 0.004	7.4% (2.6, 12.3), 0.003
Antimicrobial resistance by day 29 n/N****, %	22/449 (4.9%)	36/483 (7.5%)	18/384 (4.7%)
Antimicrobial resistance risk difference (95%CI), p-value	Ref	2.6% (-0.5, 5.6), 0.102	-0.2% (-3.1, 2.7), 0.882

*The specific question was: Out of all the study medication tablets we gave you, are there any remaining?

**Denominator is the number of participants who completed the study medication adherence questionnaire in audio computer assisted self-interview (ACASI) at day 8

***Adherence to treatment defined as not missing any single study drug and not taking any non-study antibiotic before or on day 8

****Adherence to treatment defined as not missing any single study drug and not taking any non-study antibiotic by day 29

CI = Confidence Interval. Ref=reference arm.

INDEX TEST RESULT Self reported Clinical response on audio computer-assisted self-interview conducted on Day 8	REFERENCE STANDARD RESULT Mycobacteriology based on Day 1 and Day 8 smear microscopy, Xpert/MTB/Rif and tuberculosis Culture)		
	POSITIVE (positive on any of the three tests)	**NEGATIVE** (none of the tests positive, but negative on at least one)	**Could not produce sputum**
POSITIVE (Reporting no improvement or worsening symptoms)	a	b	e
NEGATIVE (Reporting improvement of symptoms)	c	d	f

Primary outcome: Proportion of reference standard negative participants correctly identified by the index test (reporting improvement of symptoms) **d/(b+d)**
Secondary outcome (diagnostic specificity in broader population**):** participants who could not produce sputum at both time points classified as reference standard negative, before estimating **(d+f)/(b+d+e+f)**

Supplementary figure 1. Definition for primary and secondary outcomes of the diagnostic impact of trial-of-antibiotics versus mycobacteriology.

Supplementary table 1. Distribution of reference standard for primary outcome, by individual diagnostic test (day 1 and day 8)

		standard of care	**azithromycin**	**amoxicillin**
Reference Standard positive	One culture and any other test	26	19	13
	Any two of Xpert or Smear	0	1	1
	Only one Culture	11	6	13
	Only one Xpert	2	2	2
	Only one Smear	0	0	1
	Total	39	28	30
Reference Standard Negative	One culture and any other test	373	368	371
	Only one Xpert	19	15	16
	Total	392	383	387
Reference Standard undefined	Could not produce a sputum sample on both day 1 and day 8	90	102	97
	missed outcome assessment visit *	9	14	12
	Total	99	116	109

***Study visit day 8 where audio computer assisted self-interview was conducted to record change in symptoms compared to baseline. Culture = MTB culture. Xpert= Xpert MTB/RIF. Smear= smear microscopy.**

Supplementary table 2. Diagnostic performance of trial of antibiotics versus a reference standard containing all mycobacteriology up to day 29, with and without including clinical tuberculosis (a post hoc analysis)

Study arm (number randomised)	**standard of care**	**azithromycin**	**amoxicillin**
Reference standard: day 1, day 8, and day 29 sputum mycobacteriology			
Sensitivity % (n/N, [95%CI])	25.0% (10/40 [12.7%, 41.2%])	10.7% (3/28 [2.3%, 28.2%])	22.6% (7/31 [9.59%, 41.1%])
Specificity % (n/N, [95%CI])	82.5% (84/481 [78.8%, 85.8%])	90.5% (439/485 [87.6%, 93.0%])	91.1% (440/483 [88.2%, 93.5%])
Positive predictive value % (n/N, [95%CI])	10.6% (10/94 [5.2%, 18.7%])	6.1% (3/49 [1.3%, 16.9%])	14.0% (7/50 [5.8%, 26.7%])
Negative predictive value % (n/N, [95%CI])	93% (397/427 [90.1%, 95.2%])	94.6% (439/464 [92.1%, 96.5%])	94.8% (440/464 [92.4%, 96.7%])
Area under the receiver operating characteristic curve (95%CI)	0.54 (0.47, 0.61)	0.51 (0.45, 0.57)	0.57 (0.49, 0.64)
Reference standard: day 1, day 8, and day 29 sputum mycobacteriology plus clinical diagnosis*			
Sensitivity % (n/N, [95%CI])	26.1% (12/46 [14.3%, 41.1%])	15.8% (6/38 [6.0%, 31.3%])	23.8% (10/42 [12.1%, 39.5%])
Specificity % (n/N, [95%CI])	82.7% (393/475 [79.0%, 86.0%])	90.9% (432/475 [88.0% 93.4%])	91.5% (432/472 [88.6%, 93.9%])
Positive predictive value % (n/N, [95%CI])	12.8% (12/94 [6.8%, 21.2%])	12.2% (6/49 [4.6%, 24.8%])	20.0% (10/50 [10.0%, 33.7%])
Negative predictive value % (n/N, [95%CI])	92.0% (393/427 [89.1%, 94.4%])	93.1% (432/464 [90.4%, 95.2%])	93.1% (432/464 [90.4%, 95.2%])
Area under the receiver operating characteristic curve (95%CI)	0.54 (0.48, 0.61)	0.53 (0.47, 0.59)	0.58 (0.51, 0.64)

*Clinical tuberculosis includes: all mycobacteriology negative participants who were started on tuberculosis treatment based on abnormal chest radiograph, or other clinical criteria. CI = Confidence Interval.

6 Effect of the duration of antimicrobial exposure on the development of antimicrobial resistance

6.1 Introduction

In this chapter I describe the next stage of my research into the role of antibiotic prescriptions in development of antimicrobial resistance. Specifically, in this protocol, published with *Systematic Reviews*, I will assess the relationship between the duration of antimicrobial exposure and selection for resistance. The overarching aim is to inform the design of antimicrobial prescriptions, treatment guidelines and the behaviour of both physicians and patients, and drive stewardship strategies. Like the trial, this systematic review will target respiratory infections because they are a leading reason for antibiotic prescriptions. I will also focus the investigation on macrolides and use *Streptococcus pneumoniae* carriage as an indicator organism.

Apart from planning the scientific investigation, the development process of this protocol exposed me to the design and analysis approaches of network meta-analysis. Network meta-analysis is an approach to evidence synthesis that allows simultaneous comparison of three or more treatments and utilisation of both direct comparisons of interventions within randomized controlled trials and indirect comparisons across trials with a common comparator.

6.2 Protocol manuscript for the systematic review and network meta-analysis

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SECTION A – Student Details

Student ID Number	1700548	**Title**	Dr
First Name(s)	Titus, Henry		
Surname/Family Name	Divala		
Thesis Title	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)		
Primary Supervisor	Professor Katherine Fielding		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study being described in this protocol, wrote the study protocol, led the writing of the manuscript, and submitted it for publication

SECTION E

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Date	29 December 2020

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Date	23Feb2021

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Systematic Reviews

PROTOCOL Open Access

Effect of the duration of antimicrobial exposure on the development of antimicrobial resistance (AMR) for macrolide antibiotics: protocol for a systematic review with a network meta-analysis

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Abstract

Background: Antimicrobial resistance generates a huge health and economic burden and has the potential to become the leading cause of death globally, but its underlying drivers are yet to be fully described. The association between a microbe's exposure to antimicrobials and subsequent development of, or selection for, resistance is well documented, as are the exacerbating microbial and human factors. However, the nature and extent of this risk, and how it varies by antimicrobial class and duration of treatment, is poorly defined. The goal of our systematic review and network meta-analysis is to determine the relationship between the duration of antimicrobial exposure and selection for resistance. We will use macrolides as the antimicrobial class of interest and *Streptococcus pneumoniae* carriage as an indicator organism. Our secondary outcomes include duration of symptoms, risk of treatment failure and recurrence, and descriptions of resistance mechanisms.

Methods: We will conduct a systematic review, selecting studies if they are published randomised controlled trials (RCTs) which report the relationship between taking a macrolide for any indication and incidence of resistant *Streptococcus pneumoniae* in patients of any age group. We will use a predefined search strategy to identify studies meeting these eligibility criteria in MEDLINE, Embase, Global Health and the Cochrane Central Register of RCTs. Two authors will independently screen titles and abstracts, review the full texts and undertake data extraction. We will use the Cochrane Collaboration's tool to assess the quality of included RCTs. If feasible, we will perform pair-wise meta-analysis modelling to determine the relationship between the duration of macrolide treatment and development of macrolide resistant *Streptococcus pneumoniae*. If the identified studies meet the assumptions for a network meta-analysis (NMA), we will additionally model this relationship using indirect comparisons. Our protocol utilises reporting guidance by Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) and the extensions for protocols (PRISMA-P) and network meta-analyses (PRISMA for NMA). Our review will also report to these standards.

(Continued on next page)

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(Continued from previous page)

Discussion: Establishing the relationship between the duration of antimicrobial exposure and development of, or selection for, resistance will inform the design of antimicrobial prescriptions, treatment guidelines and the behaviour of both physicians and patients. This work will therefore be a strong contribution towards the full realisation of current antimicrobial resistance stewardship strategies.

Systematic review registration: PROSPERO CRD42018089275

Keywords: Antimicrobial resistance, Network meta-analysis, Macrolides, *Streptococcus pneumoniae*, Carriage, Treatment duration, Treatment failure, Disease recurrence, Resistance mechanisms, Prescriptions

Background

Antimicrobials—organic or synthetic molecules with cytotoxic or cytostatic abilities against microbes—are one of the greatest medical discoveries [1]. Unfortunately, their usefulness is limited by the inherent genetic capacity of microbes to rapidly develop, transfer and acquire resistance-causing mutations [2, 3]. Unnecessary prescription in medical settings, as well as extensive agricultural use, contributes substantially to overall antibiotic drug pressure globally [4–6]. The current era is characterised by sharply declining investment from the pharmaceutical industry in the development of effective new antimicrobials; far fewer new compounds are developed annually now than during the 1990s [7].

In 2016, antimicrobial resistance became one of only four health topics ever to be discussed at the United Nations General Assembly [8], reflecting its huge health and economic burden [9, 10]. Drug resistance is projected to become the leading cause of death by 2050 [11].

The development of antimicrobial resistance is, to some extent, inevitable. Billions of doses of antibiotics are taken globally each year. Each human hosts a microbiome of approximately 3.8×10^{13} bacteria [12], and there is spontaneous (i.e. unselected) drug-resistance within this microbiome at a frequency as high as 10^{-4} mutations, depending upon the type of antimicrobial [13–15]. Under drug pressure, resistance can then be amplified and transmitted through a variety of mechanisms [2]. Despite these risks, use of single-drug regimens remains standard practice for many conditions, because they are often sufficient to cure the patient and they reduce immediate costs and adverse events.

There is unambiguous evidence that the development of, or selection for, resistance occurs following antimicrobial exposure [4, 16–18]. The duration of treatment that is necessary for the development of, or selection for, resistance is poorly defined, however. This may differ by the resistance mechanism; for example, the unmasking of any resistant organisms generated during previous treatment periods may rapidly occur following a brief exposure to antimicrobials, while de novo generation may require longer durations. Better quantification of this relationship will inform prescription design, guidelines and behaviour, all of which are key factors in effective antimicrobial resistance control strategies [19].

To explore this relationship and derive high-quality evidence, it is necessary to choose an antimicrobial or class of antimicrobials with which patients are treated, an indicator organism in which drug resistance develops and a uniform method for assessing resistance. Macrolides are one of the most prescribed antimicrobials in clinical practice [20, 21], which act by inhibiting bacterial protein synthesis [22]. *Streptococcus pneumoniae*, a major aetiology of clinical illness [23–26], also harmlessly colonises the upper respiratory tract, creating a window for the assessment of circulating serotypes and resistance patterns. *Streptococcus pneumoniae* is also a popular indicator bacteria in randomised controlled trials (RCTs) as globally accepted laboratory procedures for its detection exist [27–29] and colonisation is more common than invasive pneumococcal disease [30–32]. The existence of internationally accepted laboratory standards presents opportunities for between-study comparisons.

Our aim, therefore, is to conduct a systematic review using data from published RCTs to determine the relationship between the duration of antimicrobial exposure and the development of, or selection for, resistance using carriage of *Streptococcus pneumoniae* as an indicator organism. We will evaluate studies involving healthy individuals or patients with any illness treated with macrolide antimicrobials in whom the development of, or selection for, antimicrobial resistance was assessed using *Streptococcus pneumoniae* carriage. We have opted to use network meta-analysis (NMA) as the preferred evidence synthesis method because RCTs with head-to-head comparisons of different durations of macrolides will likely be too rare for a meaningful pair-wise meta-analyses. NMAs allow the use of both direct and indirect evidence and are hence the most efficient method for making inferences.

Our primary objective is to determine whether the risk of developing macrolide resistance increases with the duration of macrolide exposure using upper respiratory tract carriage of *Streptococcus pneumoniae* as indicator

organism. Our secondary objectives include exploring the association between the duration of macrolide treatment and (1) symptom duration, (2) treatment failure and (3) disease recurrence.

Methods

Protocol and registration

The protocol for this planned systematic review is registered with the International Prospective Register of Systematic Reviews (PROSPERO), CRD42018089275.

Eligibility criteria

We will include studies that fulfil the following criteria:

1. *Population*: healthy individuals or patients of any illness or age, treated with macrolide antimicrobials. We will record participant characteristics, including age, sex and the indication for treatment.
2. *Interventions*: Any macrolide antimicrobial being given as monotherapy, via any route, for respiratory infections. We are interested in the impact of macrolide treatment on antimicrobial resistance in *Streptococcus pneumoniae* carriage. We will record the specific macrolide, dose and duration reported in each study. Registered macrolides include azithromycin, clarithromycin, erythromycin, fidaxomicin, telithromycin, carbomycin a, josamycin, kitasamycin, midecamycin, midecamycin acetate, oleandomycin, solithromycin, spiramycin, troleandomycin, tylosin, tylocine and roxithromycin.
3. *Comparators*: Other macrolides, other antimicrobials, placebo, or no treatment. Macrolides are commonly used for respiratory tract infections, and amoxicillin and doxycycline are expected to be the most frequent non-macrolide comparators.
4. *Outcomes*: The primary outcome will be the incidence/risk of macrolide resistance in *Streptococcus pneumoniae* carriage among individuals in whom this did not exist before commencing macrolide treatment. Macrolide resistance in *Streptococcus pneumoniae* results from either ribosomal dimethylation by an enzyme encoded by erm(B), efflux by a two-component efflux pump encoded by mef (E)/mel(msr(D)), or mutations of the ribosomal target site of macrolides [33]. We will include studies that utilised any established laboratory method to demonstrate evidence of macrolide resistance.

Our secondary outcomes include the duration of symptoms (number of symptomatic days from commencement of therapy), risk of treatment failure (persistence of symptoms after completing a dosage of antimicrobials) and disease recurrence (re-emergence of disease within 4 weeks of the resolution of previous symptoms).

5. *Study design*: RCTs. Restricting to RCTs will minimise confounding.
6. *Language and time limitations*: We will include studies published in any language and on any date. For articles in languages other than English that are eligible for full-text review, we will seek assistance from a native speaker who has been trained in data extraction using an article published in English.

Anticipated network geometry

In NMA geometry, competing interventions are represented by points termed nodes. In this case, nodes are the duration of exposure to any macrolide used in the included RCTs (Fig. 1). We will classify treatment duration as brief if it is ≤ 5 days, short if it is 6 to 10 days, and prolonged if it is > 10 days. These durations are based on an understanding of the current clinical use of macrolides, which are dosed for up to 5 days for community acquired pneumonia, up to 10 days for severe pneumonia, and for prolonged periods of time for inflammatory respiratory illnesses such as cystic fibrosis and non-cystic fibrosis bronchiectasis. Within the network, the lines joining nodes are termed edges and are drawn to a thickness that graphically represents the anticipated amount of evidence or number of comparisons that we expect to find between the particular nodes. For example, it is likely that more RCTs will compare a macrolide to a control than to another macrolide of a different duration. NMAs will allow us to compare different durations of macrolide exposure (brief, short and prolonged) by computing indirect comparisons, provided that any patient meeting our eligibility criteria would, theoretically, have been equally likely to be randomised to any of the interventions of the studies included in the network.

Information sources and search strategy

We will search for studies that meet the eligibility criteria in MEDLINE, Embase, Global Health, Web of Science and Cochrane Central Register of Controlled Trials (CENTRAL: The Cochrane Library). MEDLINE, Embase and Global Health will be searched using the Ovid platform. Papers will not be excluded on the basis of the language of publication and time frame in which they were published. We will only include data from peer-reviewed papers in order to ensure scientific quality.

In Table 1, we present our search strategy for MEDLINE, which we will adapt for the other databases. This strategy was reviewed by an information retrieval expert

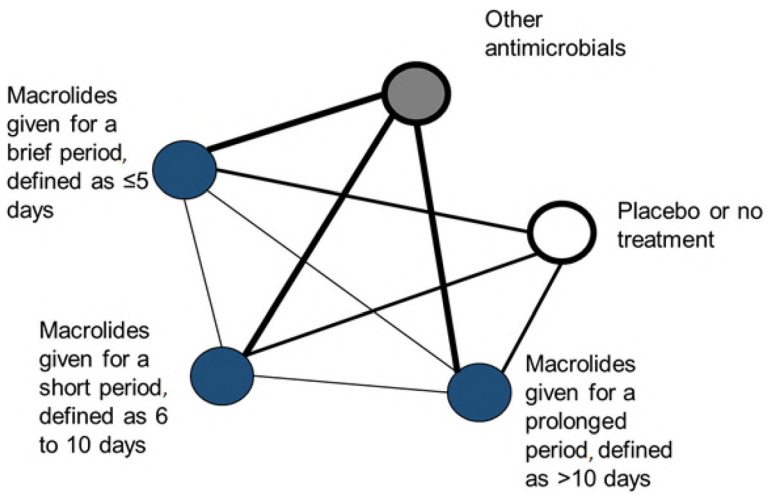

Fig. 1 Hypothetical network of anticipated randomised controlled trial data for the effect of macrolide treatment duration on the development of antimicrobial resistance. Each treatment group is a node. The lines joining nodes, termed edges, will be drawn to thickness that graphically represents the amount of direct evidence: the number of comparisons that we expect to find between a particular pair of nodes

Table 1 Search strategy for MEDLINE using Ovid platform

Theme	Line number	Searches
Antimicrobial resistance	1.	drug resista* or exp drug resistance, microbial/
	2.	bacterial resistan*.ti,ab.
	3.	antimicrobial resistan*.ti,ab.
	4.	1 or 2 or 3
Macrolides	5.	MACROLIDES/
	6.	(Azithromycin or Clarithromycin or Erythromycin or Fidaxomicin or Telithromycin or Carbomycin A or Josamycin or Kitasamycin or Midecamycin or midecamycin acetate or Oleandomycin or Solithromycin or Spiramycin or Troleandomycin or Tylosin or tylocine or Roxithromycin).ti,ab.
	7.	5 or 6
Antimicrobial resistance studies that use macrolides (*any design*)	8.	4 and 7
MEDLINE filter for clinical trials	9.	randomised controlled trial.pt.
	10.	controlled clinical trial.pt.
	11.	randomised.ab.
	12.	placebo.ab.
	13.	drug therapy.fs.
	14	randomly.ab.
	15.	trial.ab.
	16.	groups.ab.
	17.	arms.ab.
	18.	9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17
	19.	exp animals/ not humans.sh.
	20.	19 not 18
Antimicrobial resistance studies that use macrolides in clinical trials	21.	8 and 20

from the London School of Hygiene & Tropical Medicine (LSHTM) library. After running the search, we will export results to Endnote X8 and remove all duplicates. We will also include any relevant articles identified from the reference lists of included articles.

Study selection

Investigator THD will implement the search strategy, and then investigators THD and MN will screen the titles and abstracts of resulting papers against the eligibility criteria. THD and MN will independently assess the full texts of the included papers for eligibility using the above criteria. The main reason for non-inclusion at the full-text stage will be documented. Investigator KF will resolve any disagreements.

Data extraction

Publication information will be exported from Endnote into a standardised extraction form in Microsoft Excel Data will be extracted into (Additional file 1). This form is currently in draft format; it will be finalised among the study team once it has been trialled by two people extracting the same five papers. After finalisation of the form, two team members will extract the data independently. Discrepancies will initially be discussed and resolved between the two team members, with a third team member available to resolve disputes. Multiple publications arising from the same study will be combined. Where data gaps are present, the original study authors will be contacted. Once the extraction phase is complete, data will be exported into the analysis software.

Data for assessing methodological comparability of trials

In addition to the data necessary for outcome evaluation, we will extract information on any interventions, or study or population characteristics that may act as effect modifiers, as is necessary for the assessment of the assumptions of the NMA. These are:

1. Methods: study design, randomisation (individual or cluster), total duration of study, number of study centres and location, study setting, withdrawals and date of study.
2. Participants: age, number, setting, eligibility criteria and baseline antimicrobial resistance (AMR).
3. Interventions: indication of treatment, dose of both the macrolide and control interventions and duration of treatment.
4. Outcomes: authors' primary and secondary outcomes, timing for assessing AMR in relation to the treatment administration schedule and participant adherence levels. We will attempt to extract outcome data per study arm, as opposed to summary effects.
5. Additional factors: trial sponsorship, trial funding and important conflicts of interest reported by the authors.

Data from cross-over and cluster randomised trials

The units of analysis in cross-over and cluster randomised trials (CRTs) need special considerations before meta-analysis is undertaken in order to address carry-over effects and clustering, respectively. For cross-over studies we will only extract data from the first period, while for CRTs, we will extract data that accounts for the clustering.

Risk of bias assessment

We will conduct a risk of bias assessment at the level of the study. We will use the revised Cochrane risk-of-bias tool for randomised trials (RoB 2.0), the recommended method for assessing experimental studies [34]. The risk of bias assessment tool interrogates various aspects of selection and information bias. It involves assessing how the allocation sequence was generated, how it was concealed, if blinding was done, how outcomes were ascertained, the quality of follow up, and whether there was selective outcome reporting. The risk of bias assessment will be done independently by two reviewers and disagreements resolved by discussion or by third reviewer.

Data analysis

Guiding counterfactual model

Our analysis will strive, as far as possible, to mimic the counterfactual framework presented in Fig. 2. The ideal study for addressing the primary outcome is one that recruits AMR-free participants of similar demographics, randomises them (1:1:1) to receiving any of the three durations of the same macrolide antimicrobial (brief, short and prolonged), and then follows them for the same duration before assessing for AMR using the same technique. Restricting to the same type of macrolide antimicrobial would limit the impact of the inherent differences in the intervention itself. For example, within the macrolide class, the drugs have different bioavailability and half-lives; this may impact the development of, or selection for, resistance. Additionally, different dosing, routes of administration, and strength of activity against *S. pneumoniae* are other sources of variability. Furthermore, an optimal study would assess outcomes in each arm at the same time relative to the end of treatment (e.g. 1 day post-treatment), as macrolide AMR has been shown to decrease with time from last date of treatment. The use of the same technique would ensure comparability of results between arms.

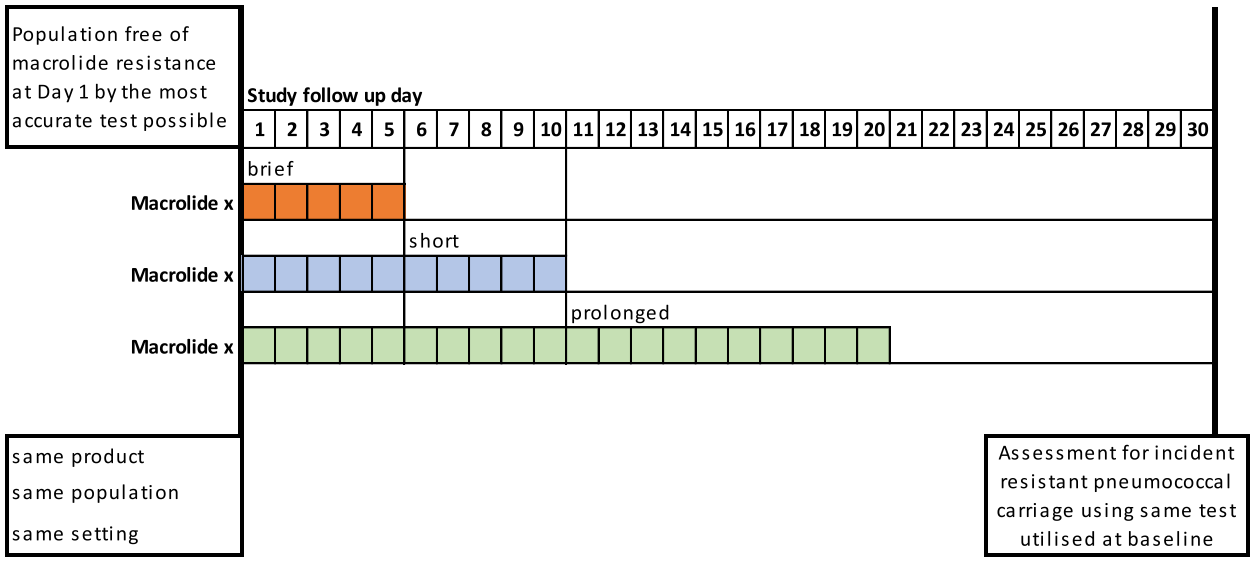

Fig. 2 Counterfactual framework guiding analysis for the primary outcome of the systematic review

While ideal, achieving all these factors in a real-life systematic review is unlikely. Our final statistical analysis plan will therefore be a calculated trade-off of these ideal conditions.

Study and network characteristics

Data will initially be analysed using descriptive statistics, including all the variables described above, in addition to reporting the comparisons performed in each study, indications for antimicrobial therapy, participant characteristics, study setting, and methodological approaches.

We will prepare a network diagram (similar to the hypothetical diagram shown in Fig. 1) in which the size of the nodes reflect the total number of patients randomised to each intervention, the thickness of edges is proportional to the number of direct comparisons, and the colour of each edge will represent the risk of bias. We will use a contribution matrix to understand and rank the influence of various comparisons in the network on the final summary data [35, 36].

Pair-wise and network meta-analysis

If sufficiently methodologically homogeneous studies are identified, we will perform pair-wise meta-analysis for the primary outcome using either fixed effects or random effects modelling approaches, depending on the extent of heterogeneity. We will assess the extent of heterogeneity using the Cochran Q^2 and I^2 statistics. We will convey the extent of heterogeneity visually using a forest plot.

Next, we will assess whether the identified studies meet the assumptions for a NMA. Apart from having reasonably homogeneous methodologies, the key assumption for ensuring validity of inferences drawn from indirect comparisons within a network is transitivity; the balance of the distributions of patient and study characteristics across studies. Initially, we will determine if this assumption is fulfilled by conducting a qualitative review of the RCT characteristics described earlier ('Data extraction' section).

For the subset of eligible studies in which the transitivity assumption holds, we will assume that each of their patients were equally likely to be randomised to any of the antimicrobial agents and treatment durations being investigated, thus establishing the basis for the indirect comparisons. Fixed and random effects NMAs will then be used to synthesise all the evidence for the primary outcome and to rank included treatments. To identify the appropriate model between fixed and random effects NMAs for our data, we will use the deviance information criteria (DIC) to assess their goodness-of-fit. The summary effect measures for all pairwise comparisons will be presented in a league table. We will rank the risk of AMR with various treatments using the surface under the cumulative ranking curve (SUCRA) and mean ranks [37].

Consistency within the network—the agreement between direct and indirect evidence—will be assessed within each loop of evidence using loop-specific approach [38] and by employing a global method for evaluating the whole network [39]. We will also estimate the I^2 for network heterogeneity and inconsistency [40, 41], but we will exercise caution when interpreting the results, considering the well-established limitations in power [42]. We will use funnel plot to assess for publication bias.

Additional analyses

We will perform subgroup, meta-regression and sensitivity NMA analyses. The subgroup analyses will involve running the NMA model stratified by study-level

characteristics, i.e. (1) the age groups of participants, (2) country in which the study was conducted, (3) treatment indications, (4) macrolide type and (5) publication calendar period. The meta-regression will include the study-level covariates described earlier ('Data for assessing methodological comparability of trials' section), in order to reduce heterogeneity. We will initially add the covariates to the NMA individually, retaining those that have a meaningful impact on the DIC and considering combinations of factors after the initial individual-level assessment. Should we identify additional relevant characteristics during data extraction or analysis for both the subgroup analyses and meta-regression, we will identify such analysis (in our publication) as post hoc. In sensitivity analyses, we will perform the NMA with and without studies that have high risk of bias.

Model implementation

We will perform our analyses and report treatment effects on both relative and absolute difference scales, stating odds ratios (ORs), risk differences (RDs) and respective 95% credible intervals (95%CrI) for all comparisons. We will model using OpenBUGS [43] and Stata release 15 (StataCorp, College Station, TX, USA). We will use binomial likelihoods with uninformative prior distributions for our Bayesian modelling. The Brooks-Gelman-Rubin diagnostic will be utilised to assess for model convergence [44, 45]. We will primarily use the mvmeta command [46] in Stata to assess inconsistency and to produce network graphs.

Credibility of the evidence

The credibility of the evidence will be evaluated with respect to its limitations, indirectness, inconsistency, imprecision and publication bias using the approach recommended by the Grade of Recommendation, Assessment, Development and Evaluation (GRADE) system [47, 48].

Dissemination of results

We will present the results of our analyses in a peer-reviewed manuscript using the reporting guidance by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [49] and the PRISMA Network Meta-Analysis extension statement [50]. This work will also form part of a PhD thesis for THD, which he will submit to the LSHTM.

Discussion

Our systematic review will use published RCTs of macrolide antimicrobials to establish the relationship between the duration of antimicrobial exposure and the development of, or selection for, resistance using upper respiratory tract carriage of *Streptococcus pneumoniae,* isolated from patients with respiratory symptoms, as indicator organism. This will inform the design of antimicrobial prescriptions, treatment guidelines and the behaviour of both physicians and patients. This work therefore will therefore be an important contribution towards the realisation of current antimicrobial resistance control strategies [19].

Where possible, through our secondary objectives, we will attempt to describe the clinical outcomes associated with different macrolide durations. Our results on these outcomes will form the basis for future, detailed, research.

The strengths of our review include publication of the full protocol with PROSPERO and in this peer-reviewed article, with detailed methodology laid out a priori. The internal validity of our review is safeguarded by our restriction of the study type to RCTs. The quality and transparency of our work are ensured by our adherence to both PRISMA and PRISMA NMA guidelines.

The conclusions of our NMA will be weakened if direct comparisons are rare, leading to an overreliance on indirect comparisons. Heterogeneity may be introduced by our broad participant population (all ages, any indication of treatment), global coverage (any setting) and unlimited study period. We will seek to limit this by adding study-level covariates to the NMA model, if required.

To our knowledge, our review and NMA will be the first attempt to systematically examine the association between the duration of exposure to macrolide antimicrobials and subsequent development of, or selection for, resistant *Streptococcus pneumonia* carriage. Therefore, our review will not only provide direction for AMR stewardship policies, but also guide future AMR research.

Additional file

Additional file 1: Data extraction form. The draft form includes the risk of bias assessment tool and documents the data which will be extracted from included studies. (DOCX 26 kb)

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Authors' contributions

All authors substantially contributed to the conception and design of the study and reviewed all documents and materials. THD developed the first draft of the protocol and manuscript, the search strategy and data extraction forms and performed the final review of the manuscript. ELC critically reviewed the protocol and the manuscript and contributed towards development of the search strategy and data extraction forms. HRS

contributed to the overall study design and the NMA methodological design and critically reviewed the final protocol and manuscript. MN and DS contributed to the study concept and reviewed the manuscript. NF contributed the scientific concept and reviewed the final manuscript. KF contributed to the conception, study design, search strategy and methodological design and critically reviewed the final protocol and manuscript. TD is the guarantor for this work. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This work will not involve direct contact with human subjects or participant identifiable data. Ethical approval is therefore not required for this study.

Consent for publication
All authors have given consent and approval for the manuscript to be submitted for publication.

Competing interests
The authors declare that they have no competing interests.

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7 Discussion

7.1 Summary of results

My first research objective was to establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis in adults presenting to primary care with prolonged cough. The systematic review described in Chapter 3 established that the available evidence base was insufficient. No randomised controlled trial had been conducted, and the identified eight observational studies had heterogenous designs, making it difficult to conduct robust and reliable meta-analysis. Pooled estimates of the diagnostic accuracy of trial-of-antibiotics against a reference mycobacteriology from the identified 8 studies showed a sensitivity of 67% and specificity of 73%. These pooled estimates were of questionable reliability, however, because of the heterogeneity in the designs of contributing studies, which was confirmed statistically (I^2 was 96% for sensitivity and 99% for specificity). The eight studies also scored poorly on QUADAS2 quality assessment conducted to check their suitability in addressing the systematic review question.

The randomised controlled trial that I conducted is described in Chapter 5 (published protocol provided in Chapter 4). This individually randomised trial examined diagnostic accuracy, being powered to estimate differences in specificity for excluding mycobacteriologically confirmed tuberculosis using trial-of-antibiotics versus no antibiotics (standard of care arm). The results showed that compared to standard of care (79.1%; 95% CI: 74.7%, 83.0%), the diagnostic specificity for mycobacteriologically confirmed tuberculosis was +8.6% (95% CI: 3.9%,13.3%) higher than standard of care in the azithromycin arm and +8.8% (95% CI: 4.0%,13.6%) higher in the amoxicillin arm.

Estimates of sensitivity of the three arms for mycobacteriology reference standard (pre-planned analysis) were: 25.6% (95% CI:13.0, 42.1) for standard of care, 10.7% (95% CI: 2.3, 28.2) for azithromycin, and 23.3% (95% CI: 9.9, 42.3) for amoxicillin. The diagnostic sensitivity remained very low and similar to standard of care (standard of care 25.0%, azithromycin 10.7%, and amoxicillin 22.6%) when all mycobacteriology from day 1, day 8, and day 29 were included in the reference standard, and did not improve (standard of care 26.1%, azithromycin 15.8%, and amoxicillin 23.8%) even after including clinical diagnosis (initiation of tuberculosis treatment in mycobacteriology- negative patients based on clinical or radiological diagnosis) (post hoc analysis, supplementary table 2). Thus we have effectively

dismissed the diagnostic value of trial-of-antibiotics, within the limitations of a single site trial.

My second research objective was to evaluate the clinical benefit of giving empirical antibiotic treatment to primary care participants with chronic cough. In the trial, clinical impact was defined using a composite measure of risk by day 29 of: all-cause mortality, hospitalisation, or missed tuberculosis (defined by persistent illness meeting a composite microbiological or clinical reference standard) that was not treated until day 29 or later. However, we reported much lower morbidity and mortality risks within the first 28 days than anticipated from previous studies at primary care level in Blantyre. This is likely to reflect the success of the scale-up of ART coverage in Malawi (as discussed in section 2.8.3) but means that this outcome was substantially under-powered: with current event rates the power to detect a difference was only 33%. The analysis found that the proportions of participants who experienced the composite clinical outcome in the azithromycin (1.1% [6/527]) and amoxicillin (2.3% [12/526]) arms were similar to that observed in the standard of care arm (1.1% [6/530]), providing reassurance that at least these most serious clinical outcomes were not adversely affected by withholding immediate antibiotics.

The third objective was to evaluate the effect of trial-of-antibiotics on antimicrobial resistance. In the trial, antimicrobial resistance was defined by day 29 nasopharyngeal isolates of *Streptococcus pneumoniae* that exhibit phenotypic evidence of resistance to any of the following commonly used antibiotics: ceftriaxone, amoxycillin, cefoxitin, azithromycin, and erythromycin. The results showed that proportions with antimicrobial resistance in the amoxicillin arm (5.1% [27/526]) were similar to standard of care arm (5.3% [28/530]). However, there was weak evidence of increased risk of antimicrobial resistance in the azithromycin arm (7.8% [41/527]) compared to standard of care arm (+2.5% [95% CI -0.5, 5.5, p value 0.10]). The difference was greater (+3.1% [95% CI: 0.2, 6.1; p value 0.04]) in a post hoc analysis restricting events to incident antimicrobial resistance (excluding participants with antimicrobial resistance at baseline). This is consistent with the high risk of acquired resistance with azithromycin reported from other studies,[1,2] and argues against empirical use of this antibiotic unless supported by evidence of clinical benefit.

7.2 Trial-of-antibiotics in the context of the end TB strategy

Achieving a 95% reduction in TB deaths and a 90% reduction in tuberculosis incidence by 2035 (the End TB Strategy) will require urgent improvement in diagnostic strategies. WHO undertook a mapping exercise to establish priorities for diagnostics, and organised the urgent needs into four priority use cases with target-product-profiles (TPPs).[3] The first, termed the biomarker test, is a point-of-care non-sputum-based test capable of detecting all forms of TB by identifying characteristic biomarkers. The second ("triage test") is defined as a simple, low-cost test that can be used by first-contact health-care providers to identify those who need further testing. The third is a point-of-care sputum-based test to replace smear microscopy for detecting pulmonary TB. The fourth is a rapid drug-susceptibility test that can be used at microscopy-centre level of the health-care system to guide regimen selection. The WHO conducted a systematic expert consultation including a Delphi survey whose output provided the standards for each of these four categories (target product profiles), which new diagnostics have to meet if they are to be deemed impactful (Table 7.1).

To establish whether trial-of-antibiotics meets the desired target product profile, one would first have to determine its category out of the four priority use cases described above. Judging by the position in diagnostic algorithms, one can consider trial-of-antibiotics either in the class of triage tests or smear microscopy-replacement tests. As shown in table 7.1, estimates of tuberculosis diagnostic performance of trial-of-antibiotics versus mycobacteriology reported in the trial for azithromycin (sensitivity 10.7%, specificity 88.7%) and amoxicillin (sensitivity 23.3%, specificity 89.4%), do not meet the target product profile requirement for either triage test (sensitivity >95%, specificity >80%) or smear microscopy-replacement (sensitivity >95%, specificity >98%) tests. It is also worth noting that when only specificity is considered, as may be argued for a rule-out diagnostic perspective, the 88.7% (azithromycin) and 89.4% (amoxicillin) fall in the same broad range as the 79% (standard of care arm) achieved without routine antibiotic prescription: they all meet or come close to the target specificity for a triage test (80%) but fall short of the target for smear microscopy replacement test (98%).

Trial-of-antibiotics also falls short on timeliness, as it would require at least 5 days for patients to complete a course of antibiotics and experience change in symptoms. The need for clinical assessment and prescription by a clinician, means that the test

cannot be used in the community or health post as may be preferred for a triage test. The level of skills needed to implement trial-of-antibiotics goes beyond the minimum requirement for a microscopy replacement test (level of a microscopist), which drives provider cost along with the requirement for antibiotics. The recommended provider costs for a new diagnostic[3] is <US$6, which is likely to be exceeded by the combined costs of drug and staff time in many settings.[4]

Table 7.1 Trial-of-antibiotics against WHO target product profile (TPP) for a community-based triage or referral test and a smear microscopy replacement test

	TPP for a tuberculosis triage test		**TPP for a tuberculosis smear microscopy replacement test**		**Trial of antibiotics (azithromycin arm of ACT-TB)**
	Minimal requirements	**Optimal requirements**	**Minimal requirements**	**Optimal requirements**	
Sensitivity	> 90%	> 95%	> 80%	> 95%	10.7% (95% CI: 2.3, 28.2)
Specificity	> 80%	> 80%	> 98%	> 98%	88.7% (95% CI: 85.1, 91.7)
Time to result	< 30 minutes	< 5 minutes	< 2 hours	< 20 minutes	7 days
Provider cost	< US$ 2.00	< US$ 1.00	< US$ 4.00	< US$ 6.00	Clinician, consultation time and antibiotics
Target user	Health workers trained to the level of auxiliary nurses	Community health workers	Similar or less than TB microscopist	Similar or less than TB microscopist	Clinically trained health worker
Setting	Health posts and primary-care	Community level or village level	primary-care	primary-care	primary-care or higher

Another set of expenses to consider when considering a diagnostic test and other tools for tuberculosis are those incurred by patients.[5] Patient costs are an important consideration in both the Universal Health Coverage and the End TB Strategy,[6] and the goal is to eliminate catastrophic costs. Defined as costs totalling ≥20% of annual household income,[7] catastrophic costs are an important barrier for diagnosis and treatment.[8] In the tuberculosis care pathway, patients experience their greatest proportion of economic burden prior to diagnosis,[8] in part because of multiple clinic appointments necessary for trial-of-antibiotics.[9,10] I will address these costs more

comprehensively as part of the economic analysis that is included in my randomised trial protocol.

In summary, trial-of-antibiotics does not meet any of the defined targets for diagnostic performance, provider cost, or timeliness and so should not be considered as an acceptable approach to providing a diagnosis under the End TB strategy. From the patient perspective, trial-of-antibiotics requires clinical evaluations and incurs patient costs. Trial-of-antibiotics is likely to be contributing substantially to both patient costs and diagnostic delays, making this approach incompatible with the aims of Universal Health Coverage and the End TB Strategy and likely to be contributing to “missing cases of tuberculosis,” and community transmission.

7.3 Impact of trial-of-antibiotics on diagnosis of tuberculosis

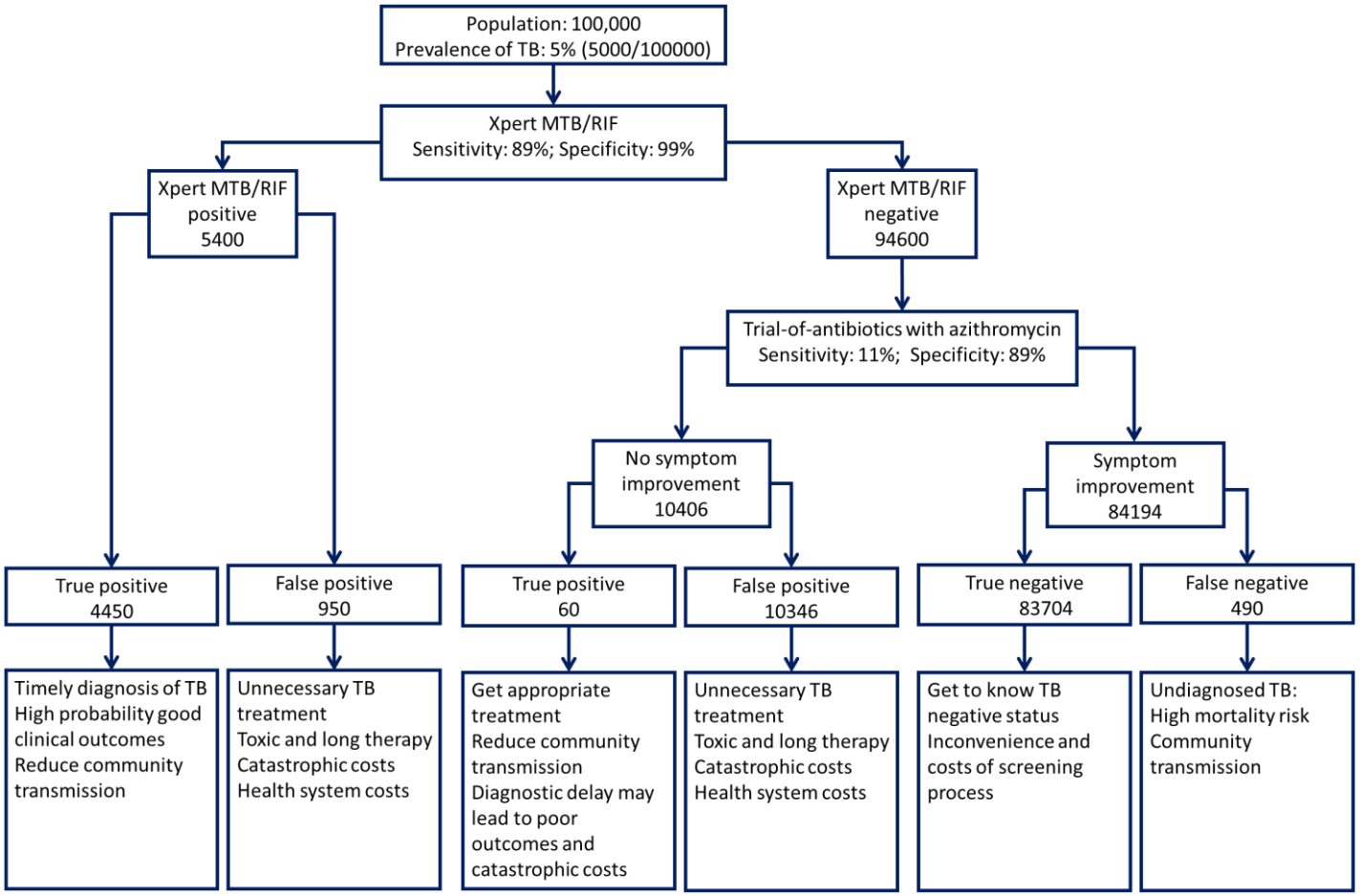
There are three common ways of using trial-of-antibiotics. In the first, antibiotics are prescribed to patients whose symptoms persist after a negative mycobacteriology test: this is the recommended approach in diagnostic algorithms as described in section 2.2.1 and figure 2.1. Only patients who have negative sputum mycobacteriology and have responded to antibiotic treatment are considered “tuberculosis-negative”, while those who remain symptomatic are deemed likely to have tuberculosis and undergo further evaluations potentially leading on to receiving tuberculosis treatment.[11-13]

The second approach prescribes trial-of-antibiotics at the same time as sputum collection. This common clinical practice is aimed at reducing the diagnostic delay and clinic visits experienced by patients in the recommended algorithms. The third approach is similar to the second, except that sputum samples for TB testing are only collected if the patient returns with progressing illness.

To demonstrate the utility and impact of trial-of-antibiotics, I took 100,000 hypothetical patients with known tuberculosis status (prevalence 5%) through these three algorithms (Figure 7.1, 7.2 and 7.3). Considering that the WHO now recommends Xpert MTB/RIF as the initial test for screening tuberculosis in symptomatic adults, I used the same in the algorithm, basing diagnostic performance for culture-positive tuberculosis from a previous systematic review (sensitivity: 89%; specificity: 99%).[14] For trial of antibiotics, I used azithromycin and

the diagnostic performance from the randomised trial (sensitivity: 11%; specificity: 89%). The outcomes described in these figures change with prevalence as described under section 7.4. To quantify antibiotic use, I used the total number of prescriptions in the randomised trial including 1) antibiotics prescribed as intervention (azithromycin [527/527 participants] and amoxicillin [526/526 participants] arms), and 2) non-study antibiotics (standard of care [93 prescriptions reported /530], azithromycin [41/527 participants] and amoxicillin [38/526 participants] arms

The results in figure 7.1 show that although the randomised trial established that trial-of-antibiotics does improve the specificity with which individuals who do not have microbiologically confirmed tuberculosis are excluded from the care pathway, the overall impact is minimal. Out of the 5000 patients with tuberculosis, Xpert MTB/RIF alone identified 4450. Screening the 94600 Xpert MTB/RIF negative patients using symptom change after a course of azithromycin yielded only an additional 60 true positives, at a very high individual and public health cost. First, a total of 10,346 patients without tuberculosis were falsely categorised as having the disease, requiring more tests and getting exposed to associated stigma, discrimination, and catastrophic costs.[7,15] This group may unnecessarily end up on tuberculosis treatment, a long and toxic chemotherapy.[16] In a further 490 patients with active tuberculosis, symptoms improve and patients were then sent back into the community without a diagnosis, putting them at risk of morbidity and mortality,[17] catastrophic costs due to further attempts to access care when symptoms resurface, and onward transmission in the population.[18]



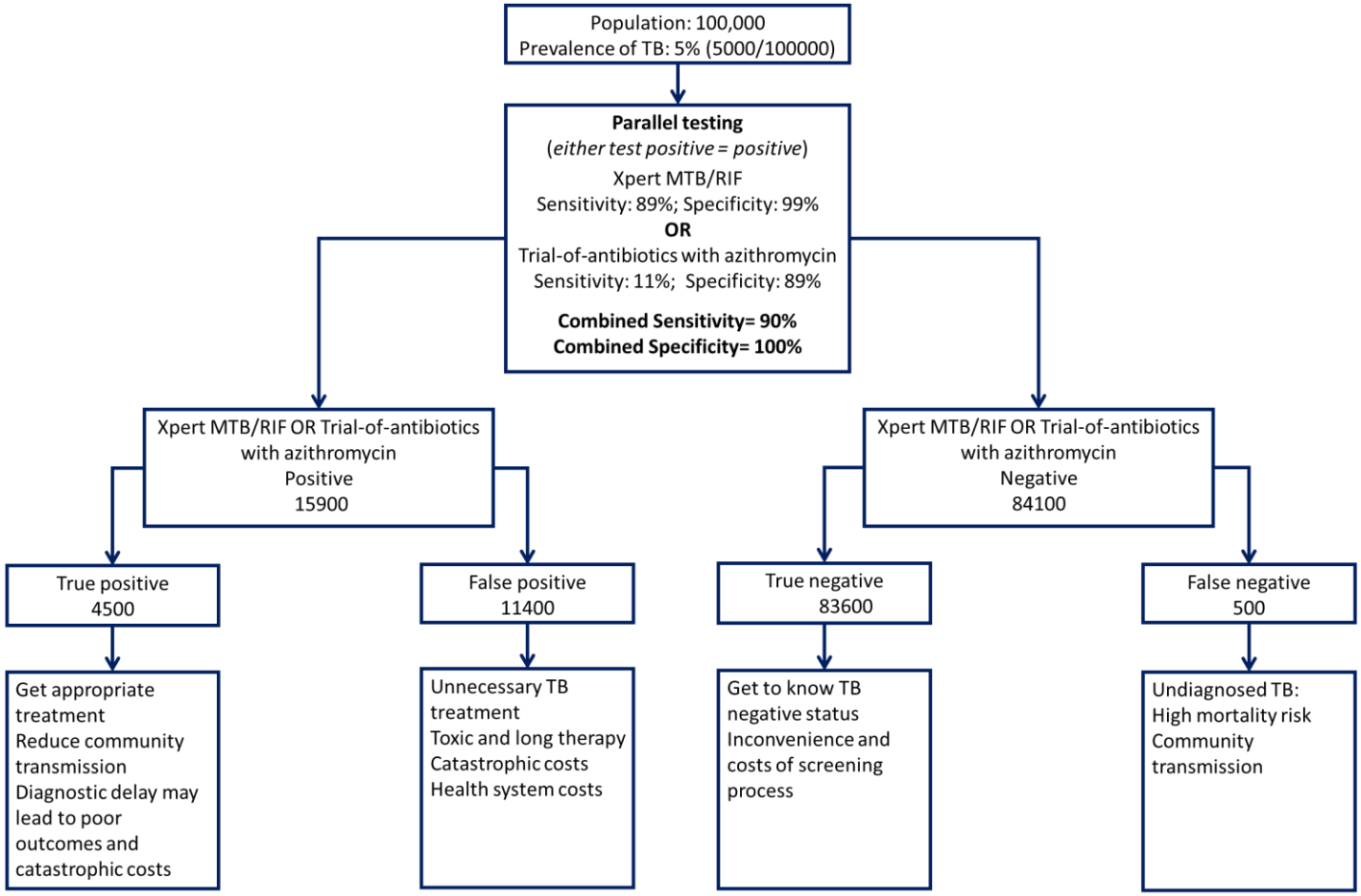
Other health system, individual, and population benefits and harms and considerations based on the above algorithm						
Total number of TB patients identified	Number needed to screen (NNS) per one TB confirmation	Total doses of antibiotics used	Antibiotic doses per confirmed TB patient	Positive antibiotic resistance by day 29	Total number of Xpert MTB/RIF done	Xpert MTB/RIF TESTS per confirmed TB
4510	22.2	102168	22.7	7379	100000	22.2

Figure 7.1 Outcomes of a diagnostic algorithm for primary care screening of tuberculosis using trial-of-antibiotics after a negative result from either Xpert MTB/RIF on a hypothetical population of 100,000 individuals with known tuberculosis status

The limited utility of trial-of-antibiotics at such extremely low sensitivity also translates into minimal impact on the efficiency of the diagnostic algorithm. For example, the number needed to treat using Xpert MTB/RIF alone was 22 (100000/4450), with this number essentially unchanged after the additional screening using trial-of-antibiotics. Health system costs are also substantial. For each confirmed case, 22.7 antibiotic courses were prescribed. The antibiotic use and costs are much higher when assessed only against the incremental yield (60 true positives) over and above Xpert MTB/RIF (1703 prescriptions per true positive identified [102168/60]). Discussion on implications for antimicrobial resistance follows in section 7.6. Other health system costs to consider are the number of Xpert MTB/RIF tests performed and the number of unnecessary tuberculosis treatment initiations and associated follow up.

The algorithm in Figure 7.2 in which both trial-of-antibiotics and Xpert MTB/RIF are implemented at the initial visit (in parallel) and a positive of either is classified as tuberculosis, produces similar number needed to screen but requires more antibiotic prescriptions.

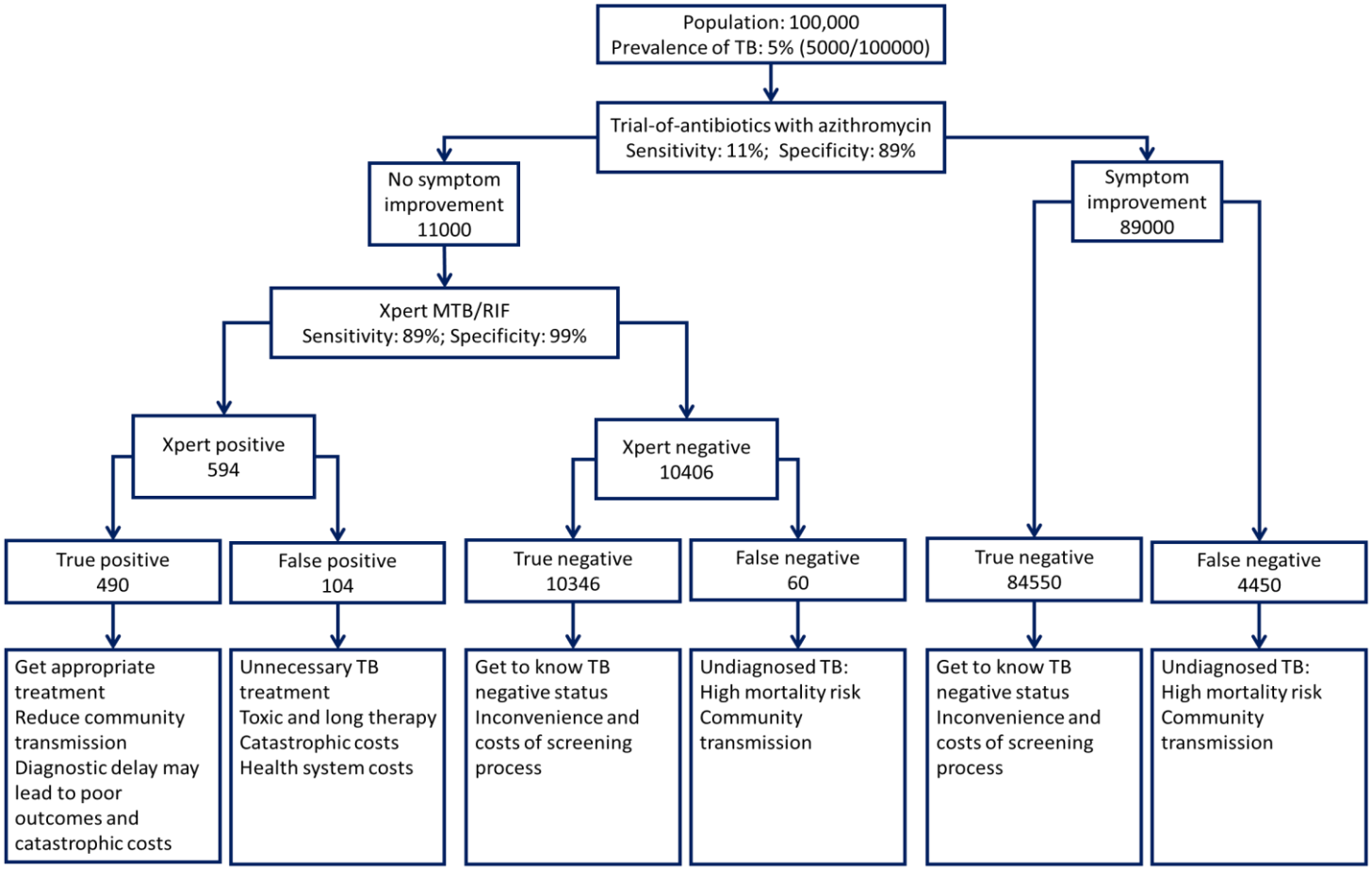
The outcomes of the algorithm in figure 7.3 underscore how inefficient the widely used clinical practice of using trial-of-antibiotics is as an initial screening test before considering either Xpert MTB/RIF or smear microscopy. The algorithm was only 9.8% (490/5000) sensitive, and its number needed to screen was extremely high (204).



Other health system, individual, and population benefits and harms and considerations based on the above algorithm						
Total number of TB patients identified	Number needed to screen (NNS) per one TB confirmation	Total doses of antibiotics used	Antibiotic doses per confirmed TB patient	Positive antibiotic resistance by day 29	Total number of Xpert MTB/RIF done	Xpert MTB/RIF TESTS per confirmed TB
4500	22.2	118000	26.2	7800	100000	22.2

Figure 7.2 Outcomes of a diagnostic algorithm for primary care screening of tuberculosis using trial-of-antibiotics in parallel with Xpert MTB/RIF on a hypothetical population of 100,000 individuals with known tuberculosis status

Figure 7.3 Outcomes of a diagnostic algorithm for primary care screening of tuberculosis using trial-of-antibiotics followed by either Xpert MTB/RIF on a hypothetical population of 100,000 individuals with known tuberculosis status



Other health system, individual, and population benefits and harms and considerations based on the above algorithm						
Total number of TB patients identified	Number needed to screen (NNS) per one TB confirmation	Total doses of antibiotics used	Antibiotic doses per confirmed TB patient	Positive antibiotic resistance by day 29	Total number of Xpert MTB/RIF done	Xpert MTB/RIF TESTS per confirmed TB
490	204.1	108000	220.4	7800	11000	22.4

7.4 Changing epidemiology of TB and relevance of trial-of-antibiotics

The prevalence of tuberculosis has been on the decline globally.[19] The changes in the epidemiology of tuberculosis and mortality in sub-Saharan Africa are secondary to improvements in HIV care.[20-22] The reciprocal improvement of HIV, tuberculosis and mortality, are described in section 2.8.3, and may explain the low morbidity and mortality in the ACT-TB trial. A 2014 study recruiting a population similar to (although with chronic cough and exclusion of patients who could not produce sputum) and at the same site as the ACT-TB trial documented a tuberculosis prevalence of 19.4%,[23] which is 13% higher than the current 6.3% (ACT-TB trial, 2020). Although slower than 2030 targets[24] and threatened by COVID-19,[25] the incidence of tuberculosis is likely to remain on a downward trend.[26] It is therefore important to review continued applicability of old diagnostics such as trial-of-antibiotics because change in prevalence affects predictive values, numbers needed to screen, and provider costs.[27]

Testing strategy	Prevalence in a population of 100,000	Number of TB patients identified (a)	Proportion of TB patients identified	Number needed to screen (NNS) (100000/a)	Number of antibiotic doses per confirmed TB patient	Number of Xpert MTB/RIF tests per confirmed TB
Azithromycin then Xpert	15%	1468	9.8%	68.1	73.6	7.5
	5%	490	9.8%	204.1	220.4	22.4
	1%	98	9.8%	1020.4	1102.0	112.2
Xpert or azithromycin (parallel)	15%	13500	90.0%	7.4	8.7	7.4
	5%	4500	90.0%	22.2	26.2	22.2
	1%	900	90.0%	111.1	131.1	111.1
Xpert then azithromycin	15%	13532	90.2%	7.4	6.8	7.4
	5%	4510	90.2%	22.2	22.7	22.2
	1%	902	90.2%	110.9	117.5	110.9

Table 7.2 The impact of changing disease prevalence on yield and costs of diagnostic algorithms containing trial-of-antibiotics on a hypothetical population of 100,000 individuals with known tuberculosis status

To describe the public health implications of using trial-of-antibiotics in the context of changing epidemiology, I used a hypothetical population of 100,000 individuals

whose tuberculosis prevalence declined from 15% to 5%, then to 1% (Table 7.2). At each of these three prevalence proportions, I calculated outcomes for three potential diagnostic approaches: 1) trial-of-antibiotics with azithromycin followed by Xpert MTB/RIF, 2) trial-of-antibiotics with azithromycin in parallel with Xpert MTB/RIF, and 3) Xpert MTB/RIF followed by azithromycin.

The implications of the outcomes modelled in table 7.2 are that over time, screening for tuberculosis is becoming more costly per patient diagnosed, with higher numbers needed to screen. Continued use of trial-of-antibiotics should therefore be weighed against increasing number of antibiotic doses per identified tuberculosis patient.

7.5 Choosing the ideal diagnostic algorithm

The lack of accurate diagnostic and the nonpromising development pipeline for new point of care diagnostics,[28] means that the only option for the foreseeable future is creative utilisation of available tools.[29,30] Careful combination of the currently available diagnostic approaches may help achieve the high sensitivity and specificity needed to minimise the “missed tuberculosis cases.” Table 7.3 uses a hypothetical population to examine the additive value of trial-of-antibiotics (using azithromycin) on Xpert MTB/RIF and Smear microscopy, the mycobacteriology tests that are widely and closest to the patient available. The total population is 10,000, prevalence of tuberculosis is 5%, the sensitivity and specificity of Xpert MTB/RIF and Smear microscopy are obtained from a systematic review and are as described in section 7.3.

In table 7.3, the approach of starting patients on antibiotics before tuberculosis screening identifies less than 10% of the patients and has extremely high number needed to screen (NNS) (204.1 when combined with Xpert MTB/RIF, and 297.6with smear microscopy). These results clearly demonstrate that trial-of-antibiotics should not be used as the initial step in a diagnostic algorithm for active tuberculosis.

Xpert MTB/RIF followed by azithromycin or in parallel (table 7.3), produce diagnostic algorithms with the highest sensitivity (90%) and lowest NNS (22) but does not present any meaningful additional value over using Xpert MTB/RIF alone (sensitivity 89%, NNS 22). The other limiting factor for the Xpert MTB/RIF followed by azithromycin (or in parallel) algorithm over Xpert MTB/RIF alone is the higher rate of misclassification with up to 12% of the screened patients being either

wrongly started on TB treatment (11,296, and 11400 -parallel testing) or having their active disease missed (490 and 500 -parallel testing).

In summary, at a prevalence of 5%, trial-of-antibiotics does not add value to primary care tuberculosis diagnostic algorithm involving either Xpert MTB/RIF or smear microscopy. The ideal approach therefore is to use and depend only on results of Xpert MTB/RIF, which is in line with the latest WHO guidance that seeks to make molecular diagnostics (Xpert MTB/RIF, Truenat MTB or Truenat MTB Plus) the initial diagnostic tests in adults with signs and symptoms of pulmonary TB.[31] However, there still remains urgent need to address the substantial misclassification that remains even when Xpert MTB/RIF is the diagnostic of choice.

Apart from misclassification, the available molecular assays are not as accessible, as rapid, or as close to the patient as trial-of-antibiotics. Therefore, research and development for new point of care diagnostics is urgently needed.

Table 7.3 Outcomes of several combinations of diagnostic algorithms for primary care screening of tuberculosis in a hypothetical population of 100,000 individuals with 5% prevalence of active disease

Initial test	Second test	Number of TB patients identified out of 5000 (a)	Proportion of true cases detected (a/5000)	Unnecessary TB treatment doses (b)	Number with undiagnosed TB & risk for onward transmission (c)	Proportion with untoward outcomes (b+c)/100000	Number needed to screen (NNS)	Antibiotic doses per confirmed TB patient
Xpert MTB/RIF	Azithromycin	4510	90%	11296	490	12%	22.2	22.7
Smear microscopy	Azithromycin	3264	65%	12141	1736	14%	30.6	31.5
Azithromycin	Xpert MTB/RIF	490	10%	104	4510	5%	204.1	220.4
Azithromycin	Smear microscopy	336	7%	209	4664	5%	297.6	321.4
Xpert MTB/RIF		4450	89%	950	550	2%	22.5	4.0
Smear microscopy		3050	61%	1900	1950	4%	32.8	5.9
Xpert MTB/RIF OR Azithromycin (Parallel)		4500	90%	11400	500	12%	22.2	26.2

7.6 Impact of trial-of-antibiotics on antimicrobial resistance

To the best of my knowledge, ACT-TB is the first randomised trial to systematically investigate the direct impact of trial-of-antibiotics on development of antimicrobial resistance. The observed effect on selection of resistant organisms was as expected,[32-34] and is discussed comprehensively in Chapter 5. The modelled antimicrobial prescriptions required to identify a single tuberculosis patient (table 7.3) indicate much higher drug pressure from trial-of-antibiotics-based algorithms (≥22 courses of antibiotics prescribed to identify one tuberculosis patient) than my initial conservative estimate of 5 courses per sputum-negative tuberculosis patient assumed when estimating global prescription of at least 13 million courses per year from trial-of-antibiotics (section 2.2.3). The number of antibiotic courses required per tuberculosis patient diagnosed will only increase over time (table 7.2), with the declining (section 2.8.3) tuberculosis disease burden (≥100 courses to identify one tuberculosis patient at 1% prevalence).

These results should inform national antimicrobial stewardship and tuberculosis policies, health worker education, and public awareness actions towards addressing the antibiotic prescription practices at the point of care. At global level, these results should add to the body of evidence supporting the urgent investment in development of new point-of-care diagnostics for tuberculosis and its wide list of differentials.[35,36] Addressing the unmet diagnostic needs for viral and bacterial respiratory tract infections may go a long way towards preventing unnecessary antibiotic prescriptions.[36,37]

Unfortunately, currently available diagnostic tests for respiratory pathogens are often laboratory–based and not available in most primary care centres of low- and middle-income countries where the burden of infectious diseases is high.[38,39] Where available, mostly in hospital settings, inefficiencies are common, leading to underutilisation, and continued overreliance on syndromic management.[40,41] Efficient use of existing diagnostics to guide patient management therefore is an integral part of antimicrobial stewardship, has been termed “diagnostic stewardship” by the WHO,[41] and is linked at programmatic level to the Global Antimicrobial Resistance Surveillance System (GLASS).[41,42]

7.7 Impact of trial-of-antibiotics on clinical outcomes

Having described the poor diagnostic value and likely adverse effect on antimicrobial resistance, clinical benefit, if established could have provided enough of an incremental value of trial-of-antibiotics to justify their continued use.[43] The 8.6% (azithromycin) or 8.8% (amoxicillin) improvement in specificity compared to standard of care may be due to successful treatment of bacterial infections, with potential risk of harm if left untreated.[44-46] Bacterial infections other than tuberculosis are a common cause of respiratory symptoms.[44-46] Although we cannot exclude benefits, such as more rapid symptom resolution, the lack of clinical benefit for the most severe outcomes (prevention of death, hospitalisation within 28 days) between arms is reassuring, especially as only 15.1% of participants randomised to the standard of care arm received non-study antibiotic prescriptions by day 29. Apart from concerns for limited power, this lack of difference suggests relatively little potential for severe harm from withholding trial-of-antibiotics at least in settings such as Malawi where newly diagnosed HIV patients and patients not receiving ART account for only a small proportion of all patients seen with respiratory illnesses at primary care level.

7.8 Strengths of the thesis

This thesis is a major contribution to the evidence-base on trial-of-antibiotics in the context of presumptive tuberculosis and includes the first systematic review and meta-analysis on this topic and the first randomised clinical trial. We summarised available observational studies and conducted a randomised trial to add the first strong evidence on the 3 key issues affecting clinical practice: diagnostic performance and the impact on clinical outcomes and antimicrobial resistance.

The research was conducted with strong adherence to public accountability and academic integrity. I registered the systematic review and trial protocols with public repositories (Prospero and Clinicaltrials.gov). I also published the study protocols before accessing data, a process that involved detailed peer review. I prepared a detailed statistical analysis plan prior to data analysis. In addition to ethics and regulatory review, the trial protocol was only implemented after review and incorporation of feedback from DSMB and TSC. The DSMB and TSC also reviewed and approved the statistical analysis plan before implementation. The trial was

monitored by the University of Malawi College of Medicine Clinical Trials Unit for adherence to protocol, standard operating procedures, and good clinical practice.

The inclusion of two antibiotic arms, one of which (azithromycin) had very high coverage against a wide range of “typical and atypical” bacterial causes of respiratory tract infection and very low prevalence of pre-existing resistance provides a strong “reference standard” that makes the lack of clinical utility reported here likely to be generalisable to most other contexts, globally, irrespective of the class of antibiotic used.

7.9 Limitations of the thesis

Although the quality of my work may be sufficient to drive decisions around diagnostic accuracy, clinical impact and antimicrobial resistance, the overall body of evidence remains quantitatively slim. As reported in the systematic review (Chapter 3), only a handful studies had investigated the role of trial-of-antibiotics in tuberculosis diagnosis, and each used a different methodology, which limits comparability of findings. None reported on clinical outcomes beyond tuberculosis diagnosis, and none investigated antimicrobial resistance. There were no previous systematic reviews, and no previous randomised controlled trials until this thesis. I have in this thesis advanced the understanding of trial-of-antibiotics by collating previous work, and conducting a randomised trial. I have also created a platform for defining “response to treatment” to facilitate comparable outcomes: using an Audio-Computer Assisted Self-Interview (ACASI), future investigators can easily compare their results to my findings.

The generalisability of my findings is limited by two factors. First, the study population, setting, and level of care demands caution when extrapolating data to the following scenarios: high HIV settings that have lower coverage of HIV diagnosis and retention on ART; outpatients with signs of severe illness; hospitalised adults. Each of these are important populations both for tuberculosis and antimicrobial stewardship for which additional studies are required to optimise clinical management. I also cannot comment on the potential impact of SARS-CoV-2 on my findings, as my follow-up completed just before the onset of the pandemic in Malawi. However, the findings and conclusions of this thesis are in line with the basic principles of good tuberculosis care and antimicrobial stewardship and should be applicable at least to the millions of outpatients presenting with cough each year

in the Africa region. This should therefore remain a useful guide in many clinical settings.

Secondly, the diagnostic yields need to be interpreted with the understanding that 1) the patient population was recruited after symptom screening, a triage test that has its own limitations as described in section 2.4.4., and 2) the diagnostic performance data I used for Xpert MTB/RIF may overestimate its effect because the reference standard in the source systematic review[14] was MTB culture, which is also an imperfect test.

7.10 Conclusions, recommendations, and research opportunities

Diagnostic value of trial of antibiotics	Recommendations for global and national programs	Research priorities
Improves specificity with a margin (+8%) too small to meet the expectations of the End-TB Strategy.	Trial-of-antibiotics should not be used for triage or diagnosing tuberculosis.	Clinical development for accurate, affordable, and point-of-care tuberculosis diagnostics that can be used at the most peripheral parts of the health system and provide results within the timeframe of a consultation.
Sensitivity is too low (<25%) for use as a triage test. Population level (5% prevalence) yield as a second test targeting patients who test negative on Xpert MTB RIF does not meaningfully reduce number needed to treat and risks rise in misclassification 8-fold over using Xpert MTB/RIF alone (11.6/1.5%). Misclassified patients experience untoward outcomes from a screening program: unnecessary tuberculosis treatment, and undiagnosed disease. Population level (5% prevalence) yield as a screening test has a number needed to treat 9 times higher than Xpert MTB/RIF alone (204/22) and 3-fold higher risk of misclassification with all the potential unintended consequences.	Xpert MTB/RIF or alternative molecular diagnostics (or smear microscopy where molecular tests are not available) should be the diagnostic of choice without use of antibiotics, repeated as necessary. Increase sensitivity and minimise misclassification of Xpert MTB/RIF based algorithm by prompt use of chest radiography, ideally with Computer-Aided Detection for tuberculosis	A randomised controlled trial investigating impact on diagnostic accuracy, yield, cost and antimicrobial consumption of various combinations of diagnostic tests. Arms could include: a) Xpert MTB/RIF b) Xpert MTB/RIF followed by repeat Xpert MTB/RIF if negative c) Digital chest radiography followed by Xpert MTB/RIF d) Xpert MTB/RIF followed by Digital chest radiography e) Options 1 to 4 with or without bacterial and viral respiratory pathogen screen point-of-care diagnostics
		Clinical development for highly sensitive and rapid tuberculosis biomarker tests
	Expand access to diagnostics for respiratory pathogens and host biomarkers that distinguish infectious and non-infectious causes of cough to as close to the patient as possible.	A systematic review to identify clinically and epidemiologically relevant respiratory pathogens and host biomarkers by region and inform development of relevant diagnostic panels.
		A randomised controlled trial investigating the impact of promising point-of-care testing for respiratory bacterial and viral pathogens and host biomarkers on clinical outcomes, antimicrobial consumption, antimicrobial resistance and cost-efficiency.

Impact of trial-of-antibiotics on antimicrobial resistance	Recommendations for global and national programs	Research priorities
May increases risk of antimicrobial resistance. Increases unnecessary antibiotic prescription and drug pressure. In a population where prevalence is 5%, a screening program that uses Xpert MTB/RIF as the initial test and routine trial-of-antibiotics to those who test negative, would need to prescribe at least 22 courses of broad-spectrum antibiotics to identify one tuberculosis patient. The number increases to 204 if the screening program uses trial-of-antibiotics as the initial test followed by Xpert MTB/RIF if symptoms do not improve.	Trial-of-antibiotics should not be used as it increases risk of antimicrobial resistance.	A modelling study estimating the global avoidable exposure to antibiotics through trial-of-antibiotics for tuberculosis diagnostic purposes, and the costs and negative outcomes of more-or-less permissive algorithms.
	Establish antimicrobial stewardship programs that target tuberculosis-related prescribing.	Implementation research investigating public health utility and impact of antimicrobial stewardship programs that target tuberculosis-related prescribing.
	Harmonise global and national tuberculosis guidelines on the role of broad-spectrum antibiotics.	Qualitative research investigating prescription drivers and practices related to tuberculosis diagnosis from clinician, patient and health system perspectives at global and national levels; and establish possible interventions.
		A randomised controlled trial investigating a range of educational and behavioural interventions (targeting clinicians and patients) for addressing inappropriate antimicrobial prescribing related to respiratory symptoms.
	Ensure that clinicians follow guidelines for use of antibiotics during primary care screening for tuberculosis.	Qualitative research investigating clinicians' acceptability and enabling factors for utilisation of tuberculosis diagnostic algorithms that do not include trial-of-antibiotics.

Impact of trial-of-antibiotics on clinical outcomes	Recommendations for global and national programs	Research priorities
Does not have impact on the risk of a composite of death, hospitalisation and missed tuberculosis, at least in stable outpatients.	At least from the perspective of severe outcomes, it appears safe to discontinue the use of trial-of-antibiotics in primary care tuberculosis screening or diagnostic algorithms for ambulatory adults without danger signs from the perspective of severe outcomes	A prospective cohort (by HIV and ART status) study investigating impact of routinely prescribed antibiotics for respiratory symptoms, on morbidity and mortality, including outcomes at immediate (7 days), short term (4 weeks) and long term (>24 weeks) outcomes including: a) less severe but still patient-important morbidity b) time to recovery c) symptom recurrence d) number of clinical consultations e) additional prescriptions f) selection for resistant organisms g) severe outcomes (hospitalisation and death)
	Unable to make recommendations due to lack of generalisability to: a) high HIV settings that have lower coverage of HIV diagnosis and retention on ART b) outpatients with signs of severe illness c) hospitalised adults	Urgent consideration of trials to investigate the role of empirical antibiotics for improving clinical outcomes in these important subgroups. The starting assumption should be that there is no TB diagnostic role, but that outcome could also be investigated, in order to strengthen the evidence-base on diagnostic value.

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8 Appendices

8.1 Results of the pilot study: Development of a tool for measuring response to treatment: an audio computer-assisted interview (ACASI) questionnaire

Development of a tool for measuring response to treatment: an audio computer-assisted interview (ACASI) questionnaire

1 Background

The main outcome measure for a trial-of-antibiotics approach is response to treatment. My systematic review established that there was no consistency in definition of response to treatment across the eight eligible studies, and that the assessment approaches were mostly subjective. I therefore set out to develop an objective and reproducible method for measuring response to treatment for use in the ACT-TB RCT and future studies.

2 Drafting the questionnaire

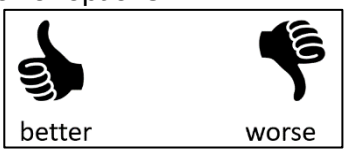
I started by drafting a questionnaire with a range of questions aimed at measuring change of illness from baseline. The first draft had three sets of questions as follows:

a) Compared to your first visit has general health changed? This was repeated for individual symptoms: cough, fever, shortness of breath and night sweats. Response options were: resolved, improved, no change, worsened, and not applicable.
b) Compared to your general health at first visit, how is your health status today? Responses were: better, much the same, worse, and much worse
c) Do you think the medication you received is helping to make you better? Responses were: yes, no, not sure, and not applicable

3 Establishing face validity

In May 2018 I convened an expert meeting comprising of members of the LSHTM TB Centre, University of Malawi Helse Nord Tuberculosis Initiative, and Malawi Liverpool Wellcome Trust, to review each question and determine if it was assessing the intended construct. The outcomes of the expert consultation were:

a) The questionnaire should be delivered via audio-computer assisted self-interview (ACASI) to minimise interviewer ascertainment and social desirability biases.
b) The questions would have to be fewer, simpler, and with fewer options
c) The responses would need to be accompanied by familiar symbols and/or colours
d) Consensus was to use thumbs up for better, and down for worse as shown here.



4 Preliminary piloting and establishing construct validity

After several iterations, I piloted the questionnaire and its translations among colleagues and three patients in August 2018, a process that helped me make the following revisions:

a) Added introductory text describing 1) how to use the tablet, 2) each question, and 3) each response.
b) Added practice questions before the real questions.
c) Added a step involving a study staff walking each participant through the introductory text and practice questions, then observing them take the practice questions on their own to confirm confidence and competence in using the ACASI.
d) Limited the interview to two questions: change in unwellness and change in cough
e) Changed from using two responses and thumbs, to using three responses and emojis as shown here.

5 Validation in the intended population

To validate the tool, I conducted a pilot study. I recruited participants attending primary care at Limbe Health Centre who met RCT eligibility criteria, and provided consent. Participants were asked about change in unwellness and in cough (the main symptom) a week after initial clinical consultation for respiratory symptoms. The two questions were delivered privately using the ACASI, and repeated in an in-person interview with study staff. The table below presented responses to both questions via both modes, and assess:

a) Internal consistency using agreement between questions
b) Test-retest and inter-rater reliability using agreement between tools.

	Unwellness on day 8 versus on day1		**Cough on day 8 versus on day1**		**Between question agreement (**internal consistency**)**		**Between approach agreement (**inter-rater and test-retest reliability**)**	
PID	**ACASI**	**Staff**	**ACASI**	**Staff**	**ACASI**	**Staff**	**Unwellness**	**Cough**
ACTP001	2	2	2	2	1	1	1	1
ACTP002	2	2	2	2	1	1	1	1
ACTP005	1	2	1	2	1	1	0	0
ACTP006	3	2	3	2	1	1	0	0
ACTP007	3	2	2	3	0	0	0	0
ACTP008	3	3	3	3	1	1	1	1
ACTP009	3	3	3	3	1	1	1	1
ACTP010	3	3	3	3	1	1	1	1
ACTP011	2	2	2	1	1	0	1	0
ACTP013	2	2	2	2	1	1	1	1
ACTP015	1	3	3	3	0	1	0	1
ACTP016	2	3	3	2	0	0	0	0
ACTP017	3	3	3	3	1	1	1	1
ACTP018	2	2	1	2	0	1	1	0
ACTP019	2	3	2	2	1	0	0	1
ACTP020	3	3	3	3	1	1	1	1
ACTP021	2	1	1	1	0	1	0	1
ACTP022	3	3	3	3	1	1	1	1
ACTP023	3	2	3	1	1	0	0	0
ACTP024	2	1	2	1	1	1	0	0
	Count of consistent responses (n=20)				**15**	**15**	**11**	**12**
	Consistency percentage (95% Confidence Interval)				**75%** (51, 91)	**75%** (51, 91)	**55%** (32, 77)	**60%** (36, 81)

6 Conclusions

The pilot study results demonstrated the validity and reliability of the ACASI approach:

a) **Internal consistency:** 75% similarity in the proportion of responses between the unwellness and cough questions (which measured the same construct), demonstrated on both ACASI and staff interviews.

b) **Test-retest reliability, and inter-rater reliability:** I did not expect excellent correlation between ACASI and staff interviews given the previously described biases associated with in-person interviews. However, the similarities in the proportion of participants giving the same response between questions (55% and 60%) provided confidence on both test-retest and inter-rater reliability.

c) **Content validity:** Three in-depth interviews (three participants), and a focus group discussion (five participants) confirmed that personal interpretations of the questions by the intended audience matched the construct expected by investigators. The eight participants also confirmed ACASI data entries as representing their intended responses and provided feedback on improving the user interface and experience.

8.2 Protocol manuscript for the systematic review and meta-analysis

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1700548	**Title**	Dr
First Name(s)	Titus, Henry		
Surname/Family Name	Divala		
Thesis Title	Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)		
Primary Supervisor	Professor Katherine Fielding		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Systematic Reviews		
When was the work published?	15 September 2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I designed the study being described in this protocol, wrote the study protocol, led the writing of the manuscript, and submitted it for publication

SECTION E

Student Signature	[redacted]
Date	29 December 2020

Supervisor Signature	[redacted]
Date	23 Feb 2021

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Systematic Reviews

PROTOCOL Open Access

Sensitivity and specificity of using trial-of-antibiotics versus sputum mycobacteriology for diagnosis of tuberculosis: protocol for a systematic literature review

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Abstract

Background: Suboptimal diagnostics for pulmonary tuberculosis (PTB) drives use of 'trial-of-antibiotics (non-tuberculosis)' in an attempt to distinguish PTB patients from those with bacterial lower respiratory tract infection (LRTI). The underlying assumption—that patients with LRTI will report 'response' to broad-spectrum antibiotics, while those with PTB will not—has minimal evidence base for such a widely used intervention. Numerous potential causes of misclassification include bacterial super-infection of active PTB, placebo effect, and antimicrobial resistance (AMR). The main aim of this systematic review is to collate available evidence on the performance of trial-of-antibiotics as a diagnostic test and to explore the timing, interpretation, and decision-making process.

Methods: We will search MEDLINE, Embase, and Global Health using the Ovid platform for published studies that recruited adults being investigated for PTB, performed trial-of-antibiotics accompanied by mycobacteriological investigations, and reported both diagnostic test outcomes at the individual level. Following article selection, two authors will independently review titles and abstracts against eligibility criteria then perform full-text screening and extraction into a spreadsheet. We will conduct a risk of bias assessment at the level of the study using QUADAS-2 (University of Bristol) tool that assesses diagnostic evaluation work in four domains: (1) patient selection, (2) the index test, (3) the reference standard, and (4) patient flow and timing of tests. We will perform a narrative synthesis and, where possible, meta-analyses addressing our primary outcome. Our protocol adheres to the standards recommended by the PRISMA-P.

Discussion: Pooling all available evidence on the accuracy, approach, and interpretation of results of trial-of-antibiotics in the context of PTB diagnosis will meet an urgent need, considering the widespread utilisation and potential for antimicrobial resistance. We therefore believe that our findings will have impact on policy and that they will inform the design of future detailed investigations into this important diagnostic approach.

Systematic review registration: PROSPERO CRD42017083915

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Background

Limitations of current diagnostics remain a challenge in the fight against tuberculosis (TB), a leading cause of infectious disease mortality with 10.4 million new cases and 1.8 million deaths annually [1]. To complement the suboptimal diagnostics, standard diagnostic algorithms in resource-limited settings include a 'trial-of-antibiotics.' This is a course of broad-spectrum antibiotics, with negligible *Mycobacterium tuberculosis* activity, given to patients with symptoms such as cough in order to 'rule--out' or 'rule in' TB [2–4]. Patients with negative sputum mycobacteriology who respond to the antibiotic treatment are considered TB negative, while those who remain symptomatic are deemed likely to have TB and undergo further evaluations leading on to receiving TB treatment.

Approximately 26.5 million antibiotics courses are prescribed in the course of diagnosis of the 5.3 million smear negative TB registrations per annum [7]. This estimate is based on assuming an average of 5 antibiotic courses per sputum-negative treatment initiation, with 2 courses given to the patients before TB treatment [5] and the other 3 courses accounting for patients whose symptoms resolved and TB was ruled out [6]. Despite this widespread use, there has been no previous systematic review of the diagnostic performance of trial-of-antibiotics. The objective of this review is to assess existing evidence for the diagnostic sensitivity and specificity of using trial-of-antibiotics compared to sputum culture for TB diagnosis.

Other important evidence gaps on this subject include the choice of non-TB antibiotics (except for avoidance of those with known anti-TB activity), timing of the treatment, number of trials, the definition of treatment response, and the exact management after knowing the treatment outcome. Lack of consolidated evidence in these may be the source of the variations of implementation of trial-of-antibiotics across national programs. We will in this review consolidate existing evidence related to these gaps as our secondary objectives.

Research question

Our study will address the following Population, Index test, Reference test, Outcome (PIRO) question.

Objectives

Primary objective

Our primary objective is to determine the sensitivity and specificity of using a trial-of-antibiotics compared to sputum mycobacteriology for diagnosis of pulmonary TB (PTB).

Secondary objectives

Our secondary objectives are as follows:

- To describe the timing of prescription of the trial-of-antibiotics in TB diagnostic algorithms as reported in included articles
- To describe the type, duration, and number of prescriptions of routine oral antibiotics
- To establish how response to trial-of-antibiotics is interpreted and the decision-making process following positive or negative results

Methods

Eligibility criteria

We will include studies in any language published after 1993 that recruited adults being investigated for PTB and performed and reported outcomes of both trial-of-antibiotics and mycobacteriology investigations as part of their diagnostics work up. We will define mycobacteriology tests as any laboratory test that identifies evidence of MTB from a sputum sample. There is no defined reference mycobacteriology diagnostic test for MTB; each of the available tests has considerable flaws. Considering the time period of the review, we expect smear microscopy, smear microscopy using a fluorescent microscope, Cepheid GeneXpert, and mycobacterial culture. The guiding PIRO (population, index test, reference test and Outcome) framework for the research question is as presented in Table 1 below.

Information sources and search strategy

We will search for studies meeting the eligibility criteria in MEDLINE, Embase, and Global Health using the Ovid platform. We will use the search strategy presented in Table 2 below to retrieve studies from the databases. We have chosen to include studies published after 1993 when the World Health Organization declared tuberculosis as a 'global emergency' greatly increasing funding and international commitment to tuberculosis research, management, and control efforts.

In Table 2 below, we have presented our search strategy for MEDLINE, which has also been adapted for Embase and Global Health (see Additional file 1). This search strategy was reviewed by an information retrieval expert from the LSHTM library (Table 2). After completing the search in these databases, we will export results to Endnote X8 and remove all duplicates. We will also include all relevant articles identified from citations and reference lists of all included articles.

Study selection and data extraction

Investigator TD will implement the search strategy, and then, investigators TD and MN will independently sift through titles and abstracts of the resulting papers against the eligibility criteria. TD and MN will independently assess full texts of the included papers for eligibility using the above criteria. The main reason for non-inclusion at

Table 1 Research question

Population	Adult patients with respiratory symptoms
Index test	Trial-of-antibiotics (any course of broad-spectrum antimicrobial given with the goal of ruling out TB in a symptomatic adult)
Reference test	Any mycobacteriology test (we expect smear microscopy, smear microscopy using a fluorescent microscope, Cepheid GeneXpert, and mycobacterial culture)
Outcome	Proportion of mycobacteriology-positive or mycobacteriology-negative participants correctly identified by trial-of-antibiotics (sensitivity and specificity)
Design	Cross-sectional, cohort, and randomised controlled studies

Table 2 Search strategy for MEDLINE using Ovid platform

Search in Ovid MEDLINE	
Search line	Search terms
Part 1	Defining study population
1.	exp Tuberculosis/
2.	tuberculosis.mp.
3.	(suspect* adj3 (TB or Tuberculosis)).mp.
4.	(presumpt* adj3 (TB or Tuberculosis)).mp.
5.	(probabl* adj3 (TB or Tuberculosis)).mp.
6.	exp Cough/
7.	tb.mp.
8.	(suspect* adj3 (TB or Tuberculosis)).mp.
9.	or/1-8
Part 2	Defining study intervention
1.	(Antibiotic* adj3 trial).mp.
2.	antibiotic*.mp.
3.	Anti-Bacterial Agents/
4.	(oral* adj3 antibiotic*).mp.
5.	(amox?cillin or erythromycin or azithromycin or doxycyclin* or Vibramycin or clavulanic acid or co-amoxiclav).mp.
6.	or/10-14
Part 3	Defining study outcome
1.	exp "Sensitivity and Specificity"/
2.	sensitivity.mp.
3.	specificity.mp.
4.	accuracy.mp.
5.	exp "Predictive Value of Tests"/
6.	((positive or negative) adj2 predictive value).mp.
7.	(ppv or npv).mp.
8.	or/16-22
Part 4	Subject combinations
1.	9 and 15 (*population and intervention*)
2.	23 and 24 (*Population and intervention and outcome*)
Part 5	Applying pre-defined limits
1.	limit 25 to yr="1993 -Current"

the full-text stage will be documented. Investigator KF will resolve any disagreements in eligibility. Investigators TD and MN will then extract data from all the eligible papers into an excel spreadsheet. Should we identify multiple publications from the same study, we will report data from one.

For studies with missing or incomplete information for meta-analysis, we will contact the authors by using the contact information provided in the publications. When attempts to contact the authors have not been successful, such studies will be excluded from the meta-analysis.

Quality assessment

We will conduct a risk of bias assessment at the level of the study using the QUADAS-2 (University of Bristol), the recommended tool for evaluating primary studies for the inclusion in systematic reviews for diagnostic accuracy. The tool, provided in Additional file 2, has four domains evaluating (1) patient selection, (2) the index test, (3) the reference standard, and (4) patient flow and timing of tests. Assessment is done with respect to risk of bias and applicability of results.

Data analysis

We will provide a narrative synthesis of our results summarising the key findings, reporting on their consistency and quality, and identifying evidence gaps or limitations. We will perform a meta-analysis for sensitivity and specificity of trial-of-antibiotics against mycobacteriology tests for all studies providing true positives, false positives, true negatives, and false negatives. Our sensitivity-specificity joint modelling will require each study to provide data for both sensitivity and specificity.

We will utilise the MIDAS module [7] in Stata statistical software (version 15.0; Stata Corporation, College Station, TX, USA), to carry out the meta-analysis. We will also report point estimates and 95% confidence intervals, for sensitivity and specificity of trial-of-antibiotics versus mycobacteriology for each study and for pooled data, using bivariate random effects meta-analysis. We will report these results using a forest plot and plot a summary receiver operating characteristics (SROC) curve. We will examine clinical utility of trial-of-antibiotics using a Fagan plot.

Subgroup analyses

We will perform the following subgroup analyses:

1. Geographical location. While sensitivity and specificity cannot be influenced by disease prevalence, in the case of trial-of-antibiotics, causes of symptoms and antibiotic susceptibility may vary from place to place. We will assess performance of trial-of-antibiotics versus mycobacteriology in the following regions: (1) sub-Saharan Africa, (2) Asia, and (3) South America.
2. Type of reference test. The goal of a reference standard test is to provide error-free classification of the disease outcome presence or absence. Since for TB, there is no test that truly meets this definition, the performance of trial-of-antibiotics may vary depending on the inherent properties of each reference standard. We will assess the performance of trial-of-antibiotics versus mycobacteriology in the following regions: (1) studies using microscopy-based approaches, (2) studies using MTB culture, and (3) studies using Cepheid GeneXpert.

Assessment for heterogeneity and publication bias

We will assess the extent of heterogeneity of diagnostic specificity and sensitivity using Cochran Q^2 and I^2 tests. Diagnostic specificity and sensitivity forest plots and a bivariate boxplot will provide visual representation of the extent of heterogeneity.

There is limited consensus on the most appropriate approach for identifying evidence of publication bias in studies of diagnostic performance. We have decided to use the Deeks funnel plot [8], where the inverse of the square root of the effective sample size is plotted against the diagnostic odds ratio, and publication bias is deemed absent if the plot achieves a funnel shape.

Result presentation and dissemination

For individual studies, we will present data as follows: author, year, country, whether a country has a low or high TB burden, population, sample size, design, TB reference standard, and results (sensitivity and specificity). We will present the results of our study selection using the approach prescribed by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [9].

We will prepare a manuscript, which we will submit for publication in a peer-reviewed journal. This work will also form part of a PhD thesis for TD, which he will submit to the London School of Hygiene & Tropical Medicine (LSHTM).

Protocol and registration

We registered this systematic review protocol with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42017083915.

Discussion

Our systematic review will be, to our knowledge, the first to pool evidence on the approach, implementation, and accuracy of using a trial-of-antibiotics for the diagnosis of tuberculosis. Trial-of-antibiotics is an integral component of diagnostic algorithms in low- and middle-income countries which, despite leading to 30 million empirical antibiotic prescriptions per annum, remains without strong evidence basis. Our findings therefore have high potential to prompt policy review as well as potentially stimulating funders and researchers to consider future studies into this component of the diagnostic algorithm.

Additional files

Additional file 1: Search strategy for Embase and Global Health in Ovid Embase. (DOCX 18 kb)

Additional file 2: Data extraction form. (DOCX 82 kb)

Acknowledgements

We would like to thank the staff of the library at London School of Hygiene & Tropical Medicine for their support especially during the development of the search strategy.

Funding

TD is funded by the Commonwealth Scholarship Commission and the Helse Nord RHF. This review is part of his PhD work at London School of Hygiene & Tropical Medicine. The funding agencies had no role in the preparation of this manuscript nor will they take part in the systematic review process or its publication.

Authors' contributions

All authors substantially contributed to the conception and design of the study and reviewed all documents and materials. THD developed the first draft of the protocol and manuscript, developed the search strategy and data extraction forms, and performed the final review of the manuscript. MN critically reviewed the protocol and the manuscript and contributed towards the development of the search strategy and data extraction forms. DJS contributed to the study concept and reviewed the manuscript. AGW contributed to the study design and development of the search strategy and reviewed the final manuscript. ELC contributed to the conception, study design, and search strategy and critically reviewed the final protocol and manuscript. KF contributed to the conception, study design, search strategy, and methodological design and critically reviewed the final protocol and manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This work will not involve direct contact with human subjects or participant identifiable data. Ethical approval is therefore not required for this study.

Consent for publication

All authors have given consent and approval for the manuscript to be submitted for publication.

Competing interests

The authors declare that they have no competing interests.

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8.3 Protocol for the randomised trial

Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)

Short title:	**A**ccuracy and **C**onsequences of using **T**rial-of-antibiotics for **TB** diagnosis
Acronym:	ACT-TB Study
Trial registration:	
Protocol version:	4.0, 27 Jan 2020
Chief Investigator:	Titus H Divala London School of Hygiene & Tropical Medicine Keppel Street, London WC1E 7HT Tel: +44 740 523 1847 Email: titus.divala@lshtm.ac.uk
Co-Investigators:	Katherine L Fielding, Neil French, Derek J Sloan, Elizabeth L Corbett
Collaborators:	Marriott Nliwasa, Augustine Choko, Ankur Gupta-Wright, Jennifer Cornic, Jon Øyvind Odland, Chisomo Msefula, Hendramoorthy Maheswaran
Sponsor:	London School of Hygiene & Tropical Medicine is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance and Integrity Office: London School of Hygiene & Tropical Medicine Keppel Street, London WC1E 7HT Tel: +44 207 927 2626, Email: RGIO@lshtm.ac.uk
Funding:	Helse Nord RHF Tromsø, Norway; Tel: +47 97065144 Email: Hanne.Husom.Haukland@helse-nord.no
Study Coordination Centre:	University of Malawi College of Medicine Private Bag 360 Chichiri, Blantyre, Malawi Tel: +2651871911 Email: rscdirector@medcol.mw

This trial will adhere to the principles outlined in the International Council for Harmonisation Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations.

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1 Abbreviations

ACASI	Audio Computer Assisted Self-Interview
AE	Adverse Event
AMR	Antimicrobial Resistance
AR	Adverse Reaction
ART	Antiretroviral Therapy
CD4	Cluster of Differentiation 4
CEACs	Cost-Effectiveness Acceptability Curves
COMREC	University of Malawi College of Medicine Research and Ethics Committee
CXR	Chest X-Ray
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data Safety and Monitoring Board
GLM	Generalised Linear Model
HIV	Human Immunodeficiency Virus
HRQoL	Health Quality of Life
LAM	Urine Lipoarabinomannan Assay
LJ	Lowenstein-Jensen
LSHTM	London School of Hygiene & Tropical Medicine
MDA	Mass Drug Administration
MGIT	Mycobacteria Growth Indicator Tube
MTB or M.tb	*Mycobacterium tuberculosis*

NMBs	Net Monetary Benefits
NTM	Non-Tuberculous Mycobacteria
NTP	National Tuberculosis Control Program
NTS	Non-Typhoidal Salmonellae
PCP	Pneumocystis Jiroveci
PLHIV	People Living With HIV
PTB	Pulmonary Tuberculosis
QALY	Quality-Adjusted Life Year
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOC	Standard of Care
STGG	Skim Milk Tryptone Glucose Glycerol
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TMG	Trial Management Group
WHO	World Health Organization
WTP	Willingness to Pay

2 Trial Investigational Team

Our investigational team includes expertise in diagnosis and management of TB; clinical evaluation of TB diagnostics; design and conduct of large randomised controlled trials; laboratory TB and AMR diagnostics; data management and analysis.

Chief investigator

Dr Titus H Divala: will be responsible for protocol development, coordination and conduct of the trial, governance, data management, data analysis and results dissemination. Dr Divala is a clinician with a career interest in clinical trials. Apart from medical training, he holds MPH and Masters of Science in Epidemiology and Preventive Medicine. In his career, Dr Divala has managed two large GCP, US-NIH-funded clinical trials, one of which was IND as the local PI supervising over 40 study staff at two sites in different cities; and coordinating protocol work across 4 laboratories in 3 cities. He has worked as a clinician for over 8 years in Malawi, a period when identifying TB cases and putting them on treatment was a daily job. This topic therefore falls in area of great personal interest above and beyond the potential benefit it has towards improving patient care in Malawi and all low and middle-income countries where 95% of the TB burden lies, where this approach is the standard.

This work will contribute towards PhD thesis for Dr Divala whose training is registered in the Infectious Disease Epidemiology Department of the London School of Hygiene & Tropical Medicine (LSHTM). The research is hosted in the Helse Nord Tuberculosis Initiative (HNTI), Department of Microbiology, University of Malawi College of Medicine where he is a Fellow. The research and his training are funded through a training grant from Helse Nord RHF of Norway which is managed by the Research Support Center (RSC).

Co-investigators and members of PhD supervisory team

Prof Katherine L Fielding: a seasoned TB statistician and clinical trialist, and Dr Divala's PhD supervisor for the CI, will be responsible for protocol development, conduct of the study, and data dissemination. Prof Fielding is also the Director of the LSHTM TB Center.

Prof Elizabeth L Corbett: a seasoned TB clinical epidemiologist and and Dr Divala's PhD co-supervisor for the CI, will be responsible for protocol development, conduct of the study, and data dissemination. Prof Corbett is a Wellcome Trust Senior Fellow.

Co-investigators and members of PhD advisory committee

Prof Neil French: a seasoned pneumococcal expert, will be responsible for protocol development, oversee all aspects of AMR work, and data dissemination.

Dr Derek J Sloan: clinician with detailed local clinical and research experience, will be responsible for protocol development, trial implementation and data dissemination.

Collaborators

Dr Marriott Nliwasa: clinician, with experience conducting studies in the study setting. He will be support the conduct of the study, linkage with the national program, and data dissemination.

Mr Augustine Choko: statistician, with expertise and experience in using ACASI. He will support ACASI development, data management and development of analysis plan.

Dr Ankur Gupta-Wright: clinician, will provide clinical input in protocol development and clinical consultation support to research coordinators during study implementation..

Dr Jennifer Cornick: microbiologist, will be support protocol development, and AMR laboratory methods and analysis, and data dissemination.

Prof Jon Øyvind Odland: Epidemiologist and honorary professor at University of Malawi College of Medicine, responsible for seeking ethical approvals from the funder appointed ethics committee.

3 Executive Summary

3.1 Study type

This proposal is for a randomised controlled clinical trial. It is individually randomised, open label and has three arms.

3.2 Background

Antimicrobial resistance (AMR) is a growing public health threat that is in part fuelled by empirical antibiotic usage. Empirical antibiotic use is often motivated by lack of point of care diagnostics a common problem in infectious diseases most of which are life-threatening. Tuberculosis (TB), the leading cause of infectious disease mortality, is one of the life-threatening illnesses without adequate diagnostics. Just over 50% of TB cases reported to WHO annually have confirmed mycobacteriological diagnosis. To complement the diagnostic gap, standard diagnostic algorithms include empirical antibiotic use. The antibiotic course, referred to as “trial-of-antibiotics”, given to mycobacteriology-negative but symptomatic adults, is often broad-spectrum aiming to provide treatment for pneumonia. The goal is to treat infectious causes of respiratory symptoms other than TB, effectively performing the role of a “rule-out” diagnostic test for TB.

3.3 Problem statement

Approximately 26.5 million antibiotics courses are prescribed in the course of diagnosis of the 5.3 million smear negative TB registrations per annum. Despite this widespread use, there is no randomised controlled trial (RCT) evidence supporting the diagnostic accuracy of antibiotic trials and their impact on AMR. It is also unknown whether this usage of antibiotics can improve clinical outcomes considering that in settings of high HIV prevalence, bacterial infection associated mortality just before and during TB treatment is high.

3.4 Objectives

3.4.1 Broad objective of the study

To determine the benefits and consequences (antimicrobial resistance) of using trial-of-antibiotics in TB diagnostic algorithms in low and middle income countries.

3.4.2 Specific objectives of the study

3.4.2.1 Primary

- To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with cough (and have a valid sputum test result) at primary care level in Malawi.
- To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with cough.

3.4.2.2 Secondary

- To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.
- To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in primary care presenting Malawian adults with prolonged cough including those without a successful sputum test (unable to submit sputum and those with invalid sputum results)
- To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.

3.5 Methods

To address the evidence gaps related to a) accuracy, b) antimicrobial resistance, and c) impact on clinical outcomes), we propose to conduct a randomised controlled clinical trial recruiting adult patients presenting to primary care centres in Blantyre, Malawi with history of cough for at least 2 weeks. After excluding those with danger signs we will randomise participants to receiving or not receiving trial-of-antibiotics (azithromycin or amoxicillin) from Day-1 to determine diagnostic accuracy (specificity) against mycobacteriology reference standard (smear microscopy, Xpert/MTB/RIF and culture). Our second primary outcome will be the between-arms difference of incidence of either death or hospitalisation or missed TB diagnosis by Day 29.

For secondary outcomes, we will compare between arms differences in incidence of antimicrobial resistance and cost-effectiveness by Day-29. To our knowledge this will be the first randomised controlled trial to address these questions in over 20 years of systematic use of trial-of-antibiotics without strong evidence base.

To adequately address the primary objective, we will need 625 participants in each of the three arms (azithromycin, amoxicillin and standard of care), a total sample size of 1875 participants.

3.6 Expected results and dissemination

The detailed understanding of the value of a trial-of-antibiotics in the context of diagnosis of TB will have invaluable impact on patient care in Malawi and the rest of the low and middle income world which hosts 95% of the global TB burden. This work will form part of a PhD thesis for Titus Divala, which he will submit to the London School of Hygiene & Tropical Medicine (LSHTM). We will share the results of this work with COMREC, LSHTM REC and Regional Committee for Health and Research Ethics at NTNU, Norway. The Malawi National TB Control Program are already aware of the study through our long standing collaborations. Apart from NTP, we will share our results with Blantyre District Health Office, the wider Ministry of Health, and the University of Malawi College of Medicine via the annual research dissemination conference. We will also prepare manuscripts for peer reviewed publications.

4 Tabular summary and schematic

Title	Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)
Design	Three arm (625 per arm) individually randomised (1:1:1), open-label controlled clinical trial investigating standard care diagnostic approach for tuberculosis. The trial will not use any unlicensed products.

Objective	**Outcomes**
Primary	
1. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with cough (and have a valid sputum test result) at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among participants with a valid result from at least one sputum TB test
2. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.	Proportion of participants experiencing at least one of the following adverse outcomes by Day 29: 1) death 2) hospitalisation 3) missed TB diagnosis
Secondary	
3 To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.	Proportion of day 29 nasopharyngeal *Streptococcus pneumoniae* isolates resistant to commonly used antimicrobials.
4. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in primary care presenting Malawian adults with cough including those without a successful sputum test (unable to submit sputum and those with invalid sputum results).	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among all randomised participants, with those who could not provide sputum or had an invalid sputum result classified as mycobacteriologically negative.

5. To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.	• Incremental cost per quality adjusted life year gained • Total direct medical costs per participant over 56 days • Eq-5D utility score
Exploratory	
Our exploratory analyses will be comparisons between the azithromycin and amoxicillin arms for all our primary and secondary outcomes.	
Population	Adults presenting to primary care centres in Malawi reporting cough.
	Inclusion criteria: • Ambulatory clinic attendees presenting with cough • Should have been ill for ≥ 14 days • Aged at least 18 years • Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period. Exclusion criteria: • Self-reported allergy to study medications • Acute danger signs defined in national TB treatment guidelines • Tuberculosis treatment or isoniazid preventive therapy in the last 6 months • Treated with antibiotics, other than co-trimoxazole prophylaxis, for the current illness or within the past 14 days
Treatment	**Arm 1**: Azithromycin 500mg once daily for 3 days commencing on randomization day. **Arm 2**: Amoxicillin 1 g 3 times daily for 5 days commencing on randomization day. **Arm 3**: Standard of care in current national guidelines for patients presenting with cough and without danger signs (No treatment until re-evaluation with with sputum TB test results)
Duration	We will give treatments on the randomisation day (Day-1) and perform follow up activities on days 8, and 29.

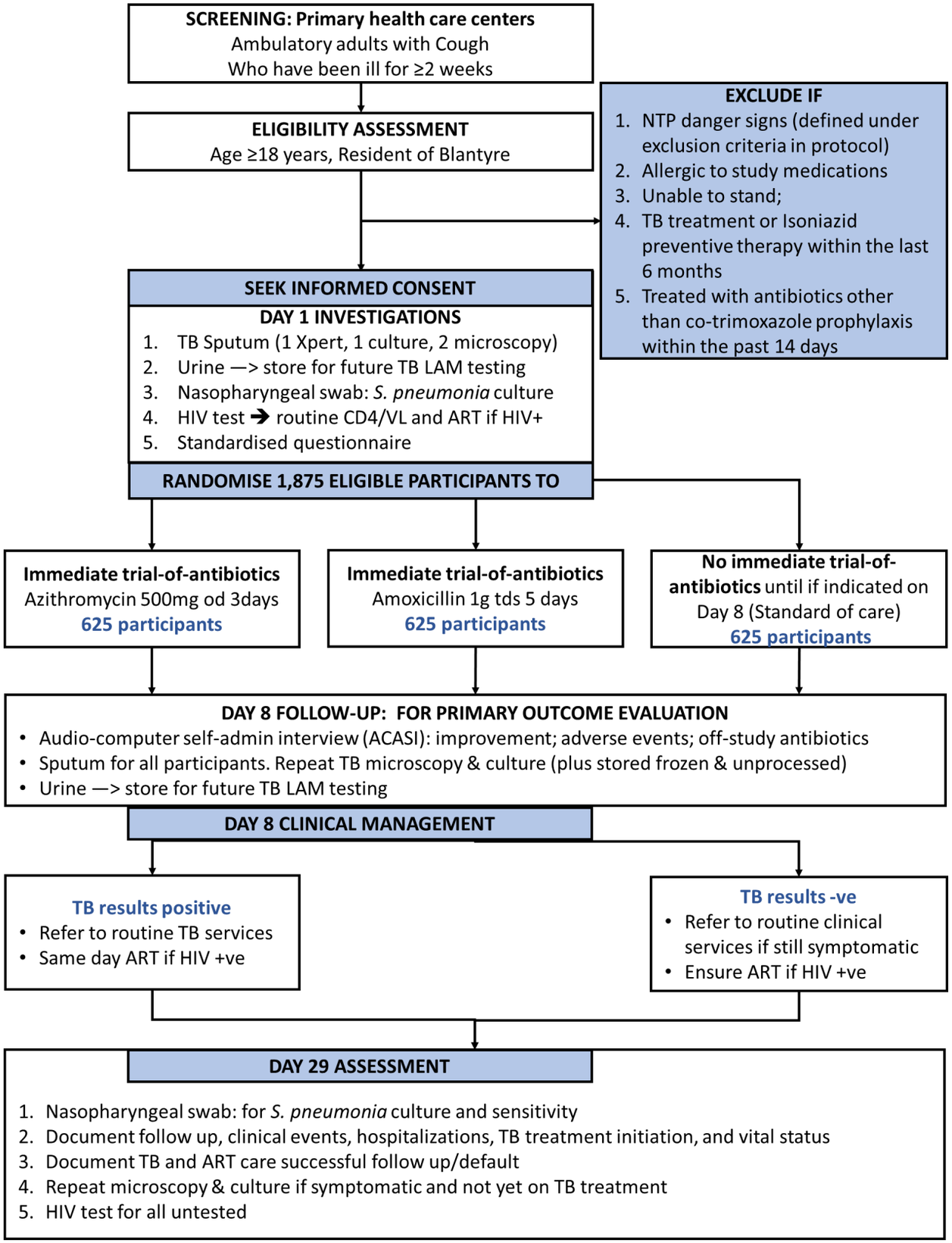


Figure 1: Flow diagram for the planned clinical trial in Blantyre, Malawi

5 Background information and introduction:

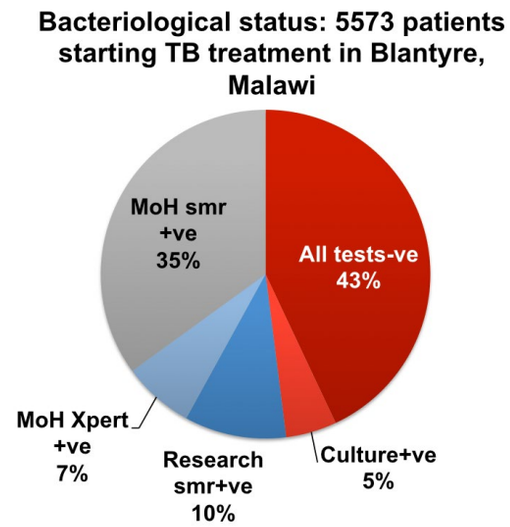
5.1 Background

Antimicrobial resistance is a growing crisis, becoming in 2016 one of only four health topics ever to be discussed at the United Nations General Assembly.[1-4] Tuberculosis is the leading global infectious cause of death in adults,[5] with approximately 10.4 million cases and 1.8 million deaths in 2015.[6] The high case-fatality rate in part reflects suboptimal diagnostics(Figure 2).[7-10]

Diagnosis method	Percentage
Mycobacteriology	57%
Clinical	43%

A. WHO annual TB notifications (WHO 2017 TB Report)

B.



Nliwasa et.al

Figure 2: method of diagnosis for TB notifications globally (A) and in Blantyre, Malawi (B)

To complement the suboptimal diagnostics, standard diagnostic algorithms in resource-limited settings include a “trial-of-antibiotics” (Figure 3). This is a course of broad-spectrum antibiotics, with negligible *Mycobacterium tuberculosis* activity, given to patients with symptoms such as cough in order to “rule-out” or “rule in” tuberculosis.[11-13] Patients with negative sputum mycobacteriology and responded to antibiotic treatment are considered tuberculosis negative while those who remain symptomatic are deemed likely to have tuberculosis and undergo further evaluations leading on to receiving tuberculosis treatment.

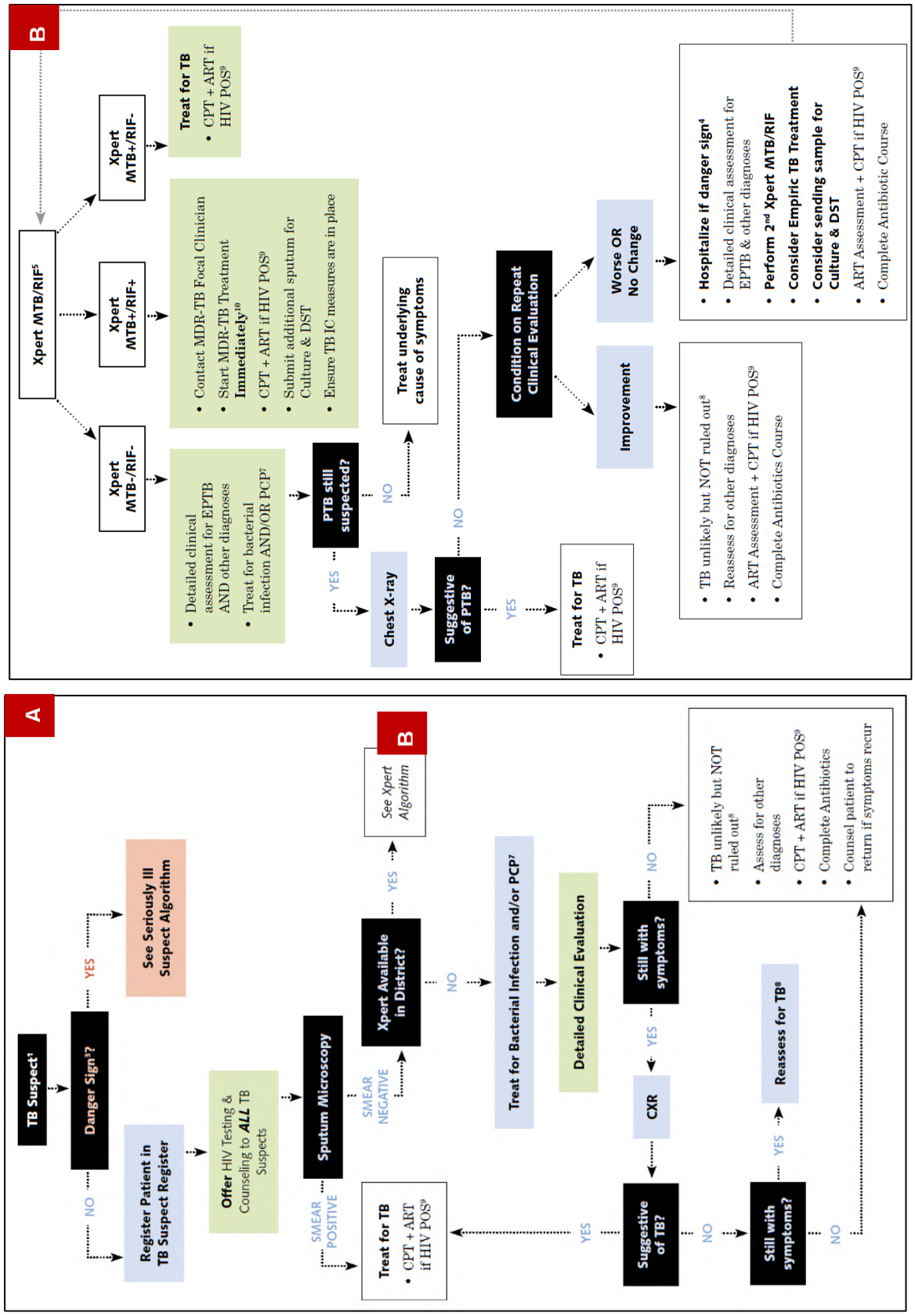


Figure 3: Implementation of trial-of-antibiotics (marked with red boxes) in Malawi TB diagnostic algorithm, National TB control program (NTP)

Approximately 26.5 million course of antibiotics are prescribed in the diagnosis of the 5.3 million smear negative tuberculosis registrations per annum (Figure 4).[6] This estimate is based on an average of 5 antibiotic courses per sputum-negative treatment initiation, with 2 courses given to the patients before

tuberculosis treatment,[8] and the other 3 courses accounting for patients whose symptoms resolved and tuberculosis was ruled out.[14].

Wilkinson et al[14] prescribed 120 + 74 courses of trial-of-antibiotics to diagnose 40 smear-negative TB patients (a typical ratio of ~1:5).[8] If generalizable, then for 5.3 million annual smear-negative TB registrations globally ~5 x 5.3 million trial-of-antibiotics courses (26.5 million) will have been prescribed.

Enrolled	**280**	
TB smear microscopy positive		160
Given trial-of-antibiotics (amoxicillin)	**120**	
Improved, declared TB negative		46
Given trial-of-antibiotics (erythromycin)	**74**	
Improved, declared TB negative		34
Treated for smear negative TB	**40**	

Wilkinson et.al Int J Tuberc Lung Dis. 2000

Figure 4: Quantifying number of trial-of-antibiotics courses prescribed per year using data from Wilkinson et.al and WHO TB Report 2016

Despite this widespread use, there is no randomised controlled trial evidence supporting the diagnostic accuracy of trial-of-antibiotics. There is also a dearth of evidence on their impact on antimicrobial resistance or patient clinical outcomes.

5.2 Systematic literature review

We performed a systematic literature review to determine the sensitivity and specificity of using a trial-of-antibiotics compared to sputum mycobacteriology for diagnosis of PTB. We also wanted to describe how trial-of-antibiotics tits into TB diagnostic algorithms: timing of prescription; type, duration, and number of antibiotic prescriptions; and how response to treatment is measured. We searched MEDLINE, Embase, and Global Health using the Ovid platform to identify studies meeting the following criteria:

Population	Adult patients with symptoms suggestive of pulmonary tuberculosis
Intervention	Routinely prescribed broad-spectrum oral antibiotics without MTB activity, and given as part of evaluation of pulmonary TB
Outcome	sensitivity and specificity of the intervention in comparison with any mycobacteriology test
Study design	Any design with prospective component allows evaluation of the outcome of the intervention
Time frame	Studies published after WHO declaration of TB as a 'global emergency' (1993)
Language	English, lack of translation capacity

We identified 7,064 articles from a systematic search on MEDLINE, Embase, and Global Health using the Ovid platform. Of these studies, 12 were eligible for narrative synthesis and seven had suitable data for meta-analysis. None of the studies was an RCT and all the observational studies were small and not primarily designed to address the benefits and consequences of trial-of-antibiotics. Unlike our proposed RCT, most of the published work was from hospital setting or in specialised clinics. Most studies used amoxicillin and some studies prescribed a subsequent course of antimicrobials either before or after assessing for improvement. The definition of improvement from baseline clinical state was largely subjective: it was based on self-report, clinical examination, radiological assessment or a combination.

There is no consensus on the sensitivity and specificity of trial-of-antibiotics across studies with estimates ranging from 43% to 91% for sensitivity and 41% to 82% for specificity (shown below).

Population, Study		Sensitivity, 95% CI			Specificity, 95% CI		
237	Wilkinson et al 1997	0.5	0.37	0.64	0.82	0.76	0.87
120	Wilkinson et al 2000	0.83	0.71	0.91	0.56	0.44	0.67
204	Kudjawu et al 2006	0.91	0.83	0.96	0.65	0.56	0.73
1000	Kamran et al 2006	0.72	0.62	0.8	0.41	0.38	0.44
264	Soto et al 2011	0.43	0.32	0.55	0.68	0.63	0.72
439	Soto et al 2013	0.46	0.34	0.58	0.6	0.53	0.66
440	Padmapriyadarsini et al 2	0.7	0.55	0.8	0.69	0.64	0.73

We could not identify any RCT, the current literature only has small studies, with trial-of-antibiotics not being the primary focus of investigation in most cases. There is limited data for primary care settings as most of the work was in hospital setting. None of the studies addressed AMR. Therefore, despite widespread use, the approach, the value and consequences of having trial-of-antibiotics in TB diagnostic algorithms, remains to be established.

5.3 Planned study

To address the evidence gaps related to a) accuracy, b) antimicrobial resistance, and c) impact on clinical outcomes), we propose to conduct a randomised controlled clinical trial recruiting adult patients presenting to primary care centres in Blantyre, Malawi with history of cough for. After excluding those with danger signs we will randomise participants to receiving or not receiving trial-of-antibiotics (azithromycin or amoxicillin) from Day-1 to determine diagnostic accuracy (specificity) against mycobacteriology reference standard (smear microscopy, Xpert/MTB/RIF and culture).

For secondary outcomes, we will also compare between arms differences in antimicrobial resistance and clinical outcomes (risk of death, hospitalisation, and missed TB diagnosis) at Day-29. To our knowledge this will be the first randomised controlled trial to address these questions in over 20 years of systematic use of trial-of-antibiotics without strong evidence base.

6.1.1 Accuracy of trial-of-antibiotics

As an approach that is being used on such a large scale, trial-of-antibiotics should ideally have a strong evidence-base (supported by reference mycobacteriology) of how much diagnostic and/or clinical improvement it brings to the TB diagnostic algorithm.[15,16] This will be among the most important considerations when deciding whether it is worth the trade-off with potential for AMR. Such evidence could come from an RCT or a well-designed prospective study.[15-17] However, despite being in use for more than 20 years, we have not identified any such clinical trial, and even the observational evidence is highly limited and of insufficient quality and quantity to definitively address the question.

There is also no guidance on antibiotic choice beyond a recommendation to avoid those with anti-tuberculosis activity (like fluoroquinolones). Another key area that lacks clarity is lack of a clear definition for clinical resolution when determining the outcome of trial-of-antibiotics. Clinical resolution is the basis for decisions that follow (i.e. discontinue follow up or proceed Antimicrobial resistance and trial-of-antibiotics

Antimicrobial resistance can be either intrinsic or acquired. The risk of acquired resistance relating to antibiotic use during evaluation for suspected tuberculosis has not been previously investigated, although previous work has shown that empirical antibiotics can drive rapid emergence of AMR.[18,19] For example, co-trimoxazole prophylaxis for HIV-positive patients, introduced in 2005, was followed by near-universal resistance in bloodstream infections by 2010.[20] Mass drug administration of azithromycin for trachoma control initially reduces nasopharyngeal carriage of *Streptococcus pneumoniae*, but with increased macrolide-resistance 6 months later.[21,22]

In our study, the AMR risks of empirical antibiotic prescriptions (azithromycin and amoxicillin arms of the RCT) are justified because of the widespread use of this approach for amoxicillin, and the low potential clinical impact and short-lived effects of use of azithromycin on AMR, given the limited use of macrolides in Malawi. Mathematical modelling work suggests that macrolide resistance can successfully be eliminated by intra-species competition alone (fitness cost) within 5 years of last use.[23]

6.1.2 Antimicrobial resistance and trial-of-antibiotics

Antimicrobial resistance relating to antibiotic use during evaluation for suspected tuberculosis has not been investigated before. Previous work has shown that empirical antibiotics can drive rapid emergence of antimicrobial resistance.[18,19] Co-trimoxazole prophylaxis for HIV-positive patients, introduced in 2005, was followed by near-universal resistance in bloodstream infections by 2010[20] also shown in Table 1. Mass drug administration of azithromycin for trachoma control initially reduces nasopharyngeal carriage of *Streptococcus pneumoniae*, but with increased macrolide-resistance 6 months later.[21,22]

We will investigate antimicrobial resistance in nasopharyngeal *S. pneumonia* by randomisation arm and cumulative antibiotic exposure to assess the extent to which brief exposure drives antimicrobial resistance during diagnostic work-up for tuberculosis. An ecological niche for many bacterial species,

the upper respiratory tract also presents a convenient window for investigating antimicrobial resistance.[24] *S. pneumonia* is the organism of choice not only for being an important cause of respiratory tract infections but also because it often colonises the upper respiratory tract and has well documented laboratory investigation procedures in place.[25] As exploratory analyses, we will also assess nasopharygeal colonization and antimicrobial resistance in relation to tuberculosis treatment and HIV status.

Table 1 Resistance patterns of common aetiologies of pneumonia to commonly used antimicrobials in Blantyre, Malawi

		Gram positive		Gram negative			
Organism		Streptococcus pneumoniae	Staphylococcus aureus	Haemophilus influenzae	Klebsiella pneumoniae	Escherichia coli	Pseudomonas aeruginosa
Prevalence		15.6%	6.6%	0.9%	4.4%	0.1%	1.5%
Resistance percentage	amoxicillin	*	*	58%	100%	94%	100%
	penicillin	21%	*	*	*	*	*
	co-trimoxazole	98%	40%	100%	92%	94%	75%
	chloramphenicol	21%	2%	92%	48%	61%	100%
	Erythromycin	2%	30%	*	*	*	*
	tetracycline	38%	35%	*	*	*	*
	Ceftriaxone	*	*	0%	90%	30%	100%
	Ciprofloxacin	*	*	NA	705	31%	24%

*not routinely tested

2016 data from hospitalised febrile patients at Queen Elizabeth central hospital (Blantyre, Malawi) as reported by the MLW Clinical research laboratory (unpublished).

6.1.3 Potential benefits of antibiotics

In areas with high HIV prevalence, empirical antibiotics during tuberculosis investigations could be life-saving: mortality immediately before and after tuberculosis diagnosis is high, [7,26] and is often secondary to severe bacterial infections.[26-28] The leading aetiologies of infection and death on tuberculosis treatment as well as among outpatients with tuberculosis-like symptoms are *Streptococcus pneumoniae* and non-typhoidal salmonellae (NTS): both can present with cough (primary cause) or as co-morbidities (super-infections) in patients presenting with active *Mycobacterium tuberculosis* (*M.tb*) disease.[26-28] If effective treatment of this type of life-threatening primary/super-infections reduces mortality during the diagnostic work-up of suspected TB in people living with HIV (PLHIV), then empirical use of broad-spectrum antibiotics would be indicated for this purpose alone, irrespective of any diagnostic contribution to TB treatment decisions. In this context, azithromycin may be the most effective arm, as Salmonella infections are highly sensitive to azithromycin, but not to amoxicillin.[29]

6.1.3.1 Measures of clinical benefit of trial-of-antibiotics

In this study, we will investigate the overall clinical benefit of trial-of-antibiotics by comparing the risk of any of death, hospitalisation, or missed TB diagnosis by Day 29. Although all these events are potential consequences of trial-of-antibiotics, grouping them as a single composite endpoint may

only appropriately represent the effect of the intervention 1) there are similarities in the importance patients would attach to each of its components and 2) the components occur with similar frequencies in the patient population.[30]

The impact of antibiotics on hospitalisation and mortality causing illnesses is as described above. Both these outcomes are important with their similarity hinged on the fact that hospitalisation event predicts mortality. In patients with chronic cough, frequencies of mortality and that of hospitalization over a two months period are similar, ranging from 2 to 6%.[31]

TB misdiagnosis becomes a concern because of the potential for misclassification in either direction –false positive or false negative. False positive diagnosis in the context of trial-of-antibiotics would occur when the underlying pathology for the respiratory symptoms is not responding to the antibiotic, which can be secondary to either AMR or the illness not being of bacterial origin. On the other hand, patients would be prone to a false negative result had both TB and a susceptible bacterial infection. If the symptoms were largely driven by the susceptible bacterial infection, their symptoms will improve and would be declared TB negative. TB is a life-threatening illness, missing its diagnosis can therefore lead to death which is more important to an individual patient than taking TB chemotherapy with a false positive TB diagnosis. We will therefore include only missed TB diagnoses in the composite clinical outcome. Unpublished data from Blantyre shows that the frequency of missed TB diagnosis under routine care settings is approximately 5% which is similar to that of death and hospitalisation.

6.1.4 Important subgroups

Response to trial-of-antibiotic- in patients with bacteriologically confirmed tuberculosis (i.e. false-negatives/low sensitivity from the perspective of TB diagnosis) may relate to multiple super-infections and so this phenomenon may vary by HIV status, since multiple concurrent infections are a hallmark of advanced HIV immunosuppression, and commonly identified in patients with suspected TB in the pre-ART era.[8,27] More recently, in Malawi, 45% of adults who presented to primary care with prolonged cough (≥2 weeks) were HIV-positive, of whom only ~20% started TB treatment on the basis of positive mycobacteriology.[30] As such, the benefits and consequences of trial-of-antibiotics may vary by HIV status and by subsequent TB treatment decisions. We will, therefore, include a pre-specified sub-analysis of trial outcomes stratified by HIV and ART status.

6.2 Choice of study interventions

Our trial will compare azithromycin and amoxicillin to standard of care. We propose 2 different antibiotic arms for the following reasons: -

a) Macrolides, including azithromycin, are rarely used in Malawi because of their higher manufacturing costs. However, they do provide a more effective treatment of community-acquired pneumonia than the standard antibiotic by Ministry of Health for trial-of-antibiotic (amoxicillin), because of low levels of acquired macrolide-resistance in bacterial isolates in Malawi,[29] reflecting

low rates of past exposure to this class of drugs, and also better intrinsic coverage of "bacterial cause of pneumonia including "atypical" intracellular organisms such as *mycoplasma* species.

Although viral pneumonias, *Pneumocystis jiroveci* (PCP) and non-infectious causes of cough will still not be expected to respond to azithromycin, this arm should then provide the highest possible diagnostic discrimination for bacterial vs mycobacterial causes of cough. The starting point of low pre-existing (acquired) resistance will also facilitate investigation of AMR acquired during trial-of-antibiotics. However, the trial will have limited national relevance in Malawi without comparison to an antibiotic in programmatic use.

b) Amoxicillin is low cost option that is still a recommended treatment for community-acquired pneumonia in most settings, including UK, despite potential treatment failure from bacterial pneumonia due to organisms with intrinsic ("atypicals") or acquired (common in gram-negative organisms, and *Staphylococcus aureus*) penicillin resistance.[29] This arm reflects the true standard of care (SOC) currently in widespread use in Malawi and many other low-income countries, and so provides data of immediate programmatic relevance and also a starting point to investigate exacerbation of pre-existing AMR pressure. If there is a marked difference between the azithromycin and amoxicillin arms, then there will also be important health economic considerations of relevance to many national TB programmes beyond Malawi.

Azithromycin provides effective treatment for community-acquired pneumonia[31-33] and has negligible activity against *M.tb.*[34,35] As discussed above, macrolides are not commonly used in Malawi. Azithromycin has an excellent safety profile and is used for mass drug administration (MDA) in communities prone to trachoma. Azithromycin used for MDA in Ethiopia reduced inter-current infections[21,36] and death in children,[37,38] supporting the safety of using this drug for our trial.[7]

Amoxicillin is the first line treatment for outpatient management of pneumonia in Malawi and is commonly used for trial-of-antibiotics. We anticipate higher specificity for azithromycin than amoxicillin, due to broader coverage of "atypical pneumonia" organisms, and salmonella species, but with the 2 antibiotics arms having "equipoise" due to lack of previous head-to-head comparison.[27]

6.3 Nasopharyngeal pneumococcus for AMR

Streptococcus pneumonia is a major cause of morbidity and mortality in children and adults.[20,29,39,40] Asymptomatic nasopharyngeal carriage of *S. pneumoniae* is common and a prerequisite for the occurrence and transmission of invasive pneumococcal disease.[41,42] Since carriage is more common than the invasive *S. pneumoniae* disease it forms a basis for establishing circulating serotypes, resistance patterns, and evaluation of vaccine effectiveness.

The other key advantage is the existence of globally accepted laboratory procedures for assessing and interpreting pneumococcal resistance. Our laboratory (in Malawi-Liverpool Wellcome Trust) has carried out pneumococcal work for decades with outstanding quality assurance reputation.

7 Objectives and outcomes

7.1 Broad objective of the study

To determine the benefits and consequences (antimicrobial resistance) of using trial-of-antibiotics in TB diagnostic algorithms in low and middle income countries.

7.2 Specific objective of the study

In Table 2 below, we present study objectives together with corresponding outcomes. We have clarified the outcomes with detailed definitions and planned analyses under "statistical approach" section.

Table 2: study objectives and outcomes

Objective	**Outcome**
Primary	
1. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with cough (and have a valid sputum test result) at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among participants with a valid result from at least one sputum specimen
2. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with cough.	Proportion of participants experiencing at least one of the following adverse outcomes by Day 29: 1) death 2) hospitalisation 3) missed TB diagnosis
Secondary	
3. To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.	Proportion of Day 29 acquiring nasopharyngeal *Streptococcus pneumoniae* isolates resistant to any of the commonly used groups of antimicrobials by Day-29.
4. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in primary care presenting Malawian adults with including those without a	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a

successful sputum test (unable to submit sputum and those with invalid sputum results).	mycobacteriology reference standard, among all randomised participants, with those who could not provide sputum classified as mycobacteriologically negative.
5. To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.	• Incremental cost per quality adjusted life year gained • Total direct medical costs per participant over 56 days • Eq-5D utility score
Exploratory	
Our exploratory analyses will be comparisons between the azithromycin and amoxicillin arms for all our primary and secondary outcomes.	

8 Study design, participants, and statistical approach

8.1 Study design

This is a three arm (625 per arm) individually randomised (1:1:1), open-label controlled clinical trial investigating accuracy and broader clinical, and antimicrobial resistance impact of using trial-of-antibiotics to “rule out” tuberculosis among adults presenting with cough at primary care centres in Malawi.

8.2 Study setting

We will screen adults aged at least 18 presenting to primary care centres in Blantyre, Malawi. Blantyre has an estimated adult HIV prevalence of 12.7% (95% CI: 11.9 to 13.6) and an estimated tuberculosis prevalence of 1,014 per 100,000 (95% CI: 486 to 1,542).[43]

8.3 Standard of care

The standard of care in national guidelines from the NTP for primary care patients presenting with cough and are otherwise well (no danger signs) is to take sputum x 2 for smear microscopy or Xpert and ask them to return for results, typically 3 days - 1 week later (Figure 3 and 5). The Malawi tuberculosis diagnostic algorithm recommends use of broad-spectrum antibiotics as trial-of-antibiotics after negative sputum tests are provided to the patient, if they remain symptomatic.

However, more commonly this algorithm is adapted in the outpatient setting to combine prescription of antibiotics (usually amoxicillin) with sputum collection at the first visit, to save the patient from making separate visits: thus, our amoxicillin arm is the most common standard-of-care in Malawi, while the no-antibiotic arm is the NTP recommended standard-of-care.

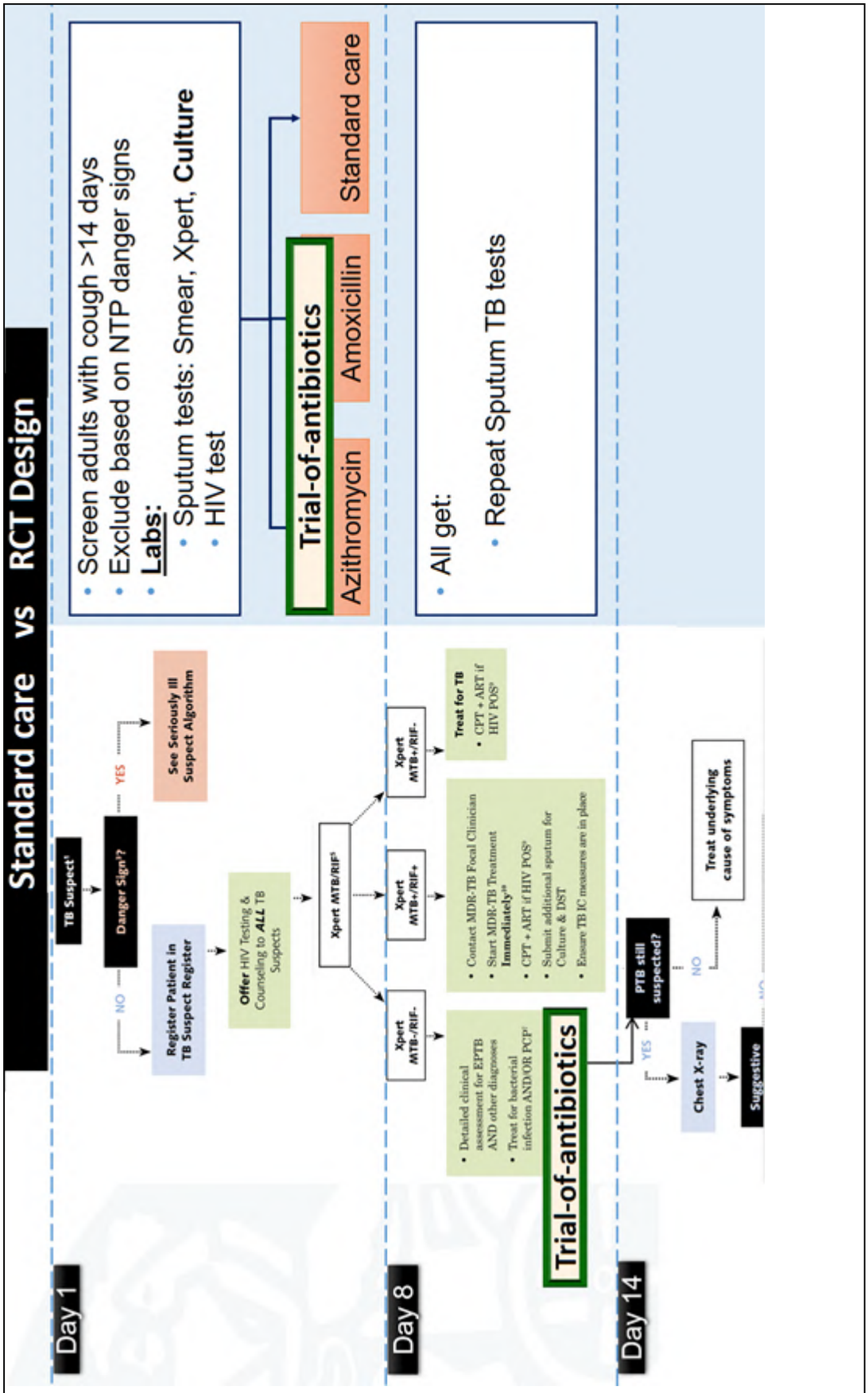


Figure 5: A comparison of standard of care (Malawi NTP guidelines) and the RCT design with respect to timing of trial-of-antibiotics and study procedures

8.4 Eligibility criteria

We will offer enrolment to patients who satisfy the following inclusion and exclusion criteria.

8.4.1 Inclusion Criteria

- Ambulatory clinic attendees presenting with cough
- Should have been ill for at least 14 days
- Aged at least 18 years
- Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period.

8.4.2 Exclusion Criteria

- Self-reported allergy to study medications
- Danger signs (WHO/Malawi NTP): respiratory rate > 30/min, temperature >39°C, Heart rate >120/minute, confused/agitated, respiratory distress, systolic blood pressure <90 mmHg, inability to walk unassisted
- Treated with antibiotics other than co-trimoxazole prophylaxis within the past 14 days
- TB treatment or Isoniazid preventive therapy within the last 6 months

8.5 Interventions

We will have two active study arms receiving trial-of-antibiotics at enrolment (azithromycin and amoxicillin) and a standard of care arm of no trial-of-antibiotics. In this study, the goal is to investigate the role of these antibiotics as they are used in TB diagnostic algorithms, as "trial-of-antibiotics," to exclude TB in symptomatic patients. The study is likely to be underpowered to detect differences between the 2 antibiotic arms will only be compared for exploratory outcomes.

8.5.1 Name and description of intervention arms

The study will have three arms as follows:

- Arm 1: Immediate trial-of-antibiotics with Azithromycin 500mg once daily for 3 days.
- Arm 2: Immediate trial-of-antibiotics with Amoxicillin 500 mg 3 times daily for 5 days.
- Arm 3: Standard of care

8.5.2 Legal status of drugs used in intervention arms

Both azithromycin and amoxicillin are registered for use in Malawi and United Kingdom, with both Arms 1 and 2 regimens being UK-recommended community-acquired pneumonia treatment.

8.5.3 Summary of Product Characteristics

Appendix 3 includes current versions of package insets for azithromycin and amoxicillin. We will review and update (when applicable) the package inserts annually with each ethics continuing review.

8.5.4 Drug Storage and Supply

We will procure study products from Durbin PLC (DURBIN PLC 180 Northolt Road South Harrow Middlesex HA2 0LT). Azithromycin will be manufactured by Sandoz limited or other pharmaceutical companies recognised in United Kingdom where Durbin is based. Amoxicillin will be manufactured by Medopharm private limited or other pharmaceutical companies recognised in United Kingdom where Durbin is based. Both azithromycin and amoxicillin are stable at room temperature. We will therefore ship and store in ambient conditions.

8.5.5 Preparation and labelling of study drugs

Study products will be stored at Malawi Liverpool Wellcome Trust Pharmacy. The pharmacy team will be responsible for packing and labelling.

8.5.6 Known drug reactions (adverse events)

Azithromycin and amoxicillin are already widely used in Malawi and are well tolerated. Rare side effects for azithromycin include nervousness, dermatologic reactions including Stevens–Johnson syndrome, anaphylaxis and prolonged QT interval. Rare side-effects for amoxicillin are mental state changes, light-headedness, photosensitivity and severe allergic reactions.

8.5.7 Concomitant medication and interaction with other therapies

We do not have any restrictions with respect to concomitant medications apart from those listed in the exclusion criteria. We expect some participants to be on HIV antiretroviral drugs and some may subsequently start tuberculosis therapy. Important interactions therefore would be those with HIV antiretroviral drugs and tuberculosis therapy. There is no moderate or major interaction between either azithromycin or amoxicillin with the classes of HIV antiretroviral drugs, tuberculosis therapy, and antimalarial drugs used in Malawi.

8.5.8 Trial restrictions

We do not require participants to have any dietary restrictions. We will also accept co-administration with contraception. Our trial interventions can safely be used in pregnancy, so we will include pregnant women should they be eligible.

8.5.9 Assessment of compliance

On Day-8, we will document self-reported compliance adherence of study products.

8.5.10 Withdraw of interventions

The investigator may also terminate a participant from study product if indicated by an adverse reaction. If a participant stops taking study product either voluntarily or by investigator decision, they will be encouraged to remain in follow up and their data will form part of intention to treat analyses.

8.6 Statistical approach

We will summarise the processes of recruitment including non-eligibility and reasons of exclusion in a CONSORT flow chart. We will describe the study participants by their baseline characteristics

which we will report for each arm. We will perform analyses of all our outcomes based on an intention to treat analysis (using the arm patient was randomised to), adjusting for centre. We will make the following comparisons:

i) azithromycin or amoxicillin versus standard of care

ii) azithromycin versus standard of care

iii) amoxicillin versus standard of care

We will perform data cleaning and analysis using Stata release 15 (Stata Corp, College station, Texas, USA).

The following are descriptions of each outcome and corresponding statistical approach. The statistical approach will be expanded in a detailed statistical analysis plan, separate to the protocol, which will be finalised before unblinding the study data.

8.6.1 Primary outcome

The clinical trial has two separately powered, and distinctly assessed primary outcomes, one for diagnostic evaluation (Primary outcome 1: Day 8) and the other for clinical impact (Primary outcome 2: Day 29) of the intervention.

8.6.1.1 Primary outcome 1: Specificity of day 8 symptom change versus mycobacteriology

Investigational test The investigational test is change in symptoms at Day 8 categorised as: improved or not improved (no change plus worsened) in response to the following question: *on day 1, you reported that you were unwell; compared to that day, has your illness worsened, remained the same, or improved?*

To minimise ascertainment bias in ascertaining this endpoint, the evaluation of improvement of baseline symptoms will be captured using a self-interview platform: Audio Computer Assisted Self-Interview (ACASI). After orientation, the participant will be left alone in the room to interact with the computer. ACASI on Day-8 will precede all other interaction with research staff and clinical assessment/decision making. We will report ACASI interview outcome as:

- **ACASI-test-negative** if the participant reports improvement
- **ACASI-test-positive** if the participant reports no change or worsening.

Reference test

Mycobacteriology reference standard will be defined in participants with at least one specimen with a valid result on days 1 and 8 as:

- **Sputum-test -POSITIVE**: if at least one positive smear microscopy, Xpert/MTB/RIF, or MTB culture on sputum samples taken.

- **Sputum-test-NEGATIVE**: none of the day 1 and day 8 sputum samples are positive on smear microscopy, GeneXpert MTB/RIF, or MTB culture.

To minimise bias, the mycobacteriology will be performed by a high-quality research laboratory in the University of Malawi College of Medicine by staff with no access to participant treatment allocation information or ACASI results.

The diagnostic assessment outcome

Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 ACASI against a mycobacteriology reference standard (b+d in Figure 6). Using the investigational test and reference test described above, this can be rewritten as: proportion of sputum-test-negative participants who are ACASI-test-negative.

-

		Reference Result: *any positive smear microscopy, Xpert/MTB/RIF, or MTB Culture from sputum samples collected on Day 1 and Day 8 visit defines tuberculosis-test-positive*	
		Sputum-test-positive	Sputum-test-negative
ACASI* Response on Day 8 *ACASI test is defined by response to the following question asked using ACASI on Day 8: on day 1, you reported that you were unwell; compared to that day, has your illness worsened, remained the same, or improved?*	ACASI-test-positive (worse or no change)	**a**	**b**
	ACASI-test negative (Improved)	**c**	**d**

Primary outcome: specificity, calculated by **d / (b+d)**

*Audio Computer Assisted Self-Interview (ACASI) in which the participant, after a how-to-use test session, responds to the prescribed question on a database-linked android tablet, without any human interaction, and in private.

Figure 6: Ascertainment of diagnostic value of trial-of-antibiotics

Estimation of measures of effect

We will use a generalised linear model (GLM) with identity link to estimate risks differences and the GLM with log link to estimate risk ratios for the three comparisons, adjusting for center. For each comparison, we will report 95% Confidence Intervals and Chi-square p-values. In pre-specified subgroup analysis, we will estimate the treatment effects stratifying by baseline HIV status. If the GLM model does not converge, we will use logistic regression to estimate the treatment effect using an odds ratio.

Participants without valid sputum mycobacteriology result

Primary analyses will be limited to participants who have at least one valid sputum sample result from all samples collected on visits Day-1 and Day-8. However, in real-life, ~15% fail to produce sputum, we will as a secondary outcome, perform all the analyses described for primary outcome with these participants defined as mycobacteriology negative. Further sensitivity analyses with urine lipoarabamannan antigen (LAM) results will include them in mycobacteriology definition.

8.6.1.2 Primary outcome 2: Clinical benefit of trial-of-antibiotics

Outcome definition

Proportion of participants experiencing at least one of the following adverse outcomes: death, hospitalisation, and missed TB diagnosis. The definitions of the components of this composite clinical outcome are defined in the table below:

Outcome component	**Definition**
death	Proportion of deaths by Day 29
hospitalisation	Proportion hospitalised for any cause by Day 29
missed TB diagnosis	Day 29 proportion of participants meeting standard **mycobacteriological and radiological** TB definitions but incorrectly classified as TB negative and not yet on TB treatment by Day 29.

Estimation of measures of effect

We will use a generalised linear model (GLM) with identity link to estimate risks differences and the GLM with log link to estimate risk ratios for the three comparisons, adjusting for primary care center. For each comparison, we will report 95% Confidence Intervals and Chi-square p-values. If the outcome is rare or if GLM does not converge, we will use logistic regression to model odds and report odds ratios for the following comparisons and their associated report 95% CIs and p-values.

8.6.2 Secondary outcomes

Outcome definitions

1) Proportion of day 29 nasopharyngeal *Streptococcus pneumoniae* isolates resistant to any of the commonly used antimicrobials.

We will define **AMR positive** as having nasopharyngeal isolates of Streptococcus pneumoniae that are resistant to any of the following commonly used antibiotics: ceftriaxone, amoxycillin, cefoxitin, azithromycin, and erythromycin as determined using disc diffusion technique; and **AMR negative** as either (1) not isolating any Streptococcus pneumoniae or (2) isolating any Streptococcus pneumoniae that is not resistant to any of the assessed antibiotics. For each arm, and at both baseline and day 29, we will report proportion of AMR positive participants. The study outcome will be the proportion of AMR positive participants at day 29.

2) Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among all randomised participants, with those who do not have a valid sputum test result classified as mycobacteriologically negative.

Estimation of measures of effect

Our secondary outcomes are anticipated to be rare, we will therefore use logistic regression to model odds and report odds ratios for the following comparisons and their associated report 95% CIs and p-values.

8.6.3 Exploratory outcome

Our exploratory analyses will be comparisons between the **azithromycin** and **amoxicillin** arms for all our primary and secondary outcomes.

8.6.4 Planned subgroup analyses

We will perform subgroup analysis for the primary outcome. The important subgroups based on rationale detailed under section 2.4.4, include HIV status, ART status, and PTB treatment. HIV and ART status will be as documented on Day-1 while PTB treatment will be either as:

- TB treatment commenced based on positive baseline (Day-1 and Day-8) mycobacteriology, *or*
- TB treatment commenced within 29 days of enrolment in patients with negative Day1 and Day-8 bacteriology.

The 29 days cut off for clinical decision to treat is to ensure that we only capture TB disease that was present at baseline. 29 days is a reasonable because: TB is a slowly progressing disease which if positive at Day-29, must have been incident on Day-1; and in routine care setting it can take over a month from presentation to diagnosis of TB.[8]

8.7 Sample size and power

8.7.1 Primary outcome1: specificity of day 8 symptom change versus mycobacteriology

We assume that trial-of-antibiotics (in azithromycin arm or in amoxicillin arm) will correctly classify 60% of mycobacteriology negative participants.[14] We have determined that 400 mycobacteriologically negative (true negatives) participants per arm will provide 80% power to detect a 10% difference in proportion of participants correctly classified as negative by amoxicillin arm or by azithromycin arm (60%) versus standard of care arm (50%). See table 3. We assume that 80% of participants randomised will have negative mycobacteriology,[30] requiring 500 participants to yield the 400 per arm. Assuming that 15% will not be able to produce sputum, and that 5% will not return for Day-8 visit, the sample size is increased to 625 per arm or 1,875 for the whole study.

For a 2:1 comparison (combining the two antibiotic arms versus the standard of care arm), 305 sputum-test-negative participants per arm will be needed to achieve 80% discriminatory power to detect a 10% difference in specificity. Accounting for TB prevalence, ability to produce and submit sputum, and loss-to-follow up increases the sample size requirement to 472 per arm or 1,416 for the whole study.

Table 3: Power and sample size estimation for primary outcome

True negatives (mycobacteriology tests negative participants) **b+d**	[1]**p (negatives correctly classified) d/(b+d)**	[2]**effect size**	**power (X^2 difference between independent proportions)**
320	0.60	0.10	69%
400	**0.60**	**0.10**	**80%**
480	0.60	0.10	86%

[1] specificity with either azithromycin or amoxicillin trial-of-antibiotics arms

[2]risk difference (azithromycin arm- standard of care arm)

8.7.2 Primary outcome 2: Incidence of adverse clinical outcome at Day-29

We will use a pilot study to determine the standard of care risk of at least one of death, hospitalisation, and missed TB diagnosis. The pilot study is described in section 5.0.

For now, we will assume that there is a 10% risk of experiencing this composite adverse outcome in the standard of care arm, and that loss to follow up by Day-29 will be 10%. With the sample size of 625 participants per arm (based on the primary outcome 1 sample size calculation), and alpha of 0.05, we will be able to detect the difference between intervention and standard of care with 80% power, if the risk in intervention arm is 6% or lower (Table 4). This estimate is applicable to the 2:1 comparison of the study arms.

Table 4: Sample size estimation for clinical benefit outcome

Participants per arm <u>based on primary</u>	**625**

10% loss to follow up by Day-29	562
Outcome risk in standard of care arm	0.10
Desired power	0.80
Alpha	0.05
Required intervention arm risk	**0.06**

8.7.3 Secondary outcome

1) Incidence of resistant *S. pneumonia* on Day-29

Study arms will be compared based proportion of participants with resistant *Streptococcus pneumoniae* on day 29. We assume 10% loss to follow up by Day-29, and the rate of *S. pneumonia* isolation from nasopharyngeal swabs in this population is expected to be ~45% at Day-29. The sample size based on the primary outcome (625 per arm) will provides ~253 *S. pneumonia* isolates/arm. In the standard of care arm, with 10% risk of resistant isolates, this translates into 25 cases. For the intention to treat population (the randomised 625 participants/arm) in the standard of care arm the 21 cases of resistant isolates translate into 4% (25/625) risk. To detect a twofold change in odds of day 29 AMR risk with at least 80% power, alpha of 0.05, and using Pearson's Chi-squared test, we will need at least 431 and 553 participants per arm for the 2:1 and pairwise comparisons respectively.

8.7.4 Exploratory outcomes

We anticipate that our sample size will be enough for hypothesis generation around our exploratory objectives but may not be enough to provide discriminatory power for comparison of outcomes between arms.

9 Pilot study

This area of research has limited evidence to guide the precise determination of sample size and the practical aspects of the clinical trial making a pilot study an invaluable tool. We have identified the following as key knowledge gaps which require exploration using a pilot study:

1) Among the adult patients presenting to primary care centres with cough for at least 2 weeks what proportion gets antibiotics:

- **a.** before clinic presentation?
- **b.** on first clinic visit?
- **c.** on follow up clinic visit after mycobacteriology results?

2) Following antibiotic treatment, how do patients report their clinical response? What are the best questions to ask patients post-antibiotic treatment to determine if they have improved or not? How best can we deliver these questions via Audio Computer Assisted Self-Interview (ACASI)? How well do these responses correlate with mycobacteriology and radiology?

3) What is the best timing for nasopharyngeal swabs for evaluating AMR in patients who receive a course of antibiotics during TB investigations?

4) In the standard of care setting, what proportion of adult patients presenting to primary care centres with cough for at least 2 weeks experience the following adverse outcomes (as defined under the clinical benefit composite endpoint)?

- **a.** death
- **b.** hospitalisation
- **c.** missed TB diagnosis
- **d.** HIV care loss to follow up
- **e.** TB care loss to follow up

9.1 Specific objectives of the pilot study

1) To determine the proportion of adults with prolonged cough who
   a. present to primary care having already had antibiotics for the index clinical complaints.
   b. receive antibiotics before sputum mycobacteriology results at first presentation
   c. receive antibiotics after negative mycobacteriology

2) To establish an objective way of documenting response to antibiotic treatment using Audio Computer Assisted Self Interview (ACASI). Assessing ACASI responses against clinical signs, outcomes of TB mycobacteriology and chest radiography.

3) To determine:

a. the prevalence of *Streptococcus pneumonia*;
b. the prevalence of resistant *Streptococcus pneumonia* isolates;
c. the optimal specimen collection timing for evaluating impact of antibiotic use on prevalence of *Streptococcus pneumonia* isolates resistant to common antibiotics

4) To establish standard of care rates of the following adverse clinical outcomes:

a. death
b. hospitalisation
c. missed TB diagnosis
d. HIV care loss to follow up
e. TB care loss to follow up

9.2 Population for the pilot study

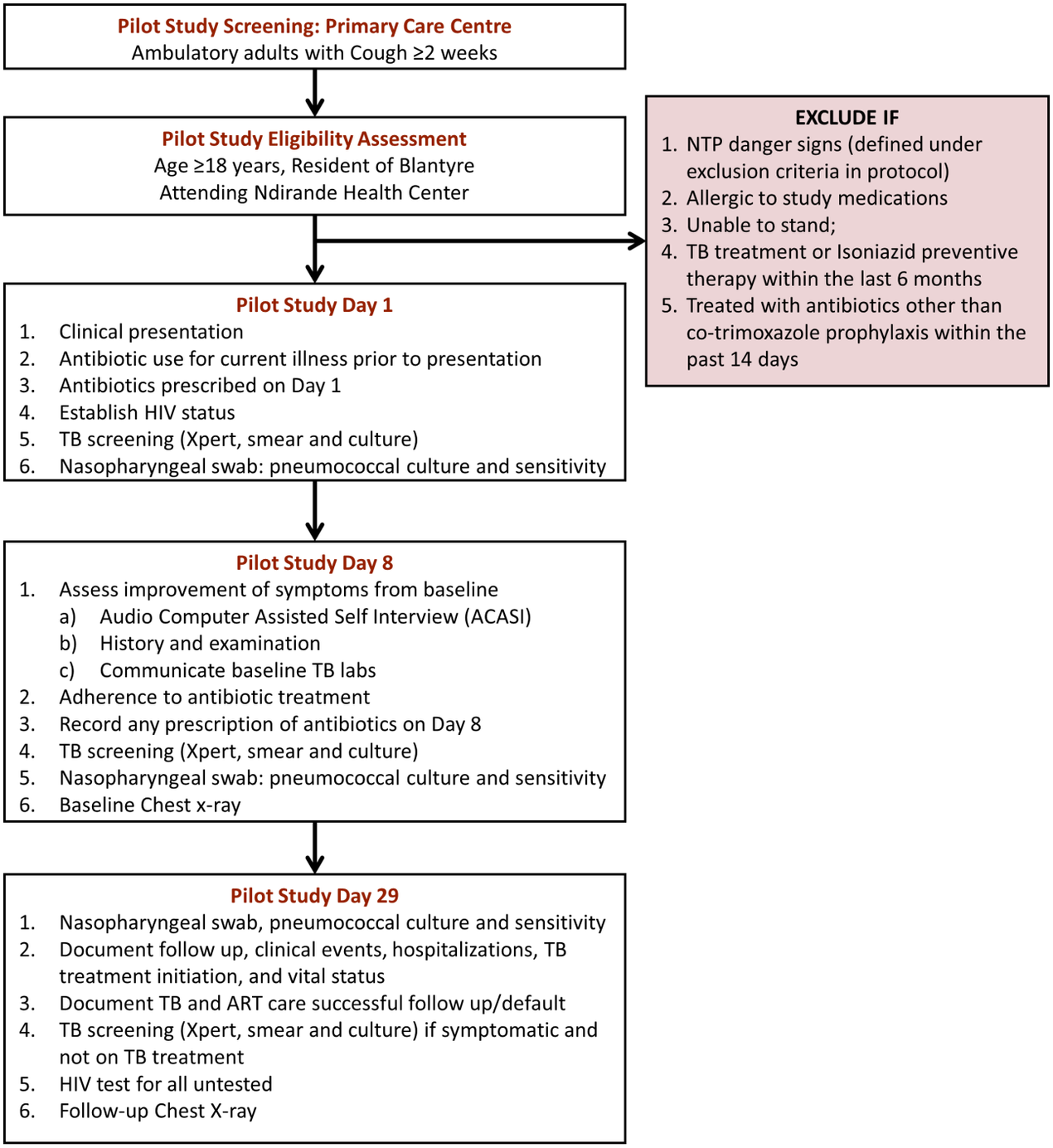
This exploratory study will include up to 400 adult (≥18 years old) patients presenting to primary care centres with cough for at least 14 days. We will exclude patients not meeting the eligibility criteria of the clinical trial.

9.3 Pilot study procedures

The pilot study procedures are outlined in the flow chart below. Following pilot study informed consent, we will use a baseline assessment questionnaire to collect clinical history, and antibiotic use for the index illness prior to the clinic visit. Throughout follow up, we will record all antibiotic use from any source. We will collect sputum samples for mycobacteriology from all participants on Day1 and Day 8.

We will establish HIV and TB diagnosis throughout the study, link participants to care services, and follow their adherence to follow up. For TB we will use a combination of Xpert, smear and culture on Day 1, 8 and whenever symptomatic suggestions of TB arise. We will also perform a chest x-ray on Day 8 and a follow up film on Day 29.

We will collect nasopharyngeal swab samples, for antimicrobial resistance assessment using *Streptococcus pneumonia* culture and sensitivity, on Day 1, Day 8, and Day 29. We will assess change in symptoms and well-being from Day 1 to Day 8, by using various combinations of questions and answers delivered via Audio Computer Assisted Self Interview (ACASI) on Day 8. We will ask participants which sets of questions they found easy to understand. We will also collect clinical information on all study visits including illness events, hospitalisations and vital status.



Pilot study flow diagram, summarizing the study procedures at each visit.

9.4 Data analysis

We will report the proportions of participants who used any antibiotics prior to primary care and during work-up for Tuberculosis. We will determine the best ACASI question and response combinations by participant reported ease of use, and by assessing correlation with clinical findings, mycobacteriology and radiological outcomes. The optimal time for assessing AMR will be determined by comparing incidence of resistant *Streptococcus pneumonia* isolates at days 8 and 29.

We will comparing participants exposed to antibiotics to those not exposed to antibiotics by estimating and reporting relative risk and 95% confidence intervals for:

1) Day 8 and Day 29 of resistant Streptococcus pneumonia
2) Composite adverse outcome of experiencing any of: death, hospitalisation, missed TB diagnosis, HIV care loss to follow up, and TB care loss to follow up

10 Study procedures

10.1 Screening

At the designated primary health care centres, study staff will approach patients with symptoms of pulmonary tuberculosis (including cough of any duration, fever, weight loss, and night sweats) with information about the study. Those willing to be screened for eligibility will be assessed against the study inclusion and exclusion criteria.

10.2 Informed consent

We will seek written informed consent (Appendix 1) from all patients who meet eligibility criteria before any trial-specific procedures. Screening for tuberculosis symptoms will not be considered as part of the study procedures, as it is already a fundamental component of the routine clinical assessment and history taking. A member of the study team will hand an informed consent form to a potential participant in their preferred language (Chichewa or English) detailing background, procedures, risks, benefits and participant expectations should they choose to join the study. The consent form will also state that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, and no obligation to give reason for the withdrawal.

If they choose to join as a study participant, we will then request them to sign two copies of informed consent form. If a potential participant does not know how to read or write, we will perform the informed consent process in the presence of a witness. In such cases, if they agree to participate in the study, we will ask them to sign using a thumb-print in the presence of their witness and a study team member. We will keep one copy of the signed informed consent forms and hand the participant the other copy.

10.3 Baseline procedures

After consenting, we will on the same visit request participants to provide 2 on the spot sputum samples for smear microscopy, Xpert and culture collected at least one hour apart. Those unable to spontaneously produce sputum will be instructed in the physiotherapy manoeuvre of “huffing” (forced expiration technique) for inducing mucus clearance from the airways.

Patients still unable to provide at least one mucoid sputum sample of >1 ml will initially will be given a sputum container and asked to return it the next day. If they do not manage to produce sputum at home, their mycobacteriology results will be treated as missing. We expect ~15% of participants to fall in this category[30] and have accounted for them in the sample size estimation. For participants who produce less than the needed quantity of sputum, we will process them for the planned tests in the following priority order: 1) Xpert MTB/RIF, 2) MTB culture, 3) smear microscopy. The Xpert MTB/RIF is the single most important guide for immediate clinical diagnosis and the MTB culture is the single most accurate reference diagnostic.

We will also collect a urine sample which we will store for subsequent lipoarabamannan antigen detection (LAM); and a nasopharyngeal swab for pneumococcal culture and sensitivity testing to

estimate prevalent antimicrobial resistance. We will also perform HIV tests according to the national algorithm, and if positive we will do HIV viral load. After completing all Day-1 visit procedures, we will link the newly tested positive participants to routine HIV care and will document when they start ART (Malawi National Program provides same-Day-ART initiation to all newly-diagnosed or untreated HIV-positive patients). After the sample collections, we will collect the following information:

- Demographic data, including precise geographic locator information using ePAL geolocation software (to aid follow-up). The locator information will also include phone numbers for the participant and for up to 3 family, or friends they nominate as alternative contacts.
- Clinical history including information on tuberculosis symptoms and health care seeking for HIV and tuberculosis care services including ART, cotrimoxazole, isoniazid preventive therapy, and past TB treatment.
- Vital signs including height and weight

After completing all these baseline procedures, we will randomise the participants to the three study arms.

10.4 Assignment of interventions

Step 1: An independent statistician based at LSHTM and without contact with participants or the study staff that see participants, will use the ralloc command in Stata (StataCorp LLC, College Station, Texas USA. Release 15.0) to prepare a random allocation sequence in advance of study recruitment efforts. Randomisation will be 1:1:1 to the three arms of the trial, block-randomised with variable block sizes, and stratified by primary care centre.

Step 2: Each treatment allocation will be printed alongside a randomisation number onto a pdf document.

Step 3: The statistician will email the pdf document to an independent designee within the University of Malawi who will print and place the randomisation assignments in envelopes labelled with randomisation numbers. The independent designee will hand the envelopes directly to the study pharmacist who will also receive a shipment of study medications. The pharmacist will store the envelopes in a secure location within the pharmacy.

Step 5: The pharmacist will pre-pack 625 each of protocol doses of azithromycin and amoxycillin without any reference to the allocation sequence. There is no need to refer to the allocation sequence for this step because the dosage for both treatments is the same and the total number of allocation for each treatment is known.

Step 6: At the beginning of each working week and upon request from study site staff, the study pharmacist will hand to site coordinators of each primary care center, a recruitment-rate-driven working stock of 1) the sequentially numbered sealed opaque envelopes containing randomization numbers and corresponding treatment allocations, and 2) study drugs.

Step 7: Study staff from each site will conduct patient eligibility assessments. Patients meeting the quick criteria of age and cough for ≥14 days, will be assigned screening IDs before being taken through the full eligibility criteria and consenting process. Participants will be considered eligible and ready for randomisation after they meet all criteria and sign consent.

Step 8: Upon signing consent, the participant will be taken to the site-coordinators (nurse or clinical officer) who will assign them the next available study ID number and document it on their paper and electronic eligibility checklist and enrolment CRF. The study ID number will be the number on the treatment allocation envelope plus a site-specific code. They will then open the envelope, document the treatment assignment, to the participant's enrolment paper and electronic case report forms as well as on a study card that will be pasted in the participant personal health profile book.

Step 9: The coordinator will double-check to ensure that the enrolment number and the treatment assignment are recorded correctly. They will then record screening date, screening ID, randomisation date, study ID, and randomisation arm on an enrolment log. They will then administer the allocated treatment. Administering study medications will not be considered as prescribing considering that prescription to all eligible participants will have already been done by the study protocol.

Step 10: When the stock of either envelopes or study drugs runs out, the nurse-coordinators or designee will reorder a from the study pharmacist.

Additional details

All steps of receipt and utilization of the allocations and study drugs are elaborated in a detailed SOP. The SOP guides implementation of the above plan as far as possible and in line with site conditions.

The study drugs will be pre-packed blindly without any reference to treatment allocations ensuring that neither the pharmacist nor the nurse-coordinator know the treatment allocations until just before assigning to a participant.

10.5 Blinding

We will mask the treatments as far as possible. The study pharmacist will remain blinded as they will use the randomly- allocated label numbers to prepare and pack the correctly dosed study medications in opaque packaging. Study outcome assessment will occur without reference to study treatment allocation. All laboratory forms for mycobacteriology and nasopharyngeal pneumococcal work will have no reference to participant treatment allocation. On Day-8, assessment of improvement from baseline symptoms will utilize audio computer-assisted self-interview (ACASI) to minimise potential for social-mediated reporting and ascertainment biases (see Procedures Section of the protocol). All clinical endpoints assessment case report forms will bear no reference to treatment arm. However, we will keep participants, research coordinators, and routine care staff

unmasked to ensure safety of the participants and allow appropriate patient management decision-making which may be related to the trial interventions.

10.6 Participant follow up

Following enrolment and completion of baseline procedures we will ask participants to return for follow up visits on days 8, and 29. They will be given 1 sputum collection bottle when leaving the clinic on Day-1 to bring with them sputum for mycobacteriology planned for Day-8 ("morning" specimen) followed by collection of one further "spot" sputum on Day-8, 2 sputum samples in total). We will also collect a second urine sample for storage for subsequent LAM antigen testing. Patients unable to produce at least one mucoid sputum sample of >1ml on Day-8 will be assumed for purposes of analysis to be mycobacterially negative for the Day-8 sputum samples. We are performing two sets of sputum examinations (Day-1 and Day-8) for each participant to strengthen the accuracy of the reference standard. Considering that TB progresses very slowly, making a diagnosis on Day-8 is not different from that made on Day-1.

We will advise participants that their sputum TB test results will start becoming available from 48 working hours after collection, but with the last test (MTB culture) taking up to 4 weeks. Patients will be advised that they will not be routinely contacted if positive TB test results become available before their Day-8 appointment (as is standard for outpatient management without danger signs in Malawi), and so will be advised to report promptly back to the clinic (with refund of transportation given) if they experience any clinical deterioration during Days 2 to 7. In the circumstances where TB treatment is commenced before completion of antibiotics prescribed for trial-of-antibiotics (amoxicillin and azithromycin), we will ask them to carry on with their allocated intervention together with the TB treatment.

10.6.1 Day-8 activities

On Day-8, the first activity before the participants undergo all other evaluations will be documentation of self-reported improvement of baseline (Day 1) TB symptoms using a pilot-validated set of questions and answer options delivered via Audio Computer Assisted Self Interview (ACASI). We will use ACASI with the goal of eliminating inter-observer variability and patient/interviewer reporting or ascertainment biases. After a "how to use" orientation and testing session, the participant will be left alone in the room to interact with the computer. A pre-recorded interviewer will ask the participant questions related to how their symptoms have changed on that day compared to how they were on Day-1 and will offer categorised voice-recorded responses with touch screen response buttons. The ACASI questionnaire will also include questions about adherence to study arm drugs and any other medical care (including traditional medicine) sought during the previous week.

Other activities for all participants on Day-8 include:

- collection of a second sputum sample for mycobacteriology tests.

- providing participants with Day 1 smear and Xpert results linking those with positive tests, ongoing symptoms and other illnesses with routine care for appropriate management.
- clinical history detailing clinical events since enrolment.
- documentation of any medications including antimicrobials and traditional medicine outside the study
- providing a study Day-29 appointment card

For participants with negative Day-1 mycobacteriology results we will perform clinical evaluation after ACASI and will inform the patient that any positive Day-8 sputum mycobacteriology results will be reported actively (via telephone or house visit) as soon as quality-assured results become available (within 48 working hours for microscopy and Xpert). Patients who have not had complete resolution of symptoms will be referred with all available results to routine primary care management.

10.6.2 Day-29 activities

Day-29 will be the final study visit. We will on this visit, collect data on clinical impact of antibiotic treatment and risk of AMR. In line with the second primary endpoint (composite clinical impact), we will document:

1) vital status
2) hospitalisations
3) identify missed TB diagnosis by using repeat mycobacteriology and routine care radiology for the symptomatic
4) perform HIV tests for those with unknown status and eligible for routine HIV test

We will collect information on clinical events prior to and at the visit and communicate all available sputum culture results. After collecting the clinical information, we will collect nasopharyngeal swab sample for assessing antimicrobial resistance. To collect the sample, a trained study staff will swab the participants' nasopharynx and place the swab in a tube containing skim milk tryptone glucose glycerol (STGG).

10.7 Laboratory methods

10.7.1 Tuberculosis mycobacteriology

We will process mycobacteriology tests at the Malawi College of Medicine TB laboratory, a reference laboratory located in Blantyre. For sputum samples collected on Day-1, we will perform smear microscopy, Xpert MTB/RIF and MTB culture. For sputum samples collected on Day-8, we will perform smear microscopy and MTB culture. We will use Mycobacteria Growth Indicator Tube (MGIT) and Lowenstein-Jensen (LJ) culture methods for TB culture. Once isolated, we will perform speciation as *Mycobacteria tuberculosis* (MTB) or non-tuberculous mycobacteria (NTM) using MBP84 antigen testing, microscopic cording and, if necessary, morphology and growth

characteristics at different temperatures and on solid (LJ) media containing p-nitrobenzoic acid (PNB).

10.7.2 Urine antigen testing for lipoarabamannan and other MTB antigens

Urine will be collected and stored as two 1 ml aliquots at -20°C from each participant on both Day-1 and Day-8 for subsequent mycobacterial antigen testing. No appropriate product is available for immediate use, but we anticipate that a commercial product with sufficiently high analytic accuracy for use in ambulant outpatients (sensitivity and specificity) may become available during the course of, or soon after, the study. If ongoing evaluations of the FIND-sponsored FujiFilm product[44] meet or exceed pre-specified requirements for clinical utility in the outpatient context, then point-of-care LAM testing at Day-1 and Day-8 will be added to the mycobacteriological definition of TB and patient management as soon as kits have been obtained and evaluated in Malawi.

10.7.3 Antimicrobial resistance testing

We will store swabs in STGG at minus 80°C. At a later stage we will thaw them in batches, and plate them onto selective media and culture colonies consistent with *S. pneumoniae*. We will determine Minimal inhibitory concentrations (MICs) using E-test strips (azithromycin and amoxicillin), and Kirby Bauer Disc diffusion testing (azithromycin, rifampicin, tetracycline, ceftriaxone, chloramphenicol, co-trimoxazole, erythromycin and penicillin) and define resistance by EUCAST breakpoints.

We will store isolates and remaining STGG at minus 80°C to allow genotypic characterization, isolation and susceptibility testing of other key respiratory pathogens, FTD 33 respiratory pathogen diagnostic panel, metagenomics analysis, and microarrays to detect multiple carriage and macrolide resistance genes in a broader range of pathogens at a later stage.

10.8 Loss to follow-up

To minimise loss to follow up, we will at enrolment record geolocation information of participants' place of residence using ePAL android app, a high-resolution mapping system validated in Blantyre. We will also record up to 3 contact phone numbers of the participant and their nominated friends and relatives. Should a participant miss a study visit, we will contact them by phone or by visiting them at home to encourage them to attend the study visit before expiry of prescribed visit window.

We anticipate a loss to follow-up of 5% by Day-8, and 10% by Day-29. We have accounted for these assumptions in the sample size calculation. We will not replace participants who discontinue study participation or study treatment regardless of reason for withdrawal or discontinuation or the time either of these occurs.

10.9 Trial closure

We will consider the trial closed after completing follow up of the last enrolled participant, and upon recording all mycobacteriology laboratory reports. Antimicrobial resistance lab work will continue beyond trial closure. The trial may be terminated early by the trial steering committee upon

recommendation of the DSMB. The halting rule for a trial arm is an unacceptable high level of deaths assessed using an alpha determined at the first DSMB meeting.

10.10 Summary schedule for study procedures

In Table 5 below, we have summarised all key study procedures over the study period.

Table 5: key study procedures over the study period

	STUDY PERIOD			
	Enrolment	**Follow up**		
TIMEPOINT**	**Day-1**	***Day-8***		***Day-29***
ENROLMENT:				
Eligibility screen	X			
Informed consent	x			
Allocation	x			
INTERVENTIONS:				
Azithromycin	x			
Amoxicillin	x			
Standard of care	x			
ASSESSMENTS:				
Demographics	x			
History of antibiotic use	x	x		x
**History & examination*	x	x		
***Sputum collection*	x	x		
Urine for TB LAM test	x	x		
Nasopharyngeal swab	x			x
HIV test and CD4 count	x			x
Linking to routine care	x	x		x
[1]ACASI		x		
****Clinical events*		x		x
Update contact & address		x		x

*For symptomatic participants, Day-8 sputum mycobacteriology should be fast-tracked to inform care before they leave the clinic.

**Give sputum bottles at end of Day-1 visit for submission on Day-8. Also collect sputum and perform mycobacteriology at any time of the study when clinically indicated

***Illnesses, clinic visits, radiological outcomes, new HIV diagnosis, new TB diagnosis, death, hospitalisation, missed TB diagnosis, HIV care loss to follow up, and TB care loss to follow up

[1]Audio Computer Assisted Self-Interview for documenting change of symptoms on Day- 8 versus Day-1

11 Safety reporting

11.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.
Adverse Reaction (AR)	Any untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product.

11.2 DMID grading for AEs

We will adopt the events grading criteria prepared by the Division of Microbiology and Infectious Diseases (DMID) of the USA National Institutes of Health as shown in the table below.

1 MILD	2 MODERATE	3 SEVERE	4 LIFE-THREATENING
Transient or mild discomfort (< 48 hours); no medical intervention required	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention required	Marked limitation in activity, some assistance usually required; medical intervention required, hospitalizations possible	Extreme limitation in activity, significant assistance required; significant medical intervention required, hospitalization probable

11.3 Grading for expected events

The following table provides guidance for grading known important or frequent side effects of azithromycin (based on the AE grading criteria provided in the BREATHE Trial Protocol, also investigating azithromycin) and amoxicillin graded on the DMID scale. All events not mentioned here or in Appendix 2, will be graded using the DMID grading for AEs table presented above.

	1 MILD	2 MODERATE	3 SEVERE	4 LIFE-THREATENING
Side-effects	**1**	**2**	**3**	**4**
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal oedema
Rash ***Specify type, if applicable***	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis
Mental state changes	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Photosensitivity	Painless erythema covering <10%	Tender Erythema covering 10 -	Erythema covering >30% body surface area and	Life-threatening consequences; urgent intervention indicated

	1 MILD	2 MODERATE	3 SEVERE	4 LIFE-THREATENING
	body surface area	30% body surface area	erythema with blistering, requiring intervention	
Arrhythmia (by ECG or physical examination) ***Specify type, if applicable***	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Prolonged QTc Interval	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Diarrhea ≥ *1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration	Life-threatening consequences (e.g., hypotensive shock)

	1 MILD	2 MODERATE	3 SEVERE	4 LIFE-THREATENING
			indicated (e.g., IV fluids)	
Laboratory	**1**	**2**	**3**	**4**
ALT or SGPT, High ***Report only one***	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Creatinine Clearance or eGFR, Low ***Report only one***	NA	< 90 to 60 ml/min or ml/min/1.73 m2 OR 10 to < 30% decrease from baseline	< 60 to 30 ml/min or ml/min/1.73 m2 OR ≥ 30 to < 50% decrease from baseline	< 30 ml/min or ml/min/1.73 m2 OR ≥ 50% decrease from baseline or dialysis needed

11.4 Causality

When reporting on serious adverse events, the trial investigator will state whether they believe that the event is causally associated with any of the trial treatments and the strength of the causal relationship. They will also state whether the adverse event was expected and what if any action was taken.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

11.5 Reporting Procedures

11.5.1 Non-serious Adverse Events (AEs)

Adverse events will be ascertained from patient follow-up visits or reports from relatives or guardian if patient cannot be contacted for follow-up. Study clinicians will be responsible for recording of details of the event including a description of the event, date of onset, severity, assessment of relatedness to trial interventions. Adverse events will be recorded in case report forms and uploaded into the study database.

11.5.2 Serious Adverse Events (SAEs*)*

All serious adverse events (SAEs) will be recorded on the relevant study CRFs and reported immediately to the Principal Investigator who will ensure that they are compiled in aggregate form and reported to COMREC and the DSMB once every 6 months. The DSMB will review SAE reports at their 6 monthly meetings and issue recommendations which will be shared with ethics committees. Events relating to a pre-existing condition or any planned hospitalisations for elective treatment of a pre-existing condition will not be reported as SAEs.

12 Economic evaluation

12.1 Objective

The objective of the economic evaluation is to undertake a cost-utility analysis to estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other. We will systematically compare costs and consequences associated with the interventions.

12.2 Outcomes

We will perform a within trial comparison of the three treatment arms to estimate the incremental cost per quality-adjusted life year (QALY) gained for the azithromycin or amoxicillin arm in comparison to standard of care. Costs will be estimated from the Malawian Ministry of Health perspective. Health outcomes will be quantified in QALYs, estimated from participants' responses to the Chichewa version of the EQ-5D-3L, a Health quality of life (HRQoL) measure.[45,46] We will adopt a time horizon matching the length of participant follow-up to achieve the within trial evaluation.

12.3 Data collection

The health economic data collection will be undertaken alongside planned clinical data collections. We will administer the Chichewa version of the EQ-5D-3L to all trial participants at baseline (Day1), Day 8 and Day 29. The Chichewa EQ-5D-3L was prepared in accordance with international and EuroQoL guidelines. The EQ-5D uses a descriptive system and a visual analogue scale (VAS). HRQoL on the day of response is defined using the descriptive system in terms of the following dimensions: 1) mobility, 2)self care, 3)usual activities, 4)pain/discomfort, and 5) anxiety or depression. The responses are then split into the following ordinal levels: 1) no problems; 2) some or moderate problems; and 3) severe or extreme problems.

The EQ-5D has 243 health states to which each response is allocated and converted to an EQ-5D utility score using a tariff. Tariff sets are derived from national surveys and currently no Malawian EQ-5D tariff exists. Zimbabwe, a setting similar to Malawi, has EQ-5D tariff set. In this study, we will use the Zimbabwean set to derive EQ-5D utility scores[47] an acceptable practice considering the similarities in how the two populations value health.[48] The EQ-5D utility scores in the Zimbabwean tariff, range from 1.0 (which means no problems in the five dimensions) to -0·29 (defined as severe problems in all five dimension).

We will capture all healthcare resources used by trial participants from recruitment into the trial till Day 29. This will be undertaken on Day 1, Day 8 and Day 29. Healthcare resources will be translated into direct medical costs using previously estimated costs[46,49,50] and the wider literature. Drug prices will be based on International market prices.[51] The health resource use questionnaire will at a minimum capture:

- Outpatient clinic visits

- Days of inpatient hospital care
- Medications
- Investigations and procedures

12.4 Data analysis

Our primary analysis will focus on direct intervention and the broader healthcare costs. We will define direct intervention costs as the costs associated with the application of the interventions. We will plot health state values measured by the EQ-5D-3L against time assuming that the health states reported at each time point are linearly connected. We will estimate QALYs associated with participant health profile by area under the plotted curve as calculated using the trapezium rule.

We will use a range of analytical methods depending on whether baseline covariates (EQ-5D utility values) are balanced between the trial arms or not. If they are balanced, we can obtain unbiased cost-effectiveness estimates by using non-parametric bootstrap approaches; if imbalance exists regression methods will be the approach of choice.

We will explore a range of estimators and undertake model diagnostics to determine the optimal model because the distributions of costs and QALYs are commonly skewed, often bimodal, or truncated. We will estimate mean costs and outcomes for each intervention together with respective mean incremental cost-effectiveness ratio. We will for each estimate report respective measures of uncertainty (standard errors and confidence intervals). We will also estimate the net monetary benefits (NMBs) for a range of different willingness to pay (WTP) thresholds. To identify the optimal intervention at different WTP thresholds, we will construct cost-effectiveness acceptability curves (CEACs) based on the NMB framework.

12.5 Missing data

For each participant we will collect complete data as far as possible but in cases of missing values, a common occurrence in trials, we will perform additional analyses to explore the impact of and account for the missingness.

13 Data management

13.1 Source Data

We will consider a document as source if it is where data were first recorded, and from which we obtained participants' case report forms (CRF) data. These will include hospital records, health center records, participant health passport, laboratory and pharmacy records, diaries, radiographs, and correspondence. We will consider CRF entries as source data if the CRF is the site of the original recording.

We will on all study-specific documents, other than study ID code list, the signed consent forms household locator form and, refer to the participant by their trial participant identification number, not by name. We will keep study ID code list, consent and locator forms separate from the rest of the participant file to avoid linkage between participant name and the study ID.

13.2 Data collection methods

We will collect data using standardised, pre-tested CRFs in two forms:

- programmed into android tablets using Open Data Kit (ODK) platform (opendatakit.org) with paper back-ups.
- optical mark recognition readable forms which will be read and extracted using TELEFORM system (Cardiff Software, Inc., Vista, CA), an optical-character-recognition software.

13.3 Data management

Any participants' identifiable data collected by the Study Coordination Centre will be stored securely and their confidentiality protected in accordance with the Data Protection Act 1998.

To ensure data security and maintenance of participant confidentiality, we will take several strict measures. All the study data collection tablets and computers will be encrypted, password protected and stored in a fireproof lockable cabinet inside a locked room. The principal investigator, study coordinator and data manager will be responsible for the maintenance of the tablets as well as all other computers, and their security from viruses and theft. All users will check in with the study coordinator and sign for data entry tablets every time they are taken out to for data entry and upon return. Whenever not in use, the devices will be kept in their locked cabinet.

We will keep all paper records in a locked space only be accessible to the principal investigator, co-investigators and delegated study staff. Study databases will be encrypted, password protected and will be stored on dedicated servers within the University of Malawi College of Medicine. We will keep all electronic and paper records securely for up to 10 years after the end of the trial in accordance with LSHTM Records Retention & Disposal Schedule guidelines.

13.4 Quality control and quality assurance

We will apply quality control at each stage of data handling in accordance with GCP requirements to ensure that all data are reliable and have been processed correctly. We will manual review all paper CRFs for completeness, accuracy and legibility before scanning. Our ODK data entry system will include automatic pre-programmed real-time data validation. The TELEFORM system will also have pre-programmed automatic data validation capabilities. We will perform data quality assurance (QA) on a random 10% of all participant files. The QA process will involve examining database entries and for paper source documents, verification of database entries and source.

13.5 Access to data

We will upon request, provide direct access to authorised representatives from the Sponsor, host institution and the regulatory authorities to allow smooth running of trial-related monitoring, audits and inspections.

14 Data monitoring and quality assurance

14.1 Data monitoring

Site monitoring for safety will be conducted to ensure human subject protection. The study will be monitored just before commencing enrolment, then once every 6 months by a monitoring team from the University of Malawi College of Medicine. The objective will be to ensure that study procedures, study products administration, and data collection processes are of high quality and meet ethical and regulatory guidelines. The regular monitoring will focus on the following areas: 1) protocol adherence, 2) informed consent documentation, 3) trial endpoints, 4) treatment discontinuation, 5) regulatory documents, 6) compare source documents and case report forms for accuracy, and 7) documentation practices in general.

14.2 Audits and Inspections

The study will be subject audit by the London School of Hygiene & Tropical Medicine under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

14.3 Data Safety and Monitoring Board (DSMB)

We will set up a DSMB before commencing trial activities. The DSMB will provide independent review of the study conduct, progress and findings. It will comprise 3 members including a chairperson who will be responsible for collating and communicating the views of the DSMB. The DSMB will consist of an independent statistician and two clinicians, at least one of them a physician, with research experience and expertise in the management of tuberculosis and HIV in Africa. The proposed data safety monitoring plan will be discussed in a teleconference including the DSMB members and the key investigators prior to the study starting.

The proposed meeting schedule is 6 monthly. Two weeks before a 6 monthly DSMB meeting, the study team will prepare a report covering study progress, study approvals, any obstacles, and recruitment statistics, adverse events, withdrawals and trial outcome measures. The DSMB will, through its chairperson, provide written feedback to the principal investigator who will be responsible for passing it on to ethics committees.

14.4 Trial Management Group (TMG)

A Trial Management Group (TMG) will be appointed and will be responsible for overseeing the progress of the trial. The day-to-Day-management of the trial will be co-ordinated through the University of Malawi College of Medicine.

15 Ethics and dissemination

We will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki and in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice E6 (R2) of November 2016.

15.1 Risk assessment

This is a low risk study as it is using already licensed antibiotics with good safety profile in a population defined by national clinical guidelines as clinically stable and not requiring other intervention but TB investigations. Our work complements standard of care by bringing in detailed TB diagnostics. In our study, the standard of care equivalent of the antibiotics we will prescribed on Day-1 to those randomised to either azithromycin or amoxicillin arms, are in standard of care prescribed on Day-8 only to mycobacteriology negative symptomatic patients (similar to the no antibiotic or standard of care arm of our trial). So, participants randomised to no antibiotic at Day-1 will not be receiving inadequate care but the recommended standard management of withholding antibiotics until after the TB results are available (Figure 1). To maintain participant safety and continuity of their care while on study interventions, we will not blind routine care clinical team and they will be free to manage the participants on their clinical judgement and national guidelines.

15.2 Research ethics approval

We will seek ethical approval for the trial protocol, informed consent forms, participant information sheet, any advertising material, and amendments to any of these documents, from the University of Malawi College of Medicine Research and Ethics Committee (COMREC), the LSHTM Research Ethics Committee, and Regional Committee for Health and Research Ethics, NTNU-Midt, Norway (on behalf of the funder). We will seek regulatory approval from the Malawi Pharmacy, Medicines, and Poisons Board (PMPB). Every year when the trial is active, we will seek continuous ethical review and approval before expiry of previous year's approval. In the event of an amendment, the changes will only be implemented upon ethical and regulatory approval.

15.3 Indemnity

London School of Hygiene & Tropical Medicine holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial.

15.4 Sponsor

London School of Hygiene & Tropical Medicine will act as the main sponsor for this study. Delegated responsibilities will be assigned locally.

15.5 Declaration of interests

The study team declares that they have no conflict of interest in conducting this clinical trial.

15.6 Cost of participation, ancillary and post-trial care

During the study, participant will benefit from frequent interaction with clinical study staff and associated optimised management of illnesses. There are minimal risks including discomfort associated with collection of nasopharygeal samples, and side-effects of study interventions. We will reimburse participant transport for attending study visits.

15.7 Dissemination policy

This work will form part of a PhD thesis for Titus Divala, which he will submit to the London School of Hygiene & Tropical Medicine (LSHTM). This work will form part of a PhD thesis for Titus Divala, which he will submit to the London School of Hygiene & Tropical Medicine (LSHTM). We will share the results of this work with COMREC, LSHTM REC and Regional Committee for Health and Research Ethics at NTNU, Norway. The Malawi National TB Control Program are already aware of the study through our long standing collaborations. Apart from NTP, we will share our results with Blantyre District Health Office, the wider Ministry of Health, and the University of Malawi College of Medicine via the annual research dissemination conference. We will also prepare manuscripts for peer reviewed publications.

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator.

Members of the TMG and the DSMB will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy

16 Study requirements, budget and justification

		CURRENCY	
Study staff	**Salary/month**	**GBP**	**MWK**
Clinical officers: 1 for 15 months	484	7,260	7,623,000
Nurses: 3 for 15 months	484	21,780	22,869,000
Clinic assistants: 4 for 12 months	300	18,000	18,900,000
Laboratory Technicians: 1 for 12 months	482	7,230	7,591,500
Data Clerks: 1 for 12 months	482	7,230	7,591,500
Subtotal study staff		**61,500**	**64,575,000**
Materials and consumables			
Trial participant costs: Stationary, printing, photocopying		1,000	1,050,000
Internet costs		1,500	1,575,000
Telephone airtime for for RCT staff/participant follow-up		1,500	1,575,000
International shipping (30% of UK consumable costs)		625	656,250
Transportation of field worker, airport transfers etc		3,000	3,150,000
Participant transport reimbursement		11,250	11,812,500
Antimicrobial resistance testing at MLW (paid to LSTM via LSHTM)		0	0
TB Xpert/MTB/RIF (Paid through LSHTM)		0	0
TB smr+culture @ £9.00 (subsidised by PhD supervisor)		6,300	6,615,000
Chest X-ray @ £5.00 (All participants Day 8, 15% on D56)		8,625	9,056,250
Subtotal of materials and consumables		**33,800**	**35,490,000**
Misclaneous costs			
MLW pharmacy fees (PhD student discount)		3,629	3,810,823
Data Safety & Monitoring Board & TSC		600	630,000
Medical indemnity @30/staff		420	441,000
Health and Safety including PEP		1,000	1,050,000
Translation costs		300	315,000
Subtotal of Other		**5,949**	**6,246,823**
Other direct costs in COM			
Infrastructure at CoM (3 lockable cabinets)		750	787,500
COMREC submission fees & amendments		300	315,000
Recruitment of clinical and non-clinical support staff		500	525,000
Protocol Training (for study staff)		1,000	1,050,000
GCP Training Facility		1,000	1,050,000
Clinical Trial Monitoring by COM RSC		1,500	1,575,000
Subtotal of COM Costs		**5,050**	**5,302,500**
Total before overheads		**106,299**	**111,614,323**
Indirect costs			
COM 10% Overheads		**10,630**	**11,161,432**
Malawi Pharmacy, Medicines and Poisons Board Trial Registration		5,532	5,808,871
Fronting Insurance (required for Malawi Trial Participants)		1,073	1,126,210
Subtotal indirect costs		**17,235**	**18096512.9**
Grand total		**123,534**	**129,710,835**

16.1 Budget justification

16.1.1 Study staff

The study will require the services of 2 clinical officers, 2 research nurses, 2 clinic assistants, 1 laboratory technician, and 1 data clerk. The chief investigator a PhD student, will not draw a salary from the study budget as he already has a stipend. None of the co-investigators will draw a salary from the study as they are already paid by their respective institutions.

16.1.2 Materials and consumables

We will need to cover costs for stationary, printing and photocopying of research tools for the project. These include paper-based questionnaires, information sheets and consent forms. We will need funding to cover communication needs (telephone and internet) throughout the study. This will allow maintainance of supervision of the study team, and facilitate participant follow up. We will need to cover local ground transportation for study staff and participant tracing. We will reimburse participants transport costs during the study period.

We will perform various laboratory tests. All TB tests will be done at the COM-MLW TB reference lab in Microbiology building. The costs of all TB tests have been subsidised by PhD supervisor. One of the TB tests, Xpert/MTB/RIF, will be paid from the LSHTM component of the funding. For antimicrobial resistance analysis, we will collect 1578 nasopharyngeal swabs on day 1 and day 29 and perform pneumococcal culture and drug susceptibility testing in MLW laboratory. Payment for this will be paid to Liverpool School of Tropical Medicine from LSHTM.

16.1.3 Miscellaneous costs

We will pay for use of the MLW pharmacy. We will cover costs for coordination of Data Safety & Monitoring Board and Trial steering committee meetings. We will conduct protocol, GCP and human subjects training for study staff before commencement of the study. We will have funds for Health and Safety (including PEP) related costs. We will utilise clinical trial monitoring from COM-RSC. We will need some COM infrastructure support.

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18 Appendix 1: Informed consent

Included as a separate document on headed pages.

19 Appendix 2: Division of Microbiology and Infectious Diseases (DMID) adult toxicity table

TABLE VERSION: November 2007

ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal LLN = Lower Limit of Normal R_x = Therapy Req = Required

Mod = Moderate IV = Intravenous ADL = Activities of Daily Living Dec = Decreased

- **ESTIMATING SEVERITY GRADE**

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

GRADE 1 **Mild** Transient or mild discomfort

(< 48 hours); no medical intervention/therapy required

GRADE 2 **Moderate** Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required

GRADE 3 **Severe** Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible

GRADE 4 **Life-threatening** Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THIS TABLE

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of participants in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide for Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	9.5 - 10.5 gm/d L	8.0 - 9.4gm/dL	6.5 - 7.9 gm/d L	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/ mm^3	750-999/ mm^3	500-749/ mm^3	<500/ mm^3
Platelets	75,000- 99,999/ mm^3	50,000- 74,999/ mm^3	20,000-49,999/ mm^3	<20,000/ mm^3
WBCs	11,000-13,000/ mm^3	13,000- 15,000 / mm^3	15,000- 30,000/ mm^3	>30,000 or <1,000 / mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	----------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL ----------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/ ml	41-50 mcg/ ml	51-60 mcg/ ml	> 60 mcg/ ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20.0 %

CHEMISTRIES				
	Grade 1	Grade 2	Grade 3	Grade 4
Hyponatremia	130-135 mEq/ L	123-129 mEq/ L	116-122 mEq/ L	< 116 mEq/ L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/ L	151-157 mEq/ L	158-165 mEq/ L	> 165 mEq/ L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/ L	2.5 - 2.9 mEq/ L	2.0 - 2.4 mEq/ L or intensive replacement therapy or hospitalization required	< 2.0 mEq/ L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/ L	6.1 - 6.5 mEq/ L	6.6 - 7.0 mEq/l	> 7.0 mEq/ L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/d L or abnormal glucose *with* mental

				status changes or coma
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/d L	251 - 500 mg/dL	> 500 mg/d L or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/d L	11.6 - 12.5 mg/d L	12.6 - 13.5 mg/d L	> 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/ L	1.1 - 0.9 mEq/ L	0.8 - 0.6 mEq/ L	< 0.6 mEq/ L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	> 1.75 x ULN

increase in other liver function test)				
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/d L	12.1 – 15.0 mg/d L	>15.0 mg/d L
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required
EN ZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
URINALYSIS				
	Grade 1	Grade 2	Grade 3	Grade 4
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day

Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion
CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/ Hg; no treatment	recurrent, chronic increase > 20mm/ Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral flu id treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment

Pericarditis	minimal effusion	mild/ moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused
RESPIRATORY				
	Grade 1	Grade 2	Grade 3	Grade 4
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring Oxygen therapy
GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4

Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV flu ids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/Day-or mild diarrhea last < 1 week	moderate or persistent; 5-7 loose stools/Day-or diarrhea lasting >1 week	>7 loose stools/day or bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	hypotensive shock or physiologic consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink flu ids; requires IV fluids
NEUROLOGICAL				

	Grade 1	Grade 2	Grade 3	Grade 4
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at	sensory loss involves limbs and trunk; paralysis; or seizures

	focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	ankles) and/or joint position or mild impairment that is not symmetrical	least mod degree in multiple different body areas (i.e., upper and lower e xtremities)	
MUSCULOSKELATEL				
	Grade 1	Grade 2	Grade 3	Grade 4
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint distruction
Myalgia	myalgia with no	muscle	severe muscle	frank

	limitation of activity	tenderness (at other than injection site) or with moderate impairment of activity	tenderness with marked impairment of activity	myonecrosis
SKIN				
	Grade 1	Grade 2	Grade 3	Grade 4
Mucocutaneous	erythema; pruritus	diffuse, maculo-papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm	
Erythema	< 15mm	15-30 mm	>30mm	
Edema	< 15mm	15-30 mm	>30mm	

Rash at Injection Site	< 15mm	15-30 mm	>30mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	
SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25-50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

20 Appendix 3: Package insert for Azithromycin and amoxicillin

To be included during ethics submission.

AMOXICILLIN CAPSULES BP

CATEGORY
ANTIBIOTIC - ANTIBACTERIAL

COMPOSITION

Dosage form	Strength
Capsules containing Amoxicillin Trihydrate BP equivalent to Amoxicillin	250 mg / 500 mg per capsule

INDICATIONS
Used in the treatment of E.N.T., U.T.I., G.I. and soft tissue infections due to gram positive & gram negative bacteria & surgical infections.

CONTRA-INDICATIONS & SIDE EFFECTS
Hypersensitivity to penicillin. Infectious mononucleosis. Other rarely observed side effects include anaphylactic shock, pseudomembranous colitis, G.I. upset, diarrhoea, sore mouth or tongue.

PRECAUTIONS
Patients allergic to other penicillins must be assumed to be allergic to Amoxicillin. It should be given with care to renal or hepatic dysfunction patients.

DOSE
One to two capsules, two to three times daily or as directed by the physician.

STORAGE
Store in a dry place, below 25°C, protect from light.
Keep out of reach of children.

PRESENTATION
Jars containing 100/250/500/1000 capsules.
Box containing 10 x 10, 50 x 10, 100 x 10 Strips / Blisters, or as required.

Manufactured by:
medopharm pvt. ltd.
INDIA.

PENC0508

MEDOPHARM	Editorial :	ERP :	RAD :
Brand Name:	Client: DURBIN PLC	Date: 21-12-2015 / Item Code: PENC0508	Head QA :
Generic Name: AMOXICILLIN CAPSULES BP	Type of Material: LEAFLET	Software: CDR X5 / Language: ENGLISH	Head CQA :
Size: H-145mm x L-105mm		Packing:	
Colors: BLACK			Head QC :
Spec.: 60GSM / MAPLITHO			Head Prodcuction : & Packing
Remarks : FOLDING SIZE : H-43mm x L-55mm			

SANDOZ

PACKAGE LEAFLET: INFORMATION FOR THE PATIENT

SZ00000LT000

Azithromycin 500 mg Tablets

Azithromycin

Read all of this leaflet carefully before you start taking this medicine because it contains important information for you.
- Keep this leaflet. You may need to read it again.
- If you have any further questions, ask your doctor or pharmacist.
- This medicine has been prescribed for you only. Do not pass it on to others. It may harm them, even if their signs of illness are the same as yours.
- If you get any side effects, talk to your doctor or pharmacist. This includes any possible side effects not listed in this leaflet (see section 4).

What is in this leaflet:

1. WHAT AZITHROMYCIN TABLETS ARE AND WHAT THEY ARE USED FOR

Azithromycin Tablets is an antibiotic. It belongs to a group of antibiotics called macrolides. It is used to treat infections caused by bacteria.

This medicine is usually prescribed to treat:
- chest infections such as chronic bronchitis, pneumonia
- infections of the tonsils, throat (pharyngitis) and sinuses
- ear infections (acute otitis media)
- skin and soft tissue infections, with exception of infected burn wounds
- urethra and cervix infections caused by chlamydia.

2. WHAT YOU NEED TO KNOW BEFORE YOU TAKE AZITHROMYCIN TABLETS

Do not take this medicine if you are allergic (hypersensitive) to:
- azithromycin
- erythromycin
- any other macrolide or ketolide antibiotic
- any of the other ingredients of this medicine (listed in section 6).

Warnings and precautions
Talk to your doctor or pharmacist before taking Azithromycin Tablets:
- Liver problems: your doctor may need to monitor your liver function or stop the treatment.
- Kidney problems: if you have severe kidney problems, the dose may have to be adjusted.
- Nervous (neurological) or mental (psychiatric) problems.
- A certain type of muscle weakness called myasthenia gravis.

Since azithromycin may increase the risk of abnormal heart rhythm please tell your doctor if you have any of the following problems before taking this medicine:
- Heart problems such as a weak heart (heart failure), very slow heart rate, irregular heart beat, or something called “long QT syndrome” (found by an electro-cardiogram)
- Low potassium or magnesium in your blood.

Other medicines and Azithromycin Tablets
Tell your doctor or pharmacist if you are taking, have recently taken or might take any other medicines. This includes any medicines obtained without a prescription. It is especially important to mention before taking this medicine:
- **Theophylline** (used to treat asthma): the effect of theophylline may be increased
- **Warfarin** or any similar medicine to prevent blood clots: concomitant use can increase the risk of bleeding
- **Ergotamine, dihydroergotamine** (used to treat migraine): ergotism (ie. itching in the limbs, muscle cramps and gangrene of hands and feet due to poor blood circulation) may occur. Concomitant use is therefore not recommended
- **Cyclosporin** (used to suppress the immune system to prevent and treat rejection of an organ or bone marrow transplant): if concomitant use is required, your doctor will check your blood levels regularly and may adapt the dose
- **Digoxin** (for heart failure): digoxin levels may increase. Your doctor will check your blood levels
- **Antacids** (for indigestion): see section 3
- **Cisapride** (for stomach problems), terfenadine (used to treat hay fever): concomitant use with azithromycin may cause heart disorders
- **Medicines for irregular heart beat** (called anti-arrythmics)
- **Nelfinavir** (used to treat HIV infections): concomitant use can increase the side effects of azithromycin
- **Alfentanil** (used for narcosis) or **astemizol** (used to treat hay fever): concomitant use with azithromycin may increase the effect of these medicinal products.

Azithromycin Tablets with food and drink
The tablets may be taken with or without food.

Pregnancy and breast-feeding
If you are pregnant or breast-feeding, think you may be pregnant or are planning to have a baby, ask your doctor or pharmacist for advice before taking this medicine. You should not use this medicine during pregnancy and when you are breast-feeding unless your doctor has specifically recommended it.

This medicine goes into human milk. So, you should stop breast-feeding until 2 days after you have finished taking this medicine.

Driving and using machines
This medicine may cause side effects such as dizziness or convulsions. This may make you less able to do certain things, such as driving or using machines.

Azithromycin Tablets contain soya lecithin
If you are allergic to peanut or soya, do not use this medicine.

3. HOW TO TAKE AZITHROMYCIN TABLETS

Always take this medicine exactly as your doctor or pharmacist has told you. Check with your doctor or pharmacist if you are not sure. These doses are for adults and children weighing more than 45 kg. Children weighing less than this should not take these tablets.

The recommended dose is:
Azithromycin Tablets is taken as a 3 or 5 day course
- 3 day course: Take 500 mg (two 250 mg or one 500 mg tablet) once each day
- 5 day course:
  - Take 500 mg on Day 1 (two 250 mg tablets)
  - Take 250 mg (one 250 mg tablet) on Days 2, 3, 4 and 5.

For urethra & cervix infections caused by chlamydia, it is taken as a 1 day course:
- 1 day course: 1,000 mg (four 250 mg tablets or two 500 mg tablets).
  Take the tablets together on one day only.

Patients with kidney or liver problems
You should tell your doctor if you have kidney or liver problems as your doctor may need to alter the normal dose.

Swallow these tablets whole with a drink of water.
- You can take these tablets with or without food.

Taking Azithromycin Tablets with medicines for indigestion
- If you need to take a medicine for indigestion, such as an antacid, take your tablets at least one hour before or two hours after the antacid.

If you forget to take Azithromycin Tablets:
- If you forgot to take a dose, take it as soon as possible. Then go on as before. Do not take more than one dose in a single day.

If you take more of Azithromycin Tablets than you should:
If you take too many tablets you may feel unwell. You also may experience other side effects such as deafness and

diarrhoea. Tell your doctor or talk to your nearest hospital casualty department immediately. If possible, take your tablets or the box with you to show the doctor what you have taken.

If you stop taking Azithromycin Tablets:
Always keep taking the tablets until the course is finished, even if you feel better. If you stop taking the tablets too soon, the infection may come back. Also, the bacteria may become resistant to the medicine and will then be more difficult to treat. If you have any further questions on the use of this medicine, ask your doctor or pharmacist.

4. POSSIBLE SIDE EFFECTS

Like all medicines, this medicine can cause side effects, although not everybody gets them.

Serious side effects:
If you have any of the following symptoms of a severe allergic reaction stop taking this medicine and **tell your doctor immediately** or go to the casualty department at your nearest hospital:
- sudden difficulty in breathing, speaking and swallowing
- swelling of the lips, tongue, face and neck
- extreme dizziness or collapse
- severe or itchy skin rash, especially if this shows blistering and there is soreness of the eyes, mouth or genital organs

If you experience any of the following side effects **contact your doctor as soon as possible**:
- diarrhoea that is serious, lasts a long time or has blood in it, with stomach pain or fever. This can be a sign of a serious bowel inflammation. This is something that can rarely happen after taking antibiotics.
- yellowing of the skin or whites of the eyes caused by liver problems
- inflammation of the pancreas, which causes severe pain in the abdomen and back.
- increased or reduced urine output, or traces of blood in your urine caused by kidney problems
- skin rash caused by sensitivity to sunlight
- unusual bruising or bleeding
- irregular or rapid heart beat

These are all serious side effects. You may need urgent medical attention. Serious side effects are uncommon (may affect up to 1 in 100 people), rare (may affect up to 1 in 1,000 people) or the frequency cannot be estimated from the available data.

Other possible side effects:

Very common side effects (may affect more than 1 in 10 people):
- diarrhoea.

Common side effects (may affect up to 1 in 10 people):
- headache
- vomiting, stomach upset, stomach cramps, feeling sick
- low numbers of lymphocytes (type of white blood cells), higher number of eosinophils (type of white blood cells), low blood bicarbonate, higher number of basophils, monocytes and neutrophils (types of white blood cells).

Uncommon side-effects (may affect up to 1 in 100 people):
- yeast and bacterial infections especially of the mouth, throat, nose, lung, bowel and vagina
- low numbers of leukocytes (type of white blood cells), low number of neutrophils (type of white blood cells), higher number of eosinophils (type of white blood cells)
- swelling, allergic reactions of various severity
- loss of appetite
- nervousness, sleeplessness
- dizziness, drowsiness, taste disturbance, tingling or numbness of the hands or feet
- visual disturbances
- impaired hearing, spinning sensation
- pounding heart beat
- skin rash, sweating (hot flush)
- difficulty breathing, nose bleeds
- constipation, wind, indigestion, inflammation of the stomach, difficulty in swallowing, bloating, dry mouth, eructation, mouth sores, increased salivary flow
- inflammation of the liver
- itchy rash, inflammation of the skin, dry skin, sweating
- joint inflammation, muscle, back and neck pains
- difficulty and pain when passing urine, kidney pain
- uterine bleeding, testis disorder
- skin swelling, weakness, generally feeling unwell, tiredness, swelling of the face, chest pain, fever, pain
- abnormal laboratory test values (e.g. blood, liver and kidney function test results)
- problems after treatment.

Rare side-effects (may affect up to 1 in 1,000 people):
- agitation, a feeling of loss of identity
- abnormal liver function
- being sensitive to sunlight.

Side effects of not known frequency (frequency cannot be estimated from the available data):
- reduction in blood platelets, which increases risk of bleeding or bruising
- reduction in red blood cells which can make the skin pale yellow and cause weakness or breathlessness
- feelings of aggression, anxiety, severe confusion, hallucination
- fits, fainting, decreased skin sensitivity, feeling hyperactive, disturbed sense of smell, loss of sense of smell or taste, muscle weakness (myasthenia gravis)
- poor hearing, deafness or ringing in the ears
- abnormal electrocardiogram (ECG)
- low blood pressure
- staining of the tongue
- joint pain.

Reporting of side effects
If you get any side effects, talk to your doctor or pharmacist. This includes any possible side effects not listed in this leaflet. You can also report side effects directly via the Yellow Card Scheme (www.mhra.gov.uk/yellowcard). By reporting side effects you can help provide more information on the safety of this medicine.

5. HOW TO STORE AZITHROMYCIN TABLETS

Keep this medicine out of the sight and reach of children.

Do not use this medicine after the expiry date which is stated on the carton after EXP: The expiry date refers to the last day of that month.

This medicinal product does not require any special storage conditions.

Do not throw away any medicines via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help to protect the environment.

6. CONTENTS OF THE PACK AND OTHER INFORMATION

What Azithromycin Tablets contains
- The active substance is azithromycin monohydrate equivalent to 500 mg azithromycin.
- The other ingredients are in the:
  - Core: microcrystalline cellulose, pregelatinised maize starch, sodium starch glycolate Type A, colloidal anhydrous silica, sodium laurilsulfate, magnesium stearate,
  - Coating: polyvinyl alcohol, titanium dioxide (E 171), talc, soya lecithin, xanthan gum.

What Azithromycin Tablets looks like and contents of the pack
Azithromycin 500 mg Tablets are white to off-white, oblong, film-coated, deep break line on one side and score line on other side. The tablet can be divided into equal halves.
Azithromycin 500 mg Tablets are packed in a PVC/PVdC//Aluminium-blister.

The 500 mg tablets are packed in the following pack sizes:
Carton box with blister(s) containing: 2, 3, 6, 12, 24, 30, 50, or 100 film-coated tablets. Not all pack sizes may be marketed.

Marketing Authorisation Holder and Manufacturer
Marketing authorisation holder: Sandoz Ltd., Frimley Business Park, Frimley, Camberley, Surrey, GU16 7SR, UK.
Manufacturer: Sandoz GmbH, Biochemiestrße 10, 6250 Kundl, Austria ***or*** Lek d.d., Pharmaceuticals, Verovškova 57, 1526 Ljubljana, Slovenia ***or*** Sandoz S.R.L., Livenzeni Street no 7A, Targu Mures, RO – 540472, Romania.

This leaflet was last revised in 06/2013.

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8.4 Information sheets and consent forms the randomised trial

What is the benefit and unintended consequences of using antibiotic treatment as a way of excluding tuberculosis disease in patients with cough?

Introduction

We would like to invite you to take part in a research study. Joining the study is entirely up to you. Before you decide, you need to understand why the research is being done and what it would involve. One of our team will go through this information sheet with you, and answer any questions you may have. Ask questions if anything you read is not clear or you would like more information. Please feel free to talk to others about the study if you wish. Take time to decide whether or not to take part.

What is the purpose of the study?

Tuberculosis (TB) is a disease that causes a long illness and cough with sputum. Although curable TB is difficult to detect. When they fail to detect TB after testing sputum, clinicians give antibiotic treatment that can cure all other causes of TB symptoms but not TB. In this approach, TB is considered ruled out if patient gets better and it is considered likely if they do not get better. The goal of this research study is to develop understanding of how well the antibiotics help distinguish TB patients from those who do not have it, whether giving antibiotics carries other health benefits, and whether it leads to development of disease causing organisms which are resistant to drugs.

We will learn about this by comparing a group of patients given antibiotics on the first day of the study to another group not given antibiotics. There will be two groups receiving antibiotics as follows: 1) Azithromycin taken as one tablet once a day for 3 days, and 2) Amoxicillin 4 capsules taken three times a day for 5 days. The group you will go into, out of the three, will be decided by chance so you can fall into any group.

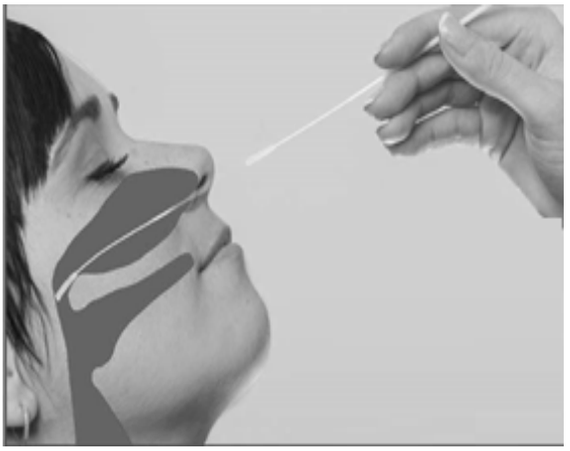
What will be involved if I accept to participate in the study?

We are considering you for participation in this study because you told us that you have a cough. Any patient who has been coughing for at least 2 weeks, is at least 18 years, and lives within Blantyre, is eligible to participate in this study if they do not have signs consistent with serious illness. Apart from you, we will recruit 1,874 other individuals.

Study activities will be performed the first day, at 1 week (Day 8), and at one month (Day 29). At each of these study visits, we will ask you questions about your contact details, your health, use of medications, and any illnesses or hospitalisations you may have had in between study visits. We will also document relevant details from your health passport and other clinical documentation you may have.

On Day 1 and at 1 week, we will ask you to submit sputum and urine samples for TB tests. If you are not able to give sputum on Day 1, we will give you containers so that you can bring them the following morning. Some of the sputum TB tests results will become available after 7 days and we will pass them to health center clinicians who will make a plan for your care, the other results may take up to 4 weeks so you will get them at the 1 month visit. Urine TB test results will not be available for your clinical care.

We will also do an HIV test. If the results are confirmed to be HIV positive we will do a viral load test, and at the end of the study activities on Day 1, we will link you to HIV management team here at the health center who will start you on treatment. Should we make a diagnosis of TB or HIV at any other point during the study, we will link you with the responsible health center team for treatment services.



On day 1 and at 1-month visit, we will swab the back of the inside of your nose as shown in this picture to collect germs that live there. We will test the germs for drug resistance. Results of this test are not relevant to your care.

On 1-week visit, we will ask you to report how your health has changed in comparison to how you were on day 1. These questions will be read to you by a computer and you will answer them by choosing various options which it will display during the interview.

The 1 month visit will be the final study visit where we will also provide you with results for TB culture and ask if you have TB symptoms. If you are in HIV or TB care, we will ask how your follow up is going. The appointment with you at 1 months is very important because it will help you to know the results of the TB tests and it will also help us know the status of your health.

The number of clinic visits you will make for this study is at least three. Here we count Day 1, one visit after one week, and another visit at one month. If you have not been able to come here for any of the visits, we will remind you by phone call or we will use the permission and information you will give us to visit you at your home. The first visit will take about 60 minutes and the later visits will take about 30 minutes each.

Will there be any risks involved in this study?

This study is a low risk study. There are no risks involved in submitting sputum or urine for the study. You may feel some discomfort during swabbing of the back of the nose and during blood collection for HIV and viral load tests. Azithromycin and amoxicillin are already widely used in Malawi and rarely cause problems. Rare side effects for azithromycin include feeling nervousness, skin reactions and disturbance of heart function. Rare side-effects for amoxicillin are mental state changes, feeling light-headed, and reactions to sunlight.

The London School of Hygiene and Tropical Medicine holds insurance policies which apply to this study. If you experience harm or injury as a result of taking part in this study, you may be eligible to claim compensation.

Will there be any benefits in this study?

The key benefit of this study is that you will have access to a more detailed TB evaluation process than usual. This will help you know if you have TB and to have the opportunity to start TB treatment. The study is also beneficial to health care providers because it will address important questions about use of antibiotics during the TB diagnostic process.

Will the findings in the study be confidential?

Your identity in this study will be treated as confidential. The results of the study, including laboratory or any other data, may be published for scientific purposes but will not give your name or include any identifiable references to you. Information about TB test result and HIV test results will be recorded using an identification number. However, any records or data obtained as a result of your participation in this study may be used by LSHTM who are sponsoring this study, regulators of health research (COMREC), or by members of the research team. These records will be kept in a locked space in the University of Malawi College of Medicine. Information and samples collected in this study will be retained for up to 10 years after the end of the trial, according to our institution recommendations. These collected samples and other information may also be used for future studies if you give us that consent.

Can I withdraw from the study anytime and will this affect my treatment?

You are free to choose whether or not to participate in this study. While we would like you to participate in the study to the very end, withdrawing at any point is an option that is freely available to you without any penalty or loss of any entitled benefits. You will be provided with any significant new findings developed during the course of this study that may relate to or influence your willingness to continue participation.

What are the financial benefits of participating in this study?

There will be no payment given to you for participating in the study. The study will provide at least MK8,000 as compensation for your costs of attending the study visits. We will give this money in instalments on scheduled study visits.

Is this study approved by an ethics committee?

The study has been approved by the London School of Hygiene & Tropical Medicine Research Ethics Committee, and the College of Medicine Research Ethics Committee (COMREC).

Who do you ask if you have questions regarding the study?

If you have any questions concerning participation in this study, please feel free to ask me. Alternatively, you can contact the following people by phone or post:

	Name	Telephone	Postal address
Study investigators	Dr Titus Divala Dr Marriott Nliwasa	0999478376 0888681948	Helse Nord Tuberculosis Initiative University of Malawi College of Medicine Private Bag 360, Chichiri, Blantyre 3, Malawi
COMREC	Administrative officer, COMREC Secretariat	01 877 245 01 877 291	University of Malawi College of Medicine Private Bag 360, Chichiri, Blantyre 3, Malawi

Approved by College of Medicine
21-May-2019
(COMREC) Research and Ethics Committee

What is the benefit and unintended consequences of using antibiotics treatments as a way of excluding tuberculosis disease in patients with cough?

Patient declaration

Statement	Initial or thumbprint each box
I confirm that I have read the above information sheet for the above named study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily. OR I have had the information explained to by study personnel in a language that I understand. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
I understand that relevant sections of my medical notes and data collected during the study may be looked at by authorised individuals from LSHTM, University of Malawi College of Medicine, and COMREC, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
I understand that data about me may be shared via a public data repository or by sharing directly with other researchers, and that I will not be identifiable from this information	
I understand that the tissue sample collected from me will be used to support other research in the future, and may be shared anonymously with other researchers, for their ethically-approved projects	
I agree to take part in the above named study	

Printed name of participant	**Signature/thumb print of participant**	**Date**

Printed name of impartial witness*	**Signature of impartial witness***	**Date**

I attest that I have explained the study information accurately to_____, and was understood to the best of my knowledge by, the participant and that he/she has freely given their consent to participate* in the presence of the above named impartial witness (where applicable).

Printed name of staff obtaining consent	**Signature of staff obtaining consent**	**Date**

*Impartial witness should be someone the participant trusts. The impartial witness can write the participant name but cannot sign for them. Instead, illiterate participants should use thumbprint in place of signature and the impartial witness should go ahead and sign in designated space.

Kodi kugwiritsa ntchito mankhwala opha tizirombo toyambitsa matenda ena ngati njira yothandizira kufifuza chifuwa chachikulu kuli ndi phindu kapena kuipa kotani?

Chiyambi

Tikukukupemphani kuti mutenge nawo mbali mu kafukufuku. Ndi chifuniro chanu kulowa mu kafukufukuyu. Musanapange chiganizo, mukuyenera kumvetsa chifukwa chimene kafukufukuyu akuchitikira komanso zimene zitadzachitike. M'modzi mwa anthu a gulu logwira ntchito mu kafukufuku awerenga chikalatachi pamodzi ndi inu, ndipo ayankha mafunso ena aliwonse amene mungakhale nawo. Funsani mafunso ngati simukumvetsa zomwe mwawerenga kapena ngati mukufuna uthenga owonjezera. Muli omasuka kulankhula ndi ena zokhudza kafukufukuyu ngati mukufuna. Ganizani mofatsa musanavomereze kutenga nawo mbali kapena ayi.

Kodi cholinga cha kafukufukuyu ndi chiyani?

Chifuwa chachikulu (TB) ndi matenda amene munthu amkhala chidwalire kwa nthawi yaitali. Odwalayo, amapanga makhololo. Ngakhale chili chochizika, chifuwa chachikulu ndi chovuta kuchipeza. Pamene njira zoyeza makholoro zalephera kupeza chifuwa chachikulu, achipatala amapereka mankhwala opha tizirombo toyambitsa matenda amene angathane ndi zonse zimene zimayambitsa zizindikiro za matenda ofanana ndi chifuwa chachikulu. Ngati odwala apeza bwino ndi njira imeneyi amaganiziridwa kuti alibe matenda a chifuwa chachikulu koma ngati sanapeze bwino amaganiziridwa kuti ali ndi chifuwa chachikulu. Cholinga cha kafukufuku ameneyu ndi kufuna kumvetsa za m'mene mankhwala amenewa amathandizira kusiyanitsa odwala matenda a chifuwa chachikulu ndi amene alibe matendawa, ngati mankhwalawa ali ndi phindu lina kwa odwala, komanso ngati kupereka mankhwalawa kukubweretsa tizirombo tosamva makhwala.

Tiphunzira zimenezi pakusiyanitsa gulu la anthu odwala amene apatsidwa mankhwala opha tizirombo toyambitsa matenda patsiku loyamba la kafukufukuyu ndi gulu lina limene silinapatsidwe mankhwalawa. Pakhala magulu awiri olandira mankhwala opha tizirombo motere: 1) Azitrhomycin omwedwa pilisi imodzi kamodzi patsiku kwa masiku atatu, komanso 2) Amoxicillin makapusolo anayi omwedwa katatu patsiku kwa masiku asanu. Gulu limene mulowe, mwa magulu atatuwa, lisankhidwa mwa mayere choncho mukhoza kupezeka mu gulu lina lirilonse.

Kodi chidzachitike ndi chiyani ngati ndingavomereze kutenga nawo mbali mu kafukufukuyu?

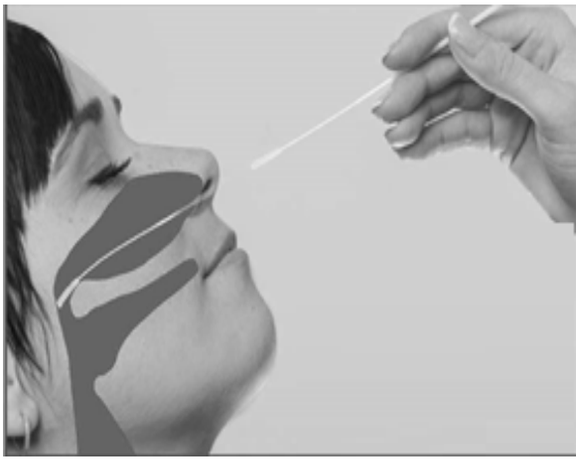
Tikukupemphani kuti mutenge nawo mbali mu kafukufukuyu chifukwa mwatiuza kuti muli ndi chifuwa. Odwala wina aliyense amene wakhala akukhosomola kwa masabata osachepera awiri, ali ndi zaka zosachepera 18, ndipo amakhala mu Blantyre muno, atha kutenga nawo mbali mu kafukufukuyu ngati alibe zizindikiro zosonyeza kudwalika kwambiri. Kupatula inu, tilemba anthu ena okwanira 1,874.

Zochitika za kafukufukuyu zidzapangidwa patsiku loyamba, pa sabata imodzi (Tsiku 8), ndi pamwezi umodzi (Tsiku 29). Pa masiku a kafukufuku onsewa, tidzakufunsani mafunso okhudzana ndi m'mene tingalumikizirane nanu, thanzi lanu, kagwiritsidwe ntchito ka mankhwala, ndi matenda ena aliwonse kapena kugonekedwa mu chipatala komwe kungakuchitikireni. Tidzalembanso zinthu zofunikira

kuchokera mu bukhu lanu la kuchipatala komanso zolembedwa zina za chipatala zimene mungakhale nazo.

Patsiku loyamba ndi pakutha pasabata yoyamba, tidzakufunsani kuti mupereke makhololo komanso mkodzo pofuna kuyeza matenda a chifuwa chachikulu. Ngati simungakwanitse kupereka makhololo patsiku loyamba, tidzakupatsani mabotolo kuti mudzawabweretse m'mawa wa tsiku lotsatira. Zotsatira zina za makhololo zidzatuluka pakutha pa masiku asanu ndi awiri ndipo tidzazipereka kwa matodolo a chipatala chino kuti akuthandizeni, zotsatira zina zidzatenga pafupi-fupi masabata anayi choncho mudzazilandira pa ulendo wa pamwezi umodzi. Zotsatira zanu zoyesa mikodzo ku matenda a chifuwa chachikulu sizidzakhalapo ku nkhani ya chisamaliro chanu cha kuchipatala.

Tidzayezanso kachirombo ka HIV. Ngati zotsatirazi zasonyeza kuti muli ndi kachirombo ka HIV tidzayeza kuchuluka kwa tizirombo ta HIV, komanso kukutumizani kolandilira chithandizo chamatendawa. Ngati tingakupezeni kuti muli ndi matenda a chifuwa chachikulu kapena kachirombo ka HIV panthawi ina iliyonse mkati mwa kafukufukuyu, tidzakutumizani kolandilira zithandizo zamatendawa pompano pachipatala.



Patsiku loyamba komanso pa ulendo wa mwezi woyamba, tidzapukuta kumbuyo kwa mkati mwa mphuno mwanu ngati m'mene zikuonekera pachithunzichi kuti titenge tizirombo timene timakhala m'menemo. Tidzayeza tizirombo timeneti kuti tione ngati tikumva mankhwala. Zotsatira zimenezi sizidzagwiritsidwa ntchito kuchisamaliro chanu chaku chipatala.

Pa ulendo wa sabata yoyamba, tidzakupemphani kuti mutiuze m'mene thanzi lanu lasinthira kuyerekeza ndi m'mene munaliri patsiku loyamba. Mafunso amenewa adzawerengedwa kwa inu kudzera pa makina a kompyuta ndipo mudzawayankha pakusankha mayankho angapo amene makinawa adzawonetse panthawi yomwe azidzafunsa.

Ulendo wa pa mwezi umodzi udzakhala wotsiriza umene tidzakupatseninso zotsatira za zoyesa za matenda a chifuwa chachikulu komanso tidzakufunsani ngati muli ndi zizindikiro za matenda a chifuwa chachikulu. Ngati panthawiyi mudzakhale kuti mukulandira Thandizo la HIV kapena TB, tidzakufuna kudziwa kuti zikuyenda bwanji. Kukumana ndi inu patatha mwezi umodzi ndikofunikira kwambiri chifukwa zidzakuthandizirani kuti mudziwe zotsatira za zoyeza za matenda a chifuwa chachikulu ndipo zidzatithandiziranso kudziwa zam'mene thanzi lanu liliri.

Maulendo a kuchipatala amene mudzayende a kafukufukuyu ndiwosachepera atatu. Pamenepa tikuwerenga tsiku loyamba, ulendo umodzi pakutha pa sabata imodzi, ndi ulendo umodzi pa mwezi umodzi. Ngati simunakwanitse kubwera kuno pa ulendo wina uliwonse tidzakukumbutsani pokuyimbirani lamya kapena tidzagwiritsa ntchito chilorezo ndi uthenga umene mudzatipatse kuti tikuyendereni kunyumba kwanu. Patsiku loyamba tidzakhala nanu kwa mphindi makumi asanu ndi imodzi, pamene paasiku ena onse, tidzakhala nanu kwa mphindi makumi atatu.

Kodi padzakhala ziopsezo zina zilizonse zochitika mu kafukufukuyu?

Kupanga nawo kafukufukuyu sikuika moyo wanu pa chiopsyezo chochuluka. Palibe chiopsezo pa kupereka makhololo kapena mikozo mu kafukufukuyu. Mukhoza kusamva bwino panthawi yopukuta kumbuyo kwa mphuno komanso panthawi yotenga magazi oyeza za kachirombo ka HIV ndi kuchuluka kwa tizirombo toyambitsa matendawa. Azithromycin ndi amoxicillin ndi mankhwala oti akhala akugwiritsidwa ntchito kwa nthawi yayitali m'Malawi ndipo sikweni-kweni kuyambitsa mavuto. Patali-patali azithromycin amapangitsa kumva nthumazi, ziwengo, komanso kusokonekera kwa kagwiridwe ntchito ka mtima. Patali-patali amoxicillin amapangitsa kusakhazikika mmanganizo, kumva chizungulire, komanso kutuluka ziwengo munthu akakhala padzuwa.

A London School of Hygiene ndi Tropical Medicine ali ndi thumba landalama zachipukuta misozi lokhudzana ndi kafukufukuyu. Ngati mwapweteka kapena kuvulala chifukwa chotenga nawo mbali mu kafukufukuyu, mudzakhale omasuka kupempha chipukuta misonzi.

Kodi padzakhala zopindula zina zilizonse mu kafukufukuyu?

Chopindulitsa chodziwika cha kafukufukuyu ndi chakuti mudzakhala ndi mwayi oyezedwa matenda a chifuwa chachikulu mozama kuposa m'mene zimakhalira nthawi zonse. Zimenezi zidzakuthandizirani kudziwa ngati muli ndi matenda a chifuwa chachikulu komanso kukhala ndi mwayi oyamba kulandira thandizo la mankhwala a chifuwa chachikulu. Kafukufukuyu ndi opindindulitsanso kwa opereka chisamaliro cha kuchipatala chifukwa adzayankha mafunso ofunikira okhudzana ndi kagwiritsidwe ntchito ka mankhwala opha tizirombo toyambitsa matenda panthawi ya ndondomeko yoyeza matenda a chifuwa chachikulu.

Kodi zotsatira za mukafukufukuyu zidzakhala za chinsinsi?

Chizindikiritso chanu mu kafukufukuyu chidzatengedwa kukhala cha chinsinsi. Zotsatira za kafukufukuyu, zikhoza kudzasindikizidwa ndi cholinga cha sayansi koma dzina lanu kapena chizindikiritso chilichonse chokhudzana ndi inu chidzabisidwa. Uthenga okhudza zotsatira zoyesa matenda achifuwa chachikulu kapena HIV zidzalembedwa pogwiritsa ntchito nambala yanu yakafukufuku. Komabe, zina zomwe mungatifotokozere zitha kudzagwiritsidwa ntchito ndi amene ali oyang'anira za kafukufuku wa zaumoyo (COMREC) komanso LSHTM. kapena ndi mamembala a gulu la kafukufukuyu. Zolembedwazi zidzasungidwa mumalo otsekedwa bwino ku sukulu ya ukachenjede ya Malawi College of Medicine. Uthenga ndi zoyesa zotengedwa mu kafukufukuyu zidzassungidwa kwa zaka pafupi-fupi khumi (10) pakutha pakuyesaku, malingana ndi ndondomeko ya bungwe lathu. Zoyesa zotengedwazi ndi mauthenga ena zikhoza kugwiritsidwanso ntchito pa kafukufuku wamtsogolo ngati mutatipatsa chilolezo chimenecho.

Kodi ndikhoza kusiya kafukufukuyu nthawi ina iliyonse ndipo zimenezi zingadzakhudze thandizo langa la mankhwala?

Muli ndi ufulu kusankha kutenga nawo mbali kapena kusatenga nawo mbali mu kafukufukuyu. Ngakhale tingakonde kuti mutenge nawo mbali mu kafukufukuyu mpaka ku mapeto, kutuluka nthawi iliyonse mukafukufuku ndi chisankho chanu popanda chilango chilli chonse kapena kuluza kulandira thandizo lililonse lomwe mukuyenera kulandira. Munthawi yakafukufukuyu, tidzakudziwitsani patati

patuluka mauthenga ena a sayansi ofotokoza zinthu zimene zingakupangitseni kuti mulingalirenso zachisamkho chanu chotenga nawo mbali.

Kodi pali phindu la ndalama lotani pakutenga nawo mbali mu kafukufukuyu?

Sipadzakhala kupatsidwa malipiro chifukwa chotenga nawo mbali mukafukufukuyu. Ndalama yomwe tidzakupatseni ndi yokwana MK8,000. Ndalamayi tizikupatsani pangonopango pamasiku anu akafukufuku..

Kodi kafukufukuyu ndiwovomerezeka ndi komiti yowona za ufulu wa anthu mukafukufuku?

Kafukufukuyu wavomerezedwa ndi London School of Hygiene & Tropical Medicine Research Ethics Committee, ndi College of Medicine Research Ethics Committee (COMREC).

Kodi mungafunse ndani ngati muli ndi mafunso okhudzana ndi kafukufukuyu?

Ngati muli ndi mafunso ena aliwonse okhudza kutenga nawo mbali mukafukufukuyu, chonde khalani omasuka kundifunsa. Munjira ina, mukhoza kulumikizana ndi anthu otsatirawa pa lamya kapena polemba kalata kumakeyala awa:

	Name **Dzina**	**Telephone** **Lamya**	**Postal address** **Adilesi**
Study investigators Akulu-akulu akafukufuku	Dr Titus Divala	0999478376	Helse Nord Tuberculosis Initiative University of Malawi College of Medicine
	Dr Marriott Nliwasa	0888681948	Private Bag 360, Chichiri, Blantyre 3, Malawi
COMREC			
	Administrative officer, COMREC Secretariat	01 877 245 01 877 291	University of Malawi College of Medicine Private Bag 360, Chichiri, Blantyre 3, Malawi

Approved by
College of Medicine
21-May-2019
(COMREC)
Research and Ethics Committee

Kodi pali phindu lotani komanso zotsatira zosayembekezereka zotani pogwiritsa ntchito mankhwala opha tizirombo toyambitsa matenda ngati njira yothana ndi matenda a chifuwa chachikulu mu anthu amene ali ndi chifuwa?

Chitsimikizo cha odwala

Lembani mubokosi liri kumanjali mawu oyamba adzina lanu kapena dindani ndi chala ngati mukuvomereza

Mfundo yachitsimikizo	
Ndikutsimikiza kuti ndawerenga chikalata cha uthenga wa kafukufuku amene watchulidwa m'mwambamu. Ndakhala ndi mwayi woganizira za uthengawu, kufunsa mafunso komanso ndayankhidwa mokhutira. KAPENA Ndafotokozeredwa uthengawu ndi akafukufuku mu chilankhulo chimene ndikuchimvetsa. Ndakhala ndi mwayi woganizira za uthengawu, kufunsa mafunso komanso ndayankhidwa mokhutira.	
Ndikumvetsa kuti kutenga nawo mbali kwanga ndikosakakamizidwa ndipo ndili ndi ufulu kusiya panthawi ina iliyonse popanda kupereka chifukwa china chilichonse, popanda kukhudza chisamaliro cha kuchipatala kapena ufulu wanga.	
Ndikumvetsa kuti magawo ofunikira a zolembedwa zanga za ku chipatala komanso mu kafukufukuyu kuwonedwa ndi anthu ovomerezeka aku LSHTM, University of Malawi College of Medicine komanso COMREC, pamene kuli kofunika kutenga nawo mbali mukafukufukuyu. Ndikupereka chilolezo kwa anthu amenewa kuti athe kuwona za zolembedwa zanga.	
Ndikumvetsa kuti zomwe atolere akafukufuku zokhudza ine zikhoza kugawilidwa kwa anthu ena opanga kakafukufuku, ndipo kuti sipadzakhala chizindikiro chilichonse chosonyeza kuti zinachokera kwa ine.	
Ndikumvetsa kuti zoyeza za mthupi mwanga zimene zidzatengedwe kwa ine zidzagwiritsidwa ntchito kuthandizira kafukufuku wina mtsogolo, ndipo zikhoza kudzagawidwa mwachinsinsi ndi akafukufuku ena, pa ntchito yawo yovomerezeka ndi malamulo aowona zakafukufuku.	
Ndikuvomereza kutenga nawo mbali mu kafukufuku amene watchulidwa pamwambayu.	

Dzina la wotenga nawo mbali **Sayini/chidindo cha chala cha wotenga mbali** **Tsiku**

Dzina la mboni yopanda mbali* **Sayini ya mboni yopanda mbali** **Tsiku**

Ndikutsimikiza kuti ndafotokoza za uthenga wa kafukufukuyu molondola kwa _____, ndipo zinamveka monga mwakudziwa kwanga ndi, wotenga nawo mbali komanso kuti apereka chilolezo chawo kuti atenge nawo mbali* pamaso pa mboni yopanda mbali imene yatchulidwa pamwambapa (ngati kuli koyenera).

Dzina la wotenga chilolezo **Sayini ya wotenga chilolezo** **Tsiku**

*Mboni yopanda mbali ikuyenekera kukhala yokhulupiridwa ndi munthu ofuna kutenga nawo mukafukufukuyo. Mboni ikhoza kulemba dzina la munthu ofuna kutenga nawo mukafukufukuyo koma siingasayine mmalo mwake. Munthu ofuna kutenga nawo mukafukufuku, ngati samatha kuwerenga ndi kulemba, asayine ndi chidindo cha chala chake ndipo mboni isayine dzina ndi sayini, pamalo ambone.

Mpatseni otenga nawo mbali chikalata chimodzi kuti chikhale chake

Dzina la kafukufuku: Kodi kugwiritsa ntchito mankhwala opha tizirombo toyambitsa matenda ena ngati njira yothandizira kufufuza chifuwa chachikulu kuli ndi phindu kapena kuipa kotani?

8.5 Letters of support for the randomised trial

8.5.1 Letter of support from the National Tuberculosis Program

Prior to applying for funding, I presented the research question and study design to the Malawi National Tuberculosis program (NTP) to ensure that it is addressing an important evidence gap. The NTP reviewed, provided feedback, and the following letter of support.

In reply please quote No: NTP/003/.....

Programme Manager
National TB Control Programme
Lilongwe
Telephone:
(+265) 1 756828
(+265) 1 750089

Fax: (+265) 1 757782

Communications should be addressed to:
The Programme Manager
National TB Control Programme

Ministry of Health
National TB Control Programme
Community Health Sciences Unit
P/Bag 65
Lilongwe

30th January 2017

To: TO WHOM IT MAY CONCERN

RE: LETTER OF SUPPORT FROM THE NTP FOR TITUS DIVALA TO APPLY FOR A NEW RESEARCH PROJECT TO CONDUCT A STUDY ON A "TRIAL-OF-(NON-TB) ANTIBIOTICS" TO EXCLUDE TB AS A CAUSE OF PROLONGED COUGH IN PRIMARY CARE ATTENDEES:

The broad objective of the project is to investigate the effectiveness, safety, and broader impact of a trial-of-antibiotics to "rule out" tuberculosis (TB) among symptomatic adults greater than 16 years.

This approach is International Policy, a ubiquitous component of National TB Programme (NTP) diagnostic algorithms including Malawi NTP, and widely practiced at global level. However, there is minimal evidence-base on accuracy, and no previous research on broader clinical outcomes and antimicrobialresistance (AMR).

The purpose of this letter is to confirm that the National TB Control Programme highly supports Titus Divala in his project research application.

We look forward to getting updates on the progress of this important research project application.

[redacted]
Dr. James Mpunga

NTP PROGRAMME MANAGER

8.5.2 Letter of support from the Blantyre District Health office

After acquiring funding, I presented the trial design to the Blantyre District Health Office under whose authority the research sites belong to. The District Health Management team provided feedback (which I incorporated into the protocol) and a letter of support (below). Next I presented the study to staff of Limbe and Ndirande Health centres. They provided additional feedback including supporting the design of patient flow and linkage to existing services such as tuberculosis registers, XPERT /MTB/RIF testing, and HIV testing.

Telephone: Blantyre 0 1875332 / 01 877 401
Fax: 01 875 430 / 01 872 551/01878 539

Communication should be addressed to:
The Director of Health and Social Services
0882002533 :gkawalazira@yahoo.co.uk

UNITY AND FREEDOM

In reply please quote No. ……..

DISTRICT HEALTH OFFICE
P/BAG 66
BLANTYRE
MALAWI

Ref No: BT DHO/MED/9 **5th April, 2018**

The Chairman,
COMREC,
Private Bag 360,
BLANTYRE

Dear Sir,

RE: LETTER OF SUPPORT FOR A STUDY TITLED: "RANDOMIZED CONTROLLED TRIAL INVESTIGATING IF BENEFITS OF USING RESPONSE TO BROAD SPECTRUM ANTIBIOTICS AS AN EXCLUSION DIAGNOSTIC FOR TUBERCULOSIS IN PRIMARY CARE ADULT PATIENTS OUTWEIGH THE RISK OF ANTIMICROBIAL RESISTANCE".

Blantyre District Health Office renders its support to the above study which is to be conducted by Dr. Titus Divala as part of his PhD studies.

We look forward to the findings from this study and hope the findings will help in policy direction.

Yours Sincerely ,

[redacted]

District Health Officer
5 APR 2018
Private Bag 66
Blantyre

Dr. Gift Kawalazira
DIRECTOR OF HEALTH AND SOCIAL SERVICES

8.6 Ethical approvals for the randomised trial

The trial was reviewed and approved by the University of Malawi College of Medicine Research and Ethics Committee, the LSHTM Research Ethics Committee, and Regional Committee for Health and Research Ethics, NTNU-Midt, Norway. The following are certificates of approval.

UNIVERSITY OF MALAWI

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.04/18/2381 - Accurancy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study) by Titus H Divala

On 03-Jul-18

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC as indicated on the next page

[redacted] 03-Jul-18

Dr. YB. Mlombe - Chairperson (COMREC) Date

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Dr Titus Divala
LSHTM

9 May 2018

Dear Titus ,

Study Title: RCT investigating if benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis in primary care adult patients outweigh the risk of antimicrobial resistance

LSHTM ethics ref: 15232

Thank you for your application for the above research, which has now been considered by the Interventions Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Safety Information	ACT_PackageInsertAzithromycin	14/03/2013	JUNE 2013
Safety Information	ACT_PackageInsertAmoxicillin	21/12/2015	DEC 2015
Sponsor Letter	2018-KEP-077_Sponsor Confirmation_13.03.18	13/03/2018	1
Other	GCP Cert_LSHTM_TDivala_21.03.18	21/03/2018	1
Investigator CV	ACT-CV1_TitusDivala	30/03/2018	1
Investigator CV	ACT-CV2_KatherineFielding	30/03/2018	1
Investigator CV	ACT-CV3_LizCorbett	30/03/2018	1
Information Sheet	ACT-20180330InformedConsentEnglish	30/03/2018	1.0
Information Sheet	ACT-20180330InformedConsentChichewa	30/03/2018	1.0
Protocol / Proposal	ACT-20180330Protocol	30/03/2018	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord			06.11.2018	2018/1964/REK nord
			Deres dato:	Deres referanse:
			25.09.2018	

Vår referanse må oppgis ved alle henvendelser

Jon Øyvind Odland
Institutt for samfunnsmedisin

2018/1964 En kontrollert klinisk studie for å undersøke responsen til bredspekret antibiotika som en eksklusjonsdiagnose i allmenpraksis mot risiko for antibiotika resistens

Forskningsansvarlig institusjon: UiT Norges arktiske universitet
Prosjektleder: Jon Øyvind Odland

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK nord) i møtet 25.10.2018. Vurderingen er gjort med hjemmel i helseforskningsloven (hforsknl) § 10.

Prosjektleders prosjektomtale
Det skal undersøkes, ved hjelp av en randomisert, kontrollert studie om respons til bredspekret antibiotika kan brukes som eksklusjonsdiagnose for tuberkulose. Dette skal vurderes opp mot risiko for resistensutvikling knyttet til behandingen. Tre grupper (625 per gruppe) individuelt randomisert (1:1:1), med åpen informasjon undersøkes med standard diagnostisk tilnærming. Kun voksne deltakere, med full brukermedvirkning og informert samtykke. Studien kan forenkle dignostikk og klinikk for en utsatt gruppe mennesker. Studien skal i sin helhet foregå i Malawi. Alle etiske godkjenninger fra Malawi og London er vedlagt.

Komiteen vurderte at Hanne Husom Haukland var inhabil og hun fratrådte møtet da saken ble behandlet, jf. fvl. § 6.

Om prosjektet
Prosjektet er en del av en PhD. Studenten skal avlegge sin PhD i London.

Dette er et samarbeidsprosjekt mellom Universitetet i Malawi, London School of Hygiene and Tropical Medicine, og Universitetet i Tromsø.

Prosjektet skal gjennomføres i Malawi og er et «Tuberkulose initiativ» fra Helse Nord.

Det foreligger godkjenning fra London School of Hygiene and Tropical Medicine, og University of Malawi.

Data
Det skal hentes data fra pasientjournal og fra lokale poliklinikker. Data er standard medisinske opplysninger knyttet til infeksjoner og sykehistorie.

Nye helseopplysninger er respons på behandling av infeksjoner.

Besøksadresse: MH-bygget UiT Norges arktiske universitet 9037 Tromsø
Telefon: 77646140
E-post: rek-nord@asp.uit.no
Web: http://helseforskning.etikkom.no/

Data avidentifiseres med koblingsnøkkel. Database oppbevares i 5 år i Malawi.

Deltakere/rekruttering
Voksne deltakere fordeles i tre grupper voksne deltakere (625 per gruppe), individuelt randomisert. Deltakere rekrutteres gjennom behandling på poliklinikkene.

REK har ingen innvendinger til prosjektet

Vedtak
REK har gjort en helhetlig forskningsetisk vurdering av alle prosjektets sider og godkjenner det med hjemmel i helseforskningsloven § 10. REK forutsetter at prosjektet også har godkjenning fra Malawi.

Sluttmelding og søknad om prosjektendring
Prosjektleder skal sende sluttmelding til REK nord på eget skjema senest 21.03.2022, jf. hfl. § 12. Prosjektleder skal sende søknad om prosjektendring til REK nord dersom det skal gjøres vesentlige endringer i forhold til de opplysninger som er gitt i søknaden, jf. hfl. § 11.

Klageadgang
Du kan klage på komiteens vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes til REK nord. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK nord, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Kopi til:jon.oyvind.odland@uit.no

8.7 Regulatory approval for the randomised trial

The Malawi Pharmacy and Medicines Regulatory Authority (formerly Pharmacy, Medicines, and Poisons Board) reviewed and issued a no-objection letter paving way for the implementation of the trial.

PHARMACY, MEDICINES & POISONS BOARD

Mission: To promote and improve the health of the population of Malawi through the regulation of Pharmacy Personnel, Pharmacy Businesses, Medicines and Allied Substances

ALL CORRESPONDENCE SHOULD BE ADDRESSED TO THE REGISTRAR

Head Office:
Off Paul Kagame/
Chilambula Road
P.O.Box 30241
Capital City
LILONGWE 3, MALAWI

Phone: (+265) 01 755 165
Fax : (+265) 01 755 204
Email: info@pmpb.mw
Web: www.pmpb.mw

PMPB/CTRC/III/14062018102
DATE: 4th July, 2018

Department of Infectious Disease Epidemiology
London School of Hygiene and Tropical Medicine
Keppel St
London

Attn.: Dr. Titus Divala

RE: ACCURACY AND CONSEQUENCES OF USING TRIAL-OF-ANTIBIOTICS FOR TB DIAGNOSIS (ACT-TB STUDY).

Refer to your application to register the above mentioned clinical trial with the Pharmacy, Medicines and Poisons Board (PMPB).

The Clinical Trial Review Committee (CTRC), at its meeting held on 22nd June, 2018, issued a **No Objection** to the implementation of the trial after members agreed that the nature of the trial was seen to be outside the scope of trials that should be regulated by PMPB through the CTRC.

Please contact the undersigned if there are any issues that need further clarification.

Yours faithfully,

[redacted]

M. Kawaye
ACTING REGISTRAR

PHARMACY MEDICINES & POISONS BOARD
REGISTRAR
04 JUL 2018
P.O. BOX 30241, LILONGWE 3

8.8 Data management plan for the randomised trial

Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)

Data Management Plan

Version V1.0, 27 June 2019

1 Approvals

Author	Name	Titus Divala	Signature		Date
	Role	Chief Investigator			
Reviewer	Name	Lingstone Chiume	Signature		Date
	Role	Data Manager			
Reviewer	Name	Katherine L Fielding	Signature		Date
	Role	Co-Investigator			
Reviewer	Name	Elizabeth L Corbett	Signature		Date
	Role	Co-Investigator			

2 Table of contents

3 Abbreviations

CRF	Case report form
DM	Data Manager
DMP	data management plan
LSHTM	London School of Hygiene & Tropical Medicine
MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
SOP	Standard Operating Procedure
OMR	Optical Mark Recognition
OCR	Optical Character Recognition
ODK	Open Data Kit
DMP	data management plan

4 Purpose and scope

4.1 Objectives and Scope of the Data Management Plan

The ACT-TB Study Data Management Plan (DMP) describes the activities and methods used to collect, validate, and process data obtained by the study in line with Good Clinical Data Management Practices. This plan also describes study-specific data management roles and responsibilities, data flow, and timelines, serving as a guideline and reference document for persons involved in all study-related data management processes.

4.2 Version Control

The Investigators and the Data Manager will on a regular basis review and update this DMP over the lifetime of the study, making sure it reflects the current required processes and procedures at all time. To ensure the correctness of the DMP, any updates in the study affecting data management must be communicated to the study Data Manager as soon as possible.

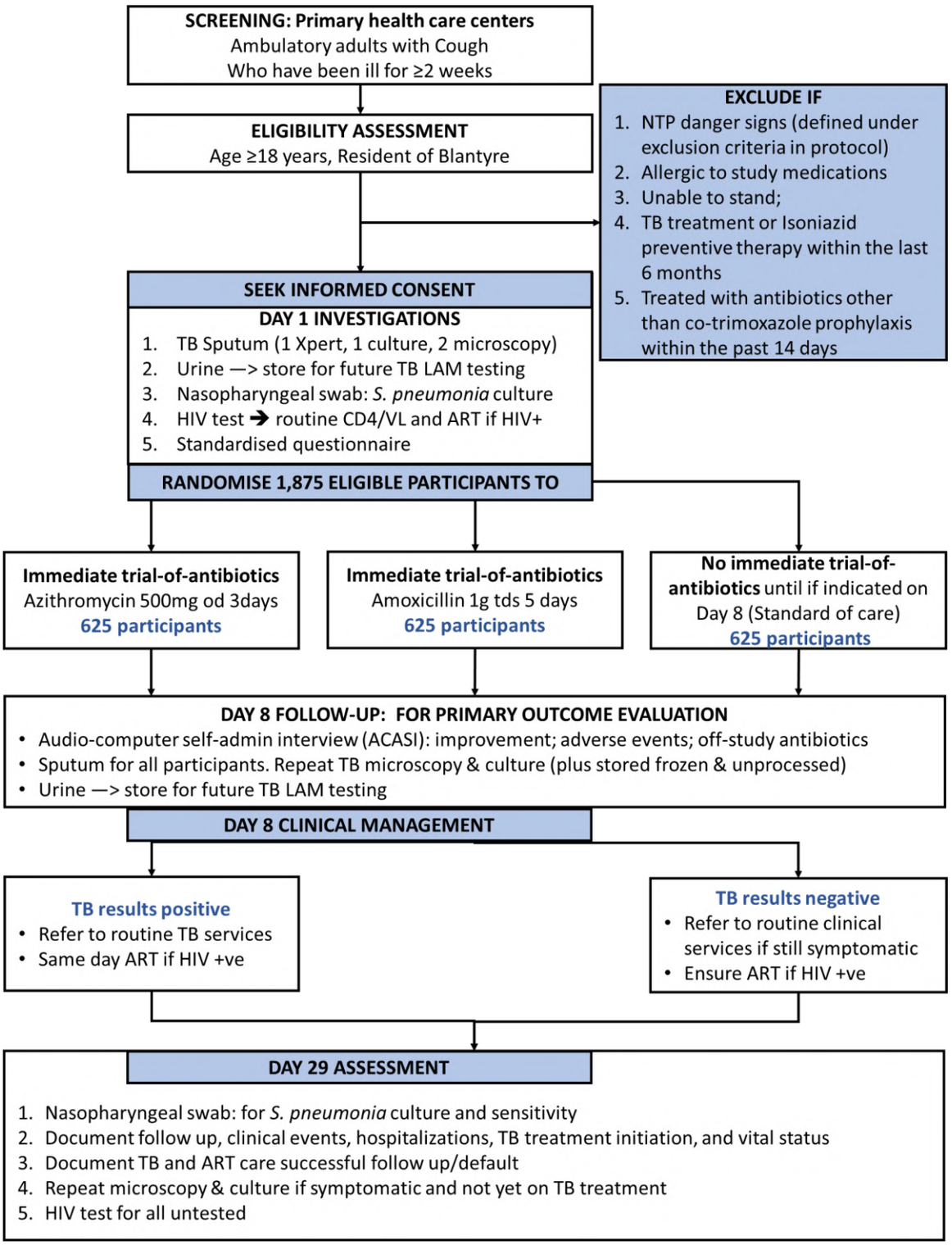
5 Study Description and Timetable

5.1 Study Description

Title	Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)
Design	Three-arm (625 per arm) individually randomised (1:1:1), open-label controlled clinical trial investigating standard care diagnostic approach for tuberculosis. The trial will not use any unlicensed products.
Objective	**Outcomes**
Primary	
1. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary	Proportion of participants correctly classified as PTB negative based on report of improvement

tuberculosis (PTB) in adults with cough at primary care level in Malawi.	of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among participants submitting at least one sputum specimen
2. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.	Proportion of participants experiencing at least one of the following adverse outcomes by Day 29: 1) death 2) hospitalisation 3) missed TB diagnosis 4) HIV care loss to follow up 5) TB care loss to follow up
Secondary	
3. To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.	Risk of acquiring nasopharyngeal Streptococcus pneumonia isolates resistant to any of the commonly used groups of antimicrobials by Day-29.
4. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with cough at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among all randomised participants, with those who could not provide sputum classified as mycobacteriologically negative.
5. To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.	• Incremental cost per quality adjusted life year gained • Total direct medical costs per participant over 56 days • Eq-5D utility score
Exploratory	
Our exploratory analyses will be comparisons between the azithromycin and amoxicillin arms for all our primary and secondary outcomes.	
Population	Adults presenting to primary care centres in Malawi reporting cough.
	Inclusion criteria: • Ambulatory clinic attendees presenting with cough • Should have been ill for ≥ 14 days

	• Aged at least 18 years • Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period. Exclusion criteria: • Self-reported allergy to study medications • Acute danger signs defined in national TB treatment guidelines • Tuberculosis treatment or isoniazid preventive therapy in the last 6 months • Treated with antibiotics, other than co-trimoxazole prophylaxis, for the current illness or within the past 14 days
Treatment	**Arm 1**: Azithromycin 500mg once daily for 3 days commencing on randomization day. **Arm 2**: Amoxicillin 1 g 3 times daily for 5 days commencing on randomization day. **Arm 3**: Standard of care in current national guidelines for patients presenting with cough and without danger signs (No treatment until re-evaluation with sputum TB test results)
Duration	We will give treatments on the randomisation day (Day-1) and perform follow up activities on days 8, and 29.



Flow diagram for the planned clinical trial

5.2 Data Management SYSTEMs

ACT-TB Study data will be collected using TeleForm and Open Data Kit systems (ODK). TeleForm is a data capture system that utilises optical character recognition to extract data from specially designed paper forms into a database to reduce data entry and manual processes associated with paper-based forms. ODK is a suite of tools that allows data collection using mobile devices and data submission to an online server, even without an internet connection or mobile carrier service at the time of data collection. In our case we will use android tablets for data collection, mobile carrier for data transmission, and the data hub at Malawi-Liverpool Wellcome Trust (MLW) for online server. We will use ODK for all forms, TeleForm use will be limited to screening form, laboratory forms, and all forms for collecting clinical endpoints. TeleForm use in these forms is to ensure future availability of a paper backup for clinical data.

5.3 Timelines

Activity/ Deliverable	Planned Date
Protocol Approved v2.0	June 2018
Database Development Complete	20 Feb 2019
First Participant In	25 Feb 2019
Last Participant In	30 Mar 2020
Last Participant Out	30 Apr 2020
Last Participant last data available from site	15 May 2020
Clean file (all data submitted; all queries resolved)	20 May 2020
Final Database Lock	30 May 2020

5.4 Study Identification Numbers

5.4.1 Site Codes:

There will be 2 study sites:

Limbe Health Centre, Blantyre, Malawi, assigned site code is 1.

Ndirande Health Centre, Blantyre, Malawi, assigned site code is 2.

5.4.2 Screening Identification Number (SID):

All patients screened for the ACT-TB study are assigned a unique screening ID number to identify eligibility screening data for those screened but not enrolled. Participant study numbers consist of three key components and take the format:

A 9 B B B B

A= Site number

9= To identify the ID as a screening ID and differentiate from participant ID

BBBB = Unique 4-digit participant number, assigned in chronological order upon screening.

5.4.3 Participant Identification Number (PID):

Enrolled ACT-TB Study participants are assigned a unique study number that will be used to identify all participant data. Participant study numbers consist of three key components and take the format:

A-B B B B-C

A= Site number

BBBB = Unique 4-digit participant number, assigned in chronological order upon determining eligibility and enrolment into the study.

C = Check digit.

The participant identifier is assigned at the time of Enrolment (completion of ACT01). Only the participant ID is used when capturing the data into the system to fully identify the participant in the ACT-TB study.

6 Data Management deliverables

6.1 Data Life Cycle

Task	Person Responsible	Details
CRF Development and Sign-off	Chief Investigator, Co-Investigators, and Data Manager	Preparation of all study data collection tools (CRFs, logs, SOPs, checklists, etc.) with input from the study team.
Database Development	Data Manager	From the final CRFs in Teleform and ODK, the Data Manager develops database, tables and code lists
CRF Completion/ Scanning	Study physician, Study Nurse, Research Assistant, Data officer	Completed CRFs will be checked by Investigators, Study physician or Site coordinator, and data officer. The data officer will scan checked CRFs, and verify them and address errors, in the TeleForm software.
Validation and Querying	Data Manager, Site Coordinators, Chief investigator	The Data Manager will ensure that all query systems are working correctly. Data queries will be run weekly by the Data Manager at the MLW data hub, including checking the same data queries as at the site, and further ad hoc queries. These will be fed back to the local sites for resolution. The Chief Investigator will on a monthly basis receive blinded data and run queries to make sure that there is no outstanding problem.
Database Lock and Final Analysis	Data Manager/ Chief Investigator	After last participant out, when all data is entered, and queries resolved the database is locked for entry and prepared for final analysis and archiving. The database cannot be unlocked after locking.
Data Archiving	Data Manager/ Chief Investigator	Once the Data Manager and Study Investigators have no further questions, paper records will be sent to LSHTM/MLW for archiving. Electronic records will be archived on the MLW/LSHTM servers

6.2 Schedule of CRFs

		Format
	Visit Day 1	
1	ACT-01 Screening Form	TeleForm
2	ACT-02 Baseline Characteristics Form	ODK
3	ACT-03 Sputum TB Form	TeleForm
4	ACT-04 TB Laboratory form for Urine LAM test	TeleForm
5	ACT-05 MLW Lab Form for Nasopharyngeal Swab Microbiology	TeleForm
6	ACT-06 Socioeconomic status (SES) Form	ODK
7	ACT-07 Quality of Life Assessment Form	ODK
8	ACT-08 Participant Locator Form	ODK
9	ACT-09 Randomization Documentation Form	ODK
10	ACT-10 Screening and Enrolment Log	ODK
	Visit Day 8	
1	ACT-11 Audio Computer Assisted Interview (ACASI) Form	ODK
2	ACT-12 Day 8 Clinical Assessment Form	ODK
3	ACT-16 Brief Chest X-ray Report Form	TeleForm
	Visit Day 29	
1	ACT-07 Quality of Life Assessment Form	ODK
2	ACT-13 Adverse events log	ODK
3	ACT-14 Concomitant medications log	ODK
4	ACT-18 Day 29 Clinical Assessment Form	ODK
5	ACT-16 Brief Chest X-ray Report Form	TeleForm
7	ACT-19 ART Adherence Form	TeleForm
8	ACT-20 TB Treatment Adherence Form	TeleForm
9	ACT-21 Study Exit Form	TeleForm

6.3 Case Report Forms

CRFs will be drafted by the Chief Investigator based on the approved study protocol. Co-Investigators and site study staff review CRF drafts. The Data Officer will prepare CRFs for ODK and TeleForm systems, and the Data Manager will review, finalise and deploys them for use. The CRFs will be reviewed and checked by the Chief Investigator and site staff before finalisation.

6.4 Database Development

After the CRFs have been agreed upon and created in TeleForm and in ODK systems the Data Manager with assistance of Data officer and MLW database team, will compile data variables, code lists and system queries as specified by the study team during the CRF development process. Once the CRFs are finalized in TeleForm and ODK, the database and tables (one table per CRF) will be created (this is an automated process as part of the ODK and TeleForm systems) and exported to Microsoft SQL Server. Once the system goes live, any changes to questions, answers, validation, etc. must be reviewed and approved by the Data Manager in discussion with the study team.

Access to the database for data entry, query resolution, and reporting will be controlled by the Data Manager. All changes to the database in the process of query resolution will be marked with ID of operator and date/time to create an audit trail within SQL Server. The database will be stored in computers at the MLW data hub, a secure office which is always locked when not in use. The databases will be encrypted, and password protected. The only people with access will be the Data Manager, and the Chief Investigator. All patient identifiable information will be removed before extraction of the databases and uploading to the secure cloud storage (for sharing with Statisticians and other investigators).

The data manager will provide data entry and CRF training to the study staff with duties related to data collection and management. In-depth training for Data officer in all data systems will include data entry, CRFs, scanning, verification, committing data to database, data synchronisation with the data hub, running data queries and resolving queries and generating study reports.

6.5 Data upload and backup

TeleForm CRFs will be collected from study sites and scanned once every week while ODK data is uploaded to the MLW data hub, via mobile data, at the end of each working day. The study database at the MLW data hub will also be backed up on the MLW servers. All databases will be encrypted, and password protected.

6.6 Post-Data Entry Validation (Queries)

The data officer in consultation with the data manager will run the following data checks for each patient (identified by screening ID, and later by PID) on the study database once weekly:

General (All CRFs**)**	1. All required fields should not have missing values. 2. Date checks (valid date, no future dates, dates within expected ranges e.g. during follow-up period) 3. Range checks (e.g. for laboratory or clinical values) 4. Expected CRFs for each visit should be completed and submitted within expected period 5. Cross-CRF checks of data fields that are repeated on more than 1 CRF.
Patient eligibility (ACT01-Screening Form**)**	1. A05 If date of birth OR A06 age gives age as <18 years at enrolment, A08 and A26, eligibility check must be answered NO 2. All questions A08-A16 should be complete 3. If any inclusion criteria (A07 to A12) are NO, A26 eligibility check must be answered NO 4. If any exclusion criteria (A13 to A16 and A21) are YES, A26 eligibility check must be answered NO

	5. If informed consent question (A22) is NO, A26 eligibility check must be answered NO 6. A26 Eligibility check- if YES, **A07 to A12 and A22** must all be answered YES and **A13 to A16 and A21** must be answered NO 7. A26 Eligibility check- if NO, one of **A07 to A12 and A22** must be answered NO OR one of **A13 to A16 and A21** must be answered YES 8. A27 must bear PID barcode sticker if A26 is answered YES.
Visit Day 1 CRFs	**ACT03-Sputum sample Form** 1. C03 should have a PID barcode 2. C04 and C09 should be complete to properly identify the study visit 3. C27, C28 and C29 should be complete for an adequate result report **ACT04-Urine LAM Form** 1. D01 should have a PID barcode 2. D03 and D16 should be complete to properly identify the study visit 3. D14 should be complete for an adequate result report **ACT05- Nasopharyngeal Swab Form** 1. E01 should have a PID barcode 2. E03 and E04 should be complete to properly identify the study visit 3. E11 and E12 should be complete for confirmation of sample storage **ODK CRFs** 1. ACT02, ACT06, ACT07, ACT08, and ACT09 should be uploaded within 24 hours of enrolment 2. There should be no missing or incomplete PID or other required data (variable, value) in CRFs ACT02, ACT06, ACT07, ACT08, and ACT09
Visit Day 8 CRFs	**ACT11- ACASI Form** 1. ACT 11 should be uploaded by 8 days from upload date of ACT09 2. K01 should have a PID 3. K08 and K09 should be complete to document clinical improvement **ACT 12 Day 8 Clinical assessment form** 1. ACT 11 should be uploaded within 8 days of uploading ACT09 2. There should be no missing or incomplete PID or other required data (variable, value)
Visit Day 29 CRFs	**ODK CRFs** 1. ACT07, ACT13, ACT14, and ACT18 should be uploaded within 28 days of uploading ACT09 2. There should be no missing or incomplete PID or other required data (variable, value) in CRFs ACT07, ACT13, ACT14, and ACT18 3. Participant who has completed Day 29 visit should have two sets of ACT07 (one at Day 1 and the other at Day 29) **ACT-19 ART Adherence Form** 1. U01 should have a PID barcode 2. U03 and U04 should be complete to properly identify the study visit 3. U10 must be complete to confirm that patient is a registered ART client 4. U21 and U23 must be completed as an indicator question for treatment adherence 5. U24 must be completed as an indicator question for duration of adherence/nonadherence

	ACT-20 TB Treatment Adherence Form 1. V01 should have a PID barcode 2. V03 and U04 should be complete to properly identify the study visit 3. U08 must be complete to confirm that patient is a registered TB treatment client 4. V20 and V22 must be completed as an indicator question for treatment adherence 5. V23 must be completed as an indicator question for duration of adherence/nonadherence **ACT21- Study Exit Form** 1. W01 should have a PID barcode 2. W03 and W04 should be complete to properly identify the study visit 3. W05 should be complete for documentation of exit reason 4. W22 should be complete to document study physician or PI review

The data queries will be exported to an excel based data query form (sorted by study staff member who completed the CRF containing the query and PID) and printed for completion. The query will highlight, in addition to the staff member completing the CRF, the date and time query run, the CRF number, the data field being queried and the nature of the query. The staff member (or site coordinator if staff member is on leave) will be responsible for addressing the query- they will document the resolution on the generated query form and the CRF by indicating the correct value for the data field being queried, and return to the data officer. The Data officer will update the database with the data query resolution. The expected timeline for resolution of data queries is 72 hours, and queries not resolved in this time will be reported to the Data Manager and highlighted to the Chief Investigator.

Further queries may also arise as part of the cleaning and analysis process from the Data Manager and/or study Investigators/Statisticians. The Data Manager will liaise with the trial Site Coordinators and Data officer to ensure resolutions of these queries. The Chief Investigator and Data Manager will work closely to define and anticipate potential areas for data error and manage them proactively in order to prevent delays in the final study reporting.

6.7 Database Documentation

The Data Manager prepares database documentation at the following time points:

1. Pre go-live for study team review and approval.
2. Go-live for initial study documentation.
3. For any mid-study update (e.g. database version change)
4. At study close-out for documentation.

It is the study team's responsibility to review this document thoroughly to ensure that all protocol requirements are met.

6.8 Weekly study status report

Study status reports will be generated weekly by the data officer in consultation with the Data Manager. The study status report will be based on data uploaded up to the end of the previous week. The reports will be generated from the study database, and the output will be exported to an excel file. Requests for *ad hoc* reports are submitted to the Data Manager

with a description of the priorities for the proposed report. The report (excel file) will contain the following data by study site:

Report	Description
Screening	Total number of participants screened since study start (*based on a count of screening IDs from ACT01 CRFs*)
	Number of participants screened per study week
Enrolment	Total number of participants randomised since study start (*based on a count of randomisation numbers from ACT09 CRFs*)
	Number of participants randomised per study week
Participant retention	Number of participants who, based on randomisation date, are expected to have reached Day 8 (*PIDs and randomisation date identified from all received ACT09 CRFs, expectation of completing Day 8 based on randomisation date plus 7 days*).
	• Total number out of the expected participants who turned up for Day 8 (*based on PIDs **with** ACT 11 CRF in the database)*
	• List of PIDs of those who are yet to attend visit Day 8 (*based on PIDs **without** ACT 11 CRF in the database)*
	Number of participants who based on enrolment date are expected to have reached Day 29. (*PIDs and randomisation date identified from all received ACT09 CRFs, expectation of completing Day 8 based on randomisation date plus 28 days*).
	• Total number out of the expected participants who turned up for Day 28 (*based on PIDs **with** ACT 18 CRF in the database)*
	• List of PIDs of those who are yet to attend visit Day 29 (*based on PIDs **without** ACT 18 CRF in the database)*
Study endpoint data	Total number of deaths recorded since study start (*Response to W05 on ACT21*)
	Total number of SAEs recorded since study start (*Response to M12 on ACT13*)
	Total number of mycobacteriologically confirmed TB cases (GeneXpert or smear microscopy or culture positive) recorded since study start (*Response to C25 t0 C27 on ACT03*)
	Total number of participants on TB treatment recorded since study start (*Response to V05 on ACT20*)
Data queries	List of PIDs with missing forms
	List of PIDs with other unresolved queries

6.9 Clean data status

Clean data status is declared individually for each participant, completed or early withdrawals, via a separate field within the database. The Data Manager is responsible for moving participants to clean data status, expected to be within 14 days after exiting the study, but no later than 30 days from the participant's final visit date.

7 Quality ASSURANCE

Quality assurance procedures are in place at several levels. All CRFs will be checked by either the study physician or site coordinator and the data officer prior to scanning and verification. Any errors will be resolved immediately by the staff member who completed the CRF. The Chief Investigator will conduct query-driven data monitoring/verification once a month.

The Clinical Trial external monitor (Research Support Centre Trials Unit, University of Malawi-College of Medicine) will make scheduled visits to each site on 6-monthly basis to review data quality. Each monitoring visit will be documented with regards to errors found/corrected and any operational issues that arise as part of the visit.

8 Serious Adverse Events

Serious Adverse Events for this study will be documented and reported on a 6-monthly basis to ethics committees, the Data and Safety Monitoring Board and the trial steering committee.

9 Protocol Deviations

All protocol deviations will be documented and reported on the protocol deviation logs.

10 Access to study data

10.1 Access prior to clean file

The Data Manager has the responsibility of granting access to the study database (password protected and encrypted) and will restrict access to the study database during the study. Any data required can be generated by the Data Manager as an ad hoc report if given enough notice, and if it does not contain the study arm allocation or participant identifiable information.

10.2 Locking of final study database

The final study database is locked to changes after all patient records have been declared ‘clean’. Once the database has been locked, no data can be changed.

11 Extraction of study data

The Data Manager will on a bimonthly basis extract a dataset (excluding study arm, or locator/patient identifiable information) for Chief Investigator access. In addition, *ad hoc* extractions can be performed at the request of the study team for presentations, papers, etc. The study team must provide one weeks’ notice prior to *ad hoc* extraction to ensure that any outstanding queries are resolved prior. Any *ad hoc* extractions should be understood by those analysing it to be ‘dirty’ data, i.e. not to the clean standard of a data freeze or lock. Any ad hoc extraction must not contain the study arm allocation.

12 Training and Support

All personnel involved in data management for the study will receive appropriate training prior to receiving access to the study database. Training will be provided by the Data Manager. CRF training will be provided by the Chief Investigator, study physician, or site coordinators with input from the Data Manager.

13 Archiving

13.1 Documents

All data management documents are archived according to sponsor/funder and GCP recommendations. Participant CRFs will be archived in individual participant files. Participant files should be kept in numeric order and by CRF code order within each participant file.

13.2 Final Study Database

The final study database will be stored as software datasets on the MLW and LSHTM servers and burned to disk for hardcopy storage.

13.3 Programs

Study specific programs (i.e. STATA .do files) are also stored on the MLW and LSHTM servers.

14 Communication

14.1 Methods of communication

The Data manager, data officer and Chief investigator will communicate regularly throughout the study. There will be twice monthly data management meetings as part of the weekly study meeting.

15 Data sharing and reuse

We will not provide access to data beyond study team membership prior to publication. Upon publication the subset of the data required for the purposes of verifying research findings will be available for sharing, and will be placed in the institutional research data repository established by LSHTM Research Data Management Support Service. This repository enables directly download of records with full annotation enabling use and replication of the analyses.

Fuller sharing of all data with any group requesting access to individual records will be ensured within 12 months of completion of my thesis analyses, with all data and study tools made available by that time through the LSHTM Institutional Research Data Repository.

Anonymised data will be held for sharing as original databases stored with a soft copy of the fully annotated questionnaires and the STATA files used for recoding and analysis. Personal identifiers, such as names, will not be held, with ID numbers used instead.

16 References

- ACT-TB Study Protocol v3.0
- ACT-TB Study Standard Operating Procedures

17 Acknowledgements

This data management plan, along with a number of data collection tools, was developed based on that of the STAMP Trial.

8.9 Data collection tools for the randomised trial

A01 SID	Screening ID	PLACE BARCODE HERE	**A02 IID** Interviewer ID	
A03 DOI	Date of interview	d d - m o n - y y y y		
A04 SEX	Gender	☐ Male (1) ☐ Female (2)		
A05 DOB	Date of birth	d d - m o n - y y y y	*Leave DOB blank if not known; Est. age below.*	
A06 AGE	Age (in years)		*Only complete if DOB is not known.*	

INCLUSION CRITERIA

A07 AGEINC	Is the participant's age 18 years or older?	☐ Yes (1) ☐ No (2)
A08 UNWELL	Have you been unwell for at least 14 days? *M'masabata awiri apitawa mumamva bwanji mthupi? Munali ndi zizindikiro zilizonse za kudwala?*	☐ Yes (1) ☐ No (2)
A09 COUGH	Do you have a cough? ***Response should be YES only if the cough is either the main or among the key reasons for coming to the clinic today.***	☐ Yes (1) ☐ No (2)
A10 WALK	Are you able to walk on your own?	☐ Yes (1) ☐ No (2)
A11 RES	Do you live within Blantyre?	☐ Yes (1) ☐ No (2)
A12 RES2	Will you be able to return to the clinic to attend 2 more visits in the next 4 weeks?	☐ Yes (1) ☐ No (2)

EXCLUSION CRITERIA

A13 TBRX	Were you taking treatment for TB at any point in the last 6 months? *By TB treatment I mean registered at one of the TB clinics and receiving 6-8 months of treatment with a TB card?*	☐ Yes (1) ☐ No (2)
A14 IPT	Were you taking Isoniazid Preventive Therapy (IPT) at any point in the last 6 months?	☐ Yes (1) ☐ No (2)
A15 ABRX	Did you take any antibiotics in the last 14 days? **By this, I am referring to all other antibiotics except daily bactrim prophylaxis given given as part of HIV care package.**	☐ Yes (1) ☐ No (2)
A16 ALRGY	Have you ever had any allergic reaction after taking either azithromycin or amoxicillin or has any clinical staff ever told you not to tka ethese drugs?	☐ Yes (1) ☐ No (2)

If YES to ALL inclusion criteria, and NO to ALL exclusion criteria, proceed to A17. If the patient is not eligible, thenk them for their time and complete Screening Log.

A28 IDI Staff ID/Initials:

COLLECT VITAL SIGNS ACCORDING TO STUDY SOP X

A17 BP Blood pressure ___ / ___ **mmHg**

A18 PR Pulse rate ___ **beats/minute**

A19 RR Respiratory rate ___ **cycles/minute**

A20 TEMP Temperature ___ . ___ **Degrees Celcius**

A21 DNGR Has any of the following?

	Yes (1)	No (2)
Respiratory rate > 30.min	☐	☐
Temperature > 39°C	☐	☐
Heart rate > 120/minute	☐	☐
Systolic blood pressure < 90mmHg	☐	☐
Confused/agitated	☐	☐
Respiratory distress	☐	☐

If YES to ALL inclusion criteria, and NO to ALL exclusion criteria, and NO to ALL danger signs, conduct informed consent process.
If the patient is not eligible, them for their time and complete Screening Log.

OUTCOME OF INFORMED CONSENT PROCESS

A22 ICF Did the patient consent to enrolment?
☐ Yes (1)
☐ No (2)

A23 NOCON If no, reason for not consenting
☐ Too busy (1)
☐ needs permission from influential other (2)
☐ Already in another study (3)
☐ Not interested (4)
☐ Study staff unable to complete consent process (5)
☐ No reason provided (6)
☐ Other (9) ***If Other, please specify:***

A24 SUSE Did patient consent to future use of specimens?
☐ Yes (1)
☐ No (2)

A25 DATAS Did patient consent to data sharing?
☐ Yes (1)
☐ No (2)

SCREENING CONCLUSION

A26 ELIG Eligible to participate in study?
(***To be eligible, ALL inclusion criteria should be answered "YES", and all exlusion criteria should be answered "NO".***)
☐ Yes (1)
☐ No (2)

If the patient is not eligible, thank them for their time, and refer them to the clinic awaiting room so that they can continue with routine care team.
Complete the Screening Log and Enrollment Log.

If participant is eligible and consented, assign Participant ID. Place barcode below.

A27 PID Participant ID

PLACE BARCODE HERE

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to the ACT02A-TB study (Baseline Characteristics form) . Please swipe forward to continue.
b01apid	B01. Scan the participant ID:
b0test	Did the barcode scan successfully?
b01cpid	Enter the PID as it appears on the label
b02iid	B02. Staff ID
b03doi	B03. Date of interview
visit	Study Visit
sympto	Ask the participant if they have any of the following symptoms
b04fever	B04. Do you have fever or hot body?
b05sweat	B05. Do you have excessive sweat at night to the extent of drenching your clothes or beddings?
b06chestp	B06. Do you have pain in the chest?
sympto	
b07acghdur	B07a. How long have you been coughing?
b07sput	B07b. Are you coughing up sputum?
b08bsp	B08. Does your sputum have blood in it?
b09wtloss	B09. Have you lost weight unintentionally?
tbhist	TB History
b10tbdx	B10. Have you ever been given a TB registration card and treated for TB?
b11ntbdx	B11. How many times have you had TB and given a new TB registration card?
b12itbrx	B12. When was the last time you took TB treatment?
b13dtbrx	B13. How long did you receive treatment for TB on your most recent episode?
tbhist	
preghist	Pregnancy history
b14preg	B14. If female, are you pregnant?
preghist	
hivhist	HIV Testing, Counselling and treatment
b15evrtest	B15. Have you ever been tested for HIV?
b16yrtest	B16. Have you been tested for HIV in the last 12 months?
b17phivt	B17. The last time you tested for HIV, what was the result?

b18poshiv	B18. Have ever had a positive HIV test?
b19curart	B19. Are you currently taking ART drugs?
b20evart	B20. Have you ever taken ART drugs?
b21doart	B21. When did you start taking ART?
b22artreg	B22. Which ART medication are you currently taking?
artreothr	If taking other ART medication, please specify
note5	If HIV positive and not on ART, refer participant to ART initiation. Give them a copy of test results and a note about VL sample collection.
hivhist	
anthro	Anthropometric measurements
wt	Weight
b25ht	B25. Height
anthro	
samples	Have you ever collected each of these samples and completed respective laboratory forms?
b26asamples1	B26A. Sputum
sputreas	If No sputum, provide reason
b26bsamples 2	B26B. Nasopharygeal swab
nasoreas	If No NPS, provide reason
b26csamples3	B26C. Urine
urinreas	If No urine, provide reason
samples	
note8	This is the end of the first part of the Baseline Characteristics form

name	label
today	
starttime	starttime
endtime	endtime
b01bpid	B01B. Scan the participant ID:
b0test	Did the barcode scan successfully?
b01dpid	Enter the PID as it appears on the label
b02biid	B02B. Staff ID
b03bdoi	B03B. Date of interview
visit	Study Visit
b27needtest	B27. Does the participant need to be offered an HIV test?
rest	
b28testreas	B28. Reason for HIV test
b29testyes	B29. Staff: Has the participant accepted HIV test?
b30testresul	B30. Staff: What is the HIV test result?
samples2	
b31cd4samp	B31. Have you collected CD4 sample and completed respective
b31breason	If No CD4 sample, provide reason
b32vlsamp	B32. Have you collected viral load sample and completed respective laboratory forms?
b32breason	If No viral load sample, provide reason
samples2	
rest	
note	This is the end of the second and last part of the Baseline

ACT03 - ACT-TB Study Sputum

Microbiology Request Form

C01 Name of study: A C T - T B

C02 Study Site:

C03 Patient Study ID: PLACE BARCODE OR WRITE HERE

C04 Visit:
- ☐ Day 1 (1)
- ☐ Day 8 (2)
- ☐ Day 28 (3)

Client Details

C06 Age:

C07 Gender:
- ☐ Male (1)
- ☐ Female (2)

C08 Specimen Type:
- ☐ Smear Microscopy (1)
- ☐ MTB Culture (2)
- ☐ GeneXpert (3)

C09 Date Collected: DD - MON - 2 0 2 YYYY

C10 On Tb Drugs:
- ☐ Yes (1)
- ☐ No (2)

C11 *If yes--give weeks*

Lab to fill this section

C12 Lab No.: - -

C13 Date Specimen Recieved: DD - MON - 2 0 2 YYYY

C14 Lab # Barcode: PLACE BARCODE OR WRITE HERE

Microscopy & Culture

C15 Auramine direct:
- ☐ Sca (1)
- ☐ Neg (2)
- ☐ 1+ (3)
- ☐ 2+ (4)
- ☐ 3+ (5)
- ☐ ND (6)

C16 Concentrate:
- ☐ Sca (1)
- ☐ Neg (2)
- ☐ 1+ (3)
- ☐ 2+ (4)
- ☐ 3+ (5)
- ☐ ND (6)

C17 Check ZN:
- ☐ Pos (1)
- ☐ Neg (2)
- ☐ ND (3)

C18 Culture:
- ☐ MGIT (1)
- ☐ LJ (2)
- ☐ TLA (3)
- ☐ None (4)
- ☐ Other (9)

C19 Wk

C20 Result:
- ☐ +ve (1)
- ☐ -ve (2)
- ☐ NA (3)

C21 Wk

C22 Result:
- ☐ +ve (1)
- ☐ -ve (2)
- ☐ NA (3)

C23 Final Result:
- ☐ Positive (1)
- ☐ Scanty (2)
- ☐ Negative (3)
- ☐ Cont. (4)
- ☐ ND (5)

Identification

C24 MPT64Ag:
- ☐ Pos (1)
- ☐ Neg (2)
- ☐ ND (3)

C25 Cording:
- ☐ Pos (1)
- ☐ Neg (2)
- ☐ ND (3)

C26 PNB:
- ☐ PNB (1)
- ☐ Temp (2)
- ☐ Growth (3)
- ☐ No Growth (4)
- ☐ ND (5)

C31 ID:
- ☐ MTB (1)
- ☐ Non TB mycobateria (2)
- ☐ Unidentified ZN +ve (3)
- ☐ ND (4)

C32 Genotype DST:
- ☐ Hain (1)
- ☐ GXP (2)
- ☐ Other (3)
- ☐ ND (4)

C33 Culture DST:
- ☐ MGIT (1)
- ☐ LJ (2)
- ☐ Other (3)
- ☐ ND (4)

Final Report

C27 Smear:
- ☐ Pos (1)
- ☐ Sca (2)
- ☐ Neg (3)
- ☐ ND (4)

C28 Xpert:
- ☐ Pos (1)
- ☐ Neg (2)
- ☐ ND (3)
- ☐ Invalid (4)
- ☐ Error (5)

C29 Culture:
- ☐ Pos (1)
- ☐ Sca (2)
- ☐ Neg (3)
- ☐ Cont. (4)
- ☐ ND (5)

C30 Colonies (if scanty)

	Result	*Comments*
C35 Rif:		
C36 INH:		
C37 EMB:		
C38 PZA:		

C34 Interpretation:

C39 Interpretation:

C40 Date reported: DD - MON - 2 0 2 YYYY

C41 Tech. Sign & Code:

C42

PART A: To be filled by research assistants *(Complete separate form for each sample)*

D01 PID Participant ID — PLACE BARCODE HERE

D02 IID Staff ID ☐☐

D03 DOI Date urine sample collected ☐☐ - ☐☐☐ - ☐☐☐☐ (d d - m o n - y y y y)

D16 VIS Study visit
- ☐ Day 1 (1)
- ☐ Day 8 (2)

D04 BCODE Paste TB Lab barcode on sample container and here: SAMPLE BARCODE HERE

SAMPLE TRANSPORTATION

D05 TRANS Sample packed in cooler box to send to research lab?
- ☐ Yes (1)
- ☐ No (2)

PART B: Sample Reception by Laboratory Staff

D06 RCPT URINE specimen received?
- ☐ Yes (1)
- ☐ No (2)

D07 VOL Volume of URINE sample received ☐☐ mls

D08 STORE URINE samples stored?
- ☐ Yes (1)
- ☐ No (2)

D09 DOP Date URINE sample was processed for storage ☐☐ - ☐☐☐ - ☐☐☐☐ (d d - m o n - y y y y)

URINE sample locations

D10 LOC1 Vial 1 ☐☐☐ Box No. ☐☐☐ Position

D11 LOC2 Vial 1 ☐☐☐ Box No. ☐☐☐ Position

PART C: Sample Analysis

D12 DOC Date URINE sample was tested ☐☐ - ☐☐☐ - ☐☐☐☐ (d d - m o n - y y y y)

D13 BRAND Brand of urine LAM test
- ☐ Brand name 1 (1)
- ☐ Brand name 2 (2)

D14 LAMRS Urine LAM result
- ☐ Negative (1)
- ☐ Grade 1 (2)
- ☐ Grade 2 (3)
- ☐ Grade 3 (4)
- ☐ Grade 4 (5)
- ☐ Grade 5 (6)

D15 NOTDN If URINE LAMnot done, what was the reason?
- ☐ No test strips available (1)
- ☐ Not enough urine (2)
- ☐ Other (9) **If other, please specify below:**

PART A: To be filled by research assistants *(Complete separate form for each sample)*

E01 PID Participant ID — PLACE BARCODE HERE

E02 IID Staff ID

E03 DOI Date nasopharyngeal sample collected — d d - m o n - y y y y

E04 VIS Study visit
- ☐ Day 1 (1)
- ☐ Day 29 (3)

E05 LIMS Paste Lab Number on sample container and here: — SAMPLE BARCODE HERE

SAMPLE TRANSPORTATION

E06 TRANS Sample added to STTG tube, and packed in cooler box for sending to MLW lab?
- ☐ Yes (1)
- ☐ No (2)

PART B: Sample Reception by Laboratory Staff

E07 RCPT NASOPHATYNGEAL specimen received?
- ☐ Yes (1)
- ☐ No (2)

E08 DOREC Date specimen was received — d d - m o n - y y y y

E09 STORE NASOPHARYNGEAL samples stored?
- ☐ Yes (1)
- ☐ No (2)

E10 DOP Date NASOPHARYNGEAL sample was processed for storage — d d - m o n - y y y y

NASOPHARYNGEAL sample locations

E11 LOC1 Vial 1 — Box No. / Position

E12 LOC2 Vial 1 — Box No. / Position

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to ACT-05B MLW lab Form for Nasopharyngeal Swab Microbiology. Please swipe forward to continue.
e01sid	E01. Sample ID
e00	Did the barcode scan successfully?
e01bsid	E01. Sample ID
e02iid	E.Staff ID
e03b01	E03. Date sample processed
e04b02growth	E04. Day 2 record of growth
e05b03growth	E05. Day 3 record of growth
e06boptochin	E06. If E05b=1, what is the day 3 record of disc diffusion results for optiochin
e06boptochin_conf	Please confirm day 3 record of disc diffusion results for optiochin
e06diff	Day 3 record of disc diffusion results for optiochin and the confirmation entered are not the same,Please go back and enter it again
e07bamoxycilin	E07.Day 4 record of Disc diffusion results for AMOXYCILIN (2 ug) (mm)
e07bamoxycilin_cor	Please confirm Day 4 record of Disc diffusion results for AMOXYCILIN (2 ug) (mm)
e07diff	Day 4 record of disc diffusion results for AMOXYCILIN and the confirmation entered are not the same,Please go back and enter it again
e08bceftriaxone	E08. Day 4 record of Disc diffusion results for CEFTRIAXONE (30 ug) (mm)
e08bceftriaxone_co	Please confirm Day 4 record of Disc diffusion results for CEFTRIAXONE (30 ug) (mm)
e08diff	Day 4 record of disc diffusion results for CEFTRIAXONE and the confirmation entered are not the same, Please go back and enter it again
e09bazithromycin	E09. Day 4 record of Disc diffusion results for AZITHROMYCIN (15 ug) (mm)

e09bazithromycin_c	Please confirm Day 4 record of Disc diffusion results for AZITHROMYCIN (15 ug) (mm)
e09diff	Day 4 record of disc diffusion results for AZITHROMYCIN and the confirmation entered are not the same, Please go back and enter it again
e10berythromycin	E10. Day 4 record of Disc diffusion results for ERYTHROMYCIN (15 ug) (mm)
e10berythromycin_	Please confirm Day 4 record of Disc diffusion results for ERYTHROMYCIN (15 ug) (mm)
e10diff	Day 4 record of disc diffusion results for ERYTHROMYCIN and the confirmation entered are not the same, Please go back and enter it again
e11bcefoxitin	E11. Day 4 record of Disc diffusion results for CEFOXITIN (2 ug) (mm)
e11bcefoxitin_conf	Please confirm Day 4 record of Disc diffusion results for CEFOXITIN (2 ug) (mm)
e11diff	Day 4 record of disc diffusion results for CEFOXITIN and the confirmation entered are not the same, Please go back and enter it again
e12bcomm	Comment
note2	End of Questions

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to the ACT-TB study (Socioeconomic status (SES) Form) . Please swipe forward to continue.
f01pid	F01. Participant ID
f0test	Did the barcode scan successfully?
f01bpid	F01B. Enter PID as it appears on the label.
f02iid	F02. Staff ID
f03doi	F03. Date of interview
f04visit	F04. Study visit
hhprep	Interviewer to Participant: "Chonde ganizirani za munthu amene ndi mkulu wa khomo lanu. Mutha kukhala inu kapena, munthu wina."
f05hhsex	F05. Mutu wa banja ndi mwamuna kapena mkazi?
f06hhage	F06. Mutu wa banja ali ndi zaka zingati
f07hhedu	F07. Mutu wa banja unapita patali bwanji ndimaphunziro?
f08bed	F08. Kodi mkulu wa khomo lanu amagona poatani?
f09ount	F09. Kodi pa khomo lanu pamakhala anthu angati?
co	
f10power	F10. Kodi muli ndi magetsi amene amagwira ntchito m'nyumba yanu?
f11bank	F11. Kodi inu, panokha kapena ndi munthu wina wa khomo lanu, kapena wakhomo lina, mukusunga ndalama ku banki, ku bungwe lokongoza ndalama, ku bungwe la m'mudzi losungitsa ndi kubweleketsa ndalama (banki m'khonde), kapena ku bungwe la za ndalama lililonse?

f12food	F12. Pa masiku asanu ndi awiri apitawa, munakhalapo ndi nkhawa kuti pakhomo lanu simukhala ndi chakudya chokwanira?
co	
f13cloth	F13. Mokhudzana ndi zovala za anthu a pa khomo lanu, kodi ndi chiti mwa zotsatirazi chomwe ndi chowona?
cd	
f14dvd	F14. Kodi pa khomo lanu muli ndi choimbira chogwiritsa ntchito kaseti (tape) kapena chimbale (CD/DVD).
f15sofa	F15. Kodi pa khomo lanu muli ndi mipando yokhala ndi zotsamilitsilapo manja kapena mipando ya sofa?
f16iron	F16. Kodi pa khomo lanu, muli ndi simbi (aironi) yositira zovala?
cd	
f17rank	F17. Taganizilani kuti pali masitepe asanu ndi limodzi, amene kumayambililo kwake, sitepe yoyamba ikuimilira anthu asaukitsitsa ndipo kumapeto kwake, sitepe ya chisanu ndi chimodzi ikuimilira anthu olemera. (ONETSANI CHINTHUNZI CHA MASITEPEWA). Kodi inu muli pa sitepe iti panopa?
note1	This is the end of Socioeconomic status (SES) Form

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to ACT-07 Quality of life Form. Please swipe forward to continue.
g01pid	G01. Participant ID
g0test	Did the barcode scan successfully?
g01bpid	Enter the PID as it appears on the label
g02iid	G02. Staff ID
g03doi	G03. Tsiku
g04visit	G04. visit
g05eqmob	G05. Mayndedwe: kodi pazonenedwa zotsatirazi, ndi ziti zomwe zikufotokoza bwino za umoyo wanu lero lino?
g06eqsc	G06. Kudzisamalira ndekha (mwachitsanzo, kusamba ndi kudziveka ndekha): Chongani mu gulu lirilonse pansipa, chonde sonyezani mfundo zimene zikufotokoza bwino za umoyo wanu.
g07equsu	G07. Zochitika za tsiku ndi tsiku (monga kugwira ntchito za pakhkomo, za m'banja kapena kuchita zimene zimandisangalatsa): Chongani mu gulu lirilonse pansipa, chonde sonyezani mfundo zimene zikufotokoza bwino za umoyo wanu.
g08eqpain	G08. Ululu / kuphwanya mthupi kosowetsa mtendere: Chongani mu gulu lirilonse pansipa, chonde sonyezani mfundo zimene zikufotokoza za umoyo wanu.
g09eqanx	G09. Nkhawa/kukhumudwa? (Osasangalala): Chongani mu gulu lirilonse pansipa, chonde sonyezani mfundo zimene zikufotokoza bwino za umoyo wanu.

g10eqohs	*G10. Kuti tithandize anthu kunena za umoyo wawo, tajambula mlingo woyesera (chofanandi choyesera kuzizila/kutentha kwa m'thupi) momwe:* *Umoyo wabwino (wokoma/wosangalara) wayerekezedwa ndi chizindikiro cha 100 ndipo umoyo wosakoma wayerekezedwa ndi chizindikiro cha 0.* *Tikufuna mutisonyeze pa mlingowu mmene umoyo wanu uliri lero kuti uli bwino kapena suli bwino mmene inu mukuganizira.* *Lembani mzere kuchokerapa pansipa (umoyo osakoma) kukwera mpaka pa mlingo umene ukuimila kwa mmene umoyo wanu uliri lero.*
note1	This is the end of Quality of life Form

name	label
today	-
starttime	starttime
endtime	endtime
intronote	Welcome to the ACT-TB study. Please swipe forward to continue.
h01pid	Scan the participant ID:
qpid	Did the barcode scan successfully?
h01pid2	Enter the participant ID:
h02iid	Staff ID
h03doi	Date of interview
h04afname	What is your first name?
h04bsname	What is your surname?
h04coname	What other names are you known by?
phon	Phone numbers
h05aphone	What is your phone number?
h05baltph1	What other phone number can we reach you by?
h05caltp1name	Who is the owner of this line?
h05daltph2	What other phone number can we reach you by?
h05ealtp2name	Who is the owner of this line?
h06messg	If we speak to a person other than you, we will tell them the following: "Iam < staff name >, a staff at < clinic name >. I was calling to remind < participant name > of their clinic visit which was planned for < date >. Please inform < participant name > that they should come to the clinic at their earliest convenience."
note1	Household identification
h07plt	What is the name of your plot?
h08hsn	What is the surname that your household is known by?
h09onm	What other names are you known by?
h10phyad	May you describe the physical address of your home. (include important features)
epal	Physical Location
h11epal	May you point the location of your home on this map.
epal	
h12area	Is the participant coming from the ePAL area?
prm	In the event you do not return to the clinic for a study visit, by what means should we contact you?
h13acall	By phone

h13bsms	By phone message
h13chome	By home visit
prm	

name	label
i01pid	Participant ID
i01bpid	Enter the PID as it appears on the label
i02iid	Staff ID
i03doi	Date of interview
i04eligconf1	All inclusion criteria questions are answered Yes
i04eligconf2	All exclusion criteria questions are answered No
i04eligconf3	Participant sign informed consent
i04eligconf4	Participant verbally confirms willing to participate in study
i04eligconf5	Completed baseline questionnaire
i04eligconf6	Sputum sample collection attempted
i04eligconf7	Nasopharygeal sample collection attempted
i04eligconf8	Urine sample collection attempted
i04eligconf9	Socio-economic status form completed
i04eligconf10	Quality of Life Assessment Form completed
i04eligconf11	Locator form completed
i05rand	Open the next available envelope and select the allocation study arm of these options:
i06randon	Randomisation number
i07descr	Have you described the allocated study arm to the participant?
i08dose1	If in arm 2 or arm 3, have you observed the participant take their first dose?
i09instr	Is the participant able to say back to you how they will discharge the expectations of their treatment arm?

name	label
k01pid	[K01] Scan the participant ID:
k01pid2	[K01] Enter the participant ID:
k02iid	[K02] Staff ID:
k03doi	[K03] Date of interview
k04visit	Visit
k05prearm	[K05] Preselect the participant's treatment arm
k06test1a	[K06] Kusiyanitsa ndi mbuzi, kodi ng'ombe ndiyayitali, yotalika chimodzimodzi, kapena ndiyayifupi?
k07test1b	[K07] Kusiyanitsa ndi phala, nsima ndi yofewa, ndiyolimba chimodzimodzi, kapena ndiyolimbirapo?
k08well	[K08] Mu ulendo wanu woyamba, munanena kuti simumamva bwino thupi. Kuyerekeza ndi tsiku limene lija, panopa matenda aonjezekera, sizinasinthe, kapena mukupezako bwino?
k09cough2	[K09] Mutabwera tsiku loyamba kafukufuku lija munanena kuti mumakhosomola. Kuyerekeza ndi tsiku limene lija, panopa kukhosomola kwaonjezekera, sikunasinthe, kapena kwachepa?
k10test2a	[K10] Kodi munabadwa chaka chapitachi?
k11test2b	[K11] Kodi kuno ndi ku chipatala?
k12remainde	[K12] Pamankhwala omwe tinakupatsani aja, alipo omwe atsala?
k13anymiss	[K13] Kodi inuyo liripo tsiku lomwe munadumphitsa kumwa mankhwala kamodzi kapena kuposera apo?
k14miss2dys	[K14] Kodi masiku omwe munadumphitsa kumwa mankhwala akhoza kukwana awiri kapena kuposera apo?

L01 PID Participant ID — PLACE BARCODE HERE

L02 IID Interviewer ID

L03 DOI Date of interview __ __ - __ __ __ - __ __ __ __ (d d - m o n - y y y y)

L04 VISIT Study visit ☒ Day 8(1)

SELF-REPORTED (TO CLINICAL STASFF) IMPROVEMENTS FROM BASELINE SYMPTOMS

Have any of the following symptoms changed since the day you started the study?

	Symptom	Available at baseline?	Any change today?		
L05AA	Cough	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)
L05BA	Blood in sputum	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)
L05CA	Fever	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)
L05DA	Night sweats	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)
L05EA	Weight loss	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)
L05FA	Chest pain	☐ Yes (1) ☐ No (2)	☐ Resolved (1) ☐ Improving (2)	☐ No change (3) ☐ Worse (4)	☐ Much worse (5)

DOCUMENT RESULTS OF, AND COMMUNICATE PREVIOUS LAB RESULTS

STUDY STAFF: *In this section, you will need to gather applicable of the following laboratory results, document them, and communicate results to the participant:*

1. **GeneXpertfrom Health Centre**
2. **GeneXpert Rifampicin resistance**
3. **CD4 COunt**
4. **Viral Load**

L06 XPERT What is the result for Xpert/MTB/RIF test?

☐ Positive (1) ☐ Error (4)
☐ Negative (2) ☐ Result not yet available (5)
☐ Invalid result (3) ☐ Not done (reason) (6)

If not positive (not 1) but done (not 6), skip to L08.

L07 XPRIF Was Rifampicin resistance detected? ☐ Yes (1) ☐ No (2)

L08 VLDN Was HIV viral load done?
☐ Yes (1)
☐ No (2)
If no, give reason and skip to L11.

L08 BRSN Reason if not done:
☐ Not required for this participant (1)
☐ Not available at this facility (2)
☐ Other (3) *Specify in the box*

L09 A Viral load result available?
☐ Yes (1)
☐ No (2)
If no, why?

L09 CVLRS Viral load result (copies/ml): ____ ____ ____ ____ ____ ____ ____

L10 CDT Date of sample collection __ __ - __ __ __ - __ __ __ __ (d d - m o n - y y y y)

L11 CD4DN Was CD4 Count done?
☐ Yes (1)
☐ No (2)
If no, give reason and skip to L13.

L11 BRSN Reason if not done:
☐ Not required for this participant (1)
☐ Not available at this facility (2)
☐ Other (3) *Specify in the box*

L12 A CD4 count result available?
☐ Yes (1)
☐ No (2)
If no, why?

L12 CCDRS CD4 result: ____ ____ ____ ____ ____ cells/mm³

L13 CDT Date of sample collection ☐☐ - ☐☐☐ - ☐☐☐☐
d d m o n y y y y

PROTOCOL PROCEDURES

Conduct the following procedures:

L14 AA *Sputum sample collection*
☐ Done (1)
☐ Not done (2) *If not done, give reason below*

L14 BA *Chest X-Ray*
☐ Done (1)
☐ Not done (2) *If not done, give reason below*

L14 CA *Urine for TB LAM*
☐ Done (1)
☐ Not done (2) *If not done, give reason below*

Check if the participant needs routine care linkage and plan for these illnesses:

		Yes (1)	No (2)
L15 A	TB treatment and follow-up	☐	☐
L15 B	ART Clinic	☐	☐
L15 C	Hypertension Clinic	☐	☐
L15 D	Diabetes Clinic	☐	☐
L15 E	Other chronic illness follow-up	☐	☐

name	label
m01pid	[M01] Participant ID
m01bpid	[M01b] Enter the PID as it appears on the label.
m02iid	[M02] Staff ID
m03doi	[M03] Date of interview
m04visit	[M04] Visit
m05since	[M05] Since study start and apart from the illness you came here with initially, have you experienced any other illness?
m06aeterm	[M06] What illness(es) did you experience?
m07aeonset	[M07] When did it start?
m08aeseverity	[M08] Assessor: What is the severity of the illiness?
m09aerelate	[M09] Assessor: Is this event related to study product? (judgement to be aided by the list of known side-effects of the participant's study treatment)
m10aecause	[M10] Assessor: If Not associated with study treatment, is the event is related to:
m10baecothr	*[M10b] If other please specify*
m11aealtcause	[M11] Assessor: If not associated with study treatment, specify the most likely alternative etiology
m12aesae	[M12] Assessor: Is this event a serious adverse event (SAE)?
m13saetp	[M13] SAE type
m14seatoth	[M14] If other please specify
m15saedt	[M15] SAE onset date
m16saeds	[M16] Description of event
m17review	[M17] Study coordinator or Principal Investigator designee ID
m18doreview	[M18] Date study cordinator or PI reviewed the event
m19outcome	[M19] What is the outcome of the event?
m20resoldate	[M20] Date of outcome status
m21otherae	[M21] Apart from the illness we have discussed, is there another illness you experinced since the beginning of the study?

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to ACT-14 concomintant Medications Form for Day 29. Please swipe forward to continue.
n01pid	N01. Participant ID
n00	Did the barcode scan successfully?
n01bpid	N01b. Enter the PID as it appears on the sticker.
n02iid	N02. Staff ID
n03doi	N03. Date of form completed
would	I would like you to look back from the day you joined the study to this final day and tell me any medication you took apart from the -- (Study medication name) -- we gave you on the first day. Include all tablets, capsules, injections you may have received from this clinic or other government or private clinic. Include any traditional medications you may have received since you started the study.
n05anymed	N05. Since the study start and apart from study medications, have you taken any other medications?
rest	
n06medname	N06. What are the names of all medications, document the first on this form and each of the rest on own fresh forms.
n07medstart	N07. When did you start taking the medication?
n08medtype	N08. Assessor: What type of medication is it?
n08medother	If other, please specify
no9medsource	N09. Where did you receive this medication from?
n10medindic	N10. Why did you take this mdication?
doses	
n11meddose	N11. How much were you taking at once?
n11meddquant	Choose unit
n11cunit	If other, specify unit
doses	
n12medfreg	N12. How often were you (are you) taking this medication (how many times per day/week)?
n12medfothr	If other, please specify
n13medongoing	N13. Are you still taking medication?
n14medend	N14. When was the last time you took the medication?

n15othermeds	N15. Apart from the medication we have discussed, is there another medication you took since the beginning of the study?
rest	
note1	Thi is the end of ACT-14 concomintant Medications Form for Day 29

Q01 PID	Participant ID	PLACE BARCODE HERE	**Q02 IID** Staff ID	
Q03 DOI	Date patient sent for CXR	d d - m o n - y y y y		
Q04 VISIT	Study visit	☐ Day 8 (2) ☐ Day 29 (3)		

PERFORMING CXR: COMPLETED BY RADIOGRAPHER

Q05 RADG	Radiographer initials	
Q06 CONF	Was chest Xray done?	☐ Yes (1) ☐ No (2)
Q07 DOX	Date on film	d d - m o n - y y y y
Q08 CAD4	What is the CAD4TB score?	

REAL-TIME CXR REVIEW BY CLINICIAN

Q09 CLID	Clinician ID / Initials	
Q10 QUAL	CXR quality	☐ Good (1) *If good quality, go to Q12* ☐ Poor (2)
Q11 RPT	CXR Repeated and good quality obtained?	☐ Yes (1) ☐ No (2)
Q12 XRES	Assessment result	☐ X-Ray normal (1) ☐ X-Ray abnormal suggestive of TB (2) ☐ X-Ray abnormal not suggestive of TB (3)

Link participants with abnormal CXR to routine clinical team for management

COMMENT

Q13 COMM	Other comment	

name	label
today	
starttime	starttime
endtime	endtime
intronote	Welcome to ACT-TB study day 29 Clinical Assessment Form. Please swipe forv
swip	
t01pid	[T01] Participant ID
t0test	Did the barcode scan successfully?
t01pid2	[T01] Enter the participant ID:
t02rid	[T02] Staff ID
t03doi	[T03] Date of interview
tb	
Review	Review and communicate any expect previous laboratory results
t04tbres	[T04] Have any pending TB results been communicated to the participants?
t04tbres1	[T04] Have any pending TB results been communicated to the participants?
t04tbres2	[T04] Have any pending TB results been communicated to the participants?
t04tbres3	[T04] Have any pending TB results been communicated to the participants?
tb	
vita	Vital signs
t05temp	[T05] Temperature
t06wt	[T06] Weight
new1	
new	New adverse events and concomintant medications
t07nweas	[T07] Were any diagnoses made on Day 29?
t08nmed	[T08] Were any medications prescribed on Day 29?
new1	
hts	Participant HIV Status
t17evrtst	Have you been tested for HIV since the start of the study?
t18phivt	The time you tested for HIV, what was the result?
t19curart	Have you ever been registered to start ART? By ART I mean medications for HIV infection which one takses for the rest of their life.
hts	
tbdiag	Participant TB diagnosis
t20tbdx	Since the beginning of the study, were you at any point found to have TB and registered for treatment? By TB treatment I mean registered here or another TB clinic and informed that you will receive 6-8 months of treatment with a TB card?
tbdiag	

cond	Protocal procedures
prot	Conduct the following procudures
t09proce	[T09] 1. Chest X-ray
t10proce1	[T10] 2. Nasopharyngeal swab
cond	
check	Routine Care Linkage
link	Check if the participant needs routine care linkage and plan for TB and HIV
t11link1	[T11] TB treatment and follow up
t12link2	[T12] ART clinic
t13link3	[T13] hypertension clinic
t14link4	[T14] Diabetes Clinic
t15link5	[T15] Other chronic illness follow-up
t16link6	[T16] Specify other chronic illnesss
check	
swip	
note1	This is the end of day 29 Clinical Assessment Form

IDENTIFIERS

U01 PID Participant ID — PLACE BARCODE HERE

U02 IID Staff ID

U03 DOI Date of interview — d d - m o n - y y y y

U04 VISIT Study visit — ☒ Day 29 (3)

U08 ARTCONF Confirm that participant was once registered for ART.

	Yes (1)	No (2)
Inspected Health Passport	☐	☐
Inspected ART Clinic Register	☐	☐
Inspected ART Tablets	☐	☐

U09 ARTCLIN At which clini did you register for ART?

- ☐ Limbe (1)
- ☐ Bangwe (2)
- ☐ Ndirande (3)
- ☐ Chilomoni (4)
- ☐ QECH (5)
- ☐ Zingwangwa (6)
- ☐ Other (7)

If other, please specify:

DETAILS OF ART

U10 ARTREG What is your ART registration number

U11 DOART When did you start ART? — d d - m o n - y y y y

U12 ARTTYP What is your ART regimen?(Select the corresponding number from the list)

- ☐ Regimen 0 (ABC+ 3TC + NVP) (1)
- ☐ Regimen 1 (D4T + 3TC + NVP) (2)
- ☐ Regimen 2 (AZT + 3CTC + NVP) (3)
- ☐ Regimen 3 (D4T + 3TC + EFV) (4)
- ☐ Regimen 4 (AZT + 3TC + EFV) (5)
- ☐ Regimen 5 (TDF + 3TC + EFV) (6)
- ☐ Regimen 6 (TDF + 3TC + NVP) (7)
- ☐ Regimen 7 (TDF + 3TC + ATV/r) (8)
- ☐ Regimen 8 (AZT + 3TC + ATV/r) (9)
- ☐ Regimen 9 (ABC + 3TC + LPV/r) (10)
- ☐ Regimen 10 (TDF + 3TC + LPV/r) (11)
- ☐ Regimen 11 (AZT + 3TC + LTV/r) (12)
- ☐ Regimen 12 (DRV + r + RAL) (13)
- ☐ Regimen 13 (TDF + 3TC + DTG) (14)
- ☐ Other (99)

If others please specify:

COMPLIANCE WITH ART CLINIC VISIT SCHEDULE

U13 LASTAPPT When did you last attend an ART clinic appointment? ***(Confirm with participant records)*** d d - m o n - y y y y

U14 NXTAPPT When is your next clinic appointment? ***(Confirm with participant records)*** d d - m o n - y y y y

U15 NXTAPPT Will you attend the next ART clinic appointment?
- ☐ Yes (1)
- ☐ No (2)

U16 REFIL When did you last pick up ART medication? ***(Confirm with participant records)*** d d - m o n - y y y y

ESTABLISHING COMPLIANCE WITH ART DOSAGE

The next section of the questionnaire asks about how you took your HIV medications over the las four days. Most people with HIV have many pills to take at different times during the people find it hard to always remember their pills:

- **Some people get busy and forget to carry their pills with them.**
- **Some people find it hard to take their pills according to all the instructions, suc meals," or "on an empty stomach," "every 8 hours," "with plenty of fluids."**
- **Some people decide to skip doses to avoid side effects or to just not be taking pill**

We need to understand how people with TB are really doing with their pills. Please tell us what you are actually doing. Don't worry about telling us that you don't take all your pills. We need to know what is really happening, not what you think we "want to hear."

The next section of the questionnaire asks about the ART that you may have missed taking over the last four days. Please complete the following table by filling in the boxes below.

If you took only a portion of a dose on one or more of these days, please report the dose(s) as being missed.

U17 1DCOMP How many doses did you miss yesterday? ☐ **doses**

U18 2DCOMP How many doses did you miss day before yesterday (2 days ago)? ☐ **doses**

U19 3DCOMP How many doses did you miss 3 days ago? ☐ **doses**

U20 4DCOMP How many doses did you miss 4 days ago? ☐ **doses**

U21 COMP4D During the past 4 days, on how many days have you missed taking all your doses?
- ☐ None (1)
- ☐ One day (2)
- ☐ Two days (3)
- ☐ Three days (4)
- ☐ Four days (5)

U22 DOSGEADH Most anti-HIV medications need to be taken on a schedule, such as "2 times a day" or "3 times a day" or " every 8 hours." How closely did you follow your schedule over the last four days?
- ☐ Never (1)
- ☐ Some of the time (2)
- ☐ About half of the time (3)
- ☐ Most of the time (4)
- ☐ All of the time (5)

U23 WKENDADH Some people find that they forget to take their pills on the weekend days. Did you miss any of your ART last weekend - last Saturday or Sunday?
- ☐ Yes (1)
- ☐ No (2)

U24 LASTMISS When was the last time you missed taking any of your ART?
- ☐ Within the past week (1)
- ☐ 1-2 weeks ago (2)
- ☐ 2 to 4 weeks ago (3)
- ☐ More than 4 weeks ago (4)
- ☐ Never missed ART (5)

IDENTIFIERS

V01 PID Participant ID — PLACE BARCODE HERE

V02 SID Staff ID

V03 DOI Date of interview — d d - m o n - y y y y

V04 VISIT Study visit — ☒ Day 29 (3)

PARTICIPANT TB DIAGNOSIS

V06 TBCONF Assessor: How was the TB treatment status confirmed? (Answer All)

	Yes (1)	No (2)
Inspected TB treatment card	☐	☐
Inspected facility TB register	☐	☐
Inspected TB medication or tablets	☐	☐

V07 TBCLIN At which TB treatment center did you register for treatment? (Confirm with participant records)

☐ Limbe (1) ☐ QECH (5)
☐ Bangwe (2) ☐ Zingwangwa (6)
☐ Ndirande (3) ☐ Other (99)
☐ Chilomoni (4)

If other please specify

DETAILS OF TB TREATMENT

V08 TBREG What is your TB registration number (Confirm with participant records)

V09 DOTBRX When did you start taking TB treatment (Confirm with participant records) — d d - m o n - y y y y

V10 TBFOCUS Tuberculosis disease site (Check one)

☐ Pulmonary (1)
☐ Extra-pulmonary (2)

V11 LASTAPPTB When did you last attend a TB clinic appointment? (Confirm with participant redords) — d d - m o n - y y y y

V12 NXTAPPTB When is your next TB clinic appointment? (Confirm with participant records) — d d - m o n - y y y y

V13 ATTNXTTB Will you attend the next TB clinic appointment?

☐ Yes (1)
☐ No (2)

V14 LASTREFILTB When did you last pick up TB medications? (Confirm with participant records) — d d - m o n - y y y y

ESTABLISHING COMPLIANCE WITH TB MEDICATION

The next section of the questionnaire asks about how you took your TB medications over the last four days.

Most people have many pills to take at different times during the day. Many people find it hard to always remember their pills:

- **Some people get busy and forget to carry their pills with them.**
- **Some people find it hard to take their pills according to all the instructions, such as "wit meals," or "on an empty stomach," "every 8 hours," "with plenty of fluids."**
- **Some people decide to skip doses to avoid side effects or to just not be taking pills that day.**

We need to understand how people with TB are really doing with their pills. Please tell us what you are actually doing. Don't worry about telling us that you don't take all your pills. We need to know what is really happening, not what you think we "want to hear."

The next section of the questionnaire asks about the TB medications that you may have missed taking over the last four days. Please complete the following table by filling in the boxes below.

If you took only a portion of a dose on one or more of these days, please report the dose(s) as being missed.

V16	**1DCOMPTB**	How many doses did you miss yesterday	☐ **doses**
V17	**2DCOMPTB**	How many doses did you miss day before yesterday (2 days ago)?	☐ **doses**
V18	**3DCOMPTB**	How many doses did you miss 3 days ago?	☐ **doses**
V19	**4DCOMPTB**	How many doses did you miss 4 days ago?	☐ **doses**
v20	**COMP4DTB**	During the past 4 days, on how many days have you missed taking all your dosese	☐ None (1) ☐ One day (2) ☐ Two days (3) ☐ Three days (4) ☐ Four days (5)
v21	**DOSGEADHTB**	Most TB medications need to be taken on a schedule, such as "2 times a day" or "3 times a day" or " every 8 hours." How closely did you follow your specific schedule over the last four days?	☐ Never (1) ☐ Some of the time (2) ☐ About half of the time (3) ☐ Most of the time (4) ☐ All of the time (5)
V22	**WKENDADHTB**	Some people find that they forget to take their pills on the weekend days. Did you miss any your TB medications last weekend - last Saturday or Sunday?	☐ Yes (1) ☐ No (2)
V23	**LASTMISSTB**	When was the last time you missed taking any of your TB medication?	☐ Within the past week (1) ☐ 1-2 weeks ago (2) ☐ 2 to 4 weeks ago (3) ☐ More than 4 weeks ago (4) ☐ Never missed ART (5)

END OF CRF

IDENTIFIERS

W01 PID Participant ID — PLACE BARCODE HERE

W02 IID Staff ID

W03 DOI Date of interview — d d - m o n - y y y y

W04 FVIS Final visit attended
- ☐ Day 1 (1)
- ☐ Day 8 (2)
- ☐ Day 29 (3)

EXIT INTERVIEW

W05 EXITREAS Reason for leaving the study
- ☐ Death (1)
- ☐ Withdrew from study (2)
- ☐ Lost to follow-up (3)
- ☐ Completed follow-up (4)

DEATH

W06 DOD What was the data of death? — d d - m o n - y y y y

W07 ESTD Is this date an estimate?
- ☐ Yes (1)
- ☐ No (2)

W08 DREP Who reported the dealth (enter the most important source)
- ☐ Spouse (1)
- ☐ Sibling (2)
- ☐ Parent/Caretaker (3)
- ☐ Other household member (4)
- ☐ Neighbour/Friend (5)
- ☐ Healthcare worker (6)
- ☐ TB or other registry (7)
- ☐ Vital registration (8)
- ☐ Other (99)

If other please specify

W09 RXDOD Whas the participant on study treatment at the time of dealth?
- ☐ Yes (1)
- ☐ No (2)

W10 DSAE Has the dealth been recorded as an SAE?
- ☐ Yes (1)
- ☐ No (2)

WITHDREW FROM STUDY

W11 WTHDREAS If withdrawn form study, please give reason why.
- ☐ Not interested (1)
- ☐ Advised by influential other (2)
- ☐ Time consuming (3)
- ☐ Uncomfortable with sample collection (4)
- ☐ Other (99)

If other please specify

LOST TO FOLLOW UP

W12 1TRACE	Fist attempt type	☐ Telephone (1) ☐ Home visit (2)
W13 DO1TRACE	Date	d d - m o n - y y y y
W14 1TRACEREP	Comments:	
W15 2TRACE	Second attempt type	☐ Telephone (1) ☐ Home visit (2)
W16 DO2TRACE	Date	d d - m o n - y y y y
W17 2TRACEREP	Comments:	
W18 3TRACE	Third attempt type	☐ Telephone (1) ☐ Home visit (2)
W19 DO3TRACE	Date	d d - m o n - y y y y
W20 3TRACEREP	Comments:	
W21 LSTALV	Date last known to be alive	d d - m o n - y y y y

ALL STUDY EXIT FORMS MUST BE SIGNED OFF BY STUDY COORDINATOR OR PI

W22 SID	Staff ID	
W23 SIGN	Signature	

8.10 Data and safety monitoring board charter

Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)

Data Safety Monitoring Board (DSMB) charter

1 Introduction

1.1 Trial name

Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)

1.2 Trial registration

The study is registered with clinicaltrials.gov (*registration number pending review*)

1.3 Ethics reference number

ACT-TB study was reviewed and is registered by three ethics committees as follows:

University of Malawi College of Medicine Research and Ethics Committee (COMREC)

- Reference: P.04/18/2381

LSHTM Research Ethics Committee

- Reference: 15232

Regional Committee for Health and Research Ethics, NTNU-Midt, Norway

1.4 Sponsor

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT

1.5 Funder

Helse Nord RHF, Norway

1.6 Trial summary

1.6.1 Study type

ACT-TB study is a three-arm, open-label individually randomised controlled clinical trial.

1.6.2 Background

Antimicrobial resistance (AMR) is a growing public health threat that is in part fuelled by empirical antibiotic usage. Empirical antibiotic use is often motivated by lack of point of care diagnostics a common problem in infectious diseases most of which are life-threatening. Tuberculosis (TB), the leading cause of infectious disease mortality, is one of the life-threatening illnesses without adequate diagnostics. Just over 50% of TB cases reported to WHO annually have confirmed mycobacteriological diagnosis. To complement the diagnostic gap, standard diagnostic algorithms include empirical antibiotic use. The antibiotic course, referred to as "trial-of-antibiotics", given to mycobacteriology-negative but symptomatic adults, is often broad-spectrum aiming to provide treatment for pneumonia. The goal is to treat infectious causes of respiratory symptoms other than TB, effectively performing the role of a "rule-out" diagnostic test for TB.

1.6.3 Problem statement

Approximately 26.5 million antibiotics courses are prescribed in the course of diagnosis of the 5.3 million smear negative TB registrations per annum. Despite this widespread use, there is no randomised controlled trial (RCT) evidence supporting the diagnostic accuracy of antibiotic trials and their impact on AMR. It is also unknown whether this usage of antibiotics can improve clinical outcomes considering that in settings of high HIV prevalence, bacterial infection associated mortality just before and during TB treatment is high.

1.6.4 Objectives

1.6.4.1 Primary

- To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with prolonged cough at primary care level in Malawi.
- To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.

1.6.4.2 Secondary

- To evaluate using nasopharyngeal *Streptococcus pneumonia* carriage, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.
- To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.

1.6.5 Methods

We will conduct a randomised controlled clinical trial recruiting adult patients presenting to primary care centres in Blantyre, Malawi with history of cough for at least 2 weeks. After excluding those with danger signs we will randomise participants to receiving or not receiving trial-of-antibiotics (azithromycin or amoxicillin) from Day-1 to determine diagnostic accuracy (specificity) against mycobacteriology reference standard (smear microscopy, Xpert/MTB/RIF and culture). The second primary outcome (clinical benefit of empirical antibiotics) will be evaluated by comparing the proportion of participants experiencing at least one of the following adverse outcomes by Day 29: death, hospitalisation, missed TB diagnosis, HIV care loss to follow up and TB treatment loss to follow up between arms. For secondary outcomes, we will compare between arms differences in antimicrobial resistance at Day-29 and estimate the incremental cost-effectiveness. To adequately address the trial objectives, we will need 625

participants in each of the three arms (azithromycin, amoxicillin and standard of care), a total sample size of 1875 participants.

1.7 Scope of charter

The purpose of this document is to describe the roles and responsibilities of the independent DSMB for the ACT-TB trial, including the timing of meetings, methods of providing information to and from the DSMB, frequency and format of meetings, statistical issues and relationships with other committees.

2 Roles and responsibilities

2.1 Aims of the DSMB committee

The aim of the DSMB is to protect the safety of study participants, to assist and advise the Chief Investigator (CI: Dr Titus Divala, a PhD student), Co-Investigators (Prof Katherine Fielding, Prof Elizabeth Corbett, Prof Neil French and Dr Derek Sloan), collaborators and the Trial Steering Committee (TSC) so as to protect the validity and credibility of the trial, and monitor the overall conduct of the clinical trial.

2.2 Terms of reference

The DSMB will

1) receive and review the progress and accruing trial data;
2) advise the TSC on the conduct of the trial;
3) review and comment on the statistical analysis plan.

2.3 Specific roles of the DSMB

1) monitor adverse events to determine if there is evidence for harm
2) monitor recruitment figures and losses to follow-up
3) suggest additional data analyses
4) review and comment on the statistical analysis plan
5) advise on protocol modifications relevant to data aspects as suggested by investigators or sponsors (eg to inclusion criteria, trial endpoints, or sample size)

3 Before the trial starts

The DSMB membership will review and provide feedback on the trial protocol, and hold their first meeting before commencement of trial recruitment. The objective of the first meeting is to review and discuss the protocol including adverse event reporting, trial design, and analysis plans with the chief investigator and co-investigators. The first meeting will also involve planning future meetings and reviewing what the contents of the DSMB data report should be (dummy tables in annex 3 and 4).

4 Composition

The members of the DSMB for this trial are:

1) Professor Tim Peto, University of Oxford, Oxford, UK
2) Dr Angela Crook, University College London, UK London, UK

3) Professor Victor Mwapasa, University of Malawi College of Medicine, Blantyre, Malawi

The DSMB membership includes statistical and clinical expertise. Professor Peto will be the chairperson.

5 Preparation of DSMB Reports

This work being his PhD, the CI will prepare the open report and will write the stata code, which will be used for data analysis planned for the closed report. The CI will be blinded, so he will not run the analysis for the closed report. The role will be performed by an independent statistician from the Infectious Disease Epidemiology Department at LSHTM.

6 Relationships

Trial governance and management are described in the protocol.

The DSMB has an advisory role for this trial.

DSMB members will not be paid for their services.

DSMB members are asked to disclose information about any competing interests.

7 Organisation of DSMB meetings

DSMB meetings will be conducted once every six months. The DSMB meetings will be timed in such a way that they precede TSC meetings.

The meetings will be conducted by teleconference.

The meeting will have an open and a closed session:

1) Open session: Introduction and any “open” parts of the report
2) Closed session: DSMB discussion of “closed” parts of the report

Attendance by the study investigational team (primarily the CI) will be restricted to the open session.

The closed session will be for DSMB members and Prof Katherine Fielding (co-investigator and PhD supervisor) who will be unblinded. From time to time, the DSMB may invite others to the closed sessions when such a need arises.

8 Trial documentation and procedures to ensure confidentiality and proper communication

An outline of the intended content of material to be available in open sessions will be prepared and agreed at the first DSMB meeting.

The DSMB will receive the reports at least 1 week before any meetings.

The CI will take minutes of the open session and Prof Fielding or a designee will take minutes for the closed session.

The DSMB members shall destroy their closed reports following each meeting.

The DSMB will report its recommendations in writing to the TSC.

9 Decision making

The DSMB may make recommendations such as:

1) No action necessary, trial should continue as planned
2) Early stopping of a trial arm due, for example, to increased risk of harm, or external evidence

The CI and co-investigators will, ahead of a planned meeting, coordinate with all DSMB members and identify an agreeable meeting date. Two members including the Chair (unless otherwise agreed) can still run a DSMB meeting if one has indicated that they cannot make it at a short notice. If the DSMB is considering recommending major action after such a meeting the DSMB Chair should talk with the absent members as soon after the meeting as possible to check if they agree. If they do not, a further teleconference should be arranged with the full DSMB. If the report is circulated before the meeting, DSMB members who will not be able to attend the meeting may pass comments to the DSMB Chair for consideration during the discussions.

If a member does not attend a meeting, it will be ensured that the member is available for the next meeting. If a member does not attend a second meeting, they will be asked if they wish to remain part of the DSMB. If a member does not attend a third meeting, they will be replaced.

10 Reporting

The DSMB will report their recommendations by email letter to the Trial Steering Committee within 1 week of the meeting. Prof Fielding will combine open and close session minutes of the meeting and send to the DSMB Chair for approval.

11 After the trial

DSMB members will be named and their affiliations listed in trial report, unless they explicitly request otherwise. A brief summary of the timings and conclusions of DSMB meetings may also be included in this report.

12 Annex 1: Data Monitoring Committee members register of their assent

I, **Professor Tim Peto** of University of Oxford, Oxford, UK agree

1) to be on the ACT-TB Trial DSMB committee
2) with the contents of the ACT-TB Trial DSMB Charter
3) to keep ACT-TB Trial DSMB data reports and meeting outputs confidential

Signature: _____ Date: _____

I, **Dr Angela Crook** of University College London, UK London, UK agree

4) to be on the ACT-TB Trial DSMB committee
5) with the contents of the ACT-TB Trial DSMB Charter
6) to keep ACT-TB Trial DSMB data reports and meeting outputs confidential

Signature: _____ Date: _____

I, **Professor Victor Mwapasa** of University of Malawi College of Medicine, Blantyre, Malawi, agree

1) to be on the ACT-TB Trial DSMB committee
2) with the contents of the ACT-TB Trial DSMB Charter
3) to keep ACT-TB Trial DSMB data reports and meeting outputs confidential

Signature: _____ Date: _____

13 Annex 2: Suggested competing interests form

Potential competing interests of Data Safety Monitoring Board members

Possible competing interest(s) should be disclosed.

Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict eg strong prior belief in the trial's intervention arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication

Please complete the following section and return to the Chief Investigator.

☐ **No,** I have no competing interests to declare

☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: **Professor Tim Peto**

Signed: _____ Date: _____

Potential competing interests of Data Safety Monitoring Board members

Possible competing interest(s) should be disclosed.

Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict eg strong prior belief in the trial's intervention arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication

Please complete the following section and return to the Chief Investigator.

☐ **No,** I have no competing interests to declare

☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: **Dr Angela Crook**

Signed: _____ Date: _____

Potential competing interests of Data Safety Monitoring Board members

Possible competing interest(s) should be disclosed.

Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict eg strong prior belief in the trial's intervention arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication

Please complete the following section and return to the Chief Investigator.

☐ **No,** I have no competing interests to declare

☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: **Professor Victor Mwapasa**

Signed: _____ Date: _____

14 Annex 3: Data for open session

All data will not indicate study arm and the following will be presented

- Enrolment and accrual

Study week	Number screened	Target enrolment	Actual enrolment	Accrual

- Participant retention

Study week	Number lost to follow up or withdrawn consent	Reason for loss	Updates to retention strategies

- Participant baseline characteristics

Characteristics	Proportion among the enrolled
Age	
Sex	
HIV Status	

15 Annex 4: Data for closed session

Part 1: Aggregate data presented with statistical comparisons (for determining safety)

- Cumulative participant losses by study arm

Arm 1 (standard of care)	Arm 2	Arm 3	Arm 1vs arm 2 95% CI, p-value	Arm 1 vs arm 3 95% CI, p-value

- Mortality by study arm

Arm 1 (standard of care)	Arm 2	Arm 3	Arm 1vs arm 2 95% CI, p-value	Arm 1 vs arm 3 95% CI, p-value

Part 2: Aggregate data presented without statistical comparisons (for data monitoring)

- Diagnostic performance of trial-of-antibiotics

	True positives	False negatives	False positives	True negatives	Total
Arm 1					
Arm 2					
Arm 3					

- Proportion of participants experiencing composite adverse outcome by Day 29

Outcome	**Arm 1 (standard of care)**		**Arm 2**		**Arm 3**	
	Number	Proportion	Number	Proportion	Number	Proportion
death						
hospitalisation						
missed TB diagnosis						
HIV care loss to follow up						
TB care loss to follow up						
Any one of above						

8.11 Trial steering committee charter

Randomised controlled clinical trial investigating benefits of using response to broad spectrum antibiotics as an exclusion diagnostic for tuberculosis (TB) in primary care adult patients versus risk of antimicrobial resistance (AMR)

Trial Steering Committee Charter

(developed using MRC Clinical Trials Unit template TSC Charter version 1.02, 13-Mar-2006)

1 Introduction

1.1 Trial name

Accuracy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study)

1.2 Trial registration

Clinicaltrials.gov registration number: NCT03545373

1.3 Ethics reference number

ACT-TB study was reviewed and is registered by three ethics committees as follows:

University of Malawi College of Medicine Research and Ethics Committee (COMREC)

- Reference: P.04/18/2381

LSHTM Research Ethics Committee

- Reference: 15232

Regional Committee for Health and Research Ethics, NTNU-Midt, Norway

- Reference: (pending)

1.4 Sponsor

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT

1.5 Funder

Helse Nord RHF, Norway

1.6 Trial summary

1.6.1 Study type

ACT-TB study is a three-arm, open-label individually randomised controlled clinical trial.

1.6.2 Background

Antimicrobial resistance (AMR) is a growing public health threat that is in part fuelled by empirical antibiotic usage. Empirical antibiotic use is often motivated by lack of point of care diagnostics a common problem in infectious diseases most of which are life-threatening. Tuberculosis (TB), the leading cause of infectious disease mortality, is one of the life-threatening illnesses without adequate diagnostics. Just over 50% of TB cases reported to WHO annually have confirmed mycobacteriological diagnosis. To complement the diagnostic gap, standard diagnostic algorithms include empirical antibiotic use. The antibiotic course, referred to as “trial-of-antibiotics”, given to mycobacteriology-negative but symptomatic adults, is often broad-spectrum aiming to provide treatment for pneumonia. The goal is to treat infectious causes of respiratory symptoms other than TB, effectively performing the role of a “rule-out” diagnostic test for TB.

1.6.3 Problem statement

Approximately 26.5 million antibiotics courses are prescribed in the course of diagnosis of the 5.3 million smear negative TB registrations per annum. Despite this widespread use, there is no randomised controlled trial (RCT) evidence supporting the diagnostic accuracy of antibiotic trials and their impact on AMR. It is also unknown whether this usage of antibiotics can improve clinical outcomes considering that in settings of high HIV prevalence, bacterial infection associated mortality just before and during TB treatment is high.

1.6.4 Objectives and outcomes

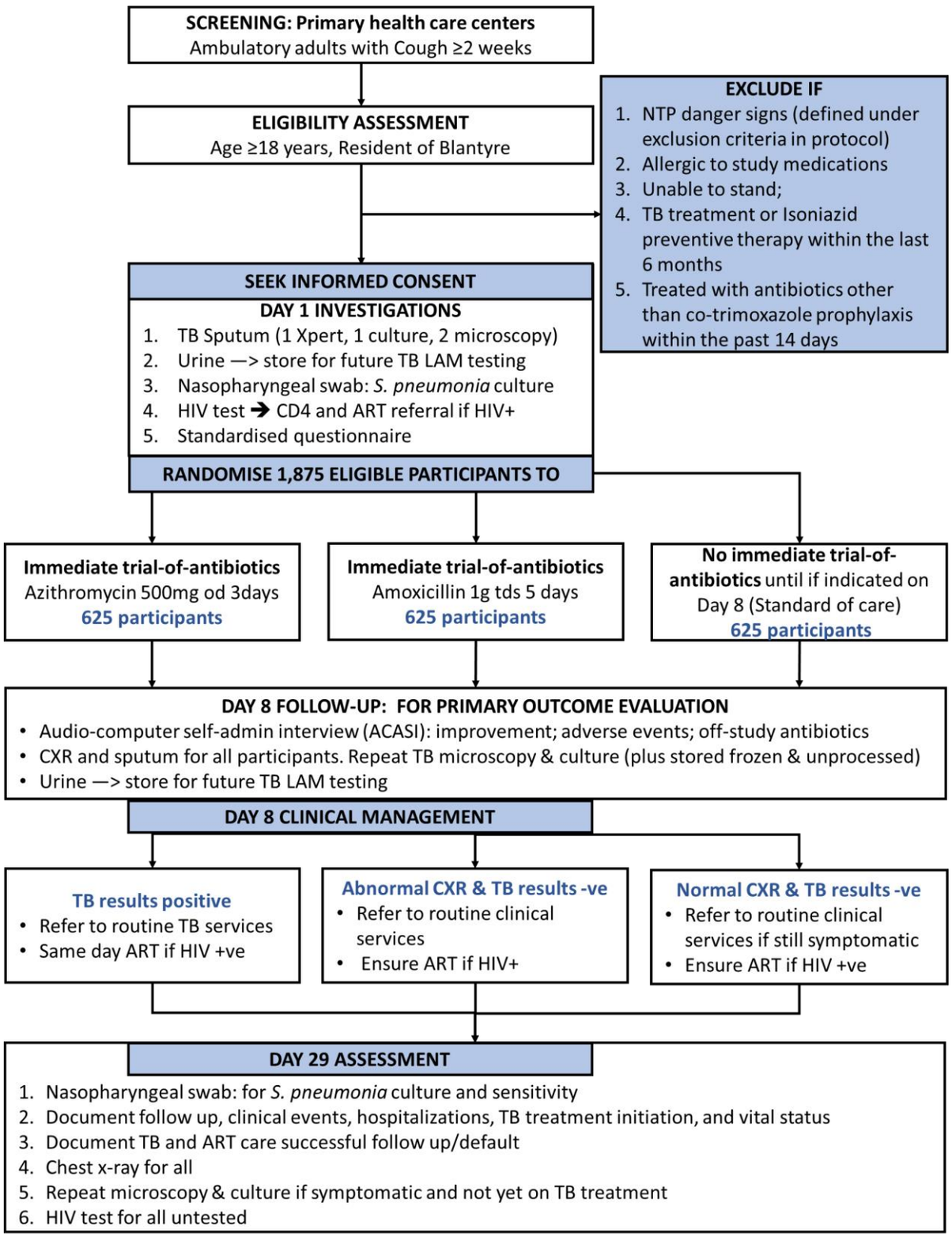
Objective	**Outcomes**
Primary	
1. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with prolonged cough at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among participants submitting at least one sputum specimen
2. To determine the overall clinical benefit of giving empirical antibiotic treatment in primary care participants with chronic cough.	Proportion of participants experiencing at least one of the following adverse outcomes by Day 29: 1) death 2) hospitalisation 3) missed TB diagnosis 4) HIV care loss to follow up 5) TB care loss to follow up
Secondary	
3. To evaluate using nasopharyngeal Streptococcus pneumonia, the effect of a trial-of-antibiotics on selection for antimicrobial resistance.	Risk of acquiring nasopharyngeal Streptococcus pneumonia isolates resistant to any of the commonly used groups of antimicrobials by Day-29.

4. To establish the diagnostic value of trial-of-antibiotics for excluding pulmonary tuberculosis (PTB) in adults with prolonged cough at primary care level in Malawi.	Proportion of participants correctly classified as PTB negative based on report of improvement of baseline symptoms on study Day-8 (i.e. after a trial-of-antibiotics if in azithromycin or amoxicillin arms, or without antibiotics if in standard of care arm) against a mycobacteriology reference standard, among all randomised participants, with those who could not provide sputum classified as mycobacteriologically negative.
5. To estimate the incremental cost-effectiveness of trial-of-antibiotics using azithromycin and trial-of-antibiotics using amoxicillin in comparison to standard of care, and to each other.	• Incremental cost per quality adjusted life year gained • Total direct medical costs per participant over 56 days • Eq-5D utility score
Exploratory	
Our exploratory analyses will be comparisons between the azithromycin and amoxicillin arms for all our primary and secondary outcomes.	
Population	Adults presenting to primary care centres in Malawi reporting cough.
	Inclusion criteria: • Ambulatory clinic attendees presenting with cough for ≥ 14 days • Aged at least 18 years • Reside in Blantyre and willing to return to the same clinic for follow up visits over the entire study period. Exclusion criteria: • Self-reported allergy to study medications • Acute danger signs defined in national TB treatment guidelines • Tuberculosis treatment or isoniazid preventive therapy in the last 6 months • Treated with antibiotics, other than co-trimoxazole prophylaxis, for the current illness or within the past 14 days
Treatment	**Arm 1**: Azithromycin 500mg once daily for 3 days commencing on randomization day. **Arm 2**: Amoxicillin 1 g 3 times daily for 5 days commencing on randomization day. **Arm 3**: Standard of care in current national guidelines for patients presenting with cough and without danger signs (No treatment until re-evaluation with sputum TB test results)
Duration	We will give treatments on the randomisation day (Day-1) and perform follow up activities on days 8, and 29.

1.6.5 Methods

We will conduct a randomised controlled clinical trial recruiting adult patients presenting to primary care centres in Blantyre, Malawi with history of cough for at least 2 weeks. After excluding those with danger signs we will randomise participants to receiving or not receiving trial-of-antibiotics (azithromycin or amoxicillin) from Day-1 to determine diagnostic accuracy (specificity) against mycobacteriology reference standard (smear microscopy, Xpert/MTB/RIF and culture). The second primary outcome (clinical benefit of empirical antibiotics) will be evaluated by comparing the proportion of participants experiencing at least one of the following adverse outcomes by Day 29: death, hospitalisation, missed TB diagnosis, HIV care loss to follow up and TB treatment loss to follow up between arms. For secondary outcomes, we will

compare between arms differences in antimicrobial resistance at Day-29 and estimate the incremental cost-effectiveness. To adequately address the trial objectives, we will need 625 participants in each of the three arms (azithromycin, amoxicillin and standard of care), a total sample size of 1875 participants. Study activities are as presented in the following flow diagram.



1.7 Scope of charter

The purpose of this document is to describe the membership, terms of reference, roles, responsibilities, authority, decision-making and relationships of the Trial Steering Committee (TSC) for this trial, including the timing of meetings, methods of providing information to and from the TSC, frequency and format of meetings and relationships with other trial committees.

2 Roles and responsibilities

2.1 Aims of the TSC

The role of the TSC is to provide oversight for the trial. It should also provide advice through its independent Chairman to the London School of Hygiene & Tropical Medicine (sponsor), and Helse Nord RHF (funder) on all aspects of the trial.

2.2 Specific roles of the TSC

1) provide expert oversight of the trial
2) maintain confidentiality of all trial information that is not already in the public domain
3) make decisions as to the future continuation (or otherwise) of the trial
4) monitor recruitment rates and encourage investigators to develop strategies to deal with any recruitment problems
5) receive reports from the DSMB and consider their recommendations
6) assess the impact and relevance of any accumulating external evidence
7) monitor follow-up rates and review investigators' strategies for dealing with problems
8) approve any amendments to the protocol, where appropriate
9) oversee the timely reporting of trial results
10) review the statistical analysis plan
11) review the main trial manuscript
12) approve external or early internal requests for release of data or subsets of data or samples including clinical data and stored biological samples

3 Before the trial starts

3.1 Whether the TSC will have input into the protocol

The TSC membership will review and provide feedback on the trial protocol, and hold their first meeting before commencement of trial recruitment. The objective of the first meeting is to review and discuss the protocol including trial design, and analysis plans with the chief investigator and co-investigators. TSC members should be constructively critical of the ongoing trial, but also supportive of aims and methods of the trial.

3.2 Whether members of the TSC will have a contract

TSC members will not be asked to formally sign a contract but should formally register their agreement to join the group by confirming (1) that they agree to be a member of the TSC and (2) that they agree with the contents of this Charter. Any potential competing interests should be declared at the same time. Members should complete and return the form in

Annexes 1 or 2. Any observers (attendees who are not members) will sign a confidentiality agreement on the first occasion they attend a meeting.

4 Composition

4.1 Membership of the TSC

The majority of members of the TSC, including the Chair, should be independent of the trial. Non-independent members will also be part of the TSC.

The members of the TSC for this trial are:

1) Professor Bertie Squire, Liverpool School of Tropical Medicine – Independent member and chair person
2) Dr Henry Mwandumba, Malawi-Liverpool Wellcome Trust (MLW) – Independent member
3) Professor Mia Crampin, Malawi Epidemiology and Intervention Research Unit (MEIRU), London School of Hygiene & Tropical Medicine– Independent member
4) Dr Titus Divala – Chief Investigator and TSC facilitator
5) Professor Katherine Fielding – Co-Investigator
6) Professor Elizabeth Corbett – Co-Investigator

4.2 The Chair, how they are chosen and the Chair's role.

The Chair should have previous experience of serving on trial committees and experience of Chairing meetings, and should be able to facilitate and summarise discussions; knowledge of the disease area would be beneficial.

4.3 The responsibilities of the CI

The CI is an important member of the TSC and no major decisions should be made without their involvement.

4.4 The responsibilities of the Facilitator

The Facilitator will be responsible for arranging meetings of the TSC, coordinating reports, producing and circulating minutes and action points. The Facilitator will be the central point for all TSC communications between the TSC and other bodies, will be copied into all correspondence between TSC members and will be kept aware of trial issues as they arise. This work being his PhD, the CI will also serve as the facilitator.

4.5 The responsibilities of the observers

Additional observers may be in attendance through (parts of) the TSC meetings in order to provide input on behalf of the trials unit, the trial's Sponsor/Funder or to provide specific relevant expertise.

5 Relationships

Trial governance and management are described in the protocol.

The TSC is the oversight body and is delegated the roles in Section 2 by the Sponsor.

TSC members will not be paid for their services.

TSC members are asked to disclose information about any competing interests.

6 Organisation of TSC and DSMB meetings

TSC meetings will be conducted once every six months by teleconference. The DSMB meetings will be timed in such a way that they follow DSMB meetings.

Effort will be made for all members to attend. If, at short notice, any TSC members cannot attend then the TSC may still meet if at least two independent members, including the Chair (unless otherwise agreed), will be present as well as a representative of the trial team. If the TSC is considering a major action after such a meeting the TSC Chair should communicate with the absent members, including the CI, as soon after the meeting as possible to check they agree. If they do not, a further teleconference should be arranged with the full TSC.

If an independent member does not attend a meeting or provide comments when requested between meetings, it should be ensured that the independent member is available for the next meeting. If an independent member does not attend the next meeting or provide comments when next requested, they should be asked if they wish to remain part of the TSC. If an independent member does not attend a third meeting, strong consideration should be given to replacing this member.

7 Trial documentation and procedures to ensure confidentiality and proper communication

A short report will be prepared by the CI on accrual and any matters affecting the trial. Additionally, the material may include a report from the DSMB, or draft publications. No trial outcome measure data will be presented by arm unless explicitly authorised by the DSMB (eg toxicity). Where relevant, accrual, compliance with follow-up and adherence to treatment may be presented by centre. The TSC will receive the reports at least 1 week before any meetings.

TSC members would be expected to delete, destroy or store securely copies of the reports to and from the TSC, agenda and minutes, as well as copies of communications between meetings. All documentation should be considered confidential.

8 Decision making

8.1 What decisions will be open to the TSC

Based on recommendations from the DSMB, possible decisions include:-

- No action needed, trial continues as planned
- Early stopping due, for example, to clear benefit or harm of a treatment, futility or external evidence (this should generally involve a recommendation from the DSMB to unblind the TSC to this data)
- Stopping recruitment within a subgroup (this should generally involve a recommend from the DSMB to unblind the TSC to this data)

- Modifying target recruitment, or pre-analysis follow-up, based on any change to the assumptions underlying the original trial sample size calculation (but not on any emerging differences)
- Stopping one or more arms of the trial
- Sanctioning and/or proposing protocol changes

Based on other factors, possible decisions include the decisions above and:-

- Censuring centres for poor recruitment/poor data quality
- Approving proposed protocol amendments or new trial sub-studies
- Approving requests for early release of (subsets of) data
- Approving external applications for the use of stored samples
- Approving presentation of results during the trial or soon after closure
- Approval of new centres or strategies to improve recruitment or follow-up

8.2 The role of formal statistical methods

Formal statistical methods may have been considered by the DSMB in making their recommendations to the TSC. These methods are usually used as guidelines rather than absolute rules. This is because they generally only consider one dimension of the trial. The DSMB will record reasons for disregarding a stopping guideline in the notes of their meetings and may choose to also note this in their report to the TSC if necessary.

8.3 How decisions or recommendations will be reached within the TSC

Every effort should be made to achieve consensus. The role of the Chair is to summarise discussions and encourage consensus; therefore, it is usually best for the Chair to give their own opinion last.

It is important that the implications (e.g. ethical, statistical, practical, financial) for the trial be considered before any decision is made.

8.4 When is the TSC quorate for decision-making?

At least two independent members of the TSC should be present including the Chair, plus a representative of the trials unit and, if major action is to be considered, the CI.

9 Reporting

9.1 To whom will the TSC report their recommendations/decisions, and in what form?

The TSC will report their decisions (via the Facilitator) to the CI and co-investigators who will be responsible for implementing any actions resulting. The TSC may also provide feedback to the DSMB and, where appropriate, to the Sponsor.

9.2 Whether minutes of the meeting be made and, if so, by whom and where they will be kept

Notes of key points and actions will be made by the Facilitator. This will include details of whether potential competing interests have changed for any attendees since the previous meeting. The draft minutes will be initially circulated for comment to those TSC members who were present at the meeting. The TSC Chair will sign off the final version of minutes or notes.

9.3 What will be done if there is disagreement between the TSC and other trial committees?

The TSC is the oversight body for the trial. However, the TSC should have good reason before deciding not to accept requests from the DSMB. If there are serious problems or concerns with the TSC decision following a DSMB recommendation, a joint meeting of the TSC and DSMB should be held. The information to be shown would depend upon the action proposed and each committees' concerns. Depending on the reason for the disagreement confidential data and/or data by trial and may have to be revealed to all or some of those attending such a meeting: this would be minimised where possible. The meeting would be Chaired by a senior member of staff from the LSHTM or an external expert who is not directly involved with the trial.

10 After the trial

10.1 Publication of results

The TSC will oversee the timely analysis, writing up and publication of the main trial results. The independent members of the TSC will have the opportunity to read and comment on the proposed main publications of trial data prior to submission and abstracts and presentations during the trial. This review may be concurrent to that of the trial investigators and DSMB.

10.2 The information about the TSC that will be included in published trial reports

TSC members will be named and their affiliations listed in the main report, unless they explicitly request otherwise.

11 Annex 1: Trial Steering Committee members register of their assent

I, ……. (name) of ……. (institution) agree

1) to be on the ACT-TB Study TSC

2) with the contents of the ACT-TB Study TSC Charter

3) to keep ACT-TB Study TSC data reports and meeting outputs confidential

Signature: _____ Date: _____

12 Annex 2: Competing interests form

Potential competing interests of Trial Steering Committee members

Possible competing interest(s) should be disclosed.

Potential competing interests

- Stock ownership in any commercial companies involved
- Stock transaction in any commercial company involved (if previously holding stock)
- Consulting arrangements with the sponsor
- Frequent speaking engagements on behalf of the intervention
- Career tied up in a product or technique assessed by trial
- Hands-on participation in the trial
- Involvement in the running of the trial
- Emotional involvement in the trial
- Intellectual conflict eg strong prior belief in the trial's intervention arm
- Involvement in regulatory issues relevant to the trial procedures
- Investment (financial or intellectual) in competing products
- Involvement in the publication as an author

Please complete the following section and return to the Chief Investigator.

☐ **No,** I have no competing interests to declare

☐ **Yes,** I have competing interests to declare (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: ……..

Signed: _____ Date: _____