Epidemiology of *Streptococcus pyogenes* in The Gambia: investigating carriage and disease burden, transmission dynamics and diagnostic accuracy

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Declaration

I, Edwin Armitage, declare that the work presenting in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature Date: 1st April 2025

Edwin P. Armitage

Preface

This thesis is structures as a “Research Paper Style Thesis” in accordance with the London School of Hygiene and Tropical Medicine submission guidance. Three chapters are research papers submitted for publication in peer-reviewed journals, two published and one under review. These chapters are clearly marked in the table of contents. Each research paper in accompanied by a cover sheet indicating the publication details and my contribution to the manuscript. There may be some repetition of material in the papers due to journal requirements. The remainder of the thesis consists of some chapters of linking material to provide background material on the PhD topic, descriptions of the study setting and design, and the overall PhD objectives, two results chapters which are structured as separate research studies, a discussion chapter and a future work chapter.

All material within this thesis was written by Edwin Armitage.

Abstract

Streptococcus pyogenes (*S. pyogenes;* StrepA; Group A *Streptococcus*; GAS) causes a substantial burden of disease globally ranging from superficial infections to invasive disease. It is responsible for over 500,000 deaths each year globally, disproportionately in low- and middle-income countries (LMIC). StrepA-positive pharyngitis can lead to acute rheumatic fever and rheumatic heart disease in some patients, which causes most StrepA-associated mortality. The importance of StrepA skin infections has been recognised in high-RHD settings, but data is lacking. As well as pyoderma, asymptomatic carriage of StrepA in the pharynx and on skin may be important for transmission.

The overarching aim of this thesis is to investigate the epidemiology of StrepA carriage and infection in The Gambia, the pathways by which it spreads within households, and how transmission and disease risk are shaped by individual, household, and environmental factors, while also evaluating the accuracy and utility of clinical decision rules and rapid diagnostic tests for StrepA pharyngitis in this setting.

In Sukuta, an urban area of The Gambia, two observational studies were conducted to better understand these aspects of StrepA epidemiology. The first recruited children presenting to primary healthcare with acute pharyngitis to assess the prevalence of StrepA pharyngitis, and to assess the diagnostic accuracy of clinical decision rules and rapid diagnostic tests for the diagnosis of StrepA pharyngitis in this setting.

The second, a longitudinal household cohort study conducted over one year, sought to understand the temporal relationship between StrepA carriage and disease, how it is spread from person to person, via skin or pharynx, acquired inside or outside households, and what risk factors for carriage and disease exist. The study utilised frequent microbiological swabbing visits with same-day culturing for StrepA isolates to provide robust measures of disease burden. Detailed information on socio-demographics, social-mixing behaviour, water access and sanitation, was combined with *emm*-typing of isolates to investigate risk factors for StrepA disease and for transmission. Utilising approaches never used before in Africa, this PhD thesis addresses several key gaps in relation to StrepA epidemiology in LMICs, providing vital data to inform surveillance strategies, optimise diagnostic pathways and guide the development of targeted interventions to reduce the burden of StrepA disease.

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List of abbreviations

Abbreviation	Full Term
ARF	Acute Rheumatic Fever
APSGN	Acute Post-Streptococcal Glomerulonephritis
CDR	Clinical Decision Rule
CRF	Case Report Form
DALY	Disability-Adjusted Life Year
DBS	Dried Blood Spot
ES	Environmental Swab
GAS	Group A Streptococcus
HIC	High-Income Country
HR	Hazard Ratio
ICD	Informed Consent Document
ID NOW	A type of rapid nucleic acid test (RNAT) for Strep A
iGAS	Invasive Group A Streptococcal Disease
IQR	Inter-quartile Range
LFT	Lateral Flow Test
LMIC	Low- and Middle-Income Countries
MDA	Mass Drug Administration
MRCG	Medical Research Council Unit The Gambia
MV	Monthly Visit
NSS	Normal Skin Swab
OF	Oral Fluid
OPS	Oropharyngeal swab
PharynGAS	Evaluating the utility of clinical scoring systems and rapid diagnostic tests for identifying Group A Streptococcus Pharyngitis in Gambian children study
POCT	Point-of-Care Test
RADT	Rapid Antigen Detection Test
RHD	Rheumatic Heart Disease
RNAT	Rapid Nucleic Acid Test
SDSE	*Streptococcus dysgalactiae* subsp. *equisimilis*
SpyCATS	*Streptococcus pyogenes* Carriage Acquisition and Transmission Study
StrepA	*Streptococcus pyogenes*
SSP	Study Specific Procedure
SSS	Scabies Skin Swab
SWS	Scabies Wound Swab

UV	Unscheduled Visit
WASH	Water access, Sanitation, and Hygiene
WHO	World Health Organization
WGS	Whole Genome Sequencing
WS	Wound Swab
WV	Weekly Visit

1 Chapter 1: Background and overview of *Streptococcus pyogenes* epidemiology

1.1 Overview of *Streptococcus pyogenes*

Streptococcus pyogenes (StrepA; Group A *Streptococcus*; GAS) is a beta-haemolytic Gram positive bacterium that causes a wide spectrum of disease, causing over 500,000 deaths each year globally (1-3). The clinical manifestations of StrepA infections range from common superficial conditions, such as pharyngitis and pyoderma, to severe invasive disease (iGAS), including bacteraemia, necrotising fasciitis, and meningitis. It also causes toxin-mediated diseases, such as scarlet fever and streptococcal toxic shock syndrome as well as leading to immune-mediated sequelae, including acute rheumatic fever (ARF), rheumatic heart disease (RHD), and acute post-streptococcal glomerulonephritis (APSGN).

The global burden of StrepA-related disease is substantial, with an estimated 616 million cases of pharyngitis, 111 million cases of pyoderma, and 1.8 million cases of iGAS occurring annually (2). In high-income countries (HICs), iGAS causes the most mortality, with up to 163,000 deaths annually, whereas in low- and middle-income countries (LMICs), RHD is the largest cause of StrepA-related mortality, causing over 300,000 deaths each year (4-7).

Scarlet fever and iGAS have seen a resurgence in some HIC, such as the United Kingdom, in the last decade (8). Novel virulent strains, such as the M1UK strain, and changes to clinical guidelines as well as the COVID-19 pandemic may have had an impact (9,10). In many HICs, surveillance systems, such as the mandatory notification of iGAS and scarlet fever in the UK, allow for monitoring and prevention of onward transmission through public health actions. However, surveillance systems in LMICs are weak or non-existent, leading to limited understanding of StrepA epidemiology in these settings.

RHD remains a significant contributor to global morbidity and mortality, particularly in LMICs, where it accounts for a substantial burden of disease and disability, particularly affecting young women around the time of childbirth (11). Despite its impact, RHD receives limited research attention and funding compared to other major infectious diseases (12). High prevalence rates and poor outcomes observed in LMICs highlight the urgent need for improved surveillance, better access to care, and targeted public health interventions to address this preventable condition. This thesis seeks to address some of these gaps by investigating the epidemiology and transmission of StrepA carriage and disease and the performance of various diagnostic tools in The Gambia, providing valuable data to inform the design of future research and of effective strategies to reduce the burden of StrepA-related mortality globally.

1.2 Historical background of *Streptococcus pyogenes*

1.2.1 Early history and discoveries

StrepA has a long history, with descriptions of its clinical manifestations dating back to ancient times. Descriptions of conditions resembling erysipelas and puerperal fever have been noted as early as the 4th century BC. The first detailed description of scarlet fever, then called “rossalia”, was given by Giovanni Filippo Ingrassias in 1553, describing “numerous spots, large and small, fiery and red, of universal distribution, so that the whole body appeared to be on fire”. Later, the term “scarlatina” was commonly used, and was coined by Thomas Sydenham, an English physician, in 1675 to distinguish it from other rashes, particularly measles (13).

It was also one of the earliest bacterial pathogens to be identified. In 1874, Theodor Billroth, an Austrian surgeon, first described the organism in wound infection as “small organisms” arranged in chains (14). Louis Pasteur then isolated the bacteria from the blood and uteruses of women with puerperal fever in 1879, showing that it was the aetiological agent, at a time when puerperal fever was the leading cause maternal and neonatal mortality (15). Friedrich Julius Rosenbach refined its classification in 1884, naming it *Streptococcus pyogenes* to reflect its role in suppurative infections (from the Greek “streptos” meaning chain, “kokkos” meaning berry, and “pyo” meaning pus) (13).

Ignaz Semmelweis’s work in the 1840s on puerperal fever led to a recognition of the importance of handwashing as a public health measure. Observing significantly higher mortality rates in women attended by physicians compared to midwives, Semmelweis concluded that disease was transmitted by contaminated hands, likely carrying material from autopsies. Although he did not know that StrepA was the causative agent, his intervention, requiring medical staff to wash their hands with chlorinated lime, dramatically reduced mortality. This marked one of the earliest documented public health interventions to prevent infectious disease transmission, predating Pasteur’s identification of StrepA in cases of puerperal fever by several decades (15).

Advances in laboratory methods enhanced the understanding of StrepA biology. In 1903, Schottmüller introduced blood agar, allowing for differentiation of haemolytic streptococci based on their effects on red blood cells (13). This technique paved the way for Rebecca Lancefield’s serological classification in the 1930s, which remains in use today. Lancefield’s work identified M protein types as critical determinants of the bacterium’s virulence and linked specific M types to distinct clinical manifestations, providing a foundation for modern epidemiological and diagnostic approaches (16).

1.2.2 History of StrepA vaccines

The history of vaccine development against StrepA disease dates back to the 1920s, when George and Gladys Dick demonstrated that scarlet fever was caused by a toxin produced by the bacterium. Numerous fatal outbreaks of scarlet fever in the 1800s had led to a focus on StrepA as a potential immunisation target. Building on their discovery, in 1924 the Dicks developed a toxin-antitoxin vaccine,

which initially showed promise in preventing scarlet fever during community outbreaks (17). However, inconsistent efficacy and safety concerns, including severe reactions in some recipients, led to the eventual discontinuation of the vaccine. Despite its limitations, this work laid the foundation for the concept of immunisation against StrepA diseases.

Efforts to develop vaccines targeting the broader spectrum of StrepA diseases, including ARF and RHD, continued in the mid-20th century. Early attempts focused on the M protein, a major virulence factor of StrepA and a target of protective immunity and used crude preparations of M proteins or cell wall extracts (18). However, the discovery of cross-reactive epitopes within the M protein that mimicked human tissue antigens raised concerns about vaccine-induced autoimmune complications, particularly the exacerbation of ARF and RHD. A particularly controversial trial of an M protein vaccine in 21 participants who were siblings of patients with ARF resulted in three cases of vaccine-linked ARF (19,20). Despite the flaws in the study, the safety concerns culminated in the US Food and Drug Administration (FDA) imposing a moratorium on human trials of StrepA vaccines in 1978 (21).

The moratorium effectively stalled vaccine development for over three decades. During this time, research efforts shifted towards understanding the immunopathogenesis of StrepA diseases and identifying alternative vaccine targets. Advances in molecular biology and immunology in the late 20th century enabled the identification of conserved regions of the M protein and other antigenic targets that were less likely to trigger autoimmune responses (18).

In 2005, the FDA lifted its ban on human StrepA vaccine trials, stimulating renewed interest in vaccine development (22). The decision was supported by accumulating evidence that vaccines targeting conserved epitopes of StrepA proteins could potentially provide broad protection without inducing autoimmunity. Since then, several candidate vaccines have entered preclinical and clinical development, with approaches ranging from M-protein-based designs to those targeting secreted toxins and surface proteins (23).

1.2.3 Understanding the chain from throat to heart

The mid-20th century saw significant advancements in understanding the aetiology of ARF and its progression to RHD. The work of Lewis Wannamaker and colleagues at the University of Minnesota, was key to this. In a seminal 1973 paper titled “The chain that links the heart to the throat” he stated that ARF resulted exclusively from StrepA infections of the upper respiratory tract (URT) and not from skin infections. This assertion, based on epidemiological and immunological evidence, became widely accepted dogma in the field and shaped subsequent research and public health strategies (24).

Wannamaker’s findings were supported by detailed studies of immune responses to StrepA infections, which demonstrated distinct pathways linking URT infections to ARF and RHD (25,26). However, this emphasis arguably led to the neglect of the potential contribution of skin infections to post-streptococcal

sequelae. Despite other work by Wannamaker's group on StrepA skin carriage and pyoderma, the focus on URT as the sole driver of ARF meant that the interplay between skin and pharyngeal disease and the role of pyoderma in ARF was later overlooked (27-34).

Penicillin was identified as a key intervention during this period to prevent ARF and RHD. Controlled studies in military populations, such as the Fort Warren study, demonstrated that early antibiotic treatment of StrepA pharyngitis significantly reduced ARF incidence (26). These findings established the basis for primary and secondary prophylaxis, which remain central to ARF prevention and RHD management today.

The apparent decline of ARF and RHD in HICs during the mid-20th century was attributed to a combination of reduced transmission of virulent StrepA strains, improved living conditions, and widespread antibiotic use. In contrast, these diseases persisted in LMICs where environmental and social determinants, such as overcrowding and limited healthcare access, likely facilitated ongoing transmission (35).

Studies conducted in the Red Lake Indian Reservation, though they later raised significant ethical concerns, highlighted the cyclical and seasonal dynamics of StrepA infections. Longitudinal investigations by Wannamaker's group demonstrated the transmission of nephritogenic strains through skin infections, linking these to APSGN outbreaks (25). These findings underscored the complexity of StrepA epidemiology and the need for integrated approaches to understanding its transmission pathways (31).

Despite Wannamaker's assertion that only URT infections led to ARF, a growing body of evidence supporting the role of skin infections in StrepA epidemiology has prompted renewed interest in the broader transmission dynamics of this pathogen (36).

1.2.4 Recent advancements and global efforts

The 21st century has seen renewed efforts to address the global burden of StrepA infections and their sequelae. The lifting of the FDA moratorium in 2005 was a pivotal moment enabling renewed research into safer vaccine designs, including those targeting conserved regions of the M protein and other less immunogenic antigens (37). Several vaccine candidates are now in clinical development, with an emphasis on global applicability and suitability for use in LMICs.

The World Health Organization (WHO) has taken a leadership role in defining priorities for StrepA research and vaccine development. It outlined a Research and Development Technology Roadmap in 2018, identifying key gaps and proposing actionable strategies (38). Among these are the need for longitudinal studies to better understand the natural history of StrepA infections and the progression to

autoimmune sequelae like ARF and RHD. Such studies are critical for identifying immune correlates of protection, which remain poorly characterised, and for guiding vaccine development strategies (37).

The roadmap also highlighted the establishment of centres of excellence in LMICs as a cornerstone for future progress. These centres would facilitate high-quality clinical trials, contribute to regional epidemiological surveillance, and address the scarcity of data on disease burden and strain diversity in high-burden settings. Additionally, they would ensure that vaccine efficacy and safety are evaluated in populations most affected by StrepA, ensuring equitable access to future interventions (38).

The WHO's preferred product characteristics (PPCs) for StrepA vaccines provide further guidance, prioritising vaccines that can prevent both invasive and non-invasive infections, reduce antibiotic use, and offer broad protection against diverse strains (39). Early-stage goals focus on demonstrating vaccine efficacy against pharyngitis and skin infections. These initiatives align with broader public health strategies aimed at reducing the global burden of StrepA diseases. In parallel with vaccine development, there is an ongoing emphasis on improving access to antibiotics, enhancing diagnostic tools, and addressing social determinants of health. Together, these efforts bring hope that the management and prevention of StrepA diseases may improve in the next decade, particularly in LMICs, where the burden remains disproportionately high.

1.3 *Streptococcus pyogenes* disease manifestations and epidemiology

1.3.1 Overview

StrepA causes a wide range of clinical manifestations including infections such as pharyngitis, pyoderma, iGAS, scarlet fever and the immune-mediated sequelae ARF, RHD and APSGN (Figure 1.1). Conservative estimates from 2005 suggest 1.8 million cases of iGAS, 111 million of pyoderma and 616 million of pharyngitis occur each year (2). Up to 163,000 deaths occur from iGAS, which is the largest StrepA-related cause of death in most HIC (4-7). In LMIC, where the largest burden of StrepA disease occurs, RHD is the cause of most StrepA-related mortality, causing an estimated 354,000 deaths per year (3,40,41). Despite the majority of StrepA-related disease occurring in LMIC, epidemiological data from those regions remains weak and suffer from a lack of diagnostic and surveillance systems and under-reporting (2,3). The African Strep A infection registry (AFROStrep) was recently established in South Africa to collect epidemiological data on iGAS and other StrepA infections, but notably the platform does not include asymptomatic carriage samples (7,41). The programme has been expanded to only four other African sites: Ethiopia, Nigeria, Sudan, and Mali, none of which have yet published any data. Data on asymptomatic carriage remain sparse, particularly in LMIC settings. Understanding carriage dynamics is important for assessing the full burden of StrepA infections and transmission risk, as asymptomatic carriers may serve contribute to transmission. Enhanced

surveillance and comprehensive epidemiological studies are required to inform effective public health interventions in these settings.

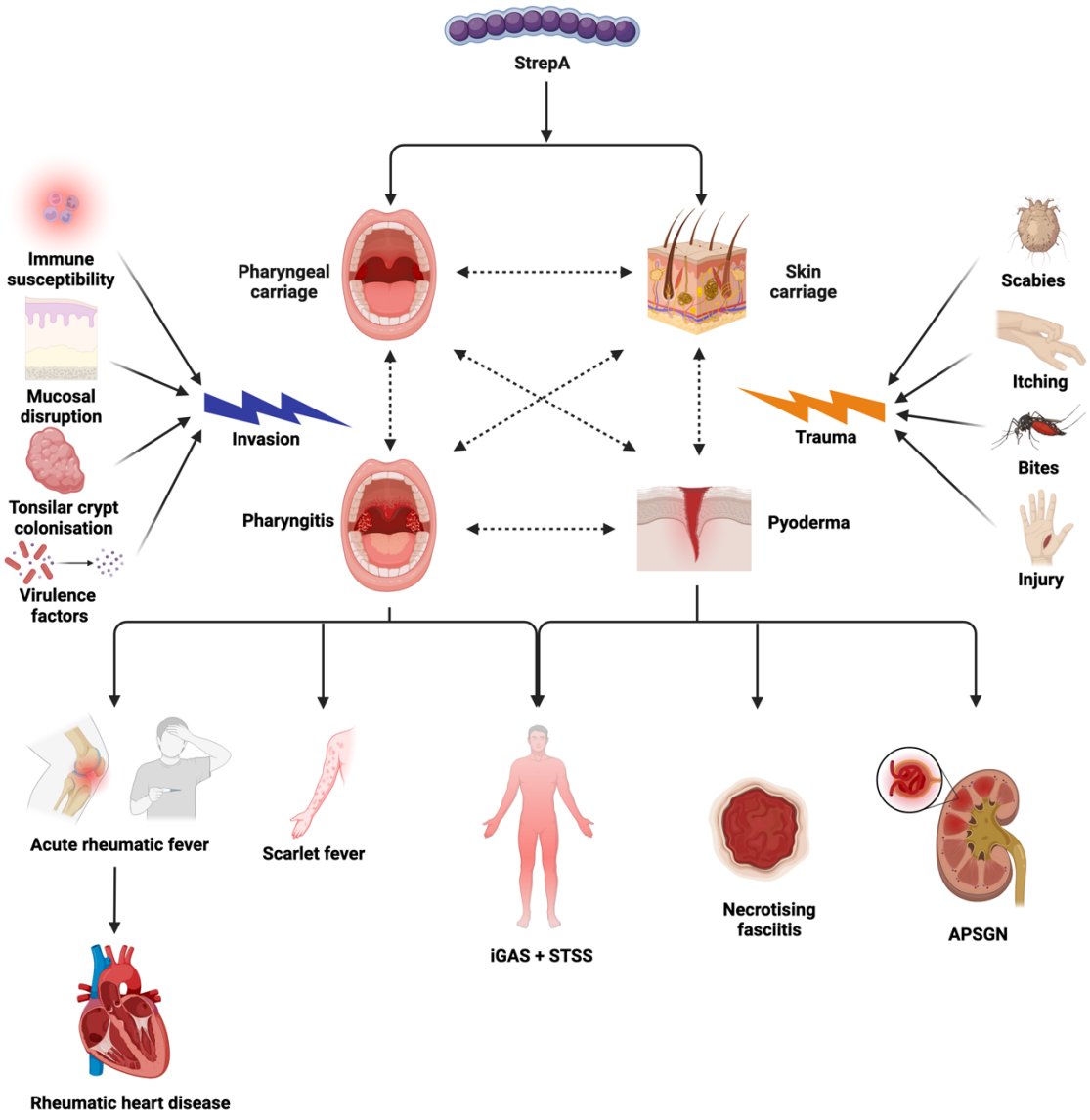

Figure 1.1. A schematic representation of the progression from asymptomatic StrepA carriage to different disease endpoints. Solid arrows indicate potential transitions influenced by host susceptibility, bacterial factors, and environmental conditions. Dotted arrows indicate potential transmission routes between and within individuals.

1.3.2 Pharyngitis

Acute pharyngitis is one of the most common presentations in primary care worldwide. It is characterised by inflammation of the pharynx and/or tonsils combined with symptoms of a sore throat (42). Incidence peaks between 4-7 years old, but can occur in adults (43). Though non-infectious causes exist, most pharyngitis is caused by viral or bacterial infections. Most infectious pharyngitis is viral, with the most common agents being Rhinoviruses and Adenoviruses (43). StrepA accounts for 20-30% of pharyngitis cases in children and 5-10% in adults (41,44). Although less common, bacterial

pharyngitis is important to distinguish from viral pharyngitis, as the pathogen causing most bacterial pharyngitis is StrepA, which requires antibiotic treatment to avoid complications such as peritonsillar abscess, iGAS, ARF and RHD (45,46).

Globally, approximately 616 million new cases of StrepA pharyngitis occur annually, with a higher burden in LMICs, potentially due to overcrowding, poor hygiene, and limited access to healthcare (2). Data from sub-Saharan Africa are particularly limited, and no data exist from The Gambia. In South Africa, historical studies reported high prevalence rates ranging from 23% to 46%, particularly in lower socio-economic settings (46). A meta-analysis of African studies identified a prevalence of 21% (17-26%) for StrepA pharyngitis among symptomatic individuals, though data were limited (41). Seasonal trends, with peaks during colder and wetter months, have been observed in several settings, including India and Africa, reflecting environmental and behavioural factors that impact transmission (41,47).

A meta-analysis into the proportion of pharyngitis casued by StrepA in OECD vs. non-OECD countries found that StrepA is the causative agent in a higher proportion of pharyngitis cases in OECD countries compared to non-OECD (24.3%, 95% CI 22.6-26.1 vs. 17.6%, 95% CI 14.9-20.7%) (44). The study also found that passive recruitment studies find a higher proportion of StrepA pharyngitis compared to active recruitment studies, possibly due to active recruitment studies including more participants with less severe (more likely viral) pharyngitis cases. Differences in health-seeking behaviour in HIC compared to LMIC therefore may influence the StrepA pharyngitis prevalence substantially. However, in children aged 5-19 in passive recruitment studies the proportion of StrepA-positive pharyngitis was similar in OCED and non-OECD countries (36.8% vs. 37.4%) suggesting a similar level of exposure to StrepA pharyngitis. Though, the proportion of StrepA-positive pharyngitis does not explain the difference in ARF incidence between HIC and LMIC therefore, suggesting that other routes of exposure, such as via the skin, are relevant in ARF aetiology. The proportion of pharyngitis caused by StrepA however does not account for the incidence of sore throat in different settings, which is higher in lower-resourced settings (48).

1.3.2.1 Clinical decision rules for acute pharyngitis

Pharyngitis is a common presentation in primary healthcare settings with most cases are self-limiting viral infections not requiring treatment. However, since a minority of cases are caused by StrepA, it is important to identify those cases and treat them to avoid ARF and RHD (42,43,45). It was shown in 1950 that treatment of acute pharyngitis exhibiting tonsillar exudate in US military recruits with penicillin significantly reduced the risk of ARF (0.3% of those treated vs. 2.1% of those untreated; p=0.0006) (26). Since then, guidelines worldwide have highlighted the importance of identifying and treating StrepA pharyngitis.

The “reference standard” for diagnosis of StrepA pharyngitis has traditionally been culture of oropharyngeal swabs (OPS), but this test has several practical limitations including cost and equipment,

the culture time of 1-2 days, relatively low sensitivity, and fears of false positives in asymptomatic StrepA carriers (43,49,50). Moreover, in LMIC many healthcare settings do not have access to culture at all. Many patients in practice (in HIC and LMIC) do not get an OPS but rather it is left to the clinical judgement of the healthcare provider to decide whether to prescribe antibiotics. As such, concerns over overprescribing of unnecessary antibiotics for viral cases, and the possible contribution to antimicrobial resistance (AMR) exist (51-53).

To enhance clinicians' ability to assess whether a presentation of pharyngitis is bacterial or viral, various clinical scoring systems or clinical decision rules (CDRs) have been developed over the last 50 years to distinguish StrepA pharyngitis from other viral causes (Table 1.1) (54-59). More recently, some have been developed specifically for use in LMIC settings including the Cape Town and Smeesters scores (60-62). These CDRs are evidence-based tools that take into account demographics and the clinical presentation to allow clinicians to stratify patients by risk of StrepA pharyngitis based on the characteristics of their presentation, and thus provide a rational basis for treatment (63).

The most commonly used scores in are CENTOR (55), FeverPAIN (59) and Modified/McIsaac score (56). However, these scores were developed and validated for use in high-income settings. One study showed that score performance varies significantly in different regions of the world, showing the importance of validating scores in local settings before implementation (64). None of the existing CDRs have been validated before in West Africa (62).

Table 2.1. Summary table of common CDRs

CDR	Country	Validation	Clinical Features	Target Population	Use in Clinical Guidelines	Reported Sensitivity (%)	Reported Specificity (%)
Centor (55)	USA	Widely validated in HICs, variable PPV	Fever, tonsillar exudate, lymphadenopathy, no cough	Adults and adolescents	NICE, IDSA guidelines	56-86	50-75
Modified Centor (McIsaac) (56)	Canada	Validated in children, good NPV	Centor criteria with age adjustment	Children and adults	Canadian guidelines	80-90	50-75
FeverPAIN (65)	UK	Validated in UK primary care settings	Fever, pus, attendance delay, inflammation, no cough/coryza	Children and adults in UK settings	NICE sore throat guidelines	75-85	60-75
Cape Town (62)	South Africa	Validated in South African primary care	Tonsillar swelling, exudate, absence of cough and rhinorrhoea	Children in South Africa	South African primary care	83.7	32.2
Smeesters (60,61)	Brazil	Validated in Brazil, aimed at LMICs	Age, viral vs bacterial signs	Children in low-resource settings	Promoted for LMIC settings	Varied, typically high	84
WHO (66)	Global	Limited validation, applied globally	Pharyngeal exudate, cervical lymphadenopathy	Children in LMICs	WHO-recommend ed	Low	Low (varies across settings)

1.3.2.2 Rapid diagnostic tests for StrepA pharyngitis

Due to the practical difficulties of the use of pharyngeal culture to diagnose StrepA pharyngitis, many point-of-care tests (POCT) exist for the rapid diagnosis of StrepA pharyngitis (67-69). Such POCTs can allow more precise targeting of antibiotic treatment. Various designs of POCT exist, but can be broadly separated into rapid antigen detection tests (RADT) and rapid nucleic acid tests (RNAT) (Tables 1.2 and 1.3). The diagnostic accuracy of different available POCTs varies, but summary estimates for sensitivity and specificity of RADTs are 85.6% and 95.4% respectively, and 97.5% and 95.1% respectively for RNATs (70-72). In various settings these are incorporated into primary healthcare guidelines, often in combination with a clinical score (72-76).

Table 1.3. Summary of different RNATs

Test Type	Technology Used	Gene Target	Example Manufacturers	Sensitivity (%)	Specificity (%)	Primary Use Setting	Time to Result
qPCR	Quantitative PCR	*speB, sdaB, spy1258*	Xpert Xpress (Cepheid), Simplexa (Diasorin), Cobas Liat	92–100	70-100	Laboratory and POC	15-45 min
LAMP	Loop-mediated isothermal amplification	*speB*	Illumigene	82-100	86-98	Laboratory and POC	10-0 min
HDA	Helicase-dependent amplification	*sdaB*	AmpliVue, Solana Strep A (Quidel)	88-99	84-97	Laboratory	20-40 min
NEAR	Nicking-enzyme amplification reaction	*cepA*	ID NOW Strep A 2 (Abbott)	96-100	56-100	Laboratory and POC	2-6 min
ssDNA	Single-stranded DNA probe	*16S rRNA*	Gen-Probe	86-93	95-100	Laboratory	30-60 min

Table 1.4. Summary of different RADTs.

Test type/Technology Used	Brands/Manufacturers	Sensitivity (%)	Specificity (%)	Primary Use Setting	Time to Result
Latex agglutination	Patho Dx, DPC	53-91	85-96	POC	5-15 min
Enzyme-linked immunosorbent assay	Abbott TestPack Strep A Plus	70-96	88–97	POC	10-30 min
Optical immunoassay	Biostar Strep A OIA, Strep A OIA MAX	71-95	81-99	POC	10-30 min
Molecular probe-based techniques	Gen-Probe	89-96	96-100	Laboratory	15-45 min

Lateral flow/Immunochromatographic assay	SD Bioline Strep A (Abbott), Quickvue+ Strep A Test	59-96	87-100	POC	5-15 min

Two POCTs relevant to this thesis, both manufactured by Abbott (formerly Alere) are: the SD Bioline Strep A strip, which is a RADT using a lateral flow assay giving a result in 5-10 minutes, can easily be performed in any clinical setting, and has a reported sensitivity of 87.3% and specificity of 95.8% against culture; and the ID NOW™ Strep A 2, which is more expensive, uses isothermal nucleic amplification technology (RNAT), requires a clean, air-conditioned laboratory environment, provides results within 10 minutes, and has a reported sensitivity of 98.5% and specificity of 93.4% against culture.

Very few studies have investigated the use of POCTs in Africa. Though studies have been done in Tunisia, Egypt and Cameroon which found high sensitivities and specificities (77-80), no POCTs have been validated for use in West Africa

1.3.3 Pyoderma

Pyoderma, encompassing all bacterial skin infections including impetigo, are responsible for an estimated 16.6 Disability-Adjusted Life Years (DALYs) per 100,000 people, with more than 162 million children estimated to be suffering from pyoderma globally at any one time, predominantly in low-income and tropical settings (1,81,82). StrepA and *Staphylococcus aureus* (*S. aureus*) are the most common infectious causes, and are often co-isolated from lesions, though the relative dominance of each varies by geography and study design (1,36).

In tropical regions, the prevalence of pyoderma is particularly high among indigenous populations and resource-constrained communities. Studies have reported prevalence rates as high as 49% in Aboriginal Australian children and 26-42% in children from Pacific Island nations, underscoring the disproportionate burden in these populations (82-84). In Africa, the prevalence is generally lower but still significant, ranging from 7% to 12% in community-based studies (1).

Pyoderma is strongly associated with scabies, an ectoparasitic infestation that predisposes individuals to secondary bacterial infections. In some studies, up to 41% of individuals with active pyoderma also had scabies, reflecting the interconnected epidemiology of these conditions (82,83). The co-occurrence of scabies and pyoderma has significant implications for disease control, as interventions targeting one condition, such as mass drug administration with ivermectin, may reduce the burden of both (82,84-87).

Beyond its immediate morbidity, pyoderma contributes to severe complications, including cellulitis, septicaemia, and immune-mediated sequelae like APSGN and likely, ARF and RHD. StrepA is implicated in up to 50% of APSGN cases in tropical settings, linking pyoderma directly to systemic health outcomes (1,36). The potential role of pyoderma in the pathogenesis of ARF and rheumatic RHD

has also garnered increasing attention. While traditionally considered to be triggered only by pharyngitis, epidemiological overlap between pyoderma prevalence and RHD, and emerging immunological evidence suggests that StrepA skin infections may independently trigger ARF and RHD, particularly in settings with endemic pyoderma (36,88-92).

The epidemiology of pyoderma in The Gambia is poorly understood, therefore in 2018 I conducted a cross-sectional study (SpyDERM) in Sukuta enrolling 1441 children under 5 years to determine the prevalence of common skin infections including StrepA pyoderma and scabies. I showed a high prevalence bacterial pyoderma (17.4%), and scabies infestation (15.9%), and specifically of StrepA culture-positive pyoderma (8.8%). I also found a significant increase in pyoderma during the rainy season (before the start of the rains vs. after: 8.9% vs. 23.1%, adjusted prevalence ratio 2.42, CI 1.39-4.23) (93).

Given that pyoderma is highly contagious (94), and common in settings with poor housing conditions, overcrowding and low socioeconomic status which are major risk factors for StrepA disease (95), it likely plays a significant role in transmission within households in The Gambia.

1.3.4 Scarlet fever

Scarlet fever is a toxin-mediated StrepA disease primarily affecting children aged 5–15 years. Although it was historically a major cause of morbidity and mortality, its incidence had declined significantly by the mid-20th century. However, recent years have seen a resurgence in scarlet fever cases in HICs, with England experiencing particularly significant increases since 2014 (96). By 2016, notifications reached their highest levels since the 1960s, with over 19,000 cases reported nationally (97). Outbreaks have also been documented in other regions, such as Hong Kong and mainland China, where incidence has similarly risen, raising questions about its global epidemiology and control (98).

In the United Kingdom, the increase in scarlet fever cases has been attributed in part to the emergence of new strains, particularly the hyper-toxigenic M1UK clone, which produces elevated levels of the scarlet fever toxin, streptococcal pyrogenic exotoxin A (SpeA) (97). Changes in clinical practice, such as evolving guidelines for managing respiratory infections in primary care, and disruptions to background immunity due to the COVID-19 pandemic, have also been implicated in exacerbating its spread (98). Despite public health measures such as mandatory notification and antibiotic treatment, the disease remains highly transmissible, with outbreaks in school and nursery settings driving localised epidemics (99).

Scarlet fever commonly presents with fever, a sore throat, and a widespread erythematous rash that feels rough to the touch. With prompt antibiotic treatment, the disease is typically mild and resolves without complications. However, delayed diagnosis or treatment can lead to serious complications,

including peritonsillar abscess, streptococcal toxic shock syndrome, and APSGN. Studies have highlighted that older children and those without the classic symptom combination are more likely to experience delays in diagnosis, underscoring the importance of clinical vigilance (98).

The spread of scarlet fever in schools and other educational settings is particularly problematic due to the intensity of transmission. Asymptomatic carriers can sustain outbreaks even after symptomatic cases are treated. Research has shown that airborne spread and high carriage rates among close contacts in classrooms contribute significantly to transmission. This highlights the importance of implementing strong infection control measures, such as better ventilation and hygiene practices, to limit the spread (99). Furthermore, household members of children with scarlet fever face a substantially increased risk of developing iGAS, with studies indicating a 20-fold increase in risk (96).

1.3.5 Invasive Group A Streptococcus infections

Invasive Group A Streptococcal (iGAS) disease occurs when StrepA penetrates sterile sites, such as the bloodstream, deep tissues, or organs, causing severe and potentially life-threatening conditions. The most severe iGAS syndromes include necrotising fasciitis, streptococcal toxic shock syndrome (STSS), and septic shock. Case fatality rates for these syndromes is high, ranging from 29% to 45%, even in settings with advanced healthcare infrastructure (100). The rapid bacterial spread and high toxicity of iGAS reflect the pathogen's diverse array of virulence factors, which enable immune evasion and extensive tissue damage. These include proteases and streptolysin toxins, such as streptococcal pyrogenic exotoxin B (SpeB) and streptolysin O (SLO), that cause cell death, superantigens that trigger a cytokine storm in STSS including streptococcal pyrogenic exotoxin A (SpeA) and streptococcal superantigen (SSA), and mechanisms that impair host defences such as neutrophilic clearance, such as the highly conserved C5a peptidase (scpA) which cleaves C5a, a component of the complement system and DNase B which degrades extracellular immune products (101-103). Together, these features make StrepA a dangerous and highly adaptive pathogen.

Although iGAS can affect individuals of any age or demographic, the highest risk groups are the very young, the elderly, and pregnant or recently postpartum individuals (puerperal sepsis) (100). In HIC, marginalised and deprived populations, such as people experiencing homelessness, prison populations and intravenous drug users, are disproportionately affected (104). These populations face barriers to timely healthcare access and are often exposed to overcrowded living conditions that facilitate bacterial transmission. These inequities highlight the need for targeted public health strategies to improve prevention and access to treatment in these vulnerable groups.

The recent resurgence of iGAS in HICs following the COVID-19 global pandemic has reignited interest in StrepA research. For example, outbreaks in the United Kingdom, Ireland, and other European nations during 2022–2023 underscored the ongoing threat posed by this pathogen (10,105,106). Factors such

as emerging hypervirulent strains, including M1UK, and potential immune shifts following the COVID-19 pandemic may have contributed to these outbreaks (14). This resurgence demonstrates that iGAS remains a critical public health concern, even in settings where the burden of other StrepA sequelae, such as RHD, is minimal.

1.3.6 Asymptomatic carriage

Most research on StrepA carriage has focused on the pharynx as the carriage site. The presence of asymptomatic StrepA carriers in the population raises difficulties in knowing whether to treat pharyngitis even when the OPS is positive (44). Identification and treatment of asymptomatic pharyngeal carriage is not recommended by Infectious Diseases Society of America (45) due to true carriers being difficult to identify, carriers being unlikely to transmit infection to others, and treatment not always being successful (107-110).

Pharyngeal StrepA carriage prevalence varies between settings and age groups with rates between 5.9% and 10.5% in children, and 2.0% and 4.6% in adults (44). Interestingly, a 2018 meta-analysis found that carriage rates were lower in children in non-OECD countries than in OECD countries, though they noted a lack of data from LMIC (44). However, two subsequent studies from Uganda have found pharyngeal carriage prevalence of 16% in children aged 5-16 years, higher than the expected rates (111,112). It is plausible therefore, that carriage rates in Gambian children may also be higher than predicted.

Despite a long history of StrepA research, surprisingly little is known about the natural history of skin carriage and its role in infection. Carriage of StrepA on normal skin was shown to be a critical step in household transmission in Native American populations in the USA in the 1970s, and that skin colonisation normally precedes pyoderma (25,27,29,31,113). However, research on StrepA carriage since has overlooked the role of skin (44,108,114,115). To our knowledge, no studies have investigated the prevalence of StrepA skin carriage in Africa.

The wide diversity of StrepA strains observed in LMIC including The Gambia (116), and the lack of tissue tropism, as observed in HIC (117-119), suggest that infection acquisition may be related to widespread carriage and transmission of StrepA within communities, rather than highly clonal single-strain outbreaks. Therefore, to develop strategies to reduce the burden of StrepA disease in settings like The Gambia, understanding the relative importance of skin and pharyngeal carriage, the typical site sequence of spread within households, and what the risk factors for carriage are in this setting is important.

1.3.7 Acute Rheumatic Fever and Rheumatic Heart Disease

Despite RHD causing most StrepA-related deaths in LMIC and a significant burden of disability-adjusted life years (DALYs) globally, it receives little research attention and funding compared to other major diseases. One study has shown that of 16 major tropical diseases RHD had the lowest research funding per DALY (12). Data on RHD disease burden in Africa is limited. One study found that prevalence of RHD in Ethiopian children is 4.6/1000, with a 12.5% annual mortality rate (120,121), whilst in Zambia prevalence was found to be 11.8/1000 (122). The INVICTUS trial followed 13,696 RHD patients across 24 LMICs, demonstrating a high mortality rate (15% at three years), primarily due to heart failure and sudden death, with limited access to life-saving valve surgery (123). Another study in Ethiopia found that subclinical RHD often persists or progressed over five years, with a higher risk among family members, yet there was poor adherence to prophylactic penicillin (124). Additional studies from South Africa, Nigeria, Malawi, and Mozambique reinforce these findings, showing that RHD remains a leading cause of cardiac morbidity, particularly in children and young adults, with substantial gaps in early detection, treatment access, and prevention efforts (125-132).

Recent work in The Gambia found that 41 of 3000 children aged 4-9 years had evidence of RHD on echocardiography [Annette Erhart *et al.,* unpublished data]. A case series of patients in The Gambia with RHD found an estimated annual case fatality rate of 19.6%, where only 48.7% had a history suggestive of ARF, 53.2% reported a history of recurrent sore throat, but only 32.2% of those had attended a healthcare facility for the complaint (133).

RHD is the result of an autoimmune response following StrepA infection, primarily affecting the heart valves. Acute Rheumatic Fever, its precursor, is a multisystem inflammatory disease typically occurring weeks after an untreated or inadequately treated StrepA infection. The clinical presentation of ARF is highly variable, but major manifestations include arthritis, carditis, chorea, erythema marginatum, and subcutaneous nodules (5). In many LMICs, arthritis and fever are the most commonly reported symptoms, yet they are often misattributed to other infections or inflammatory conditions. The long-term sequelae of ARF, particularly valvular damage leading to RHD, develop progressively and often remain undetected until the onset of symptomatic heart failure (40,126). RHD significantly impacts quality of life, disproportionately affecting young people in Africa, with most deaths occurring in early adulthood due to heart failure, stroke, or sudden cardiac death. The INVICTUS trial reported a median age of death of 28 years, with a high burden of disability before mortality, particularly among women of childbearing age due to increased cardiac stress around pregnancy and childbirth (123). Patients often experience profound limitations in daily activities, school attendance, and employment, exacerbating socioeconomic hardship (124). Stigma surrounding chronic illness, particularly in resource-limited settings, can lead to social isolation and difficulties in securing marriage or employment. Limited healthcare access further worsens outcomes, as many patients present late with severe disease, yet face significant financial and logistical barriers to accessing secondary prophylaxis, echocardiographic screening, or surgical intervention (130).

The discrepancy in ARF and RHD disease burden between high-income and low-income settings is stark. In HICs, StrepA-related mortality is largely due to invasive infections and ARF and RHD are

extremely rare, whereas in LMICs, RHD accounts for the vast majority of deaths (2,3,12,40,122,134,135). This may reflect disparities in healthcare access, timely diagnosis, and secondary prevention with penicillin prophylaxis, but it also occurs more frequently in LMIC, likely due to more frequent StrepA infections. The near-elimination of ARF in HICs has been attributed to improved living conditions, routine sore throat treatment, and secondary prevention programmes—measures that remain inaccessible in many LMICs (3,125). As a result, there is growing recognition that strengthening primary and secondary prevention of ARF is critical in reducing the global burden of RHD.

Recent findings from the Uganda GOAL trial underscore the importance of targeted screening for ARF in high-risk populations. Their studies on ARF have demonstrated that structured fever and joint pain clinics significantly improved ARF detection rates, which are typically low due to the non-specific nature of ARF symptoms and the lack of clinical suspicion among healthcare providers (136-138). This is particularly relevant for African settings, where ARF often remains undiagnosed until the development of RHD. Integrating such clinics into routine healthcare services could facilitate earlier detection, enabling prompt secondary prophylaxis and reducing disease progression.

1.3.8 Epidemiological and immunological links between StrepA and Acute Rheumatic Fever

The epidemiological connection between StrepA infections and ARF was first established through Lewis Wannamaker's team's work in the 1950s in the USA. A study of military recruits demonstrated that treatment of acute pharyngitis with penicillin significantly reduced ARF incidence from 2.1% in untreated cases to 0.3% in treated cases (26). This provided the first clear evidence that preventing or treating StrepA infections could prevent ARF.

Recent epidemiological studies have revealed that ARF pathogenesis likely requires multiple preceding StrepA exposures rather than a single infection. Serological studies examining antibody responses to type-specific StrepA antigens have shown that ARF patients typically have evidence of at least two prior StrepA infections. This has been demonstrated through both T-antigen and M-protein typing studies, with ARF cases showing broader antibody reactivity compared to uncomplicated pharyngitis cases (139,140).

The immunological basis for ARF development appears to involve both molecular mimicry and immune priming. StrepA antigens, particularly the M protein, share structural similarities with human proteins including cardiac myosin, tropomyosin, and keratin. This molecular mimicry allows antibodies generated against StrepA to cross-react with host tissues (141,142). Recent studies using multiplex antibody assays have revealed that ARF patients develop uniquely broad antibody responses compared to those with uncomplicated StrepA infections, with seropositivity to 6 or more conserved StrepA antigens being

characteristic of ARF (92). This expansive antibody repertoire likely results from repeated infections progressively boosting and broadening the immune response.

While historically ARF was thought to follow only StrepA pharyngitis, growing evidence indicates skin infections may also contribute to pathogenesis. High rates of StrepA skin infections accompanied by low rates of pharyngitis have been observed in regions with high ARF burden, such as Aboriginal communities in Australia (36,88,89). In New Zealand, skin-associated StrepA strains predominate in children with ARF, and ARF risk is elevated 5-fold in the 3-month period following either StrepA-positive throat or skin swabs (90).

Host genetic factors also influence susceptibility to ARF. HLA allele associations, particularly with HLA-DR7, have been documented in multiple populations (143,144). Polymorphisms in immune genes including those encoding inflammatory cytokines (TNF-α, IL-1, IL-6) and innate immune molecules (mannose-binding lectin) further modulate individual risk (145-147). These genetic factors likely determine which individuals develop autoimmune complications following repeated StrepA exposures (148).

The time course of ARF development, typically 2-4 weeks after StrepA infection, represents the period required for mounting pathogenic autoimmune responses (149). During this period, molecular mimicry triggers cross-reactive antibodies and T cells, while the broad antibody response suggests concurrent epitope spreading (92,141). Prevention strategies must therefore focus on interrupting this immunological cascade, either through preventing the initial StrepA infections or blocking the development of autoimmunity in susceptible individuals (37).

This updated understanding of ARF pathogenesis, incorporating evidence for multiple infection events, both throat and skin infections as triggers, and the characteristic broad immune response, has important implications for prevention strategies (36,88,92,139). Comprehensive approaches targeting both StrepA pharyngitis and skin infections will likely be required for effective ARF prevention, rather than strategies focused solely on throat infections.

1.3.9 Acute Post-Streptococcal Glomerulonephritis

Acute Post-Streptococcal Glomerulonephritis (APSGN) is an inflammatory kidney condition that arises following infection with nephritogenic strains of StrepA. Typically, APSGN manifests 1 to 2 weeks after StrepA pharyngeal infection or within 3 to 6 weeks following a skin infection like impetigo. Clinically, APSGN presents as acute nephritic syndrome, characterised by symptoms such as oedema, haematuria, hypertension, and proteinuria. Patients may notice dark, reddish-brown urine and experience swelling, particularly around the face and eyes upon waking.

Globally, APSGN remains a significant cause of acute nephritis in children, with an estimated 472,000 new cases annually, over 95% of which occur in developing countries (150). The highest incidence is observed among children aged 5 to 12 years, though older adults with chronic health conditions are also at risk. (151)

While the prognosis for children with APSGN is generally good, with most recovering fully, some may experience persistent hypertension or impaired kidney function, particularly if the diagnosis is missed, requiring ongoing medical follow-up (152). Preventative measures, including prompt and appropriate treatment of StrepA infections, are essential to reduce the incidence of APSGN. Data from Africa, where it is likely common secondary to pyoderma, are sparse.

1.4 Molecular epidemiology

1.4.1 *Emm* typing and diversity

Historically, the classification of StrepA relied on serological methods to identify different M protein types. Serological M typing involved using type-specific antisera to detect antigenic differences in the M protein on the cell surface. By using this approach, approximately 50 serotypes were identified, but the method was labour-intensive and involved production of specific antisera in rabbits and non-typeable strains were often identified due to new types lacking antisera which corresponded (153). With the development of molecular techniques, sequencing of the hypervariable *emm* gene, which encodes the M protein, has replaced serological methods as the standard serotyping method, given its much greater specificity.

Over 275 emm types are now recognised, with new *emm* types and subtypes being frequently added to the CDC library. However, there are controversies regarding the correlation between *emm* types and disease manifestations. It was previously thought that the M protein was *the* major virulence factor and determined tissue tropism of strains (154). While certain *emm* types were previously thought to be associated with invasive disease or scarlet fever, more recently, studies have shown that this does not hold true, with “invasive” *emm* types being showed to cause superficial disease, and vice versa (155,156). It is also now clear that *emm* typing is insufficient to detect transmission events in outbreaks, with significant genetic diversity within *emm* types, though may not be sufficient to properly understand StrepA transmission (157,158).

The diversity of *emm* types found in LMIC is higher than in HIC (159-164). In 2018, StrepA isolates collected in Sukuta, The Gambia, from pyoderma wounds showed a high diversity of *emm* types (46 from 107 isolates) collected from a confined geographical area (116). There are many possible factors

driving higher strain diversity, but in Brazil, a higher diversity of *emm* types was found in slums compared to neighbouring suburbs, indicating that social determinants are important (160). Poor housing conditions and overcrowding, also common in The Gambia, likely drive frequent and widespread transmission of StrepA and therefore strain diversification.

1.4.2 *Emm* clusters and tissue tropism

The *emm* cluster typing system has been developed to overcome some of the drawbacks to *emm* typing. It classifies StrepA strains into broader groups based on structural and functional similarities of the M protein rather than on sequence variation within the *emm* gene (165). This classification improves epidemiological resolution by grouping strains with shared host-binding properties and immune evasion mechanisms (166). The system defines four main cluster groups: A-C**,** D**,** E**,** and M**,** with A-C including strains commonly associated with both throat and skin infections, D clusters typically linked to invasive infections, E clusters predominantly comprising skin-tropic strains, and M clusters mostly associated with pharyngeal infections. While early studies suggested that specific *emm* types dictated tissue tropism, recent genomic and epidemiological analyses have refuted this. Whole genome sequencing studies have demonstrated significant within-cluster genetic diversity, suggesting that factors beyond the *emm* gene play a greater role in determining infection site than previously assumed (155,166). Additionally, studies in LMIC settings, where strain diversity is high, have found that the same *emm* types frequently cause both skin and throat infections, further challenging the accuracy of tissue tropism (156).

The *emm* cluster system also provides the benefit for vaccine development of allowing for selecting M proteins that are structurally representative of each *emm* cluster, which may improve the cross-protective potential of M-protein-based vaccines. By targeting conserved structural and functional features within clusters, rather than individual *emm* types, vaccine design can account for the extensive strain diversity observed in LMICs. This approach may enhance coverage across both pharyngeal and skin-associated strains, addressing a key limitation of earlier vaccine candidates that relied on traditional *emm* typing (156,166).

1.4.3 *Streptococcus dysgalactiae* subsp*. equisimilis*

A further factor potentially driving the diversity of StrepA is co-circulation with *Streptococcus dysgalactiae* subsp*. equisimilis* (SDSE; Groups C and G Streptococcus). SDSE is a closely related pathogen which shares key virulence factors and occupies the same ecological niche as StrepA, namely the skin and the pharynx (167,168). They often exhibit similar disease manifestations, particularly pharyngitis, but also invasive disease and necrotising fasciitis, though it is a less frequent cause of pyoderma (89,169,170). The relationship between SDSE and StrepA is poorly understood, but it has been shown that they can both colonise the pharynx concomitantly, and that horizonal gene transfer does occur between the two species (171,172). The significance of this is unknown but has

been suggested that co-circulation of the two pathogens, something frequently observed in high-RHD, high-StrepA diversity settings, could play a role in driving the evolution of StrepA (173-175). Though both the studies reported in this thesis did culture for SDSE in participants, these results are not presented here.

1.4.4 Whole genome sequencing

Whole genome sequencing (WGS) has significantly advanced our understanding of StrepA population structure, transmission, and genetic diversity. Compared to traditional *emm* typing, WGS offers a much higher resolution by identifying intra-*emm* type variability, distinguishing closely related strains, and detecting horizontal gene transfer events. This is particularly relevant for understanding transmission pathways, as studies in Australia have used WGS to track community spread, revealing frequent strain replacement and mixed infections (173,176).

In Africa, WGS studies remain limited but have started providing key insights. A recent study comparing Gambian isolates to UK isolates highlighted the higher genetic diversity, including strains not previously characterised in global databases and emphasised the importance of applying WGS in African settings to improve surveillance and understand the molecular epidemiology of locally circulating strains (177). Given the complex transmission dynamics in LMICs, WGS has the potential to inform targeted interventions, refine vaccine design, and guide antimicrobial resistance monitoring beyond what *emm* typing alone can achieve.

1.5 Transmission and risk factors

1.5.1 Risk factors for StrepA disease

A range of both modifiable and non-modifiable risk factors are known to exist for StrepA including pharyngitis, pyoderma and invasive disease, many of which overlap, and are more common in LMICs. Children and adolescents are known to be more susceptible to pharyngitis likely due to immunological factors and higher exposure rates in schools and other settings (42). There are also non-modifiable genetic and geographic factors that affect pharyngitis risk (194). Pyoderma is also more common in children, particularly under 5 years olds, though it is not clear if this is due to immunological factors or behavioural ones (93).

Modifiable risk factors for StrepA pharyngitis and pyoderma include overcrowding, low socio-economic status and poor access to healthcare, all of which are factors likely to increase the risk of transmission of StrepA (46). Most of these factors involve behaviours or situations in which risk of transmission of StrepA in increased due to crowding that increases person-to-person transmission, this has been noted particularly in military bases and prison populations, but also in schools, nurseries, nursing homes and

homeless shelters (195). Inadequate housing is also an important risk factor for StrepA disease with overcrowding, sharing of personal items, poor ventilation, and damp all being identified as contributing factors (160,196). Poor personal and hand hygiene, and contamination of surfaces, particularly in medical settings, has been found to increase the risk of StrepA disease with multiple outbreaks of invasive disease having been noted in hospitals due to fomite contamination (197-201). Several studies have also noted a seasonal association with StrepA pharyngitis (179,202,203).

Though most studies assessing risk factors for StrepA disease have been conducted in high-income settings, many of the important risk factors identified are prevalent in LMIC such as The Gambia, namely poor housing conditions, crowding, sharing of personal items, and poor access to hygiene facilities. The extent to which these risk factors are important for StrepA pharyngitis are unknown in The Gambia.

1.5.2 Scabies

Scabies is a WHO neglected tropical disease caused by an ectoparasitic mite, which is transmitted through close skin-to-skin contact. It affects nearly 400 million people a year globally, mainly children in LMIC (185,186). It causes a profound itch and skin lesions which impact quality of life and can lead to serious complications due to secondary bacterial infection (187,188). Superinfection of scabies lesions is an important contributing factor to the global burden of and StrepA disease (82,83,189).

Scabies presence is known to be a risk factor for StrepA pyoderma (82,83), but it is not known whether scabies infestation increases the likelihood of StrepA skin carriage. Scabies mites secrete various proteins which inhibit the complement system, creating a favourable microenvironment for StrepA and promotes growth (190,191), which may increase the likelihood of skin carriage (192).

Mass drug administration (MDA) programmes with ivermectin are highly effective at reducing scabies, pyoderma and all-cause out-patient clinic attendance (85,86,193). It is not clear whether MDA is only effective at reducing pyoderma, or whether it also has an impact on StrepA skin carriage. To better understand this, it is important to establish the relationship between scabies and StrepA skin carriage and the extent to which they are mutual risk factors.

1.5.3 Seasonal and epidemic trends in StrepA epidemiology

The epidemiology of StrepA has been seen to follow distinct seasonal epidemic patterns, likely influenced by environmental and social factors. HICs, pharyngitis caused by StrepA typically peaks during winter and early spring, possibly reflecting increased indoor crowding and school attendance during these months (178). Scarlet fever is also highly seasonality, with annual peaks typically seen in the late winter and early spring (8,179). Surveillance data from the United Kingdom indicate a marked

resurgence in scarlet fever since 2014, with significant year-on-year variation in case numbers linked to changes in circulating strains, including the emergence of the highly toxigenic M1UK lineage (8,9,97).

Studies in The Gambia have demonstrated that pyoderma peaks during the rainy season, with prevalence rates increasing significantly compared to drier months (17.4% overall prevalence, rising to 23.1% during the rainy season) (93,180). This seasonal trend likely reflects increased humidity, skin trauma from insect bites, and crowding in poorly ventilated housing during the rains. Similar trends have been observed in other African countries, with studies in Guinea-Bissau and Malawi reporting higher rates of pyoderma during periods of high rainfall (181,182).

In addition to seasonality, StrepA infections exhibit cyclical patterns of epidemic activity, with periodic surges in scarlet fever and invasive StrepA (iGAS) cases occurring every few years. These cycles are thought to result from fluctuations in population-level immunity, the emergence of new virulent strains, and changing environmental factors. For example, the UK's recent scarlet fever outbreaks have been attributed to increased circulation of M1UK, which produces higher levels of streptococcal pyrogenic exotoxins, driving increased transmissibility and disease severity (9).

In LMICs, where surveillance is limited, data on cyclical trends remain sparse. However, historical studies in Native American populations have shown that seasonal and cyclical patterns often intersect, with skin infections acting as a reservoir for StrepA transmission and subsequent outbreaks of post-streptococcal glomerulonephritis (35). The extent to which these dynamics apply to African settings remains underexplored, but available evidence suggests that the rainy season's environmental conditions play a significant role in amplifying StrepA transmission.

1.5.4 Transmission dynamics

StrepA is a highly adapted to the human host, as evidenced by the wide range of body sites affected, the ubiquity of the pathogen globally, and the vast array of adhesins and virulence factors specialised in binding to various human cell types (2,5,102,207). Human-to-human transmission therefore involves many different pathways and mechanisms that make the investigation of StrepA transmission complex (102). Furthermore, as well as host and bacterial factors affecting transmission, such as age, immunity, genetics and virulence, environmental factors such as humidity have been shown to affect bacterial longevity and viability in the environment, potentially affecting transmission (208). However, it is primarily transmitted through direct person-to-person contact, especially via respiratory droplets from infected individuals. Close household contacts of patients with iGAS infections have a significantly increased risk of developing invasive disease themselves (209). This elevated risk underscores the importance of understanding transmission dynamics within households. Asymptomatic carriers can also contribute to the spread of StrepA (95,99,171,176). Studies have identified individuals, particularly

children, who shed the bacteria heavily without showing symptoms, potentially acting as super-spreaders and sustaining outbreaks in community and educational settings (99).

Evidence from high-income settings has highlighted the complexity of transmission during outbreaks, particularly in healthcare settings where asymptomatic carriage in the throat and on the skin, as well as on fomites in the environment such as shower heads, curtains and children's toys have been identified as potential transmission sources (99,210,211). In Aboriginal Australian communities, it has been shown that in spite of historical assumptions that certain StrepA *emm* types exclusively infection either throat or skin, that bidirectional transmission occurs between throat and skin, leading to infection (176). Socio-demographic risk factors such as income, housing and exposure to skin injuries have also been shown to increase the risk of StrepA disease, with evidence suggesting that overcrowding, dampness, homelessness, sharing of belongings can increase disease risk (95,196,212). Several studies have also found a seasonal variation in StrepA disease (93,179,202).

It is now recognised that multiple *emm* type carriage does occur, particularly in endemic settings where exposure to diverse strains is frequent (171,173). This has implications for both disease surveillance and vaccine development, as co-carriage could enable genetic exchange between strains and complicate the effectiveness of type-specific interventions. These findings highlight the need for transmission models that consider heterogeneity in host infectiousness and strain interactions, rather than assuming uniform spread within populations.

Despite substantial research into StrepA transmission in high-income settings, there is a paucity of studies from Africa. In settings such as The Gambia, where StrepA skin infections are common in children, overcrowded housing and poverty are widespread, and the urban environment and the ubiquity of biting insects lead to frequent skin injuries, transmission of StrepA has not been studied, and the relative importance of different risk factors is not understood. This lack of knowledge is a barrier to the design of appropriate public health strategies aimed to prevent StrepA disease and transmission.

1.6 Vaccines and surveillance

1.6.1 Vaccine development

In 2018 the World Health Assembly voted through a resolution recognising that RHD is a significant, preventable cause of morbidity and mortality especially affecting vulnerable and poor populations, and that RHD and GAS research should be a global priority (204). The WHO then published a Group A *Streptococcus* Vaccine Development Technology Roadmap in 2018 highlighting key strategic areas for research including to improve global estimates of disease burden and epidemiology of GAS infections, and to further describe the spectrum of natural disease history (37,38).

Vaccine development has focused on two broad strategies: M-protein-based vaccines and conserved antigen vaccines. M-protein vaccines aim to induce immunity against the highly variable M-protein, while conserved antigen approaches target proteins shared across multiple strains. The M-protein is the most studied vaccine target due to its role in bacterial adhesion, immune evasion, and opsonophagocytic killing (21). Multivalent M-protein vaccines, such as StreptAnova, contain N-terminal peptide fragments from multiple *emm* types. The 30-valent StreptAnova vaccine, which underwent Phase I clinical trials in 2020, demonstrated safety, immunogenicity, and opsonophagocytic activity against vaccine-covered *emm* types (213). However, the high *emm* type diversity in LMICs presents a challenge, as coverage models based on HIC molecular epidemiology may have low coverage in LMICs (22). Other M-protein approaches focus on conserved C-repeat epitopes, such as StrepInCor, which contains a 55-amino acid peptide from the M-protein C-terminal region. This vaccine has shown high immunogenicity in preclinical studies without inducing autoimmunity and is expected to enter clinical trials soon (213,214).

To avoid the issue of *emm* type diversity, other vaccine candidates target conserved proteins essential for StrepA survival and virulence. These include SpyCEP (cell envelope proteinase), C5a peptidase, streptolysin O (SLO), and the group A carbohydrate (GAC). The Spy7 vaccine, which includes seven conserved antigens, has shown broad strain coverage and significant protection in murine models (37). Another promising candidate is the GAC-conjugate vaccine, which targets the universally expressed group A carbohydrate, an essential component of the cell wall. The GAC-conjugate has been shown to induce opsonophagocytic immunity without triggering autoimmunity (213,215). Additionally, novel peptide-based vaccines are emerging, such as J8, a modified peptide epitope from the M-protein C-repeat region, is currently in clinical trials (213).

The establishment of human challenge models is a recent breakthrough that could accelerate vaccine testing by allowing direct assessment of protection against pharyngitis (183,216). Additionally, mRNA-based vaccine approaches, similar to those developed for SARS-CoV-2, are being explored in collaboration with industry partners such as Moderna (213). The diversification of StrepA vaccine candidates represents a major step forward, with multiple clinical trials underway and increasing global investment to overcome remaining barriers (22).

1.6.2 StrepA surveillance

Surveillance programmes for StrepA carriage and disease are crucial to understand the clinical and molecular epidemiology and burden of disease as well as monitoring variation over time. For StrepA this has greater significance due to the downstream immune sequelae of StrepA disease which cause the largest burden of mortality from StrepA. In many high-income countries, routine monitoring of StrepA disease exists, including certain conditions being notifiable, such as scarlet fever and invasive StrepA disease in the UK (98,105). Routine monitoring of superficial StrepA infections (pharyngitis and

pyoderma) is usually less formal though, relying on ad hoc swab collection by general practitioners. In LMIC such monitoring systems are rare, and global burden of disease studies frequently highlight a lack of data from LMIC and Africa particularly (1-3,40,81,84).

The AFROStrep study has attempted to improve StrepA surveillance in Africa by establishing active case surveillance of StrepA pharyngitis and passive surveillance of invasive StrepA disease from sentinel sites across Africa (7). Thus far no data have been published from the study, though data are expected soon from Namibia and Ethiopia, and a key limitation is that it will not collect samples from pyoderma cases, a major source of StrepA an Africa. However, isolates from the surveillance platform have been sequenced and used to assess molecular epidemiology of StrepA in South Africa, which has contributed valuable data to inform vaccine development (217). The establishment of surveillance platforms such as AFROStrep is vital not only to provide valuable data on epidemiology, outbreaks, circulating serotypes and new sequence data, but also because it provides a base to build more research upon, bringing in researchers to the StrepA research field by providing opportunities. Building research interest and centres of StrepA and RHD research in Africa is necessary to work towards reducing the burden of RHD globally (37,38).

Aside from AFROStrep, longitudinal surveillance studies have been conducted around the world investigating StrepA over time, though their number is few. Notable examples are from Australia, New Zealand, Fiji, USA, UK and Egypt (25,29,32,89,99,108,169,176,189,218-220). To our knowledge none have been performed in sub-Saharan Africa before, and none most have not attempted to capture both pharyngeal carriage and infections as well as skin carriage and pyoderma to assess incidence and examine transmission relationship between these events. Apart from the important data that longitudinal studies can provide on the epidemiology and transmission of StrepA, they can provide data on risk factors and immunological responses to natural infection and carriage events.

The Strep A Vaccine Global Consortium (SAVAC), established to facilitate progress towards StrepA vaccines, has been key in promoting the importance of surveillance studies. Recognising the significant global burden of StrepA disease, especially in LMICs, SAVAC has done work to help establish robust surveillance systems to gather comprehensive epidemiological and health economic data. To this end a series of case definitions were published to harmonise surveillance methods globally (Table 1.4) (221-227).

Table 1.5. Summary of SAVAC StrepA case definitions.

Condition	Case Definition
Pharyngitis (nonspecific)	Acute illness in a person with the complaint of sore throat or signs of pharyngitis (e.g., erythema of pharynx and tonsils, patchy discrete exudate, and/or tender, enlarged anterior cervical nodes)
StrepA Pharyngitis	Acute clinical illness in a person (with the complaint of sore throat or clinical signs of pharyngitis) plus microbiological confirmation of StrepA in the oropharynx

Impetigo (nonspecific)	○ Clinical bullous impetigo - presence of ≥1 skin sore, defined as a large fluid-filled blister of 1–2 cm, usually in areas with skin folds such as the armpit, groin, between the fingers or toes, beneath the breast, and between the buttocks. ○ Clinical non-bullous impetigo - presence of ≥1 skin sore, defined as a round papular, pustular, or ulcerative lesion of 1–2 cm.
StrepA Impetigo	Clinical nonbullous impetigo (as defined above) with the isolation of StrepA in culture from an active impetigo lesion
Cellulitis	Hot (local warmth), erythematous (red), swollen and tender skin, for which other causes of erythema and tender inflamed skin (e.g., deep vein thrombosis, acute lipodermatosclerosis) have been excluded
Strep A cellulitis	Clinical cellulitis with laboratory-confirmation of StrepA as the etiology by one of the following: ○ StrepA isolated from culture obtained from the affected site or blood culture ○ A positive StrepA antibody detection test defined as either: ▪ A 2-fold or greater rise in antistreptolysin O (ASO) or anti-deoxyribonuclease B (ADB) titer in specimens collected at least 2 weeks apart (and preferably 4 weeks apart), with the first sample taken within 1 week of symptom onset OR ▪ A single sample taken at least 2 weeks after the onset of cellulitis that is above the upper limit of normal

In 2023, SAVAC, working with the International Vaccine Institute in Korea, identified funding to support four sentinel sites in LMICs for conducting detailed surveillance of StrepA diseases in both community and hospital settings. These sites will set up community and hospital surveillance for StrepA pharyngitis, pyoderma, cellulitis, ARF, RHD, APSGN, and iGAS based on the case definitions. Two of these sites will be helped to capacity build and prepare to be future vaccine trials. This aims to fill critical data gaps but also seeks to build research capacity in regions disproportionately affected by StrepA and RHD, whilst accelerating the development and implementation of effective vaccines. The four sites selected were India, Fiji, Malawi and the MRC Unit The Gambia.

1.7 Summary overview

Despite its long history, StrepA remains a major global cause of morbidity and mortality, with significant disparities between high-income and low-income settings. While invasive infections drive most StrepA-related deaths in high-income countries, the largest burden in LMICs is caused by RHD. The persistence of RHD as a leading cause of mortality in LMICs due to a failure in global health strategies and research to address StrepA disease. Data and isolates from LMIC are lacking, and surveillance systems are rare and immature. This lack of data has hindered the development of effective interventions tailored to high-burden settings that need them the most.

The transmission dynamics of StrepA are complex, involving multiple routes of infection, including respiratory and skin-to-skin contact, and from symptomatic and asymptomatic infections. Most studies on StrepA transmission and outbreaks have occurred in HIC where skin infections are rare, and strain diversity is lower, leaving little known about community transmission in LMICs. Studies in Aboriginal

Australian populations and elsewhere indicate that StrepA is not tissue-tropic in high-burden settings, with bidirectional transmission between throat and skin. But, the relative contributions of different transmission pathways are poorly understood, particularly in Africa. Additionally, the role of asymptomatic carriage in sustaining transmission remains uncertain, with conflicting evidence on the infectiousness of carriers. Furthermore, while pharyngeal infections were historically thought to be the primary driver of ARF and RHD, accumulating evidence suggests that skin infections may play an important role, particularly in endemic settings where pyoderma is common, though high-quality incidence data on pyoderma are rare.

Molecular epidemiology has the potential to enhance understanding of StrepA transmission, particularly through *emm* typing, which, while providing less resolution than WGS, remains a practical and scalable tool. In high-burden settings where multiple *emm* types are circulating, investigating transmission through shared *emm* types within households could provide valuable insights into strain persistence and spread. This approach has never been applied in Africa, despite the high strain diversity and StrepA burden.

The lack of robust surveillance and longitudinal epidemiological data in Africa presents a critical gap in understanding StrepA disease burden, risk factors, transmission, and natural immunity. This has direct implications for the design and use of different diagnostic tools, infection control measures, and future vaccine trials. The absence of detailed transmission data also limits the ability to develop targeted interventions that could interrupt the cycle of recurrent infections driving RHD in endemic regions. Addressing these knowledge gaps requires well-designed studies capable of capturing both the clinical and subclinical epidemiology of StrepA in high-burden settings.

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2 Chapter 2: Overview of rationale, PhD objectives, setting and study design

2.1 Project summary

Streptococcus pyogenes (StrepA) is responsible for a major disease burden globally causing a wide spectrum of disease from superficial infections of the pharynx and skin through to invasive disease (1). The greatest morbidity and mortality from StrepA disease globally results from the post-infection immune sequelae: acute rheumatic fever (ARF), rheumatic heart disease (RHD), and acute-post-streptococcal glomerulonephritis (APSGN). These disproportionately occur in low- and middle-income countries (LMIC) and within high-income countries (HIC) are confined to the most deprived communities (2-6).

The pathological immune manifestations of StrepA disease are preceded by common superficial StrepA infections, namely pyoderma (purulent or crusted infection of the skin) and pharyngitis (7). Additionally, StrepA can asymptomatically colonise normal skin and the pharynx (carriage), which may precede infection (8). Despite a history of StrepA research spanning more than a hundred years, surprisingly little is known about the natural disease course of superficial StrepA infections or the extent to which StrepA infections are influenced or preceded by asymptomatic carriage (9,10). Furthermore, most StrepA research has been conducted in HIC where the mortality from RHD has steadily declined for more that 150 years, accelerated by, but not initiated by the discovery of penicillin (11). Over 95% of global cases of ARF, APSGN and invasive infections now occur in low-resource settings, yet StrepA research from such settings is limited (1). The WHO therefore stressed, in a 2005 report, that StrepA research in sub-Saharan Africa, and other high-burden regions, should be prioritised, and that efforts should be made to establish surveillance systems (12). Some efforts have been made to establish African StrepA surveillance, such as AFROStrep, but there are large gaps (13). No StrepA surveillance system yet exists in The Gambia, and with StrepA vaccines in the development pipeline, working towards establishing a surveillance system is a priority (14). Yet questions remain around the appropriate design of such systems, such as which age groups and settings to target, what diagnostic tools are suitable, and the extent to which transmission dynamics and asymptomatic carriage should be incorporated (15).

To this end, this PhD aimed to address several questions related to StrepA surveillance and diagnostics in a high-RHD setting: The Gambia. It aimed to provide data on the incidence of StrepA carriage and infection for the first time in Africa, to investigate the interaction between skin and pharyngeal carriage and infection, to describe how StrepA is acquired and transmitted between individuals within their families and households, and to explore what risk factors exist for StrepA carriage and infection.

Furthermore, it aimed to assess a range of diagnostic approaches for StrepA disease in this setting to inform strategies to improve StrepA disease management, and the design of future StrepA surveillance programmes. These aims will be addressed through two observational field studies conducted between 2021 and 2022, both in Sukuta, The Gambia. The first, a cross-sectional diagnostic accuracy study for the StrepA pharyngitis, and the second, a longitudinal household cohort study over one year to investigate StrepA incidence, transmission and risk factors.

2.2 Rationale and knowledge gaps

Despite the significant burden of disease caused by StrepA in LMIC, efforts towards prevention and treatment of StrepA disease are lacking. In HIC, superficial StrepA infections such as pharyngitis and pyoderma are typically diagnosed early and treated with antibiotics, within well-developed primary healthcare systems, thus preventing RHD. In low-resource settings, where living conditions and environment often put people at higher risk of StrepA infections, and primary healthcare systems are under-resourced, a different model of care is required to tackle the burden of StrepA disease and its sequelae.

One way to improve the management of StrepA pharyngitis in settings such as The Gambia within the existing primary healthcare structures would be to introduce an easy-to-use, point-of-care algorithm involving a clinical score and/or a rapid diagnostic test. However, the existing scoring systems and tests are not well validated in low-income settings, and to the best of our knowledge, none have been used before in The Gambia.

Other approaches to tackle the burden of StrepA disease might include widespread public health interventions such as vaccination programmes (though no StrepA vaccines are yet licenced), mass drug administration (MDA) or improvements to water access, sanitation and hygiene (WASH). Such approaches could be effective in settings such as The Gambia, however without a better understanding of the natural history of StrepA infection, the relationship with carriage and factors affecting transmission within households, it is not clear which approach would be most effective, or in what manner these interventions should be used.

Very few longitudinal cohort studies of StrepA with active case finding have been conducted (8,16). A longitudinal study from a high-StrepA and RHD burden country in sub-Saharan Africa that combines clinical epidemiology with detailed socio-demographic and hygiene survey data, and *emm* typing to investigate carriage and disease transmission would provide vital, novel data to help meet global strategic StrepA research goals (14,17).

2.3 PhD aims and objectives

2.3.1 Overall research question and hypothesis

This PhD seeks to describe the epidemiology, carriage, and transmission of StrepA in a high-burden setting in sub-Saharan Africa, where data are severely limited. The central research question is: What are the epidemiological patterns of StrepA carriage and infection, and what are the main drivers of household transmission in The Gambia? The main hypothesis is that skin carriage and infection are underappreciated and play a more significant role in StrepA transmission and subsequent disease burden than pharyngeal carriage and infection, as seen in other high-RHD settings.

2.3.2 Aims

1. To investigate the incidence and seasonality of StrepA pyoderma and pharyngitis, and the proportion of clinical infections caused by StrepA in The Gambia (chapters 3, 5 and 6).
2. To investigate the monthly prevalence, incidence, persistence and seasonality of asymptomatic StrepA skin and pharyngeal carriage in The Gambia (chapters 5 and 6).
3. To investigate the relationship between StrepA carriage and infection and to determine socio-demographic and hygiene-related risk factors for StrepA carriage and infection within households (chapters 3, 5, 6 and 7).
4. To evaluate different clinical scoring systems and point-of-care tests for the diagnosis of StrepA pharyngitis in Gambian children (chapter 3).
5. To investigate transmission within households using data on the time of infection and carriage acquisition and *emm* type of isolates (chapters 5 and 6).
6. To compare surveillance study methodologies and diagnostic methods for StrepA to inform and optimise surveillance study design for the future (chapter 6).

2.3.3 Specific objectives

1. Measure the incidence rates of StrepA pyoderma and pharyngitis in a household cohort and determine seasonal variation over a one-year period (chapters 4 and 5).
2. Determine the proportion of pharyngitis and pyoderma clinical presentations that were positive for StrepA by culture and PCR (chapters 3, 5 and 6).
3. Determine the monthly prevalence, incidence, and persistence of asymptomatic StrepA pharyngeal and skin carriage over a one-year period (chapters 5 and 6).
4. Identify socio-demographic and hygiene related risk factors for StrepA carriage and infection events risk factors using time-to-event regression analysis (chapters 3, 5, 6 and 7).
5. Compare the diagnostic accuracy of five clinical decision rules and two rapid point-of-care tests for diagnosing StrepA pharyngitis against culture and PCR as reference standards (chapter 3).

6. Utilise data on StrepA events and *emm* types within households to map transmission pathways to identify directionality of transmission, index event type and calculate household secondary attack rates (chapter 5 and 6).
7. Compare weekly versus monthly visits and PCR versus culture for the detection of StrepA events in surveillance studies to make recommendations for future research (chapter 6).

These aims and objectives will be met through two observational studies to be carried out in Sukuta, The Gambia. The first study, PharynGAS, will seek to address the knowledge gaps around diagnosis of StrepA pharyngitis in children presenting to primary healthcare settings in The Gambia with signs and symptoms of acute pharyngitis.

The second study, SpyCATS, a longitudinal household cohort study conducted over 13 months, will seek to understand the temporal relationship between StrepA carriage and disease, how it is spread from person to person, (via skin or pharynx, acquired inside or outside households), and what risk factors for carriage and disease exist.

2.4 Research setting

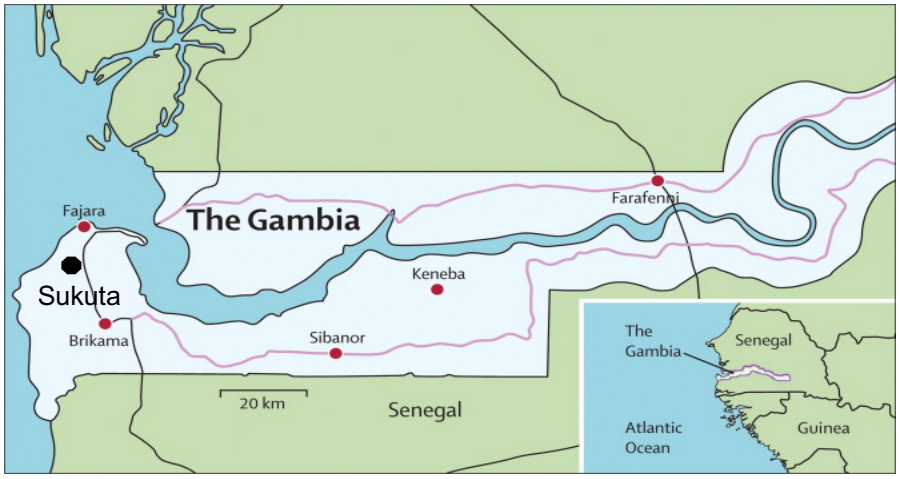

Figure 2.1. Location of Sukuta within The Gambia

2.4.1 The Gambia

The Gambia is a small country in West Africa with a population of approximately 2.2 million people. It was ranked 174th by the United Nations Human Development Index in 2022, making it one of the least developed countries in the world. It is a predominantly Muslim country, comprising several ethnic groups, the largest being Mandinka, Wolof, Fula and Jola.

Key health indicators reflect significant public health challenges. The infant mortality rate is 34 deaths per 1,000 live births, while the under-five mortality rate is 54 deaths per 1,000 live births. Neonatal

mortality accounts for 22 deaths per 1,000 live births, and the maternal mortality ratio is estimated at 433 deaths per 100,000 live births. Malnutrition remains a major concern, with 12% of children under five classified as wasted and 26% as stunted. Access to healthcare varies widely, with 57.2% of births attended by skilled health personnel. Immunisation coverage is relatively high, with 88% of one-year-old children receiving the measles vaccine. However, disparities exist in sanitation access, with 50.4% of urban populations having improved sanitation compared to just 29.0% in rural areas. (18)

The climate is sub-tropical with a long dry season from November to May, and a short rainy season between June and October each year. Seasonal variations significantly influence disease patterns, including those caused by StrepA.

2.4.2 Sukuta

Sukuta is an urban area within the Brikama Local Government Area, forming part of the broader coastal conurbation known as the Kombos, where most of the Gambian population live (see Figure 2.1). It is a majority Mandinka area, with a population of 47,048, and an average household size of 8.1 at the census in 2013, though has experienced rapid growth in the last 10 years reflecting broader urbanisation trends in The Gambia. Given the concentration of urban settlements along the Atlantic coastline, Sukuta is reasonably representative of the broader urban population in the country.

The selection of Sukuta as the primary study site was based on several practical and scientific considerations. Firstly, the area has been the site of previous epidemiological research, including the SpyDERM study, which investigated the prevalence of skin infections, including StrepA pyoderma and scabies. The presence of the Medical Research Council Unit The Gambia (MRCG) at Faraja within a reasonable distance allowed for easy access to laboratory and clinical facilities there. Furthermore, the Sukuta Health Centre serves as a primary point of care for the local population, ensuring a well-defined healthcare interface for the studies. MRCG also has a long-established satellite unit within Sukuta Health Centre, and has close links and good relationship with the community including the Alkalo (local chief) of Sukuta.

Sukuta is a majority Mandinka area, which broadly aligns with national demographics where Mandinka is the largest ethnic group. The choice to measure ethnicity in the studies was based on the need to contextualise potential differences in health-seeking behaviours, socioeconomic factors, and historical community structures. However, there is little evidence to suggest that major differences exist in the epidemiology of StrepA disease across the main ethnic groups in The Gambia. Some ethnic groups, such as the Serehule, have historically had lower socioeconomic status, which may influence access to healthcare and associated health outcomes. Nevertheless, the interplay between ethnicity, culture, and health is complex and cannot be fully captured through broad categorisations.

The climate in Sukuta follows the national pattern, with a long dry season from November to May and a shorter rainy season from June to October. Seasonal variation in infectious diseases is well-documented in The Gambia, with conditions such as pyoderma showing increased prevalence during the rainy season (19,20). This PhD seeks to investigate the epidemiology of StrepA in an urban West African setting with well-defined seasonal and demographic characteristics. Due to practical constraints, a single study site was selected, and Sukuta was chosen as it provided a representative urban population while ensuring feasibility in terms of logistics, community engagement, and access to healthcare infrastructure.

2.5 PhD studies

The PhD aims and objectives were addressed through two observational field studies conducted in The Gambia: PharynGAS and SpyCATS. An overview of the conception, implementation and my involvement in the studies is described below. More detailed introductions and methodologies can be found in the subsequent chapters.

2.5.1 PharynGAS

2.5.1.1 Overview

The PharynGAS study was a cross-sectional diagnostic accuracy study which aimed to recruit 385 children under 16 years of age presenting to Sukuta Health Centre's outpatient department with signs and symptoms of acute pharyngitis. It was carried out between June 2021 and September 2022. The study aimed to describe the proportion of participants with StrepA pharyngitis and to assess the diagnostic accuracy of five different clinical decision rules (CDRs) and two rapid point-of-care-tests for the diagnosis of StrepA pharyngitis. The reference standard for assessing diagnostic accuracy was microbiological culture primarily, though we later included PCR as a second reference standard.

2.5.1.2 Concept, funding and implementation

The PharynGAS study was originally conceived of by myself, Michael Marks and Thushan de Silva as a study to follow on from the SpyDERM cross-sectional skin infection prevalence study that I carried out in The Gambia in 2018 (19). While SpyDERM had provided useful data on StrepA skin disease burden, little was known about StrepA pharyngitis in The Gambia. We felt that anecdotally the presentation rate of children with clinical pharyngitis seemed relatively low compared to high-income settings, which we wanted to investigate further, but specifically we wanted to assess the proportion of clinical pharyngitis caused by StrepA in a primary healthcare setting, and to investigate the use of different diagnostic methods used in high-income settings, such as clinical decision rules and rapid point-of-care tests, in the Gambian setting.

The funding was obtained from an LSHTM internal call for the Wellcome Trust-funded Institutional Strategic Support Fund designed to support the development and consolidation of collaborations between LSHTM faculty and the MRC Units staff. We were successfully awarded a small project grant to carry out the study, which was originally a two-stage study involving both clinic-based pharyngeal sampling, as well as home-visits to swab family members of StrepA positive participants to investigate household transmission. The planned start date for the grant was October 2019. At the time I was an Academic Clinical Fellow at the University of Warwick and had planned to spend an academic block in The Gambia as the local principal investigator (PI). However, due to the start of the COVID-19 pandemic around that time, research activities at the MRC Unit The Gambia were suspended, so we had to delay the study start. Due to family reasons, I also took a 6-month career break from my position around that time while my wife gave birth to our twins. I was then awarded the Wellcome Clinical PhD Fellowship in April 2020, but deferred my start until 1st January 2021 due to the career break. We made the decision to delay the start of PharynGAS until I was on my PhD fellowship which I planned to spend in The Gambia full-time. I would then run the study alongside the other PhD study during 2021. We therefore applied for a no-cost extension which was granted. However, as we had hired some field staff just prior to the suspension of research activities at MRCG who were not able to be taken on by other projects, the overall budget to complete the study was reduced. We therefore decided that it was not feasible to do the household follow-up part of the study as well as the clinic-based part. As the SpyCATS study was now planned and funded as well (see below), and would include more detailed investigation of household transmission, we decided to only carry out the clinic-based part of PharynGAS.

As we had planned to include an assessment of the use of POCTs in this study, Michael Marks reached out the Abbott (formerly Alere) to enquire if they would be interested in donating some rapid antigen detection tests for use in the study. They did agree to donate their SD Bioline Strep A rapid antigen detection test kits, but also offered to donate an ID NOW™ machine and Strep A 2 detection kits. We had originally only planned to use one lateral flow RADT at the clinic site, but agreed to include the ID NOW as well, although due to the need for a sterile, air-conditioned environment, the ID NOW machine had to be installed in the laboratory at MRC Fajara, not in the clinic at Sukuta.

The original study design included only culture for use as the reference standard, but in other parallel work, we had transferred a PCR assay for StrepA to the MRCG from the University of Sheffield, which we felt would greatly add to the PharynGAS results. We decided therefore to perform the PCR on the samples as a secondary reference standard to compare the rapid tests to, to add to the strength of the study results.

2.5.1.3 My involvement and role

With Michael Marks and Thushan de Silva not based in The Gambia, I took on the role of local PI for the PharynGAS study. In this role, I presented the study to the MRCG scientific coordinating committee and led the project setup in Gambia. We had to present a letter and meet with the director of health

services at the Ministry of Health, who approved the study but directed us to meet with the regional health directorate. I was therefore invited to present the study to the regional health committee, where there were questions regarding what benefit to the health service the study would be able to bring. In order to address these questions, I worked together with the Officer in Charge (OIC) of Sukuta Health Centre, where the study was based, to deliver training on pharyngitis management and StrepA to the nurses and doctors working there.

I worked with the project's research support officer to recruit a nurse and a field worker for the field team and a scientific officer for the laboratory to work on the project. Meanwhile I wrote a series of study specific procedures (SSPs) for the delivery of the study covering clinic activities, sensitisation, informed consent, pharyngeal examination and oropharyngeal swab collection, use of the SD Bioline test, and measurement of vitals and anthropometry. I also supervised the scientific officer to write the laboratory procedures SSP covering sample reception, microbiology procedures and use of the ID NOW machine. The scientific officer and I underwent training by Abbott on the use of the ID NOW. I organised and delivered training for the field team on the implementation of the SSPs and study procedures. During this time, I also designed the case report form (CRF) for the data collection in REDCap. This involved writing all the questions for capturing the necessary data on socio-demographics, clinical features included in the five clinical decision rules, all the necessary clinical examination findings, the lateral flow test result, and various other information we captured including social mixing behaviours, risk factors and health seeking behaviours. I wrote the CRFs to include branching logic and up-front data quality controls such as ranges and sense checks to ensure data quality. The branching logic enables relevant questions to appear only when necessary, allowing more precise and efficient data collection. The CRF was then verified and approved by an MRCG data manager, who then loaded it into a REDCap project. I then loaded the project CRF onto table computers for offline data collection and tested them. After CRF testing, I then trained the study nurse on the completion of the CRF.

Upon study initiation I oversaw the field work to ensure the smooth running of the study, accurate data collection and to field any clinical queries. I remained on call for any clinical questions that arose for the duration of the study. I performed all of the data analysis and authored the manuscript (chapter 3).

2.5.2 SpyCATS

2.5.2.1 Overview

The SpyCATS study was a longitudinal household cohort study conducted over 13 months between July 2021 and September 2022. The study aimed to recruit 450 participants from 45 households, each of which was visited every month for 12 months following the enrolment visit. All participating household members underwent pharyngeal and normal skin swabbing at each monthly visit to detect asymptomatic StrepA carriage, and participants at any time complaining of symptoms consistent with pharyngitis or pyoderma underwent pharyngeal or wound swabbing to detect StrepA infection events. Clinical, socio-demographic and social mixing data were collected at each visit. Additional blood serum,

dried blood spot and salivary samples were collected at each visit to investigate immunological response to StrepA events (not reported here). Swabs were cultured and StrepA identified by latex testing. Isolates underwent PCR-based *emm* typing. PCR for StrepA was also retrospectively performed on clinical event episodes. The study aimed to investigate the epidemiology and seasonality of StrepA carriage and infection events, the household transmission of StrepA and risk factors for StrepA events. Additionally, intensive weekly visits in a sub-group and PCR of clinical episodes aimed to compare different methodologies for StrepA surveillance to inform future programme design.

2.5.2.2 Concept, funding and implementation

Following the SpyDERM study in 2018, it was clear that there was a significant and under-appreciated burden of StrepA disease in The Gambia, but several additional questions arose (19). Firstly, as a cross-sectional study design, SpyDERM was only able to provide point prevalence data, though it did suggest a seasonal trend. I started to therefore consider the feasibility of a longitudinal study design to give a better indication of disease incidence over time, and to examine the seasonal trend in more detail. Secondly, SpyDERM only enrolled children under 5 years old, so lacked any data on other age groups. As the largest burden of skin infections occurs in under 5s, but the typical age peak of pharyngitis is 6-12 years, I started to wonder about the link between these two, where the transmission occurs and what might be driving it. The household seemed a sensible place to start to investigate transmission, as the place where children under 5 and older children would usually mix. Thirdly, we saw from *emm* typing the isolates from SpyDERM that there was a remarkable level of diversity of *emm* types within a very small geographical area. This raised further questions about transmission, as a large number of serotypes circulating suggests a wide amount disease and transmission occurring, rather than the single strain outbreak patterns seen in high-income settings (21). Fourthly, the role of asymptomatic carriage in transmission seemed to be a question never before investigated in Africa. There seemed to be surprisingly little data on pharyngeal carriage, and the extant data was mostly from high-income settings, so gaining a better understanding the role of pharyngeal carriage in transmission in a high-burden, high-diversity setting appeared to be useful. But additionally, I was interested in the role of skin carriage in this setting, which had been neglected in research for decades, though had been previously shown to be an important factor in transmission and development of pyoderma. I felt that a longitudinal study to investigate the incidence of StrepA skin and pharyngeal carriage and disease would provide novel insights into StrepA epidemiology and transmission in Africa and offer many opportunities for additional questions to be studied, including investigating immunology around naturally occurring events, the incidence of other common pathogens, and risk factors for StrepA events. The study design would incorporate regular scheduled visits designed to detect asymptomatic carriage by swabbing asymptomatic participants, as well as having a system for detecting symptomatic events by active case findings.

In the preliminary planning stages for the study, I collaborated with Michael Marks and Thushan de Silva to flesh out the study visit schedule and additional samples to include. We also brought in Adam

Kucharski as we felt the study would also provide valuable data for mathematical modelling of StrepA transmission.

The original study design was built around the budgetary envelope for my application to the Wellcome Trust Clinical PhD Fellowship. To detect asymptomatic carriage, I had originally proposed to conduct scheduled visits every 3 months, however after a discussion with a group of StrepA researchers and modellers working in Australia, we adjusted the design to have monthly visits as it was felt that quarterly visits would miss most carriage episodes.

After my PhD Fellowship had been awarded, but before submitting the protocol for the MRCG Scientific Coordinating Committee and Ethics Committee, we then entered discussions to include the collection of samples to investigate immunological responses to StrepA events in the study design. Dr Alex Keeley was applying for the Wellcome Trust Clinical PhD Fellowship the following year to me and built his proposal around the collection and use of those blood and salivary samples to investigate serological responses to naturally occurring StrepA events. His proposal was also successful, which allowed for the expansion of the SpyCATS study to also investigate this immunology. This required the restructuring of my original budget to reallocate funding originally planned for whole genome sequencing to be used for the up-front sample collection instead. Funding for *emm* typing and sequencing (still in progress) was found subsequently from other sources.

Additional research questions that arose in the planning stage around carriage duration and whether monthly visits would be frequent enough to detect events led to the inclusion of two sub-studies to investigate these further. We therefore included sub-groups who were swabbed weekly to answer these questions further. We also included the possibility of use of PCR for StrepA on the samples as we felt this would be preferable to culture for sensitivity, though originally, we had not budgeted for this. The study also included the collection of in-depth social mixing and household contact data to be used in the calibration of the mathematical modelling to be performed.

During the study, I was also awarded a small grant from the Chadwick Trust to perform a nested cross-sectional survey to investigate water access, sanitation and hygiene-related risk factors for StrepA (chapter 7).

2.5.2.3 My involvement and role

As the PI for SpyCATS I oversaw every stage of the project from conception to data analysis. I acquired the funding, wrote the protocol for ethics approval, wrote the required SSPs for the field work, recruited and trained a lab and field team to conduct the study, handled procurement, wrote the CRFs and designed the database, oversaw the field work and clinical care of the participants, verified and analysed the clinical and lab data, and authored the two manuscripts (chapters 4 and 5) and two results chapters (chapter 6 and 7) included in this thesis.

During the study Dr Gabrielle de Crombrugghe, a paediatrician and PhD student, came to be based at the MRCG to collaborate. She came around the halfway point of the field work and assisted in the supervision of the field team and led the clinical care of participants for the second half of the study. Her PhD will also be derived from the study, investigating serological responses to M and M-like proteins. She also acquired funding to carry out the *emm* typing of the study isolates, which she performed in Brussels.

2.5.3 Fieldwork protocols

2.5.3.1 Sensitisation

A process of community and individual sensitisation was conducted prior to the recruitment periods to build awareness of the studies. Community sensitisation involved meetings with the Alkalo (leader) of Sukuta and invited stakeholders to explain the studies' purposes, procedures, and potential risks and benefits. Information about the studies was then disseminated to the wider community through these leaders. For SpyCATS, household and individual sensitisation occurred at least two days before the enrolment visit. Study team members visited households to discuss the studies with the head of the household and distributed information sheets. If the household head was potentially willing to participate, details of the household composition and contact information were recorded, and an appointment for an informed consent visit was scheduled.

2.5.3.2 Informed Consent

Written informed consent was obtained from all participants prior to any study activities occurring, and for SpyCATS, at least 24 hours after sensitisation. Details of the studies were explained to ensure participants understood the implications of participation. Consent was obtained from all household members meeting the inclusion criteria. For adults, the informed consent document was read to them, line by line, continuously verifying understanding, in the local language of the participant's choice by a trained nurse of field worker. Participants then signed or thumb-printed the consent form in the presence of a witness. For children aged 12-17, assent was sought in addition to parental consent. For children under 12, parental consent was sufficient. Participants were regularly reminded that they could withdraw from the studies at any time without giving a reason.

2.5.3.3 Data management

Data were collected in the field using electronic case report forms (eCRFs) on tablet computers. The questionnaires were designed in REDCap™ and included up-front data quality checks. Data were collected offline and synced with the MRCG secure database daily. All personal identifiable information were kept separate from the study database to ensure confidentiality. Unique study IDs were assigned to each participant to anonymise data. Data were stored securely and in compliance with GDPR

regulations. The anonymised data will be retained for at least 10 years post-study and made available for scientific purposes upon request.

2.5.3.4 Statistical analysis

Statistical analysis for both studies was conducted using R version 4.3.1. Data cleaning and curation were performed using the *tidyverse* suite of packages. For SpyCATS, the analysis involved preparing data into defined "events" based on the timing of positive swabs in relation to other swabs (see chapter 5 supplementary appendix) which required various bespoke functions to achieve. Setting up data for survival analysis was done using the *tmerge* function from the *survival* package. Various statistical methods were employed throughout the analysis of both studies, including descriptive statistics, hypothesis testing statistics (such as t tests and Wilcoxon tests), and regression models. The *gtsummary* package was used to create tables of regression output. The PharynGAS analysis involved receiver operator characteristics (ROC) analysis which was done using the *pROC* package. Incidence for the SpyCATS analysis used the *popEpi* function, to calculated stratified incidence rates, and the *coxph* function from the survival packages to perform Andersen-Gill regression. Data visualisation was done using *ggplot2*, *UpSetR*, and *ggplotify* packages in R, and GraphPad Prism version 10.0.3.

Cox Regression and Andersen-Gill Extension

For risk factor analysis in SpyCATS, Cox proportional hazards regression was chosen over Poisson and logistic regression due to its suitability for time-to-event data such as from longitudinal cohort studies, as we had. Although Poisson regression can be used for cohort study analysis, it assumes that the time to events is evenly spaced across follow-up, which does not leverage important time-to-event data. Additionally, Poisson regression cannot properly incorporate time-varying covariates, which were crucial for accounting for seasonality and household size in the SpyCATS study.

We chose to use the Andersen-Gill extension of the Cox model which allows for the inclusion of repeated events in the data, accounting for both individual and household clustering with robust standard errors. This flexible approach allows for incorporation of all the time-to-event data available, as well as allowing for time varying covariates and repeated events in individuals. However, it assumes that an individual's risk of an event remains the same irrespective of whether previous events have occurred, which may not be quite true in the case of StrepA events. While we may assume that immunity might reduce risk of recurrent events, individuals experiencing events might be inherently at higher risk for subsequent events, as in SpyDERM, previous reported events was the most significant risk factor for pyoderma (19). Furthermore, so little is known about conserved and type-specific immunity, particularly in highly diverse settings, we felt that it was a reasonable assumption to allow. The Andersen-Gill model was chosen over the similar Prentice-Williams-Peterson (PWP) and Wei-Lin-Weissfeld (WLW) models due to its ability to handle the complexity of recurrent event data without requiring the stratification of events by order (22). Although the PWP and WLW models account for the order of events by stratifying them, in our sensitivity analysis these methods yielded similar results to Andersen-Gill but with wider confidence intervals, providing less power. Using the *tmerge* function, data

were prepared for analysis by defining event times in a *Surv* object, creating time-varying covariates (household size and season), and specifying the outcome events.

2.5.3.5 Ethical considerations

The studies were conducted in adherence to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Approval was gained from the MRC Unit The Gambia Scientific Coordinating Committee, the Gambia Government/MRC Joint Ethics Committee and the LSHTM Research Ethics Committee. Participants were informed about the studies' purposes, procedures, risks, and benefits. Written informed consent was obtained before participation. Participants' confidentiality was maintained by anonymising data and securely storing personal identifiers. The studies aimed to contribute valuable information to the scientific community and inform public health strategies in The Gambia. Participants had the right to withdraw at any time without affecting their access to healthcare. The funders had no role in study design, data collection, analysis, or preparation of this thesis.

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3 Research Paper 1: Evaluating clinical decision rules and rapid diagnostic tests for the diagnosis of *Streptococcus pyogenes* pharyngitis in Gambian children: a diagnostic accuracy study

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Student ID Number	1806929	**Title**	Dr
First Name(s)	Edwin Peter		
Surname/Family Name	Armitage		
Thesis Title	Epidemiology of Streptococcus pyogenes in The Gambia: investigating carriage and disease burden, transmission dynamics and diagnostic accuracy		
Primary Supervisor	Prof. Michael Marks		

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

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	Darboe, Adam Kucharski, Pierre R Smeesters, Thushan I de Silva & Michael Marks on behalf of the MRCG StrepA Study Group
Stage of publication	**Submitted**

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)	The PharynGAS study was desgined by myself in collaboration with my supervisors. I oversaw all field work and lab work, was the primary author of this manuscript and performed all data clearning and analysis.

SECTION E

Student Signature	
Date	1st July 2024

Supervisor Signature	
Date	1st July 2024

3.1 Manuscript

Evaluating clinical decision rules and rapid diagnostic tests for the diagnosis of *Streptococcus pyogenes* pharyngitis in Gambian children: a diagnostic accuracy study

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Abstract:

Background

Accurate diagnosis of *Streptococcus pyogenes* (StrepA) pharyngitis is imperative in high rheumatic heart disease-burden countries. We aimed to assess the diagnostic accuracy of two rapid diagnostic tests and five clinical decision rules (CDRs) in The Gambia.

Methods

Children under 16 years presenting with signs and symptoms of pharyngitis were recruited at Sukuta Health Centre, The Gambia. A rapid antigen detection test (SD Bioline; LFT) and a rapid gene-amplification test (ID NOW™ STREP A2) were assessed for diagnostic accuracy alongside five CDRs against culture and qPCR for StrepA. Logistic regression was used to determine risk factors for StrepA pharyngitis.

Findings

Among 376 participants, StrepA positivity was 9·8% (37/376) by culture, 31·6% (119/376) by LFT, 33·3% (122/366) by ID NOW, and 32·4% (122/376) by PCR. The ID NOW had sensitivities and specificities of 94·6% and 73·6% against culture, and 93·5% and 87·6% against PCR. The LFT had sensitivities and specificities of 83·8% and 74·0% against culture and 55·7% and 80·0% against PCR. The Smeesters CDR performed best with an area under the curve (AUC) of 0·643. StrepA pharyngitis risk increased with age. Recent chest infection/cough (aOR 1·89, 1·08-3·28) and concurrent skin infection (aOR 2·11, 1·21-3·69) were associated with increased StrepA pharyngitis.

Interpretation

The LFT, culture and the CDRs had poor performance in detecting StrepA pharyngitis compared to PCR and ID NOW. There is an urgent need for novel strategies and the development affordable, highly sensitive diagnostics to accurately identify StrepA cases and guide appropriate treatment in resource-limited settings.

Funding

Wellcome Trust.

Research in context

Evidence before the study

Despite a significant disease burden of StrepA infection and its sequelae in Africa, research on StrepA pharyngitis is lacking. We searched PubMed from database inception to up to March 19, 2024, for studies on StrepA pharyngitis epidemiology and diagnostics in Africa, using the search terms “Streptococcus pyogenes”. “Group A Streptococcus”, “GAS”, “StrepA”, “pharyngeal”, “pharyngitis”, “diagnostics”, “diagnostic accuracy”, “point-of-care test”, “rapid diagnostic test”, “clinical decision rule” and “clinical score”. The proportion of pharyngitis cause by StrepA varies across African countries with data available from Ethiopia, Uganda, Cameroon, South Africa, Morocco and Tunisia. The reported prevalence ranges between 9·1% and 70% though studies are scarce and heterogenous. Various studies have evaluated the efficacy of rapid diagnostic tests and clinical decision rules, though there is no consensus on the most appropriate diagnostic tools. A global meta-analysis suggests a higher proportion of StrepA pharyngitis in higher income settings than LMIC (24·3% vs. 17·6%). No studies evaluation the utility of PCR for StrepA pharyngitis in Africa were identified.

Added value of this study

This study utilised a range of diagnostic methods, both microbiological and molecular, combined with assessing five different clinical decision scores in The Gambia. It provides the first StrepA pharyngitis prevalence data from The Gambia and shows that gene-amplification diagnostic methods have higher sensitivity than other methods. It raises the challenge in finding, within the existing array of diagnostic tools, a solution which is both sufficiently sensitive, and affordable for low-resource settings.

Implications of all the available evidence

This study contributes valuable data on StrepA pharyngitis prevalence in Africa, though surveillance programmes are necessary across the continent to accurately monitor disease burden and understand the heterogeneity. Our findings highlight a notable discrepancy between sensitivity of microbiological culture and gene-amplification methods (PCR and ID NOW), suggesting a potential large under-recognised and undiagnosed disease burden and highlighting the need for wider availability and use of molecular diagnostics for StrepA in Africa. Furthermore, the current lack of an ideal diagnostic method for low-resource settings, which is both affordable and highly sensitive, underscores an urgent need for the development of improved diagnostic tools

Background

Acute pharyngitis in children is one of the most common reasons for primary healthcare consultations worldwide. *Streptococcus pyogenes* (StrepA) is the most important bacterial cause of pharyngitis. It is responsible for between 10% and 25% of pharyngitis presentations, and causes a higher proportion of cases in high-income settings than low- and middle-income countries (LMICs) (24·3% vs. 17·6%) (1). Data from LMICs including The Gambia are lacking, though a recent study found an incidence rate of 120 cases per 1000 person years (95% CIs 57-252) in children aged 5 to 11 years of StrepA pharyngitis, with 16·7% of clinical sore throat episodes positive for StrepA by bacterial culture in that age group (2,3).

Globally, StrepA is implicated in over 500,000 deaths per year, predominantly in LMICs where immune sequelae including acute rheumatic fever (ARF) and rheumatic heart disease (RHD) are common (4,5). Although most pharyngitis cases are viral in origin, accurate diagnosis of StrepA pharyngitis and prompt administration of antibiotics is crucial in RHD-endemic settings (6). This must be balanced against concerns over antimicrobial resistance and reducing unnecessary antibiotic prescriptions for individuals with viral pharyngitis (7). In high-income settings two broad approaches, clinical decision rules (CDRs) and rapid diagnostic tests, have been widely utilised to improve the management of pharyngitis and to guide antibiotic prescribing.

CDRs offer a standardised, pragmatic approach to diagnosis based on clinical signs and symptoms. They can aid clinicians, particularly in resource-constrained settings, in making diagnostic and treatment decisions (8). Although CDRs have been widely implemented for diagnosis of StrepA pharyngitis, most were developed and validated in high-income settings, where demographic characteristics, clinical and molecular epidemiology of StrepA, and healthcare systems are substantially different to those in many African countries (9,10). The applicability of these CDRs as well as rapid diagnostic tests to LMICs such as The Gambia, is therefore uncertain.

In The Gambia, microbiological culture and rapid point-of-care diagnostic tests are not available at government health centres. Most outpatient presentations for upper respiratory tract infections are assessed by nurses with limited medical and diagnostic training. In addition, financial, and practical barriers to healthcare seeking for sore throats exist and people commonly seek out local remedies first, only seeking formal healthcare after treatment failure (11). Collectively, these factors lead to inadequate diagnosis and treatment of StrepA pharyngitis, which may contribute to the high burden of RHD. Several rapid diagnostic tests exist for point-of-care diagnosis of StrepA pharyngitis within two broad categories: rapid antigen detection tests and rapid nucleic acid (gene-amplification) tests. Meta analyses have reported a summary sensitivity for rapid antigen detection tests of 85·6% and a summary specificity of 95·4%, while for rapid nucleic acid tests the summary sensitivity is higher at 97·5% and the summary specificity is similar at 95·1% (12,13). Such tests can offer improved

diagnosis over clinical assessment while not requiring laboratories to maintain reagents or consumables. However, there are limited data on their use in low-resource settings such as The Gambia and their use has yet to be shown to be cost-effective over other strategies (14-16).

We aimed to assess the diagnostic accuracy of two rapid diagnostic tests, one rapid antigen detection test and one rapid nucleic acid test, and five commonly used CDRs for diagnosis of StrepA pharyngitis in The Gambia.

Methods

Study design and participants

This prospective diagnostic accuracy study was conducted in children under 16 years of age at Sukuta Health Centre, The Gambia. Eligible participants were those presenting with signs and symptoms of pharyngitis (including tonsillitis). The inclusion criteria allowed for the enrolment of children under five years old where non-specific symptoms were reported by parents, provided there was evidence of tonsilo-pharyngeal erythema on examination. Participants were identified by aiming to recruit a convenience sample of consecutive cases of pharyngitis presenting to the Sukuta Health Centre paediatric outpatient department.

The study was approved by the Gambia Government/MRC joint ethics committee and the LSHTM Research Ethics Committee (LEO17910). Written informed consent was provided by parents or guardians for participants. Participants aged 12 and over provided assent.

Procedures

Clinical assessment

Participants were assessed by a nurse who took a detailed clinical history of the presenting complaint and performed a thorough clinical examination. All clinical information relevant to five clinical decision rules (CDRs) were collected. Three CDRs were originally designed and validated in high-income settings: the CENTOR score (USA), Modified CENTOR/McIsaac score (Canada), and FeverPAIN (UK), and two in LMIC settings: the Cape Town score (South Africa), the Smeesters score (Brazil) (appendix p 2) (17-21). Socio-demographic data including sex, age, ethnic group, household size, mother's education level, household income, and number of siblings were gathered. Additional information on social mixing and health seeking behaviour were also collected.

Sample collection

Two swabs were held together to form a dual swab and a sample from the oropharynx was collected using standard techniques. The swabs used were Copan Transystem™ 140C (Copan, Brescia, Italy) and the SD Bioline Group A Streptococcal rapid antigen detection lateral flow test (LFT) swab (Abbott,

Yongin-si, South Korea). The LFT swab was immediately used for the SD Bioline test according to manufacturer's instructions. The Copan swab was placed in its liquid Amies transport medium and transported in a cold box to MRC Unit The Gambia laboratories at Fajara on the same day.

Laboratory procedures

The Copan swab was plated for microbiological culture on Colombia blood agar, and beta-haemolytic colonies underwent latex agglutination testing (Prolex Pro-Lab, Bromborough, UK) for the presence of Group A Streptococci. From the remaining liquid Amies, 200μl was used for the ID NOW Strep A 2 (Abbott, Scarborough, Maine, USA), rapid isothermal rapid nucleic acid amplification test, formerly known as Alere i strep A test, which targets a sequence of the *cep5* gene, encoding the C5a peptidase streptococcal virulence factor (22,23). The remaining liquid Amies was stored at -70°C until DNA extraction for PCR. DNA was extracted from 200μl of Amies using the QIAamp DNA mini kit (Qiagen) according to manufacturer's instructions following incubation with lysostaphin (1mg/mL) and lysozyme (100mg/mL) (24). Sample volume was insufficient for 30 samples, so additional buffer was added to make 200μl. Quantitative PCR was performed using Bio-Rad CFX 96 Touch Real-Time PCR detection system with primers and probes to detect the *S. pyogenes*-specific gene *speB* (forward: CTAAACCCTTCAGCTCTTGGTACTG; reverse: TTGATGCCTACAACAGCACTTTG; probe: Cy5-CGGCGCAGGCGGCTTCAAC-BHQ2) as previously described (25,26). Bacterial loads were quantified using standard curves generated by 10-fold serial dilutions of extracted DNA from *S. pyogenes* reference strain H293. The limit of detection (LOD) was determined using curve-fitting models on standard curves generated from eight serial dilutions from 10,000,000 to 1 copy per μl run in 11 replicates. The LOD was defined as the lowest concentration of DNA that could be detected at a 95% detection rate. To optimise throughput, samples were run in a single well. Based on the LOD, we defined a cycle threshold (Ct) of more than 40 to be negative, and less than 36 to be positive. Samples with a Ct between 36 and 40 were repeated to exclude contamination and determined to be positive if an appropriate amplification curve was seen and the Ct was below 40 for both runs. PCR conditions used were 50°C for 2 minutes, 95°C for 10 minutes, 94°C for 15 seconds and 58°C for 40 seconds over 45 cycles.

Statistical analysis

A sample size of 385 pharyngitis cases was chosen to detect a StrepA pharyngitis proportion of 20% with a precision of 4% and allow us to detect a sensitivity of 95% ±5% for the CDRs and rapid diagnostic tests versus the index test. The proportion of pharyngitis cases positive for StrepA by each test performed was calculated with binomial exact 95% confidence intervals (CIs). Differences in bacterial load by qPCR were assessed by Wilcoxon test with p-values adjusted for multiple testing using a Benjamini and Hochberg correction. For the primary assessment of diagnostic accuracy, microbiological culture was used as the reference standard. Given the limitations of culture (26), we also performed a secondary analysis using PCR as the reference standard. Performance of the CDRs versus culture and PCR were assessed using the area under the curve (AUC) of receiver operating

characteristic (ROC) curves. Unweighted and weighted (60% towards sensitivity) Youden's indices were calculated to identify optimal score thresholds for the CDRs. Logistic regression models were used to explore socio-demographic and other factors associated with PCR-positive StrepA pharyngitis in this setting. Unadjusted odds ratios (OR) are reported for univariable models and adjusted odds ratios (aOR) are reported for multivariable models. Marginal probabilities were calculated for each risk factor, and when adjusting age group and sex, assumed age group 5-11 and male sex. P values <0·05 were considered significant. Data were entered directly into REDCap.
Analysis was performed in R version 4.3.1.

Findings

A total of 376 participants were recruited to the study between June 9, 2021, and September 26, 2022. Participants were 55% (208/376) male, with a median age of 4 years (IQR 2-6). Median household size was 5 people (IQR 2-16) (Table 1).

Table 6. Socio-demographic and anthropometric characteristics of participants recruited. IQR = interquartile range.

Characteristic	Category	Number (%) n=376
Median age in years (IQR)	-	4 (2-6)
Age group	0-4 years old	256 (68·1)
	5-11 years old	101 (26·9)
	12-15 years old	19 (5·1)
Sex	Male	208 (55·3)
	Female	168 (44·7)
Tribe/ethnic group	Mandinka	172 (45·7)
	Wolof	52 (13·8)
	Fula	73 (19·4)
	Jola	12 (3·2)
	Other	67 (17·8)
Median household size (IQR; range)	-	5 (4-7; 2-20)
Median number of siblings from same mother (IQR)	-	3 (2-5)
Median number of siblings from same father (IQR)	-	4 (2-6)
Mother's education	None	34 (9·0)
	Arabic school only	129 (34·3)
	Primary school only	37 (9·8)
	Middle school	73 (19·4)

	Secondary school	71 (18·9)
	Further/higher education	30 (8·0)
	Don't know/unwilling to say	1 (0·3)
	Missing	1 (0·3)
Household income per month	GMD <500 (<$10)	184 (48·9)
	GMD 500-999 ($10-20)	7 (1·9)
	GMD 1000-2499 ($20-50)	83 (22·1)
	GMD 2500-4999 ($50-100)	66 (17·6)
	GMD >5000 (>$100)	19 (5·1)
	Unwilling to say	17 (4·5)
Mean height in centimetres (SD)	-	103·3 (22·6)
Mean weight in kilograms (SD)	-	16·5 (8·9)
Mean body mass index-for-age (SD)	-	-0·98 (1·15)

The most reported clinical features were throat pain (364/375, 97·1%), painful swallowing (360/375, 96·0%), a history of fever (353/376, 93·9%) and difficulty swallowing (350/375, 93·3%). The most frequently reported time of onset was less than 12 hours ago (130/353, 36·8%). On examination, 374/376 (99·5%) had tonsillar erythema, 364/376 (96·8%) had swollen anterior cervical lymph nodes, 119/376 (31·6%) had tonsillar exudate, and 361/376 (96·0%) had tonsillar swelling (appendix pp 3-4).

Proportion of participants positive for StrepA

The proportion of participants identified as StrepA positive by culture was 9·8% (37/376; 95% CIs 7.0-13·3). Both rapid tests detected a higher proportion of StrepA, with the LFT positive in 31·6% (119/376; 95% CIs 27·0-36·6) and ID NOW positive in 33·3% (122/366; 95% CIs 28·5-38·4) of cases. PCR detected StrepA in 32·4% of participants (122/376; 95% CIs 27·7-37·4) (Figure 1a).

ID NOW results were not available for 10 participants, on five occasions due to machine error, on four occasions due to the test not being done, and on one occasion due to inadequate sample volume due to spillage. Although the overall proportion of positive LFT and ID NOW tests were similar to PCR, the agreement of LFT with PCR was poor, compared to ID NOW (Figure 1b).

In StrepA PCR positive samples, the bacterial load estimated by quantitative PCR was significantly higher in samples which were culture positive compared to culture negative ($p<0·0001$), LFT positive compared to LFT negative ($p<0·0001$), and ID NOW positive compared to ID NOW negative ($p=0·0022$), though the difference was smaller (Figure 1c).

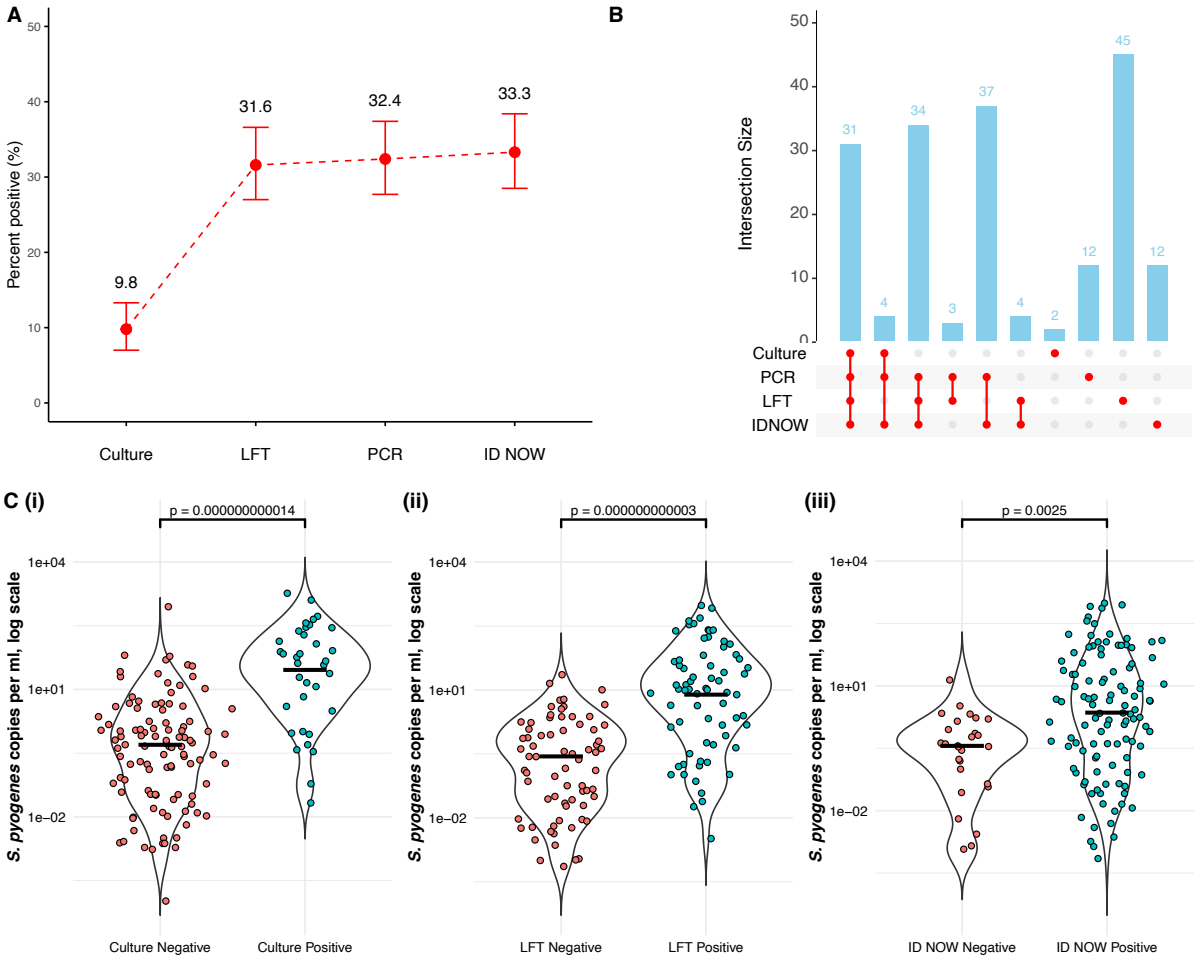

Figure 1. (A) Chart showing the percentage of participants tested who were positive for StrepA by culture, LFT, ID NOW and PCR. (B) UpSet plot showing the number of participants tested who were positive for each test and the agreement between tests. The red lines indicate the combination of positive tests that each blue bar represents. 170 participants were negative for all four tests, and ID NOW was not performed on 10 participants, so these data are excluded from the plot. (C) Violin plots showing bacterial load detected by quantitative PCR in PCR-positive samples by (i) microbiological culture status, (ii) LFT status, and (iii) ID NOW status.

Diagnostic accuracy of rapid tests

Taking the culture result as the reference standard, the LFT had a sensitivity of 83·8% and a specificity of 74·0%. PCR and the ID NOW performed almost identically with both with a sensitivity of 94.6% and specificities of 73·6% for ID NOW and 74·3% for PCR. The positive and negative predictive values of the ID NOW and PCR were higher than for the LFT (Table 2).

Table 7. Two-by-two tables of LFT and ID NOW test results against culture as the reference standard showing the diagnostic accuracy of the two tests. Sens: sensitivity; Spec: specificity; PPV: positive predictive value; NPV: negative predictive value.

	Culture +ve	**Culture -ve**	
LFT +ve	31	88	PPV: 26·1%
LFT -ve	6	251	NPV: 97·7%

	Sens: 83·8%	Spec: 74·0%	
ID NOW +ve	35	87	PPV: 28·7%
ID NOW -ve	2	242	NPV: 99·2%
	Sens: 94·6%	Spec: 73·6%	
PCR +ve	35	87	PPV: 28·7%
PCR -ve	2	252	NPV: 99·2%
	Sens: 94·6%	Spec: 74·3%	

Taking PCR as the reference standard the LFT had a sensitivity of 55·7% and a specificity of 80·0% while the ID NOW had a sensitivity of 87·6% and a specificity of 93·5%. The positive predictive value of both tests was improved when PCR was taken as the reference standard, but the negative predictive value of both tests decreased, though only marginally for ID NOW (Table 3).

Table 8. Two-by-two tables of LFT and ID NOW test results against PCR as the reference standard showing the diagnostic accuracy of the two tests. Sens: sensitivity; Spec: specificity; PPV: positive predictive value; NPV: negative predictive value.

	PCR +ve	**PCR -ve**	
LFT +ve	68	51	PPV: 57·1%
LFT -ve	54	203	NPV: 79·0%
	Sens: 55·7%	Spec: 80·0%	
ID NOW +ve	106	16	PPV: 86·9%
ID NOW -ve	15	229	NPV: 93·9%
	Sens: 93·5%	Spec: 87·6%	

Receiver operating characteristic curves of clinical decision rules against PCR

Taking culture as the reference standard, the Smeesters score performed best with an AUC of 0·694, followed by Cape Town with an AUC of 0·617. CENTOR (AUC: 0·584), Modified CENTOR/McIsaac (AUC: 0·496), and FeverPAIN (AUC: 0·600) also performed poorly against culture (appendix p 5). Similarly, with PCR as the reference standard, the performance of all the CDRs were poor (Figure 2). The CENTOR score performed best of the CDRs designed for high-income settings with an AUC of 0·583, while the Modified CENTOR/McIsaac (AUC 0·500) performed only as well as a random

classifier and FeverPAIN (AUC 0·554) performed only slightly better. Of the two CDRs designed for LMIC settings, the Smeesters score performed better with an AUC of 0·643, while the Cape Town score performed less well (AUC 0·563). Participants exhibited a diversity of score outcomes from all five CDRs tested (appendix p 6).

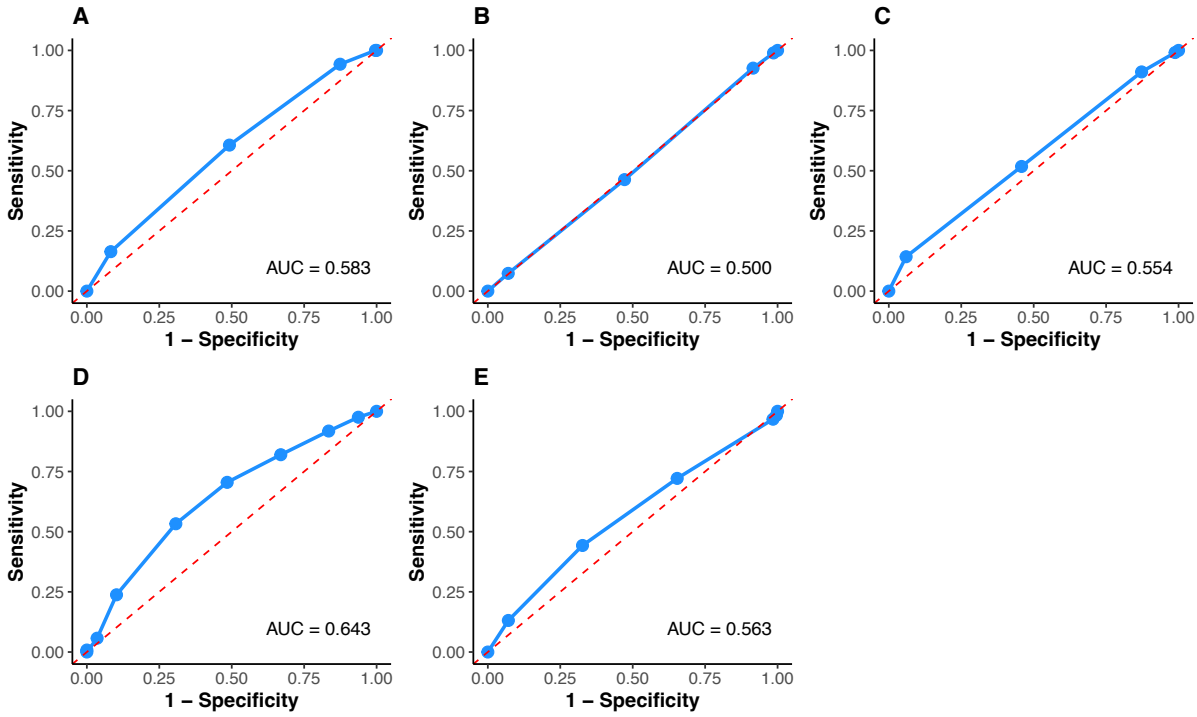

Figure 2. ROC curves for the five CDRs tested using PCR as the reference standard. (A) CENTOR score (1981), (B) Modified CENTOR/McIsaac (note 139 participants excluded due to age < 3), (C) FeverPAIN, (D) Smeesters, (E) Cape Town. The red dashed line indicates how a random classifier test would perform. The area under curve (AUC) is the area under the blue CDR line, a higher AUC suggests a higher diagnostic accuracy.

At the optimal weighted threshold, the Cape Town score had the highest combined sensitivity (83·8%) and specificity (34·2%) (appendix p 7). Combining the Cape Town score with the ID NOW into an algorithm where patients with a score above the threshold of 2·5 undergo an ID NOW test would provide an overall sensitivity of 65·6%, a specificity of 94·8%, a PPV of 86·0% and an NPV of 84·9% whilst preventing 32·4% (122/376) of patients requiring an ID NOW.

Risk factors for StrepA pharyngitis

In multivariable logistic regression models, the only socio-demographic characteristic significantly associated with increased odds of PCR-positive StrepA pharyngitis was age group (0-4 years, marginal probability 26·4% compared to 5-11 years, marginal probability 47·5%, aOR 2·52, 95% CIs 1·51-4·23, p=0·0004, and 12-15 years, marginal probability 60·5%, aOR 4·28, 95% CIs 1·50-12·68, p=0·0069) (appendix p 8). In models adjusting for age group and sex, the odds of StrepA pharyngitis by PCR were higher in participants presenting with a history of a chest infection/cough in the last two

weeks (marginal probability 56·0% vs. 40·3%, aOR 1·89, 95% CIs 1·08-3·28, p=0·024), with a concurrent skin infection seen (marginal probability 58·8% vs. 40·3%, aOR 2·11, 95% CIs 1·21-3·69, p=0·0087). Clinical presentation features associated with increased odds of StrepA were history of fever (marginal probability 44·0% vs. 18·7%, aOR 3·43, 95% CIs 1·18-12·76, p=0·038), difficulty swallowing (marginal probability 43·6% vs. 13·8%, aOR 4·84, 95% CIs 1·38-30·67, p=0·036), pharyngeal erythema (marginal probability 47·7% vs. 34·1%, aOR 1·76, 95% CIs 1·12-2·78, p=0·015) and tonsillar exudate (marginal probability 51·9% vs. 38·0%, aOR 1·76, 95% CIs 1·10-2·81, p=0·019) (appendix pp 10-12). No measures of social-mixing were significantly associated with StrepA pharyngitis risk (appendix p 13).

Discussion

This study reveals a significant prevalence of StrepA pharyngitis in Gambian children, with gene-amplification based diagnosis (PCR and ID NOW) detecting a substantially higher prevalence than traditional culture. The LFT also appeared to detect higher prevalence as well, but false positives were considerable. Whilst rapid point-of-care tests could be useful tools in LMIC settings, both tests assessed in this study had limitations that would limit their wide-spread implementation. The SD Bioline's low sensitivity and poor positive predictive value would limit its utility in high-RHD risk settings while, although the ID NOW showed high sensitivity and specificity, its high cost, and requirement for sterile, temperature-controlled, laboratory conditions may limit adoption in low-resource settings. The disparity in diagnostic sensitivity and cost between qPCR, culture, and rapid diagnostic tests like ID NOW and SD Bioline underscores the urgent need to prioritise the development and dissemination of cost-effective, high-sensitivity, point-of-care diagnostic tools for StrepA, particularly in LMICs.

The stark discrepancy between the PCR and culture results implies a possible underestimation of the global burden of StrepA based on traditional microbiological testing. However, the clinical and immunological importance of PCR-positive, culture-negative pharyngitis needs further investigation. In this study, 70·5% (86/122) of PCR-positive cases were negative on culture, and those with a higher bacterial load were more likely to be culture positive. In a region with a high RHD burden, it is vital to identify and treat symptomatic pharyngitis episodes with evidence of StrepA to limit the risk of ARF and RHD (27). The low positivity of StrepA by culture compared to PCR suggests that molecular methods may be preferable to detect StrepA in this setting. However, further work is necessary to better understand the cost-effectiveness of molecular and rapid diagnostics in this setting. Such work should consider the direct costs of the tests but also the broader economic impacts of StrepA disease and RHD, including healthcare costs, lost productivity, and the societal burden of premature mortality. Though the inherent difficulties in capturing the long-term consequences of RHD, coupled with the lack of robust surveillance data from Africa, make such analyses challenging.

While PCR demonstrated a higher detection rate of StrepA than culture, it should be noted that some of the PCR-positive cases might represent asymptomatic carriage rather than active infection. Our study did not assess serological responses, or test for alternative causes of pharyngitis, so we cannot be sure that of the aetiology in PCR-positive cases. However, our case definition aligns with the SAVAC criteria for a confirmed StrepA pharyngitis case (28). Data on the prevalence of StrepA carriage in LMICs, particularly through PCR detection, are limited, but a previous study in The Gambia indicated a StrepA pharyngeal carriage rate of 7-13% in children aged 2-4 years (25). Furthermore, significant anti-StrepA serological responses were seen in newly colonised children in that study, raising questions over whether asymptomatic carriage is immunologically silent, or whether it could be contributing to immune priming of RHD (25). Therefore, while acknowledging the possibility of asymptomatic carriage, in settings with high-RHD risk and substantial barriers to healthcare seeking for pharyngitis, the treatment of all PCR-positive cases could be warranted (11).

Clinical decision rules (CDRs) have been frequently proposed as a potential pathway to optimise the use of limited diagnostic resources. However, the low sensitivity of the CDRs assessed, even against culture, limits their utility in this setting. Many CDRs were designed in high-income settings, where clinical and molecular epidemiology of StrepA is different (10,29). None of the CDRs tested performed well enough on their own to effectively identify StrepA pharyngitis with the sufficient accuracy, underscoring the need for novel sensitive, accessible diagnostic tools. However, combining a CDR with a rapid test into a clinical algorithm could improve diagnostic accuracy while moderating test use (30).

This study has several limitations. Firstly, there was potential for selection bias by selecting participants by convenience sampling. In addition to the fact that we selected participants only from a health centre outpatient setting, thus likely missing people less likely to attend health centres. Secondly, the study was conducted in an urban area, which limits its generalisability to rural settings. Thirdly, utilisation of a non-selective culture medium may have contributed to the lower StrepA prevalence found by culture than PCR, though our methods were standard. Fifthly, by relying on parental reporting of clinical history, the data may have been subject to recall bias. Beyond these limitations, the study's findings highlight the critical gap in our understanding of the clinical presentation of StrepA infections in LMIC.

This study contributes essential insights into the diagnosis and epidemiology of StrepA pharyngitis in LMIC settings, with significant implications for global StrepA control efforts. None of the evaluated diagnostic tests nor clinical decision rules appears suitable for adoption in this setting. Identifying and validating alternative diagnostic strategies is a priority for StrepA control globally.

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Competing interests

No competing interests were disclosed.

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3.2 Supplementary appendix

Supplementary Appendix

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Table S1. Details of the five clinical decision rules assessed. *Under 3-year-olds excluded

	CENTOR	Modified CENTOR/ McIsaac*	FeverPAIN	Smeesters	Cape Town
Age		3-14 (+1); 15-44 (+0) ≥45 (-1)		≤35 months (+1) 36-59 months (+2) ≥60 months (+3)	
Fever	Hx of fever (+1)	Temp >38°C (+1)	Hx of fever in past 24hrs (+1)		
Symptom onset			≤3 days (+1)	<12 hrs (+1)	
Absence of cough	+1				+1
Absence of coryza				-1 (for presence)	+1
Absence of cough or coryza			+1		
Tonsillar exudate	+1	+1	+1		+1
Severe tonsillar swelling			+1		+2
Swollen and tender anterior cervical lymph nodes	+1	+1			
Tender anterior cervical lymph nodes				+1	
Headache				+1	
Petechia on palate				+1	
Abdominal pain				+1	
Conjunctivitis				-1	
Diarrhoea				-1	

Table S2. Clinical features of participants.

Characteristic	N (%)
Time of first symptom onset	
<12 hrs ago	130 (36.8%)
12-24 hrs ago	38 (10.8%)
24-48 hrs ago	38 (10.8%)
48-72 hrs ago	75 (21.2%)
>72 hours ago	72 (20.4%)
Don't know	23
Current skin infection?	
No	326 (86.7%)
Yes	50 (13.3%)
Cough	
No	160 (42.6%)
Yes	216 (57.4%)
Coryza (runny nose)	
No	165 (43.9%)
Yes	211 (56.1%)
Fever	
No	23 (6.1%)
Yes	353 (93.9%)
Pain of the throat	
No	11 (2.9%)
Yes	364 (97.1%)
(Missing)	1
Painful swallowing	
No	15 (4.0%)
Yes	360 (96.0%)
(Missing)	1
Difficulty swallowing	
No	25 (6.7%)
Yes	350 (93.3%)
(Missing)	1
Nausea	
No	359 (95.5%)
Yes	17 (4.5%)
Vomiting	
No	279 (74.2%)
Yes	97 (25.8%)
Abdominal pain	
No	256 (68.1%)
Yes	120 (31.9%)
Diarrhoea	
No	298 (79.3%)
Yes	78 (20.7%)
Rash (viral-like exanthema)	
No	374 (99.7%)
Yes	1 (0.3%)
(Missing)	1
Hoarseness	
No	323 (85.9%)
Yes	53 (14.1%)
Headache	
No	178 (47.3%)
Yes	198 (52.7%)
Conjunctivitis	
No	359 (95.5%)
Yes	17 (4.5%)
Any other symptoms present?	
No	350 (93.1%)

Characteristic	N (%)
Yes	26 (6.9%)
Axillary temperature >38ºC	
No	301 (80.1%)
Yes	75 (19.9%)
Tonsillar erythema (redness of the tonsils)?	
No	2 (0.5%)
Yes	374 (99.5%)
Tonsillar swelling (swelling of the tonsils)?	
No	15 (4.0%)
Yes	361 (96.0%)
Tonsillar exudate (pus/exudate on the tonsils)?	
No	257 (68.4%)
Yes	119 (31.6%)
Pharyngeal erythema (redness of the pharynx)?	
No	184 (48.9%)
Yes	192 (51.1%)
Pharyngeal swelling (swelling of the pharynx)?	
No	280 (74.5%)
Yes	96 (25.5%)
Pharyngeal exudate (pus/exudate on the pharynx)?	
No	356 (94.7%)
Yes	20 (5.3%)
Swollen anterior cervical lymph node(s) on palpation?	
No	12 (3.2%)
Yes	364 (96.8%)
Approximate size of largest anterior cervical lymph node on palpation (in centimetres)?	
< 0.5cm	136 (37.4%)
0.5-1cm	142 (39.0%)
1-1.5cm	66 (18.1%)
1.5-2cm	17 (4.7%)
>2cm	3 (0.8%)
Tenderness of anterior cervical lymph nodes on palpation?	
No	51 (13.6%)
Yes	325 (86.4%)
Petechiae on the palate?	
No	233 (62.0%)
Yes	143 (38.0%)
Skin infections seen?	
No	302 (80.3%)
Yes	74 (19.7%)
Type of infections seen?	
Pyoderma	21 (5.6%)
Fungal infection	37 (9.8%)
Other	16 (4.3%)

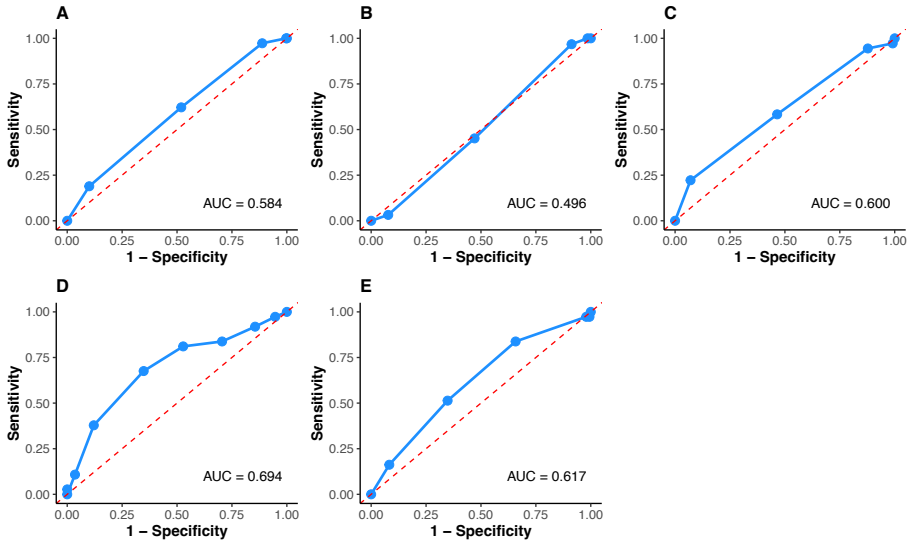

Figure S1. ROC curves for the five CDRs tested using culture as the reference standard. (A) CENTOR score (1981), (B) Modified CENTOR/McIsaac (note 139 participants excluded due to age < 3), (C) FeverPAIN, (D) Smeesters, (E) Cape Town. The red dashed line indicates how a random classifier test would perform. The area under curve (AUC) is the area under the blue CDR line, a higher AUC suggests a higher diagnostic accuracy.

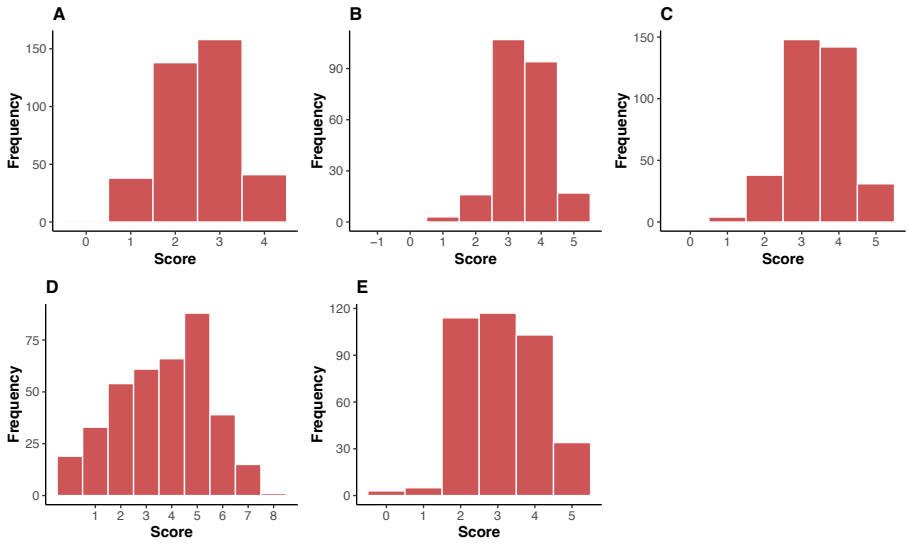

Figure S2. Histograms of frequency of the scores from the five difference clinical decision rules. (A) CENTOR score (1981), (B) Modified CENTOR/McIsaac (note 139 participants excluded due to age < 3), (C) FeverPAIN, (D) Smeesters, (E) Cape Town. NB: Data on symptom onset day were missing for 13 participants, meaning FeverPAIN score could not be calculated.

Table S3. Optimal threshold values for CDRs based on unweighted and weighted Youden's index. Unweighted Youden's index is calculated as J = sensitivity + specificity - 1 maximises both sensitivity and specificity, and weighted calculated as J = 2 * (weight * sensitivity + (1 - weight) * specificity) - 1 weighted the index towards sensitivity (0.5-1) or specificity (0-0.5).

	Unweighted Youden's index			Weighted Youden's index (60% towards sensitivity)		
	Optimal threshold	**Sensitivity**	**Specificity**	**Optimal threshold**	**Sensitivity**	**Specificity**
Centor 1981 (0 to 4 points)	2.5	62.2%	48.1%	1.5	97.3%	11.2%
Modified Centor/McIsaac (-1 to 5 points)	2.5	96.8%	8.7%	2.5	96.8%	8.7%
FeverPAIN (0 to 5 points)	4.5	22.2%	93.0%	2.5	95.0%	12.2%
Smeesters (1 to 8 points)	4.5	67.6%	65.2%	2.5	83.8%	29.5%
Cape Town (0 to 5 points)	2.5	83.8%	34.2%	2.5	83.8%	34.2%

Table S4. Univariable and multivariable logistic regression models showing the odds of PCR-positive StrepA pharyngitis for different socio-demographic characteristics.

			Unadjusted analysis				Adjusted analysis			
Characteristic	**N**	**Event N**	**Marginal Probability* (%)**	**OR**	**95% CI**	**p-value**	**Marginal Probability* (%)**	**aOR**	**95% CI**	**p-value**
Age group										
0-4 years	256	65	25.4	ref.	—		26.4	ref.	—	
5-11 years	101	46	45.5	2.46	1.52-3.99	**0.0003**	47.5	2.52	1.51-4.23	**0.0004**
12-15 years	19	11	57.9	4.04	1.57-10.85	**0.0041**	60.5	4.28	1.50-12.68	**0.0069**
Sex										
Male	208	62	29.8	ref.	—		26.4	ref.	—	
Female	168	60	35.7	1.31	0.85-2.02	0.22	33.1	1.38	0.87-2.22	0.18
Ethnic group										
Fula	73	25	34.2	ref.	—		26.4	ref.	—	
Jola	12	5	41.7	1.37	0.37-4.74	0.62	29.0	1.14	0.28-4.28	0.85
Mandinka	172	62	36.0	1.08	0.61-1.94	0.79	26.7	1.02	0.55-1.89	0.96
Wolof	52	14	26.9	0.71	0.32-1.53	0.38	19.7	0.69	0.29-1.56	0.37
Other	67	16	23.9	0.60	0.28-1.25	0.18	16.7	0.56	0.25-1.22	0.15
Mother's educational level										
Arabic only	129	43	33.3	ref.	—		26.4	ref.	—	
None	34	8	23.5	0.62	0.24-1.42	0.28	17.7	0.60	0.22-1.48	0.29
Primary only	37	12	32.4	0.96	0.43-2.06	0.92	27.5	1.06	0.46-2.40	0.90
Middle	73	29	39.7	1.32	0.72-2.39	0.36	34.6	1.48	0.78-2.82	0.23
Secondary	71	23	32.4	0.96	0.51-1.77	0.89	26.7	1.02	0.51-1.99	0.96
Higher	30	6	20.0	0.50	0.17-1.25	0.16	15.3	0.51	0.16-1.43	0.22
Household income										
GMD <500	184	62	33.7	ref.	—		26.4	ref.	—	
GMD >5000	19	6	31.6	0.91	0.31-2.42	0.85	25.0	0.93	0.28-2.87	0.90
GMD 1000-2499	83	26	31.3	0.90	0.51-1.55	0.70	23.3	0.85	0.46-1.53	0.59
GMD 2500-4999	66	18	27.2	0.74	0.39-1.36	0.34	21.9	0.78	0.39-1.53	0.48
GMD 500-999	7	3	42.9	1.48	0.28-6.89	0.62	32.0	1.31	0.22-6.96	0.75
Unwilling	17	7	41.2	1.38	0.48-3.76	0.54	24.5	0.91	0.27-2.86	0.87
Household size	376	122	0.4	1.02	0.94-1.09	0.66	-0.5	0.98	0.89-1.07	0.61
Number of siblings from same mother	376	122	0.5	1.02	0.91-1.14	0.72	-1.9	0.91	0.76-1.08	0.21
Number of siblings from same father	376	122	0.8	1.04	0.97-1.11	0.29	1.2	1.06	0.96-1.17	0.21

			Unadjusted analysis				Adjusted analysis			
Characteristic	**N**	**Event N**	**Marginal Probability* (%)**	**OR**	**95% CI**	**p-value**	**Marginal Probability* (%)**	**aOR**	**95% CI**	**p-value**

N = number of participants in the group; N event = number of PCR-positive events that occurred in the group; OR = odds ratio; CI = confidence interval; aOR = adjusted odds ratio; ref.=reference category used; p-values in bold are significant at <0.05. *For categorical variables marginal probability represents the predicted percentage of PCR-positive cases for that group, for continuous variables it represents the change in predicted percentage with an increase of 1 for the variable.

Table S5. Logistic regression models adjusted for age group and sex showing the odds of PCR-positive StrepA pharyngitis for various household and past medical risk factors.

Question wording	N	Event N	Marginal probability* (%)	aOR	95% CI	p-value
Anyone living in the household currently complaining of a sore throat?						
No	326	102	41.4	ref.	—	
Yes	48	19	45.9	1.20	0.62-2.27	0.59
Don't know	2	1	57.6	1.92	0.07-50.01	0.65
Anyone who sleeps in the same room as the participant currently complaining of a sore throat?						
No	320	99	42.4	ref.	—	
Yes	37	14	44.1	1.07	0.50-2.23	0.85
Anyone living in the household currently complaining of a skin infection?						
No	333	104	40.8	ref.	—	
Yes	42	18	52.8	1.62	0.81-3.19	0.16
Don't know	1	0	NA	NA	NA	NA
Anyone who sleeps in the same room as the participant currently complaining of a skin infection?						
No	324	100	42.0	ref.	—	
Yes	33	13	50.7	1.42	0.64-3.05	0.38
Has the participant had a sore throat in the last year?						
No	337	101	40.1	ref.	—	
Yes	39	21	49.6	1.47	0.67-3.23	0.34
Has the participant had a skin infection in the last year?						
No	318	104	42.1	ref.	—	
Yes	56	17	44.3	1.10	0.57-2.05	0.78
Has the participant ever been diagnosed with a heart condition?						
No	373	121	42.9	ref.	—	
Yes	1	0	NA	NA	NA	NA
Has the participant taken antibiotics for another reason in the last month (not including this illness)?						
No	344	113	42.9	ref.	—	
Yes	32	9	35.9	0.75	0.31-1.66	0.49
Has the participant taken antibiotics for another reason in the last six months?						
No	307	102	42.9	ref.	—	
Yes	69	20	35.9	0.86	0.47-1.53	0.60
Has the participant had a chest infection/cough in the last year?						
No	310	93	39.9	ref.	—	
Yes	66	29	53.4	1.73	0.98-3.03	0.058
Has the participant had a chest infection/cough in the last 2 weeks (not including this illness)?						
No	306	92	40.3	ref.	—	
Yes	70	30	56.0	1.89	1.08-3.28	**0.024**

N = number of participants in the group; N event = number of PCR-positive events that occurred in the group; aOR = adjusted odds ratio; CI = confidence interval; ref.=reference category used; p-values in bold are significant at <0.05. *Marginal probabilities calculated for age group 5-11 years, and male sex. NA = regression not possible.

Table S6. Logistic regression models adjusted for age group and sex showing the odds of PCR-positive StrepA pharyngitis for clinical presentation characteristics.

Characteristic	N	Event N	Marginal probability* (%)	aOR	95% CI	p-value
Time of first symptom onset						
<12 hrs ago	130	41	44.7	ref.	—	
12-24 hrs ago	38	10	33.7	0.63	0.26-1.42	0.28
24-48 hrs ago	38	10	38.7	0.78	0.33-1.75	0.56
48-72 hrs ago	75	25	48.5	1.17	0.62-2.16	0.63
>72 hours ago	72	22	44.0	0.97	0.51-1.83	0.93
Current skin infection?						
No	326	101	41.2	ref.	—	
Yes	50	21	60.7	2.20	1.15-4.16	**0.016**
Cough						
No	160	56	43.7	ref.	—	
Yes	216	66	41.2	0.90	0.57-1.42	0.65
Coryza (runny nose)						
No	165	59	41.3	ref.	—	
Yes	211	63	44.0	1.12	0.69-1.82	0.66
Fever						
No	23	4	18.7	ref.	—	
Yes	353	118	44.0	3.43	1.18-12.76	**0.038**
Pain of the throat						
No	11	0	NA	ref.	—	
Yes	364	121	42.4	NA	NA	NA
Painful swallowing						
No	15	1	11.3	ref.	—	
Yes	360	121	43.3	6.00	1.16-110.11	0.087
Difficulty swallowing						
No	25	2	13.8	ref.	—	
Yes	350	120	43.6	4.84	1.38-30.67	**0.036**
Nausea						
No	359	116	42.5	ref.	—	
Yes	17	6	41.0	0.94	0.31-2.63	0.91
Vomiting						
No	279	91	41.9	ref.	—	
Yes	97	31	44.1	1.09	0.65-1.82	0.73
Abdominal pain						
No	256	75	38.4	ref.	—	
Yes	120	47	49.4	1.56	0.98-2.49	0.062
Diarrhoea						
No	298	102	43.5	ref.	—	
Yes	78	20	35.6	0.72	0.39-1.26	0.26
Rash (viral-like exanthema)						
No	374	122	42.3	ref.	—	
Yes	1	0	NA	NA	NA	NA
Hoarseness						
No	323	105	43.3	ref.	—	
Yes	53	17	38.0	0.80	0.41-1.51	0.51
Headache						
No	178	49	37.8	ref.	—	
Yes	198	73	44.9	1.34	0.85-2.11	0.21
Conjunctivitis						
No	359	115	42.1	ref.	—	
Yes	17	7	54.3	1.64	0.56-4.51	0.35
Any other symptoms present?						
No	350	112	42.2	ref.	—	
Yes	26	10	46.2	1.18	0.49-2.72	0.71
BMI-for-age	376	122	-2.0	0.91	0.75-1.10	0.33

Characteristic	N	Event N	Marginal probability* (%)	aOR	95% CI	p-value
Axillary temperature >38ºC						
No	301	101	43.0	ref.	—	
Yes	75	21	38.7	0.84	0.46-1.47	0.54
Tonsillar erythema (redness of the tonsils)?						
No	2	2	100.0	ref.	—	
Yes	374	120	41.5	NA	NA	NA
Tonsillar swelling (swelling of the tonsils)?						
No	15	6	45.9	ref.	—	
Yes	361	116	42.2	0.86	0.29-2.75	0.79
Tonsillar exudate (pus/exudate on the tonsils)?						
No	257	73	38.0	ref.	—	
Yes	119	49	51.9	1.76	1.10-2.81	**0.019**
Pharyngeal erythema (redness of the pharynx)?						
No	184	1.76	34.1	ref.	—	
Yes	192	1.76	47.7	1.76	1.12-2.78	**0.015**
Pharyngeal swelling (swelling of the pharynx)?						
No	280	83	39.6	ref.	—	
Yes	96	39	49.2	1.48	0.90-2.43	0.12
Pharyngeal exudate (pus/exudate on the pharynx)?						
No	356	113	41.7	ref.	—	
Yes	20	9	52.4	1.54	0.58-3.95	0.37
Swollen anterior cervical lymph node(s) on palpation?						
No	12	5	49.2	ref.	—	
Yes	364	117	42.2	0.75	0.23-2.71	0.65
Approximate size of largest anterior cervical lymph node on palpation (in centimetres)?						
< 0.5cm	136	42	45.4	ref.	—	
0.5-1cm	142	50	43.1	0.91	0.53-1.56	0.74
1-1.5cm	66	20	37.8	0.73	0.37-1.42	0.37
1.5-2cm	17	4	33.8	0.62	0.16-1.91	0.43
>2cm	3	1	31.7	0.56	0.02-6.63	0.65
Tenderness of anterior cervical lymph nodes on palpation?						
No	51	11	31.6	ref.	—	
Yes	325	111	43.2	1.65	0.82-3.54	0.18
Petechiae on the palate?						
No	233	67	38.2	ref.	—	
Yes	143	55	48.5	1.52	0.97-2.4	0.069
Skin infections seen?						
No	302	92	40.3	ref.	—	
Yes	74	30	58.8	2.11	1.21-3.69	**0.0087**
Type of infections seen?						
None	302	92	39.9	ref.	—	
Pyoderma	21	8	60.5	2.30	0.86-5.86	0.084
Fungal infection	37	13	51.4	1.60	0.74-3.32	0.22
Other	16	9	70.3	3.56	1.23-10.71	**0.019**

N = number of participants in the group; N event = number of PCR-positive events that occurred in the group; aOR = adjusted odds ratio; CI = confidence interval; ref.=reference category used; p-values in bold are significant at <0.05.
*Marginal probabilities calculated for age group 5-11 years, and male sex. NA = regression not possible.

Table S7. Logistic regression models adjusted for age group and sex showing the odds of PCR-positive StrepA pharyngitis for different measures of social-mixing.

Characteristic	aOR	95% CI	p-value
Number of people the participant saw yesterday for LESS THAN 5 MINUTES, who they exchanged at least 3 words with	0.92	0.68-1.22	0.56
Number of casual contacts that the participant saw for <5 minutes yesterday, who they had any PHYSICAL CONTACT with, such as a handshake	0.98	0.7-1.35	0.88
If the participant took any public transport yesterday, number of different people they sat next to, or have close contact with	1.13	0.81-1.56	0.46
Number of people, from INSIDE OR OUTSIDE the household, who the participant had a two-way conversation lasting MORE THAN 5 MINUTES with yesterday	1.01	0.88-1.15	0.93
Number of people, from INSIDE OR OUTSIDE the household, who the participant had a two-way conversation with AND PHYSICAL CONTACT with yesterday	1.00	0.88-1.14	0.98
Number of people, from INSIDE OR OUTSIDE the household, who the participant had a two-way conversation with AND SPENT MORE THAN AN HOUR with yesterday	1.01	0.89-1.15	0.82
Number of people, from INSIDE the household, who the participant had a two-way conversation lasting more than 5 minutes with yesterday	1.01	0.88-1.16	0.84
Number of people, from OUTSIDE the household, who the participant had a two-way conversation lasting more than 5 minutes with yesterday	0.99	0.86-1.15	0.91

aOR = adjusted odds ratio; CI = confidence interval; p-values in bold are significant at <0.05.

3.3 Addendum to Chapter 3

3.3.1 SpeB gene expression and role in StrepA pathogenicity

The *speB* gene encodes streptococcal pyrogenic exotoxin B (SpeB), a cysteine protease that plays a key role in the virulence of StrepA. While *speB* is present in almost all StrepA isoaltes, its expression is tightly regulated and varies depending on environmental conditions and strain-specific factors. SpeB contributes to immune evasion by degrading host proteins, including immunoglobulins and extracellular matrix components, facilitating tissue invasion (1). However, its expression is phase-variable, with some invasive StrepA strains downregulating *speB* expression to evade host immune responses (2). Though highly specific to StrepA, SpeB homologs with proteolytic functions have been identified in other streptococcal species, though they do not share the same pathogenic significance (3).

3.3.2 Sample size considerations and study design

The primary objective of this study was to determine the proportion of pharyngitis cases caused by StrepA among Gambian children presenting with sore throat symptoms. The sample size was calculated to achieve sufficient power for this outcome, acknowledging that it was not large enough to allow for detailed age-stratified analyses. The absence of asymptomatic controls and testing for other causes of pharyngitis were due to the study's focused scope on StrepA detection. The complementary SpyCATS study was designed to provide a more comprehensive demographic breakdown and investigate broader epidemiological patterns. Culture was used to detect Groups C and G *Streptococcus* as well as Group A, and PCR has subsequently been used to look for other pathogens, but those data are not presented in this thesis.

3.3.3 Recruitment strategy

Participants were recruited using a convenience sampling approach, enrolling consecutive eligible cases presenting to the Sukuta Health Centre out-patients with pharyngitis symptoms. While this approach maximised feasibility and efficiency, it may have introduced selection bias by underrepresenting children who did not seek healthcare or who presented outside study hours. Nevertheless, given the high healthcare utilisation for childhood febrile illnesses in The Gambia and the consistency of participant characteristics with broader community demographics, the study findings remain broadly generalisable to urban Gambian children and to other similar health centres.

3.3.4 Passive vs. active surveillance in StrepA pharyngitis and age distribution

A notable limitation of the study was the high proportion of children under five years old in the cohort, an age group in which StrepA pharyngitis is less common. This may have skewed the overall prevalence estimates; however, the age distribution reflects real-world presentations to the Sukuta Health Centre, where febrile illnesses, including sore throat, are common among young children. Excluding children under five would not have been appropriate, as it would have missed an important segment of the population seeking care and potentially underestimated the burden of disease. Furthermore, given the scarcity of data on StrepA pharyngitis in The Gambia, precise estimates of prevalence in different age groups remain uncertain. The high proportion of younger children in this study suggests that if the cohort had included a greater proportion of older children, the overall prevalence of StrepA pharyngitis would likely have been higher.

This study employed passive surveillance, capturing data from children who actively sought medical care for pharyngitis. Passive surveillance may detect a higher proportion of StrepA pharyngitis, as individuals with milder symptoms might not present to healthcare facilities and more severe cases of pharyngitis are more likely to be StrepA. In contrast, active surveillance involves systematically screening individuals regardless of symptom severity, potentially identifying a higher proportion of mild or asymptomatic cases. A meta-analysis found that the prevalence of culture-positive StrepA pharyngitis was higher in clinical settings using passive recruitment methods (24.1%) compared to sore throat management programmes employing active recruitment (10.0%) (4). This suggests that passive surveillance may overestimate the prevalence of StrepA pharyngitis relative to active surveillance methodologies.

3.3.5 Health-seeking behaviour for sore throat in The Gambia

Qualitative research conducted in the same community revealed that caregivers often perceive sore throats as non-severe and typically manage them at home using traditional medicine, consulting local healers known as marabouts. This practice often delays or precludes seeking formal healthcare services. Awareness of the potential progression from untreated sore throat to severe conditions like acute rheumatic fever or rheumatic heart disease was notably low among caregivers. These findings highlight the importance of community education to improve recognition of sore throat severity and the benefits of timely medical intervention (5).

3.3.6 References

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4 Research Paper 2: *Streptococcus pyogenes* carriage acquisition, persistence and transmission dynamics within households in The Gambia (SpyCATS): protocol for a longitudinal household cohort study

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Surname/Family Name	Armitage		
Thesis Title	Epidemiology of Streptococcus pyogenes in The Gambia: investigating carriage and disease burden, transmission dynamics and diagnostic accuracy		
Primary Supervisor	Prof. Michael Marks		

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SECTION E

Student Signature	
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Supervisor Signature	
Date	1/7/24

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4.1 Manuscript

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STUDY PROTOCOL

REVISED

Streptococcus pyogenes carriage acquisition, persistence and transmission dynamics within households in The Gambia (SpyCATS): protocol for a longitudinal household cohort study [version 2; peer review: 2 approved]

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Abstract

Background

Streptococcus pyogenes (StrepA) causes a significant burden of disease globally from superficial infections to invasive disease. It is responsible for over 500,000 deaths each year, predominantly in low- and middle-income countries (LMIC). Superficial StrepA infections of the skin and pharynx can lead to rheumatic heart disease, the largest cause of StrepA-related deaths in LMIC. StrepA can also asymptomatically colonise normal skin and the pharynx (carriage),

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	1	2
version 2 (revision) 30 Oct 2023		✓ view
version 1 27 Jan 2023	✓ view	? view

1. **Shiranee Sriskandan**, Imperial College London, Hammersmith Hospitals campus,

potentially increasing infection risk. *Streptococcus dysgalactiae subsp. equisimilis* (SDSE) carriage is also common in LMIC and may interact with StrepA. This study aims to investigate StrepA and SDSE carriage and infection epidemiology, transmission dynamics and naturally acquired immunity within households in The Gambia.

Methods

A longitudinal household observational cohort study will be conducted over one year. 45 households will be recruited from the urban area of Sukuta, The Gambia, resulting in approximately 450 participants. Households will be visited monthly, and available participants will undergo oropharyngeal and normal skin swabbing. Incident cases of pharyngitis and pyoderma will be captured via active case reporting, with swabs taken from disease sites. Swabs will be cultured for the presence of group A, C and G beta-haemolytic streptococci. Isolates will undergo whole genome sequencing. At each visit, clinical, socio-demographic and social mixing data will be collected. Blood serum will be collected at baseline and final visit. Oral fluid and dried blood spot samples will be collected at each visit. Mucosal and serum anti-StrepA antibody responses will be measured.

Outcome

This study will report StrepA and SDSE clinical epidemiology, risk factors, transmission dynamics, and serological responses to carriage and infection. Detailed social mixing behaviour will be combined with phylogenetic relatedness to model the extent of transmission occurring withing and between households. The study will provide data to help meet global strategic StrepA research goals.

Keywords
Streptococcus pyogenes, asymptomatic carriage, pharyngitis, pyoderma, longitudinal cohort study, transmission modelling, The Gambia, rheumatic heart disease

London, UK

2. **Stephan Brouwer**, The University of Queensland, Saint Lucia, Australia

Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 1**

Changes and additions to the text have been made in response to reviewers' comments and suggestions.

1. The term "reservoir" regarding non-human StrepA sources has been replaced with "sources".
2. "Study design" now specifies that the household size assumption of 10 stems from our research in Sukuta, not prior census data.
3. "Exclusion criteria" provides examples for potential non-compliance with the study protocol and underscores that pregnant women and new-borns can enrol.
4. "Field activities" elaborates that new-borns born during the study can enrol with parental consent.
5. The procedure for the 16 households in "Intensified incident surveillance cohort" undergoing 6-week intensified swabbing is now clearer.
6. "Clinical and field evaluations" now includes workplace and school name in the "Socio-demographics" data.
7. "Laboratory evaluations" updates the "Culture procedures" section with: rationale for using Colombia blood agar and its limitations; clarification of storage of liquid Amies without glycerol and its limitation; morphology-based identification of S. aureus colonies; latex agglutination's role and limitations in distinguishing StrepA from SDSE; storage details of liquid Amies for *SpeB*-targeting PCR validation; usage of single colonies from the purity plate and its limitations vs. sweep; and the significance of WGS in detailing species and antigen data.
8. In "Laboratory evaluations", the "Dried blood spot processing" section elucidates the criteria for M protein, Enn, and Mrp serotype selection, reasons for specific antigen choices in the assay, and the comparability rationale of dried blood spot to serum for serological analysis with an associated reference.
9. "Study status" now provides recruitment figures.

Any further responses from the reviewers can be found at the end of the article

Introduction

Streptococcus pyogenes (Group A *Streptococcus*, StrepA) is a beta-haemolytic Gram positive bacterium that is a major cause of infectious disease burden globally, responsible for over 500,000 annual deaths[1–4]. It causes a wide spectrum of disease from superficial skin and pharynx infections to invasive disease, in addition to the immunological sequelae of acute rheumatic fever, rheumatic heart disease (RHD) and acute post-streptococcal glomerulonephritis[3,5]. Each year an estimated 1.8 million invasive StrepA, 111 million pyoderma and 616 million pharyngitis cases occur globally[2]. Most clinical StrepA infections are thought to occur in low- and middle-income countries (LMIC), though data from such countries is lacking[1,2,6,7]. Moreover, RHD, the most serious immunological consequence of StrepA infection, causes over 300,000 deaths each year, predominantly in LMIC[3], where diagnosis and surveillance is poor[3,8].

Despite the significant burden of StrepA disease and its immunological sequelae, there has been little focus on StrepA carriage and transmission in LMIC[8,9]. Furthermore, the understanding of the natural history of StrepA carriage, transmission and infection is limited[9,10]. A better understanding of carriage incidence, prevalence, persistence (duration), seasonal variation, transmission and the associated risk factors within high-disease burden settings in LMIC is crucial to design and implement interventions targeting StrepA in such countries.

The epidemiology of superficial StrepA infections in The Gambia is poorly understood. In 2018[11], a cross-sectional study in 1441 Gambian children under five years old found a high prevalence of bacterial pyoderma (17.4%), scabies infestation (15.9%), and of StrepA culture-positive pyoderma (8.8%). There was also a significant increase in pyoderma detected during the rainy season compared to before (8.9% vs. 23.1%, adjusted prevalence ratio 2.42, CI 1.39-4.23).

Whole genome sequencing (WGS) has transformed our ability to understand StrepA epidemiology, giving significantly better resolution than *emm* typing to determine linkage between strains. This has been used to gain valuable insights into transmission dynamics and to inform outbreak investigation in HICs[12–16]. In low-income settings where the molecular epidemiology of StrepA is notably different, combining WGS data, clinical and behavioural data with mathematical models can provide new insights into transmission dynamics and potential intervention strategies[17,18].

Rationale

In 2018, the World Health Assembly stated that RHD and StrepA research should be a global priority[19]. The WHO then published a Group A *Streptococcus* Vaccine Development Technology Roadmap highlighting key strategic areas for research including to improve global estimates of disease burden and epidemiology of StrepA infections, and to further describe the spectrum of natural disease history and immunity in longitudinal studies[9,10]. Our limited understanding of StrepA transmission dynamics and immunity is mostly derived from studies in high-income countries (HIC)[20–22]. However, in LMIC such as The Gambia, a higher prevalence and incidence of StrepA carriage and a wider diversity of the circulating *emm* types may underlie the higher burden of StrepA-related clinical infections and immune sequelae seen[23–25].

Very few longitudinal studies of StrepA exist[20,26,27], and high-quality longitudinal data from a high-prevalence country in sub-Saharan Africa combining classical epidemiology with detailed social mixing behaviour and next generation WGS techniques to model disease transmission will be highly informative in growing our understanding of StrepA epidemiology and meeting global strategic StrepA research goals on the road towards a StrepA vaccine.

Study objectives

Primary:

1. To determine the prevalence, incidence, duration and transmission dynamics of asymptomatic StrepA carriage and clinical StrepA infections within households.
2. To establish risk factors for pharyngeal and skin clinical StrepA infection, including detailed characterisation of the relationship with individual and household asymptomatic carriage, *emm* type and seasonality.

3. To develop a mathematical model of household StrepA transmission using clinical, behavioural and phylogenetic relatedness data to calibrate it, to allow for estimation of the relative contributions of between and within household transmission.

Secondary:

1. To determine risk factors for asymptomatic StrepA pharyngeal and skin carriage.
2. To describe the role of asymptomatic StrepA skin and pharyngeal carriage in StrepA transmission and infection.
3. To describe seasonal variation in StrepA carriage and clinical infection throughout the year.
4. To describe StrepA *emm* type diversity.
5. To investigate the extent of StrepA tissue tropism of *emm* types identified.
6. To determine the prevalence and incidence of *Streptococcus dysgalactiae subspecies equisimilis* (groups C and G streptococcus; SDSE) carriage and clinical infection.
7. To describe the prevalence, incidence and transmission dynamics of *Staphylococcus aureus* skin carriage and infection within households.
8. To describe variations in bacterial density by site, season and clinical characteristics using quantitative PCR.
9. To identify non-human sources of StrepA within households and the presence of airborne StrepA indoors using settle plates.
10. To describe the antimicrobial sensitivity of StrepA isolates identified.
11. To describe age-stratified anti-StrepA antibody titres.
12. To explore StrepA-specific serological and mucosal immune activity in response to colonisation and disease.
13. To investigate the relationship between anti-StrepA antibody titres and risk of incident colonisation and infection to explore serological correlates of protection.

Protocol

Study setting

The Gambia is a small country in West Africa with a population of approximately two million people. It was ranked 174th by the United Nations Human Development Index in 2021, making it one of the least developed countries in the world. It is a predominantly Muslim country, comprising several tribal groups, the largest being Mandinka, Wolof, Fula and Jola.

Sukuta is an urban area, part of the coastal region's sprawling conurbation, where most of the population live. It is a majority Mandinka area, with a population of 47,048, and an average household size of 8.1 in the census in 2013.

The climate is sub-tropical with a long dry season from November to May, and a short rainy season between June and October each year.

Study design

SpyCATS is a prospective, longitudinal (open) cohort study within households in Sukuta, The Gambia. Households will be recruited, and all household members present at the time of the visit will be asked to participate. Households will be followed for 12 months, with monthly visits, and more frequently for some subgroups of participants (described below).

A sample size target of 450 participants was determined (see below). With an average household size of approximately 10 people in Sukuta (based on our previous research and experience in the area given the census data is substantially out of date), 45 households will be recruited, and every available consenting household member included as an individual participant.

Selection of participants

The study will enrol participants as individuals within households. Households will be identified using a process of GPS random selection. No complete sampling frame of households exists for Sukuta, however geographic information system data exist from the 2013 census of The Gambia. These data will be utilised to obtain a random set of GPS sampling locations stratified by population density. A list of GPS coordinates for the locations will be identified and for each location and the nearest household will be approached for participation. Each location on the list will be approached in order until the desired sample size is reached. Households will only be enrolled if over 50% of household members consent to participate in the study.

For the purposes of enrolment in the study, a household will be defined according to The Gambia Demographic and Health Survey 2013 definition: "a household [is] defined as a person or a group of related or unrelated persons who live together in the same dwelling unit(s) or in connected premises, who acknowledge one adult member as the head of the household, and who have common arrangements for cooking and eating."

Inclusion criteria

Households must:

- Be within the boundary of Sukuta as determined by the 2013 census
- Have at least 3 members including at least one child under age 18

Individuals must:

- Provide signed (or thumb-printed) informed consent for study participation (obtained from a parent or guardian for children under the age of 18)
- Be willing and have capacity to participate and comply with the study protocol as judged by a member of the study team

- Be resident in the household, with no plans to move outside of the household during the period of study participation

Exclusion criteria

Households:

- Less than 50% of individuals living in the household, as defined by the The Gambia Demographic and Health Survey 2013 definition, provide consent to participate

Individuals:

- Consent not provided
- Has any condition or any other reason that may lead to difficulty or discomfort in obtaining all the necessary samples
- Is judged by the study team member to be unable or unlikely to participate and comply with the study protocol for the entire study period. Examples could include individuals with severe mental health conditions, communication barriers that cannot be overcome, or frequent absences from the household.

NB. There are no restrictions on enrolment of pregnant women or newly born babies.

Field activities

Overview. Households will be enrolled for 12 months covering both the dry and rainy seasons, with enrolment having commenced in July 2021. Every household will undergo an enrolment visit (MV0), then monthly visits (MV1, MV2, MV3 etc., up to MV12) unless practical constraints arise (see Figure 1). At each visit, the household size will be determined by the number of individuals who slept in the household the previous night, and those household members present will be asked to participate. Household members not available to be seen will be still allocated an ID number, in order to capture relevant information regarding their social mixing with other household members, and if they are present at later visits, they will be asked to consent and enrol. Participants whose baseline (enrolment) visit occurs after MV0 will be asked why they were not available previously. Reasons for missed visits and late enrolment will be captured.

At each visit an oropharyngeal swab (OPS), normal skin swab (NSS), oral fluid sample (OF) and dried blood spot (DBS) will be taken from all enrolled individuals present and data collected on socio-demographics, social mixing behaviour and clinical examination findings. In addition, a blood sample for serum (BS) will be taken at the beginning and end of the study for detailed functional immune responses. Wound swabs (WS) will also be taken from any pyoderma lesions and swabs taken from non-infected skin overlying scabies lesions (scabies skin swab, SSS). Additionally, environmental swabs (ES) will be collected from common touch points in the household and settle plates (SP) placed inside households. Throughout the study, enrolled participants reporting symptoms potentially consistent with StrepA infection will have an unscheduled visit including a physical examination, an appropriate swab (OPS or WS), OF and DBS. Swabs will be transported the

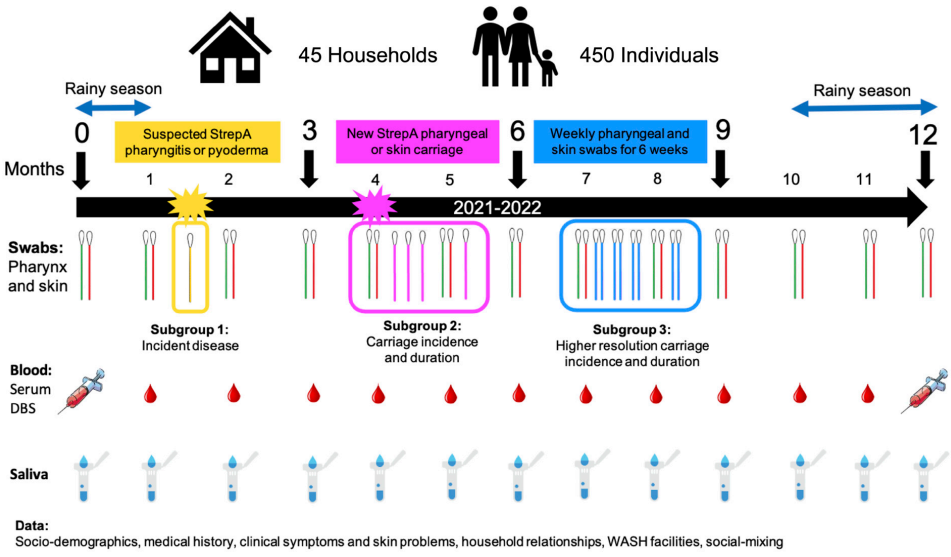

Figure 1. SpyCATS study diagram.

same day to the microbiology laboratory and plated for culture. The culture will identify the presence of catalase-negative, beta-haemolytic streptococci, and determine the group by latex agglutination testing. Antibiotic resistance will be determined, and any isolates will be stored in glycerol for later use. *Staphylococcus aureus* isolates cultured from NSS and WS will also be documented and stored for later characterisation. DBS and OF samples will be used for serological and mucosal immunology objectives. StrepA and SDSE isolates identified will undergo whole genome sequencing (WGS).

Enrolment visit. At the enrolment visit (MV0), each individual will undergo a baseline survey including participant's sociodemographic data, medical history, social mixing and behavioural factors. A physical examination will be carried out including a full body examination (taking care to maintain privacy) looking for any evidence of skin infections, and OPS and NSS will be collected. Additionally, an OF sample will be obtained, and venepuncture performed for BS and DBS. Any participant who is exhibiting signs and symptoms of pharyngitis (sore throat and pharyngeal inflammation) will be further examined and managed as they would be at an unscheduled visit for possible StrepA infection (see below). Any participant who is identified as having evidence of pyoderma (bacterial skin infection involving pus or crusts), will have a WS taken, and will be provided antibiotic treatment according to local guidelines as appropriate. Any additional abnormal finding requiring further investigation or treatment will be managed according to local practice or an appropriate referral made according to the nature of the finding.

Alongside the questionnaires on individuals, data will be collected on households such as household setup. Two ES will be taken from common touch points such as door handles and soft furnishings and a SP will be placed indoors to investigate airborne transmission.

Monthly visits (MV1, MV2, MV3 etc. up to MV12). Participants will be enrolled for 12 months undergoing an enrolment visit (MV0), then monthly visits (MV1, MV2, MV3 etc. up to MV12), though due to the open cohort design new participants can enrol at visits later than MV0 (this includes babies born during the study period if their parent/guardian consents).

At each monthly visit following enrolment, the study team will collect further survey data from each individual household member, collect an OPS, NSS, OF and DBS. Survey data collected will include socio-demographic information, social mixing, behavioural factors and clinical examination findings. Participants with any evidence of pyoderma will have a WS taken and will be offered antibiotic treatment; any other abnormal finding requiring further investigation or treatment will be managed according to WHO guidelines or best local practice or an appropriate referral made according to the nature of the finding. Additionally, any use of antibiotics or other medication, or any attendance at a healthcare setting since the previous visit will be recorded.

Unscheduled visits for possible StrepA infections. All enrolled households will be provided with an on-call number to call at any time when an enrolled household member is experiencing symptoms that could be compatible with a StrepA infection or any other acute illness. A study nurse will then complete a rapid assessment over the phone and arrange for an unscheduled visit as appropriate.

A study nurse will collect data on the history of the complaint, any medication taken and other relevant information. They will perform a physical examination, including vital signs under the supervision of a study clinician. If the symptoms are consistent with possible StrepA infection (pharyngitis or pyoderma), then a study swab will be taken (OPS or WS) for culture alongside an OF and DBS sample. For cases of pharyngitis and pyoderma, treatment will be provided empirically according to locally devised guidelines based on WHO and local guidelines and medication availability (see Table 1).

Participants presenting with symptoms of systemic infection or possible invasive disease will be urgently referred to the MRCG clinical services department for management. Participants presenting with other minor acute medical complaints will be provided appropriate treatment by the study team. All other medical presentations will be referred to the appropriate local healthcare facility.

Sub-studies

Intensified incident surveillance cohort. A subgroup of 16 randomly selected households will undergo intensified swabbing. This subgroup will be used to assess incidence and duration of StrepA and SDSE carriage and disease with greater resolution than the main cohort. During the second half of the study, the 16 households will undergo blocks of 6 weeks of intensified swabbing in batches of 4 households in turn, until all 16 have had a 6-week block of weekly swabbing. The

Table 1. SpyCATS empirical treatment guidelines for potential StrepA infections.

Diagnosis	Signs and symptoms	First-line treatment	Alternative treatment
Pyoderma	Purulent or crusted skin lesion	Cloxacillin (weight-based dose for children under 12 years; over 12 years 500mg qds 5–7 days)	Azithromycin (12 mg/kg up to max 500mg od 5 days)
Pharyngitis	Sore throat, pharyngeal/ tonsillar erythema	Penicillin V (weight-based dose for children under 12 years; over 12 years 500mg qds 5–7 days)	Azithromycin (12 mg/kg up to max 500mg od 5 days)

households will be visited weekly for each of the 6 weeks of the intensive swabbing period. All household members present will undergo swabbing (OPS and NSS) in addition to more detailed social mixing behaviour data being collected. These data will be used in combination with WGS data from any StrepA isolated to inform the household transmission model.

Estimating duration of StrepA carriage. Following MV0, any participant who becomes an asymptomatic StrepA (or SDSE) carrier (i.e. was negative at baseline or the previous visit, and then becomes positive at a monthly visit without symptoms) will have weekly swabs taken from the same site that was positive (OPS or NSS), until two negative swabs have been received in a row.

Nested cross-sectional study of personal hygiene behaviour. At a MV11 or MV12, participants will be requested to undergo an additional survey on their personal hygiene behaviours during the last week including laundry, handwashing, bathing and soap and disinfectant use. Attitudes towards wound care and usual practice of participants in response to wounds will be captured.

At the same visit, additional environmental swabs (ES) will be collected from the household including four commonly touched locations within the household and a sample of water from the main household greywater source.

These data, combined with individuals' carriage and infection data from the wider study will be used to assess the relationship between individual- and household-level hygiene behaviours and StrepA, SDSE and *S. aureus* carriage, infection and non-human source presence within households in this setting.

Clinical and field evaluations

Socio-demographics and household set-up. At the enrolment and later visits where necessary, a questionnaire will be asked of each individual participant in relation to their socio-demographic information including their date of birth, sex, tribal group, educational level and occupation. Any relevant medical information that is identifiable from ante-natal cards (ANC), or infant welfare cards (IWC) (especially for younger children) will be recorded such as birthweight, previous medical diagnoses and allergies.

For each household, data will be collected relating to the household set-up including the number of buildings, family relationships, number of rooms, accessibility for non-household members, sleeping arrangements, mosquito net use, water access, sanitation and hygiene (WASH) facilities and proximity to community meeting points.

At subsequent monthly visits, individuals will be asked to update some of their sociodemographic details such as occupation, work place, school name and attendance and any other factors that may change throughout the year, and to complete any missing data. Similarly, alterations to household set-up will also be collected.

Social mixing behaviour. For all participants at their enrolment and monthly visits, and in more detail for the intensified incidence cohort at each weekly visit, data will be collected on individuals' social mixing behaviour. Participants will be asked information about which other household members they had close contact with the previous day, and who they shared a food bowl, room and/or a bed with. They will be asked about their non-household social contacts from the previous day, including each contacts age, relationship to them, the location of meeting, whether there was physical contact and the duration. For the more detailed extended social mixing behaviour collected in the intensified swabbing cohort, the above data will also be collected for household social contacts.

Medical and drug history. At enrolment, a focused past medical history will be taken from participants including any regular medication taken, previous diagnoses and previous history of skin or throat infections and acute rheumatic fever specifically. At enrolment and at each subsequent visit a brief history of recent medication (particularly antibiotics) and current clinical symptoms will be taken, including details of any recent healthcare setting attendance including traditional healers.

At unscheduled visits, a clinical history of the presenting complaint, medication usage and healthcare attendance will be taken to capture information related to any potential StrepA infections, but also to inform immediate and subsequent medical management of other complaints.

Clinical examination and vital signs. At the enrolment visit, all participants will undergo a physical examination including vital signs to provide a baseline. Vital signs collected will include axillary temperature, pulse rate and respiratory rate. Adults (over 18 years) will also have blood pressure recorded.

Participants will then undergo a physical examination which will include an examination of the pharynx and associated lymph nodes, and a full body examination of the participant's skin, to identify any pyoderma lesions, and other relevant skin conditions (particularly scabies and fungal infections). Care will be taken to perform the full body examination with appropriate privacy and verbal consent obtained at the time. Participants' genitals will only be examined if they specifically report (or the parent reports, in the case of children) the presence of a lesion and verbally consent for the study nurse examine them.

At each subsequent visit participants will undergo the physical examination as described including throat and skin but will not have vitals recorded unless they are reporting any symptoms. If they have any medical complaint, a clinical history will be taken and fuller clinical examination of the presenting complaint will be done, in addition to recording vital signs. At unscheduled visits participants will also have their vitals recorded in the same way, and a clinical history and focused clinical examination will be done.

Clinical samples

At each visit, participants will have clinical samples collected according to the sampling schedule outlined in Table 2.

Oropharyngeal swab. Oropharyngeal swabs (Copan Transystem™ 140C rayon swabs in liquid Amies medium) will

Table 2. Visit data and sampling schedule for the various cohorts. *only at MV0 and MV12.

Visit timing	Visit window	Data and samples	Main cohort	Intensive incident cohort (16 households)	Duration cohort (new carriers)
Month 0 enrolment visit (MV0)	-	Eligibility	X	-	-
		Socio-demographics	X	-	-
		Social mixing behaviour	X	-	-
		Household setup and WASH	X	-	-
		Previous and recent medical history	X	-	-
		Pharyngeal and skin examination including vitals	X	-	-
		Oropharyngeal swab	X	-	-
		Normal skin swab	X	-	-
		Blood serum*	X	-	-
		Dried blood spot	X	-	-
		Oral fluid	X	-	-
		Environmental swabs (x2)	X	-	-
		Settle plate	X	-	-
Weekly visits for duration and intensive incident surveillance	+/- 7 days	Recent medical history	-	X	X
		Extended social mixing behaviour	-	X	-
		Pharyngeal and skin examination	-	X	X
		Oropharyngeal swab	-	X	(X) *if previously positive*
		Normal skin swab	-	X	(X) *if previously positive*
Monthly visits (MV1, MV2, MV3 etc. up to MV12)	+/- 14 days	Update socio-demographics, household setup and WASH	X	X	-
		Recent medical history	X	X	-
		Social mixing behaviour	X	-	-
		Extended social mixing behaviour	-	X	-
		Pharyngeal and skin examination	X	X	-
		Oropharyngeal swab	X	X	-
		Normal skin swab	X	X	-
		Blood serum*	X	X	-
		Dried blood spot	X	X	-
		Oral fluid	X	X	-
		Environmental swabs (x2)	X	X	-
		Settle plate	X	X	-

Visit timing	Visit window	Data and samples	Main cohort	Intensive incident cohort (16 households)	Duration cohort (new carriers)
Unscheduled visits (may occur at scheduled visits if symptoms present)	-	Clinical history and examination	X	X	X
		Wound or oropharyngeal swab	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Oral fluid	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Dried blood spot	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
		Scabies skin swab	(X) *if applicable*	(X) *if applicable*	(X) *if applicable*
Personal hygiene visit (done at another monthly visit)	-	Personal and household hygiene behaviour	X		
		Extended environmental swabs (x5)	X		

be collected from each participant by swabbing the posterior pharynx (both tonsils, posterior wall, uvula and any area of inflammation or exudation), avoiding touching the tongue, cheeks and lips. After sample collection, the swab be aseptically placed in liquid Amies transport solution and placed in a cold box until processing in the laboratory.

Oropharyngeal swabs will be collected in the same way for participants complaining of symptoms that could be consistent with acute pharyngitis at unscheduled visits

Normal skin swab. Normal skin swabs (CITOSWAB® flocked nylon fibre mini-tip swabs in 1ml liquid Amies medium) will be collected with the intention of identifying any StrepA present on the skin, rather than differentiating skin site. Therefore, to maximise sensitivity, a single swab will be used on multiple skin sites.

Swabs will be obtained using modification of a standard skin microbiota swabbing technique[28–31] in which the swab head is moistened with sterile water prior to skin swabbing. The swab will be taken from 2 by 2cm squares of skin on the forehead, then a larger area (5 by 20cm) on both forearms and both lower legs, and then placed aseptically in liquid Amies transport medium and stored in a cold box until processing in the laboratory.

SSS will be collected in the same was as NSS but from a 2 by 2cm patch of skin overlying typical non-infected scabies lesions.

Pyoderma wound swab. Pyoderma WS (Copan Transystem) will be taken at from participants any visit with evidence of pyoderma (a skin infection with pus or crusts). Pus will be expressed if necessary. WS will be placed in liquid Amies transport medium in cold boxes until processing in the laboratory.

Dried blood spot. DBS samples will be collected using dried blood spot collection cards (Whatman 903 protein saver snap-apart cards with four sample spots) from a finger prick on the participant. Four drops of blood will fill the four spots on the DBS card. The finger will be cleansed with alcohol and allowed to dry before the finger prick is made with a lancet. Following collection, the DBS card will be left to dry at room temperature before transportation. Transportation to the laboratory will be in a cold box.

Blood sampling. The study team will be trained to perform venepuncture in the field. In the case that the head of the household, all participants aged over 18, and all guardians of children under 18 verbally consent to venepuncture for blood to be taken on site, this will be performed within the household. Alternatively, an appointment will be made at a specified time to attend Sukuta Health Centre where venepuncture for blood serum will be performed by members of the study team.

Peripheral blood will be collected into serum separation tubes using aseptic technique, ensuring appropriate PPE is used. BS samples will not be obtained from participants under the age of 2 years or those who do not verbally consent.

Oral fluid samples. OF samples will be collected using an ORACOL® salivary collection device (Malvern Medical Developments, S10) from participants at the time points specified in Table 1. The oracol swab will be placed in the buccal cavity of the participant between the gums and the cheek for two minutes. Once obtained, the swab will be immediately placed in the collection tube according to the manufacturer's instructions. OF tubes will be transported to the laboratory in a cold box.

Environmental swabbing. At monthly household visits, two ES (Copan Transystem) will be taken from common touch points in the household. At the enrolment visit, the study team will identify two surfaces to swab within the household which are commonly touched by multiple people. Swabbing points might include door handles, table surfaces, curtains, benches,

chair handles etc. Once two surfaces have been decided for the household, those will be the two surfaces swabbed at each subsequent visit.

The ES tip will be soaked in sterile water prior to swabbing and will be rubbed slowly and thoroughly over the surface (up to 50cm) three times reversing direction between strokes. Once collected, the swab will be aseptically placed in liquid Amies transport medium and stored in a cold box until processing in the laboratory.

For the personal and household hygiene visit, additional environmental swabs will be collected from a wider range of common touch points in the house, and a swab will be soaked in water taken from the household greywater source.

Settle plates. A settle plate will be used at each monthly household visit to passively capture the presence of airborne StrepA within households. A culture petri dish pre-prepared with Colombia blood agar will be placed at a suitable point in the main social indoor room of the household. Ideal placement of the settle plate would be at least one metre off the floor, one metre away from the walls and other large obstacles. The plate will be left for one hour, then retrieved and stored in a cold box until processing in the laboratory.

Laboratory evaluations

Sample transport. All swabs and clinical samples taken in the field, except for DBS cards which will be dried first at room temperature, will be stored as soon as possible in a cold box maintained at approximately 2–8°C. All samples will be transported in the cold box to the MRCG Fajara laboratories for processing the same day.

Culture procedures. OPS, NSS, SSS, WS and ES will be processed in the same way. Swabs will arrive at the laboratory in 1ml of liquid Amies transport medium. On arrival at the lab, after ensuring that the swab is inside and the lid is properly closed, the swab will be briefly vortexed in the transport medium. Next the swab will be removed and streaked onto a Colombia blood agar culture plate, and then discarded. Colombia blood agar was chosen for its availability MRCG and utility in culturing both *Streptococci* and *Staphylococci*, though use of selective blood agars may have been preferrable to prevent overgrowth. The remaining liquid Amies will be stored at -70°C without the addition of glycerol for subsequent use. It should be noted that the absence of glycerol in the stored medium precludes the possibility of reculturing from these samples, and the addition of glycerol will be considered in future studies.

The culture plates will be incubated for 18–24 hours at 37°C and assessed for the presence of beta-haemolytic colonies. Colonies with clear beta-haemolysis will be picked and replated for purity for a further 18–24 hours at 37°C. Pure growth colonies will then undergo catalase testing, and if negative then latex agglutination testing for Lancefield group A, C and G. Colonies identified from the primary plate as possible *Staphylococcus aureus* from NSS, SSS and WS will be identified based on their morphology and will also be replated for purity, then if catalase positive, will be tested for *S. aureus* using staphylococcal latex testing. Latex agglutination tests will be used for initial identification of streptococcal groups due to their practicality in our setting. However, we acknowledge the limitations of this method in distinguishing between *S. pyogenes* and SDSE. Liquid Amies transport medium from the swabs will be stored for future PCR validation, specifically targeting the *speB* gene.

Single colonies identified from the purity plate of any group A, C or G streptococci isolates identified will then be stored in glycerol broth at -70°C for later revival, DNA extraction and whole genome sequencing (WGS), which will provide detailed information on species, antigen presence and carbohydrate expression. *S. aureus* colonies will also be stored for later analysis. In future studies, consideration will be given to additionally storing a sweep of colonies from the original culture plate.

Antimicrobial susceptibility testing by disc diffusion using standard CLSI procedures will also be performed on group A, C or G streptococcal isolates identified.

Whole genome sequencing (WGS). Isolates will be revived, and DNA extracted using established methods. Library preparations and WGS (Illumina short read and Oxford Nanopore technology long read platforms) will be undertaken. Quality control, *de novo* genome assembly, and core genome determination will be performed, followed by basic phylogenetic reconstruction using maximum likelihood. *Emm* and MLST typing and AMR will be performed. Genotypically-linked isolates will be determined by analysing genetic diversity and relationships between isolates.

Dried blood spot processing. Upon arrival at the laboratory, DBS cards will be dried at room temperature overnight, then stored at -20°C for later elution. To elute the blood, 6mm punches of dried blood filter paper will be obtained and eluted using a buffer solution. The resulting eluate will then undergo serological analysis for anti-StrepA antibodies, including antibodies to Streptolysin O, SpyCEP, SpyAD, GAC, DNAseB, Enn, Mrp and M protein. Responses to the hypervariable M protein will be explored by selecting three types of antigens: first, *emm*-cluster-representative M peptides; second, M peptides from emm-types identified in The Gambia; and third, M peptides from emm-cluster-representative M protein vaccine antigens. A similar framework will be applied to selecting representative Mrp and Enn proteins. The antigens were chosen to provide a mixture of well-established markers of infection and to cover antigens used in some leading vaccine candidates. Our preliminary (unpublished) evidence suggests that DBS samples are adequate for monitoring the development of immunity to StrepA infections and carriage events compared to serum, as has been shown elsewhere[32].

Serum blood. BS samples will be used to assess serological activity to StrepA antigens including Streptolysin O, SpyCEP, SpyAD, GAC, DNAseB, Enn, Mrp, and M protein at baseline

and at the end of the cohort. BS taken at the same time-points as DBS samples will additionally contribute to validation of DBS in this setting as a reliable and reproducible method for measuring anti-StrepA antibodies. Serum samples will be stored for further immunological work including streptococcal killing assays and opsonophagocytic assays to explore correlates of protection from StrepA asymptomatic carriage and clinical disease.

Oral fluid samples OF samples will be mixed with antibody stabilising buffer on the day of collection. OF samples will be used to assess for mucosal antibody activity to StrepA antigens including Streptolysin O, SpyCEP, SpyAD, GAC, DNAseB, Enn, Mrp, and M protein. Samples will be stored for further immunological work.

Modelling

Using data generated on swab positivity time, participant relationships, WGS data on phylogenetic relatedness of strains, geographic distance between households, and assortativity of social mixing in this setting, we will attempt to identify likely transmission events between individuals using R packages such as *outbreaker2* and *o2geosocial.* These models use Bayesian techniques to compute the likelihood that transmission occurred between individuals or not and hence allows for reconstruction of likely transmission chains.

Utilisation of the novel data in this project will allow estimation of relative contributions of between and within household transmission, and transmission between symptomatic and asymptomatic individuals. To our knowledge this has never been done for StrepA carriage and infection in Africa. The household model will also be valuable in evaluating potential intervention strategies for future implementation within LMICs. Once past events have been estimated, it will be possible to calibrate the model to simulate forward to predict likely onward transmission in the case of an individual with certain characteristics becoming positive.

Sample size considerations

The primary outcome measures used to determine sample size were:

1. Monthly StrepA carriage prevalence, and
2. StrepA carriage and infection incidence over 12 months.

In HIC, StrepA pharyngeal carriage prevalence in children is 2–17%[20,21], and in Uganda is 15.9%[33]. Our study also includes adults, in whom carriage is lower, but will use pooled skin and pharyngeal carriage as our outcome measure, which will likely increase prevalence in turn. We therefore estimate a pooled prevalence of 15%.

StrepA pharyngeal carriage yearly incidence in children in the US was shown to be 27–32%[20]. We found a skin infection incidence of 592/1000 child years in The Gambia during an influenza vaccine study follow-up (unpublished data) and of which ~50% are likely due to StrepA[11]. As we are including adults with a likely lower incidence, we estimate a yearly incidence of 20%.

The sample size was calculated for the primary objective, StrepA carriage prevalence, using the formula below to measure the estimated prevalence of 15% with a precision of ±5%.

$$n = \frac{Z_{\alpha/2}{}^2 \times p \times (1-p)}{e^2}$$

Where p is predicted proportion and e is desired precision.

Using Z=1.96 for α=0.05, p=0.15 and e=0.05 we require a sample of 196. Intracluster correlation is unknown, therefore we used a conservative design effect of 2, which allowing for 10% drop-out rate gives a required sample size of 431.

We therefore propose to recruit 45 households, which with an average household size of 10, will equal approximately 450 individuals for the main cohort.

This sample size would provide adequate power for precise estimates of prevalence and incidence of StrepA carriage (precision between ±4 and ±5%) and to detect risk factors for StrepA carriage with prevalence (or incidence) rate ratios of greater than 2 with 80% power.

Data analysis

The clinical epidemiology of StrepA and SDSE carriage and infection will be presented using descriptive statistics. Baseline and monthly prevalence of skin and pharyngeal StrepA and SDSE carriage will be reported, including seasonal (monthly) variation. For pharyngeal and skin infection, baseline prevalence will be reported, then monthly and annual incidence for the duration of the study. The typical patterns of transmission observed between individuals within households will be described.

Logistic regression models will be used to look for socio-demographic and medical risk factors for carriage and infection at baseline. Survival analysis (extensions to Cox proportional hazards models) will be used to explore risk factors for carriage and infection throughout the study period taking into account household clustering, repeated events and time-dependent co-variates. The impact of carriage and infection in a close contact or family member in the month prior to new acquisitions of carriage or new infection will be investigated. The relationship between SDSE carriage and StrepA carriage and infection, whether SDSE presence impacts StrepA *emm*-type diversity, whether SDSE carriage is itself a personal risk factor for StrepA will also be explored. Additional risk factors to be explored include the impact of scabies, social mixing patterns, and socio-demographic factors.

To explore the protective association of antibody titres further, regression analysis will be performed to establish the association of antibody titre for each conserved antigen with incident disease, carriage or no carriage/infection accounting for covariates including age, sex and household size.

Data collection and handling

Field data will be collected on electronic case report forms inputted on tablet computers by the field team. The

questionnaires will be designed using REDCap™ electronic data capture software hosted at MRCG. Data will be collected offline and synced with the secure database at the end of each day. Data generated in the laboratory will be inputted onto the same database. Written informed consent will be sought from all participants prior to any study activities and before any data is collected.

Questionnaires will be designed with up-front data quality checks including reference ranges and dropdown menus to minimise incorrect data entry. Additionally, after completion of the study, a data checking process will be performed running queries to check for incomplete or nonsense data.

All data will be kept confidentially, and electronic data encrypted. Each participant will be assigned a unique study ID, so that no person identifiable data will be kept on the database. Any person identifiable data will be held securely and will not be available to anyone other than those in the investigator team. Data will be handled in accordance with the data management SOPs of MRCG which is fully compliant with GDPR regulations. Anonymised data will be held in the study database for a minimum of 10 years following project completion, in compliance with LSHTM's Records Retention and Disposal Schedule. Anonymised raw study data and analysis code will be deposited in the LSHTM Data Compass repository on publication of study outputs and will be available upon request for scientific purposes.

Whole genome sequencing data will be generated using both short read and long read platforms. Raw sequence data will be in the form of fastq files and initially stored on high-performance clusters (HPCs). Data management and analysis will be performed on pipelines established on both the MRCG HPC and the University of Sheffield HPC, as well as cloud-based servers such as the MRC CLIMB platform. Raw sequence data will be archived at MRCG according to their data archiving procedures. Certain processed data fields from genomic analysis (e.g. *emm* type) will be included as variables in the study REDCap™ database. Sequence data along with links to relevant metadata will be submitted to a public sequence repository (e.g. genbank) as is standard practice, on publication of study outputs. Analysis pipeline and code will be made openly available via GitHub on publication of the study.

Ethics and informed consent

This study will be conducted in accordance with the principles set forth in the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki in its current version, whichever affords the greater protection to the participants.

Ethical approval has been obtained for the study from the MRC Scientific Coordinating Committee and the joint MRC/Gambia Government Research Ethics Committee, as well as the LSHTM ethics committee. Ethical approval reference number LEO24005.

Sensitizing potential study participants will precede the formal recruitment period to ensure that they are aware of the study as far in advance as is practical and therefore are given as much chance as possible to consider their potential involvement prior to providing informed consent. Sensitization will be approached using community and household/individual level strategies.

At the informed consent visit (at least 24 hours after sensitization), the study team will discuss the study with the household head and other household members to confirm that they have understood the consequences of study participation and to answer any remaining questions. If all the inclusion are met and none of the exclusion criteria are, the study team member will proceed to obtain informed consent from all household members.

To obtain informed consent from the household members, in the presence of a literate witness, a member of the study team will translate the informed consent document (ICD), which is in English, line-by-line into the local language spoken by the consenting individual (e.g. Mandinka or Wolof). Once the entire ICD has been translated, the study team member will answer any questions that the individual may have. If the consenting individual remain willing to participate and to provide informed consent, they will be asked to sign or thumbprint signature page of the ICD. If the participant is not literate, the witness will write the date and time and the participant will be asked to thumb-print the signature portion of the ICD. For participants under the age of 18 the child's parent will be required to sign (or thumbprint) the ICD on their behalf. Children aged between 12 and 17 years inclusive will be asked to provide assent to participate in the study in addition to the informed consent provided by the child's parent or legal guardian.

Dissemination

This observational cohort study is registered on ClinicalTrials.gov (NCT05117528). The study results along with raw data and analysis code will be published promptly in peer-reviewed journals and promoted through the MRCG communications department and through social media where appropriate. Data will be submitted as abstracts to be presented at international conferences such as the European Congress of Clinical Microbiology and Infectious Diseases and the Lancefield International Meeting on Streptococci and Streptococcal Disease.

Study status

Field work for this study is now complete. Study enrolment commenced on 27th July 2021 and the final MV12 visit was completed on 28th September 2022. 442 individuals were recruited from 44 households, with 3 households and 160 individuals being lost to follow up.

Data availability

Underlying data

No underlying data are associated with this article.

Extended data

Zenodo. REDCap data dictionary. DOI: https://doi.org/10.5281/zenodo.7463052[34]

Zenodo. SpyCATS informed consent documents (adult and child). DOI: https://doi.org/10.5281/zenodo.7501168[35]

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4.1 Addendum to Chapter 4

4.1.1 Antibiotic treatment duration

In the SpyCATS study, the prescribed antibiotic treatment duration for pharyngitis was 5 to 7 days. This duration is shorter than the standard 10-day course recommended in various guidelines, which aim to ensure complete eradication of the bacteria and prevent complications such as acute rheumatic fever (1).

The choice of a shorter treatment course was based on local treatment practices, WHO guidelines, and the local availability of medications. Participants were regularly monitored for symptoms throughout the study. Notably, the results did not indicate any relapses of pharyngitis with this treatment regimen.

However, it is important to consider that shorter antibiotic courses may carry a higher risk of relapse. Penicillin treatment failure rates for StrepA pharyngitis have been reported as high as 20-40%, influenced by factors such as patient non-compliance, beta-lactamase-producing bacteria, and intracellular persistence (2). Therefore, while the 5 to 7-day antibiotic course was consistent with local practices and WHO guidelines, and no relapses were observed during the study, the potential for higher relapse rates with shorter treatment durations must be acknowledged.

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5 Research Paper 3: S*treptococcus pyogenes* carriage and infection within households in The Gambia: a longitudinal cohort study

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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	1806929	**Title**	Dr
First Name(s)	Edwin Peter		
Surname/Family Name	Armitage		
Thesis Title	Epidemiology of Streptococcus pyogenes in The Gambia: investigating carriage and disease burden, transmission dynamics and diagnostic accuracy		
Primary Supervisor	Prof. Michael Marks		

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?	Lancet Microbe		
When was the work published?	9th May 2024		
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Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

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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)	The SpyCATS study was conceieved by myself in collaboration with my supervisors. I oversaw all field work and lab work, was the primary author of this manuscript and performed all data clearning and analysis.

SECTION E

Student Signature	
Date	1st July 2024

Supervisor Signature	
Date	1/7/24

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Streptococcus pyogenes carriage and infection within households in The Gambia: a longitudinal cohort study

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Summary

Background ***Streptococcus pyogenes*** **causes more than 500 000 deaths per year globally, which occur disproportionately in low-income and middle-income countries. The roles of *S pyogenes* skin and pharyngeal carriage in transmission are unclear. We aimed to investigate the clinical epidemiology and household transmission dynamics of both *S pyogenes* asymptomatic carriage and infection in a high-burden setting.**

Methods **We did a 1-year prospective, longitudinal, household cohort study, recruiting healthy participants from households in Sukuta, The Gambia. Households were eligible if they comprised at least three members, including one child younger than 18 years, and were excluded if more than half of household members declined to participate. Households were identified by random GPS coordinates derived from census data. At monthly visits, pharyngeal and normal skin swabs were collected for *S pyogenes* culture, and sociodemographic data were recorded by interview. Incident pharyngitis and pyoderma infections were captured. Cultured isolates underwent *emm* genotyping. The primary outcome measures were incidence of *S pyogenes* carriage and disease. Additional outcomes were prevalence of *S pyogenes* skin and pharyngeal carriage, *S pyogenes* skin and pharyngeal clearance time, *S pyogenes emm* type, risk factors for carriage and disease events, household secondary attack rate, and *emm*-linked household transmission events. The study is registered on ClinicalTrials.gov, NCT05117528.**

Findings **Between July 27, 2021, and Sept 28, 2022, 442 participants were enrolled from 44 households. The median age was 15 years (IQR 6–28) and 233 (53%) were female. We identified 17 pharyngitis and 99 pyoderma events and 49 pharyngeal and 39 skin *S pyogenes* carriage acquisition events. Mean monthly prevalence was 1·4% (95% CI 1·1–1·9) for *S pyogenes* pharyngeal carriage and 1·2% (0·9–1·6) for *S pyogenes* skin carriage. Incidence was 120 per 1000 person-years (95% CI 87–166) for *S pyogenes* pharyngeal carriage, 124 per 1000 person-years (90–170) for *S pyogenes* skin carriage, 51 per 1000 person-years (31–84) for *S pyogenes* pharyngitis, and 263 per 1000 person-years (212–327) for *S pyogenes* pyoderma. Pharyngeal carriage risk was higher during the rainy season (HR 5·67, 95% CI 2·19–14·69) and in larger households (per additional person: 1·03, 1·00–1·05), as was pharyngitis risk (rainy season: 3·00, 1·10–8·22; household size: 1·04, 1·02–1·07). Skin carriage risk was not affected by season or household size, but was lower in female than in male participants (0·45, 0·22–0·92) and highest in children younger than 5 years compared with adults (22·69, 3·08–167·21), with similar findings for pyoderma (female sex: 0·34, 0·19–0·61; age <5 years: 7·00, 2·78–17·64). Median clearance time after carriage acquisition was 4·0 days for both skin (IQR 3·5–7·0) and pharynx (3·5–7·3). The mean household secondary attack rate was 4·9 (95% CI 3·5–6·3) for epidemiologically linked *S pyogenes* events and 0·74 (0·3–1·2) for *emm*-linked *S pyogenes* events. Of the 204 carriage and disease events, *emm* types were available for 179 (88%). Only 18 *emm*-linked between-visit household transmission events were identified. Pyoderma was the most common source of *S pyogenes* household transmissions in 11 (61%) of 18 *emm*-linked transmissions. Both pharynx to skin and skin to pharynx transmission events were observed.**

Interpretation ***S pyogenes*** **carriage and infection are common in The Gambia, particularly in children. Most events are non-household acquisitions, but skin carriage and pyoderma have an important role in *S pyogenes* household transmission and bidirectional transmission between skin and pharynx occurs.**

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Introduction

Streptococcus pyogenes causes a spectrum of disease from superficial pharyngeal and skin infections to invasive disease. It results in more than 500 000 deaths each year,[1] and an estimated 1·8 million invasive infections, 111 million pyoderma, and 616 million pharyngitis cases

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Research in context

Evidence before this study

In Africa, there is a paucity of research into *Streptococcus pyogenes* and its sequelae, despite a substantial disease burden. We searched PubMed from database inception to Dec 31, 2023, for studies in any language related to *S pyogenes* carriage and infection epidemiology and transmission, using the search terms "Streptococcus", "pyogenes", "GAS", "StrepA", "carriage", "asymptomatic", "colonisation", "pharyngitis", "impetigo", "pyoderma", and "transmission". Few studies of *S pyogenes* have been conducted in Africa. Cross-sectional studies from Uganda, Ethiopia, and Zambia have documented pharyngeal carriage of 10–19% in children younger than 18 years. One review of *S pyogenes* infections in Africa found the pooled prevalence of *S pyogenes*-positive pharyngitis to be 21% and that pyoderma positivity ranged from 32–74%. In Africa, only one study has previously identified *S pyogenes* skin carriage and no longitudinal cohort studies of *S pyogenes* incidence and household transmission have been performed. In contrast to other pathogens, the relationship between *S pyogenes* asymptomatic carriage and infection is not clear. Studies conducted in the Red Lake Indian reservation in the 1960s hinted at the role of skin carriage and infection in transmission, but similar intensive sampling studies have not been repeated. In 2023, a re-analysis using whole-genome sequencing of a surveillance study conducted in Aboriginal communities in Australia has indicated the importance of pharyngeal carriage as a reservoir for *S pyogenes* transmission and shown evidence of throat-to-skin transmission.

Added value of this study

This study used frequent microbiological sampling and *emm* typing from normal skin, pharynx, and wounds in a longitudinal household design over 1 year, combined with clinical and sociodemographic data, to understand the clinical and molecular epidemiology of *S pyogenes* over time in an African setting for the first time. We describe a comprehensive overview of the epidemiology of *S pyogenes* in this setting, as well as providing evidence of important transmission routes within households.

Implications of all the available evidence

Various interventional and public health strategies could be applied to African settings to reduce the burden of *S pyogenes*-related disease, and vaccines in development will be an important addition. With so few studies into the natural history of *S pyogenes* carriage and natural infection over time, there is limited understanding of which approaches to use or how to target them. This study builds on studies from other settings and provides evidence for the first time in Africa of the importance of *S pyogenes* skin infections and asymptomatic carriage in household transmission. Evidence from this study will be useful in the design of future surveillance and interventional studies in such settings, which are vital to tackle the burden of *S pyogenes*-related disease globally.

occur globally.[1,2] The largest burden is in low-income and middle-income countries (LMICs), where the post-infection immune-mediated sequelae of acute post-streptococcal glomerulonephritis, acute rheumatic fever, and rheumatic heart disease cause substantial morbidity and mortality.[3] Rheumatic heart disease results in more than 300 000 deaths each year, predominantly in settings where diagnosis and surveillance are poor.[3,4] Despite this burden of mortality, *S pyogenes* receives little attention in global health programmes.[5] The World Health Assembly has now declared *S pyogenes* vaccine development a global research priority and the WHO roadmap for *S pyogenes* vaccines highlighted the lack of understanding of clinical epidemiology and transmission patterns as major research gaps.[6]

Asymptomatic pharyngeal colonisation (carriage) of *S pyogenes* is common and often viewed as inconsequential and not requiring treatment, and *S pyogenes* carriage on normal skin, which is known to increase pyoderma risk, is rarely studied.[7–9] Although data from the UK and Australia suggest that pharyngeal carriage could play a role in onward transmission of *S pyogenes*, its significance and that of skin carriage in Africa are unknown.[10,11]

In The Gambia, the burden of *S pyogenes* disease is largely unknown, although a substantial burden of rheumatic heart disease exists.[12] One cross-sectional study of children younger than 5 years found an 8·8% prevalence of *S pyogenes* pyoderma and suggested an increase in pyoderma risk during the rainy season.[13] Whole-genome sequencing and *emm* typing of Gambian *S pyogenes* isolates has shown a higher diversity of *emm* types than that seen in high-income countries.[9,14,15] The extent of skin carriage is unknown, but one study using quantitative PCR (qPCR) on nasopharyngeal samples from The Gambia showed high rates of *S pyogenes* carriage and an increase in antibody titres to several *S pyogenes* antigens after colonisation.[16] Widespread carriage might contribute to transmission, strain diversity, and immunity in this setting.

Longitudinal studies of *S pyogenes* carriage and infection have not been performed in Africa. We established a household cohort to understand the clinical and molecular epidemiology of *S pyogenes* and factors affecting transmission in this setting.

Methods

Study design and participants

We performed a prospective, longitudinal, household cohort study in the urban area of Sukuta, The Gambia, over a 13-month period in 2021–22. The study protocol has been published previously.[17] In brief, households containing at least three members, including one child younger than

18 years, were eligible for inclusion. Households were excluded if more than 50% of household members declined to participate. All individuals residing in the households were invited to participate, with the exclusion of those with any condition or circumstance that might cause difficulty or discomfort in sample collection, or those deemed by a study team member as unable or unlikely to adhere to the study protocol. Households were identified by random GPS selection (appendix p 14). Random GPS coordinates within the boundaries of Sukuta were derived from 2013 census data using QGIS version 3.12, stratified by low, medium, and high housing density areas. For each set of GPS coordinates, the nearest household was approached for participation, until the target number of households was met.

The study was approved by the Gambia Government/Medical Research Council joint ethics committee and the London School of Hygiene & Tropical Medicine Research Ethics Committee (LEO24005). Written informed consent was provided by adult participants and by parents or guardians for participants younger than 18 years. Children aged 12–17 years provided assent. The study is registered on ClinicalTrials.gov, NCT05117528.

Procedures

Consenting households underwent a baseline monthly visit (MV0) followed by 12 scheduled monthly visits (MV1–12). An open cohort approach was used with new household members able to enrol at any monthly visit. Sociodemographic data were collected at each monthly visit.

At monthly visits, participants were asked to provide an oropharyngeal swab (Copan Transystem 140C, Copan, Brescia, Italy) and a composite normal skin swab from skin surfaces on the arms, legs, and forehead (using flocked nylon fibre swabs, CITOSWAB, Nanjing, China). Participants who acquired new carriage were swabbed from the positive site (oropharyngeal or normal skin swab) at additional weekly visits until two consecutive negatives to estimate clearance time (clearance time cohort). 16 households, randomly selected using the *pps* package in R, underwent weekly intensive visits for 6 weeks during which oropharyngeal swabs and normal skin swabs were taken (intensive sampling cohort). Intensive visits were included in the clearance time and infection incidence analysis, but not the carriage incidence analysis. A wound swab (Copan) was taken when participants exhibited pyoderma. Participants presenting with a sore throat or skin lesions between scheduled visits were seen at unscheduled visits, at which an oropharyngeal swab or wound swab was taken as appropriate to capture incident pharyngitis and pyoderma events. Disease events were treated empirically with antibiotics (appendix p 5). Carriage events were not treated, in line with the Infectious Diseases Society of America guidelines.[9]

Swabs were placed in liquid Amies transport medium (Copan or CITOSWAB) and kept in a cold box until culture the same day. Swabs were plated on Colombia blood agar and beta-haemolytic colonies underwent latex agglutination testing (Prolex, Pro-Lab, Bromborough, UK) for group A *Streptococcus* (appendix p 4). Isolates positive for group A *Streptococcus* were assumed to be *S pyogenes*. Isolates were sent to the Molecular Bacteriology Laboratory (Brussels, Belgium; MBLB) for *emm* typing. Isolates underwent PCR-based *emm* typing as previously described.[18] *Emm* types and subtypes were assigned according to the US Centers for Disease Control and Prevention (CDC) database. New subtypes were assigned by CDC for the newly described sequences (appendix p 4).

See Online for appendix

For QGIS see https://www.qgis.org

S pyogenes events were defined as either disease (presence of *S pyogenes* with clinical symptoms of pharyngitis or pyoderma) or carriage (presence of *S pyogenes* without clinical symptoms; appendix p 2). Weekly visits were excluded from carriage incidence analysis to avoid bias. Baseline events were defined as events occurring at an individual's enrolment visit (whether at MV0 or a later monthly visit).

We defined clearance time as the time from carriage acquisition at a monthly visit or intensive visit until the midpoint between the date of the last positive swab (of the same *emm* type) and the date of the first of the two subsequent negative swabs (appendix p 6). Episodes were excluded if more than one consecutive weekly visit was missed, or if only one negative weekly visit was done at the end of an episode.

Follow-up time for carriage incidence was from enrolment until either MV12 or the midpoint between the last attended monthly visit and the first missed monthly visit. For missed monthly visits, a gap in follow-up was included from the midpoint between the last attended monthly visit and the first missed monthly visit to the midpoint between the last missed monthly visit and the next attended monthly visit. Unscheduled visits, weekly visits, and intensive visits were not included in the carriage incidence follow-up time. For disease incidence, if participants had an unscheduled visit, weekly visit, or intensive visit during a gap in follow-up, an additional 15 days of follow-up time was added (appendix pp 2–3).

We investigated the interaction between the four different event types (pharyngitis, pyoderma, pharyngeal carriage, and skin carriage) using two defined transmission windows to explore transmission within households. *S pyogenes* events that occurred within the same household within a range of 0–2 days were considered within-visit linkages, for which it was not possible to determine directionality of transmission. Events occurring within 3–42 days were considered to represent between-visit linkages. Linkages for which the *S pyogenes* isolates were of different *emm* types were considered epidemiologically linked. Linkages for which the isolates were identical *emm* types were considered *emm*-linked. Concurrent event types occurred when two event types with the same *emm* type were present within 0–2 days in an individual, and event-type changes occurred when two different event types of identical *emm* type occurred in one individual within 3–42 days.

Household secondary attack rate was calculated for between-visit linkages as the proportion of household members swabbed within 3–42 days who had an event in

that time. Household secondary attack rate was calculated for epidemiologically linked events and *emm*-linked events.

Outcomes

The primary outcome of this study was incidence of *S pyogenes* carriage and disease. Additional outcomes were baseline and monthly prevalence of *S pyogenes* skin and pharyngeal carriage, *S pyogenes* skin and pharyngeal clearance time, *S pyogenes emm* type, household secondary attack rate, risk factors for carriage and disease events, and *emm*-linked household transmission events. Monthly prevalence and incidence were stratified by sex and age group (age <5 years, 5–11 years, 12–17 years, and ≥18 years).

Statistical analysis

Detailed sample size considerations are described in the protocol.[17] Briefly, we estimated that 450 individuals would give sufficient power to detect a carriage prevalence of 15% (plus or minus 5%) and risk factors for *S pyogenes* carriage with rate ratios of greater than 2.[17] Data were entered into REDCap.[19] Analysis was performed in R version 4.2.2. Baseline carriage and disease prevalence was calculated as the proportion of participants positive at their enrolment visit with binomial exact 95% CIs. Baseline events were excluded from the incidence and regression analyses. Monthly carriage prevalence was calculated as the proportion of participants swabbed at each monthly visit with carriage with binomial exact 95% CIs. Incidence rates were calculated as events per 1000 person-years with 95% CIs, stratified by sex and age group. Clearance time was described using medians with IQRs and ranges. Wilcoxon rank-sum tests were used for differences in clearance time. The Andersen-Gill extension of the Cox model was used to identify sociodemographic risk factors for disease and carriage (appendix pp 2–3). Hazard ratios (HRs) were calculated in multivariable models including sex, age group, season, and household size (appendix p 2). Age group and sex were added to the model as fixed variables, whereas household size and season were added as time-varying covariates. Household secondary attack rate was calculated with 95% CIs. p values of less than 0·05 were considered to indicate statistical significance.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We recruited 337 participants from 44 households between July 27 and Sept 2, 2021, at MV0. An additional 105 participants from the same households were recruited at subsequent monthly visits, resulting in a total of 442 participants (figure 1). Final visits were conducted between June 28 and Sept 28, 2022. The cohort comprised 256 (58%) children younger than 18 years; the median age was 15 years (IQR 6–28), 233 (53%) were female, and the median household size was seven individuals (IQR 6–10; table 1, appendix p 7). Total follow-up time was 311·4 years (mean 0·71 years per individual [SD 0·34]) for disease events and 307·5 years (mean 0·70 years per individual [0·34]) for carriage events.

We identified 116 *S pyogenes* disease events (17 pharyngitis and 99 pyoderma) and 88 *S pyogenes* carriage acquisition events (49 pharyngeal and 39 skin). Pyoderma occurred simultaneously with pharyngitis on one occasion, with pharyngeal carriage on three occasions, and with skin carriage on four occasions. No invasive infection events, acute rheumatic fever, or other immune sequelae occurred.

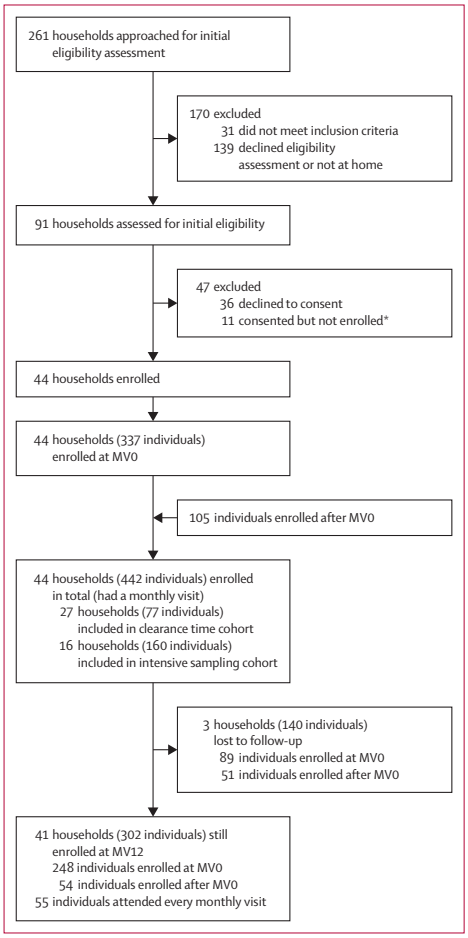

Figure 1: **Study flow diagram**
MV0=baseline monthly visit. MV12=scheduled monthly visit 12. *The study capacity was 44 households; an additional 11 households were kept in reserve in case of withdrawals.

	Participants (n=441*)
Sex	
Male	208 (47%)
Female	233 (53%)
Median age, years (IQR; range)	15 (6–28; 0–85)
Age group, years	
<5	104 (24%)
5–11	79 (18%)
12–17	73 (17%)
≥18	185 (42%)
Ethnic group	
Mandinka	311 (71%)
Wolof	30 (7%)
Fula	43 (10%)
Jola	17 (4%)
Serehule	12 (3%)
Serere	12 (3%)
Manjago	6 (1%)
Non-African	3 (1%)
Other	2 (<1%)
Missing	5 (1%)
Median household size, n (IQR; range)	7 (6–10; 4–37)†

Data are n (%) unless indicated otherwise. *Total cohort was n=442 but demographic information was missing for one participant. †Median household size for each household across all monthly visits.

Table 1: **Sociodemographic characteristics of the cohort**

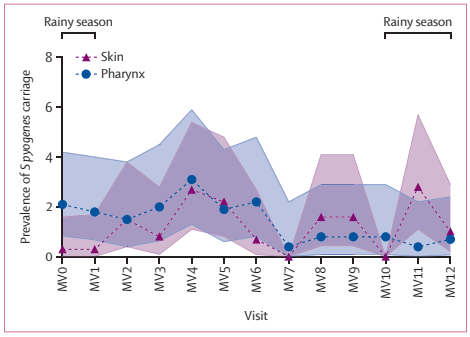

Figure 2: **Prevalence of *S pyogenes* pharyngeal and skin carriage at each monthly visit**

Shaded areas indicate 95% CIs. Carriage prevalence at MV0 is not the same as baseline because baseline included each participant's enrolment visit, which could have been after MV0. *S pyogenes*=*Streptococcus pyogenes*. MV0=baseline monthly visit. MV1–12=scheduled monthly visits 1–12.

Baseline *S pyogenes* pharyngitis prevalence was 0·2% (95% CI 0·0–1·3; one of 442) and pyoderma prevalence was 3·8% (2·3–6·1; 17 of 442). Pharyngeal carriage at baseline was 2·7% (1·4–4·7; 12 of 442) and skin carriage prevalence was 0·2% (0·0–1·3; one of 442; appendix p 8).

Monthly *S pyogenes* pharyngeal carriage prevalence ranged from 0·4% (95% CI 0·01–2·2; one of 249) to 3·1% (1·1–5·4; eight of 261) with a mean of 1·4% (1·1–1·9). Monthly *S pyogenes* skin carriage prevalence ranged from 0·0% (0·0–0·0; none of 249) to 2·8% (1·1–5·7; seven of 248) with a mean of 1·2% (0·9–1·6). There was no clear seasonal trend throughout the study period (figure 2).

The incidence of *S pyogenes* pharyngeal carriage (120 per 1000 person-years; 95% CI 87–166) and skin carriage (124 per 1000 person-years; 90–170) acquisition was similar. For disease events, of 147 episodes of symptomatic pharyngitis, 16 (11%) were *S pyogenes* positive, resulting in an incidence of 51 per 1000 person-years (31–84). Of 170 symptomatic pyoderma episodes, 82 (48%) were *S pyogenes* positive, resulting in an incidence of 263 per 1000 person-years (212–327; table 2).

Incidence of skin carriage was higher in male participants (199 per 1000 person-years, 95% CI 137–290) than female participants (64 per 1000 person-years, 35–116). Similarly, pharyngeal carriage was higher in male participants (162 per 1000 person-years, 107–246) than female participants (87 per 1000 person-years, 53–145). Pharyngeal carriage occurred most frequently in children aged 5–11 years (245 per 1000 person-years, 145–414), whereas skin carriage was most common in children younger than 5 years (239 per 1000 person-years, 156–366).

S pyogenes pyoderma was the most frequently observed event overall. It was more common in male participants (458 per 1000 person-years, 95% CI 358–587) than female participants (109 per 1000 person-years, 70–171) and, in terms of age groups, it was most common in children younger than 5 years (520 per 1000 person-years, 389–694), followed by children aged 5–11 years (412 per 1000 person-years, 276–615). *S pyogenes* pharyngitis was most common in children aged 5–11 years (120 per 1000 person-years, 57–252) and was similar in male and female participants (58 per 1000 person-years, 29–116, *vs* 46 per 1000 person-years, 23–92).

Overall, 33 *emm*-matched pharyngeal carriage episodes in 29 participants and 43 *emm*-matched skin carriage episodes in 39 participants were available. Histograms of clearance time were right-skewed (appendix p 9). Median clearance time was 4·0 days (IQR 3·5–7·0; range 3·0–42·5) for pharyngeal episodes and 4·0 days (3·5–7·3; 3·0–27·5) for skin episodes (p=0·84).

Antibiotics were prescribed at the start of two pharyngeal episodes due to pyoderma occurring concurrently. Antibiotics were given at the end of one episode and in the middle of one episode due to pharyngitis. Pyoderma occurred simultaneously with skin carriage at the start of seven skin episodes, six of which were treated with antibiotics. Of those, five were negative by the next visit. Pharyngitis occurred at the start of one skin episode, which was treated with antibiotics. Overall, antibiotics were given in four (12%) of the 33 pharyngeal episodes, compared with 10 (23%) of the 43 skin episodes (p=0·22). Clearance time length was not significantly affected by antibiotic use (p=0·15 for pharyngeal clearance and p=0·13 for skin clearance).

In multivariable Cox regression models, there was an increased risk of both pharyngeal carriage acquisition (HR 5·67, 95% CI 2·19–14·69, p=0·0004) and pharyngitis

	S pyogenes carriage		*S pyogenes* disease		
	Events	Incidence per 1000 person-years (95% CI)	Symptomatic episodes	Events	Incidence per 1000 person-years (95% CI)
Pharynx or skin					
Overall	75	244 (194–306)	309	97	311 (255–380)
Sex					
Male	49	361 (273–478)	168	70	509 (403–644)
Female	26	151 (103–222)	141	27	155 (106–226)
Age group, years					
<5	36	409 (295–568)	122	48	542 (409–720)
5–11	24	420 (282–627)	81	30	515 (360–736)
12–17	8	197 (98–393)	29	8	194 (97–387)
≥18	7	58 (27–121)	77	11	89 (49–161)
Pharynx					
Overall	37	120 (87–166)	147	16	51 (31–84)
Sex					
Male	22	162 (107–246)	55	8	58 (29–116)
Female	15	87 (53–145)	92	8	46 (23–92)
Age group, years					
<5	15	171 (103–283)	34	2	23 (6–90)
5–11	14	245 (145–414)	42	7	120 (57–252)
12–17	2	49 (12–196)	20	2	48 (12–194)
≥18	6	49 (22–110)	51	5	41 (17–97)
Skin					
Overall	38	124 (90–170)	170	82	263 (212–327)
Sex					
Male	27	199 (137–290)	120	63	458 (358–587)
Female	11	64 (35–116)	50	19	109 (70–171)
Age group, years					
<5	21	239 (156–366)	92	46	520 (389–694)
5–11	10	175 (94–325)	43	24	412 (276–615)
12–17	6	147 (66–328)	9	6	145 (65–323)
≥18	1	8 (1–58)	26	6	49 (22–108)

Incidence events do not include baseline events (positive at enrolment visit). *S pyogenes*=*Streptococcus pyogenes*.

Table 2: **Pharyngeal and skin *S pyogenes* carriage acquisition and disease incidence rates over the study period, stratified by sex and age group**

(3·00, 1·10–8·22, p=0·032) during the rainy season, whereas pyoderma and skin carriage were not affected by season (table 3).

Similarly, for each additional person in a household, we observed an increase in both pharyngeal carriage (HR 1·03, 95% CI 1·00–1·05, p=0·030) and pharyngitis (1·04, 1·02–1·07, p=0·0001) risk, whereas neither pyoderma nor skin carriage showed an association with household size. By contrast, risk of pharyngeal carriage and pharyngitis was not associated with sex, whereas the risk of pyoderma (0·34, 0·19–0·61, p=0·0003) and skin carriage (0·45, 0·22–0·92, p=0·030) was lower in female participants than male participants.

Compared with adults, the highest risk of pharyngeal carriage was in children aged 5–11 years (HR 4·80, 95% CI 1·71–13·49) followed by children younger than 5 years (2·92, 1·53–5·58). Skin carriage risk was highest in children younger than 5 years (22·69, 3·08–167·21), followed by children aged 5–11 years (18·44, 2·70–126·08). Pyoderma risk was also highest in children younger than 5 years (7·00, 2·78–17·64) followed by children aged 5–11 years (6·60, 2·77–15·74), but pharyngitis risk was not significantly associated with age (table 3).

Of 252 *S pyogenes*-positive swabs, 227 (90%) were successfully regrown and sent to MBLB for *emm* typing. One isolate failed to regrow, six were regrown but later tested as group G *Streptococcus*, and one isolate previously identified as group G *Streptococcus* was found to be group A *Streptococcus* at MBLB. Among 221 *S pyogenes* isolates that were successfully *emm* typed, 57 different *emm* subtypes were identified, including three new subtypes (data not shown). From the 204 separate carriage and disease events defined earlier, *emm* types were available for 179 (88%).

We identified 128 epidemiologically linked events that occurred 3–42 days after an index event, of which 18 (14%) were of identical *emm* type. Mean household secondary attack rates for transmission linkages are shown in table 4.

We identified 42 within-visit linkages with isolates of the same *emm* type, and 18 between-visit linkages (figure 3). For within-visit linkages, the most common event types that were linked were skin carriage with skin carriage (12 [29%] of 42 linkages), skin carriage with pyoderma (ten [24%] of 42 linkages), and pyoderma with pyoderma (eight [19%] of 42 linkages; appendix p 10). No transmissions between pharyngitis and pyoderma or skin carriage were identified. Of 18 between-visit transmissions identified, pyoderma was the source in 11 (61%), and the median serial interval was 28 days (IQR 15–29). The most common routes of transmission were pyoderma to pharyngeal carriage (three [17%] of 18 linkages), pyoderma to skin carriage (three [17%] of 18 linkages), and pyoderma to pharyngitis (three [17%] of 18 linkages; appendix p 11).

We identified eight occasions on which a different event-type occurred in the same individual within the transmission windows. Concurrent (within-visit) event types in one individual were identified five times, of which four (80%) were concurrent pyoderma and skin carriage and one was concurrent pyoderma and pharyngeal carriage. Three between-visit event-type changes were identified: two from skin carriage to pyoderma and one from pyoderma to pharyngeal carriage. No event-type changes from pharyngeal carriage to pharyngitis occurred (appendix p 13). The longest gap between events of the same *emm* type within a household was 252 days (appendix p 12).

Discussion

The findings of this longitudinal cohort study demonstrate a substantial burden of *S pyogenes* carriage and disease in The Gambia, especially in children. To our knowledge, this is the first evidence from Africa to show bidirectional household transmission of *S pyogenes* between the pharynx and the skin, that both pharyngeal and skin carriage are transmission sources, and that pyoderma is the predominant source of transmission to household contacts. However, the majority of events were not *emm* type-linked household

	S pyogenes carriage				*S pyogenes* disease			
	Pharynx (37 events)		Skin (38 events)		Pharynx (16 events)		Skin (82 events)	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Rainy season	5·67 (2·19–14·69)	0·0004	0·42 (0·09–1·91)	0·26	3·00 (1·10–8·22)	0·032	1·14 (0·34–3·84)	0·83
Sex		0·24		0·030		0·51		0·0003
Male	1 (ref)	..	1 (ref)	..	1 (ref)	..	1 (ref)	..
Female	0·71 (0·40–1·26)	..	0·45 (0·22–0·92)	..	0·75 (0·31–1·77)	..	0·34 (0·19–0·61)	..
Age group, years		0·0009		0·022		0·055		0·0001
<5	2·92 (1·53–5·58)	..	22·69 (3·08–167·21)	..	0·43 (0·11–1·69)	..	7·00 (2·78–17·64)	..
5–11	4·80 (1·71–13·49)	..	18·44 (2·70–126·08)	..	2·86 (0·95–8·58)	..	6·60 (2·77–15·74)	..
12–17	0·92 (0·18–4·64)	..	16·52 (2·58–106·93)	..	1·15 (0·38–3·43)	..	2·69 (1·18–6·12)	..
≥18	1 (ref)	..	1 (ref)	..	1 (ref)	..	1 (ref)	..
Household size	1·03 (1·00–1·05)	0·030	1·00 (0·98–1·01)	0·74	1·04 (1·02–1·07)	0·0001	1·01 (1·00–1·03)	0·14

HR and CI values are rounded to two decimal places. p values are rounded to two significant figures (to a maximum of four decimal places). *S pyogenes*=*Streptococcus pyogenes*. HR=hazard ratio.

Table 3: **Multivariable Cox proportional hazards regression models showing the impact of sociodemographic factors on *S pyogenes* carriage acquisition and disease**

transmissions, but rather appeared to be new introductions to the household. These findings provide fundamental insights into the dynamics of *S pyogenes* transmission and will inform intervention strategies to reduce *S pyogenes* transmission and disease.

Although the incidence of *S pyogenes* carriage was similar between the pharynx and the skin, we identified a higher number of pyoderma episodes than pharyngitis episodes. The role of skin infections in the development of acute rheumatic fever has been debated,[20,21] but it is likely that repeated infections of the skin contribute to immune priming ahead of an event that triggers acute rheumatic fever.[22–24] We demonstrate that *S pyogenes* pyoderma is common in the age group most at risk of immune priming and that pyoderma is a key source of household transmission. We observed substantial diversity of *S pyogenes emm* types, consistent with previous findings.[14,15] Exposure of children to the diversity of *S pyogenes emm* types seen in LMICs could be a contributing factor in immune priming for acute rheumatic fever and rheumatic heart disease.[22,25,26] The higher risk of pyoderma in male participants was unexpected and in contrast to previous findings.[13] More work is required to confirm this finding and whether it should influence pyoderma prevention strategies.

Onward transmission of *emm* types within households occurred from all four event types (pharyngitis, pyoderma, pharyngeal carriage, and skin carriage). Most events are likely to have originated from a non-household source. Nevertheless, for within-visit transmissions, there was extensive interaction between pyoderma wounds and skin carriage, and pyoderma was the source in most of the between-visit transmissions identified, suggesting *S pyogenes* on the skin could be the predominant source of transmission within households and the main source of event-type change within individuals. Relatively few household transmission events were identified, suggesting

	Index events*	Between-visit (3–42 days) transmissions			
		Epidemiologically linked events†		*emm*-linked events‡	
		Secondary events	Mean HSAR (95% CI)	Secondary events	Mean HSAR (95% CI)
Overall	169	128	4·9 (3·5–6·3)	18	0·74 (0·3–1·2)
Event					
Pharyngeal carriage	40	30	4·6 (1·4–7·8)	2	0·71 (0·0–1·7)
Skin carriage	33	20	6·0 (2·4–9·4)	2	0·58 (0·0–1·5)
Pharyngitis	15	19	6·8 (0·6–12·9)	3	0·80 (0·0–2·0)
Pyoderma	81	59	4·2 (2·4–6·0)	11	0·81 (0·1–1·6)

HSAR=household secondary attack rate. *S pyogenes*=*Streptococcus pyogenes*. *Events at which *emm* type was available and at least one household member was swabbed within 3–42 days. †Any *S pyogenes*-positive event occurring within 3–42 days of the index event. ‡*S pyogenes* positive even with identical *emm* type occurring within 3–42 days of the index event.

Table 4: **Mean HSAR for between-visit transmissions for epidemiologically linked events and *emm*-linked events**

that most acquisitions occur elsewhere. Future research should aim to better understand *S pyogenes* transmission dynamics beyond the household environment.

S pyogenes-positive pharyngitis was rare and served as the transmission source with a frequency similar to that of pharyngeal and skin carriage. Of note, we did not identify any occasions on which an individual was identified as a pharyngeal carrier and then progressed to pharyngitis. On only one occasion was an individual identified as a pharyngeal carrier after an episode of pharyngitis. These findings suggest that in this setting, pharyngitis and pharyngeal carriage do not represent different stages in the natural history of pharyngitis, but rather represent two ends of the symptomatic spectrum of pharyngeal *S pyogenes* infection. There is already increasing recognition that carriage events are not immunologically silent, which indicates that carriage might be implicated in rheumatic heart disease.[16,27] Collectively, these results raise questions about the current advice to leave asymptomatic carriage

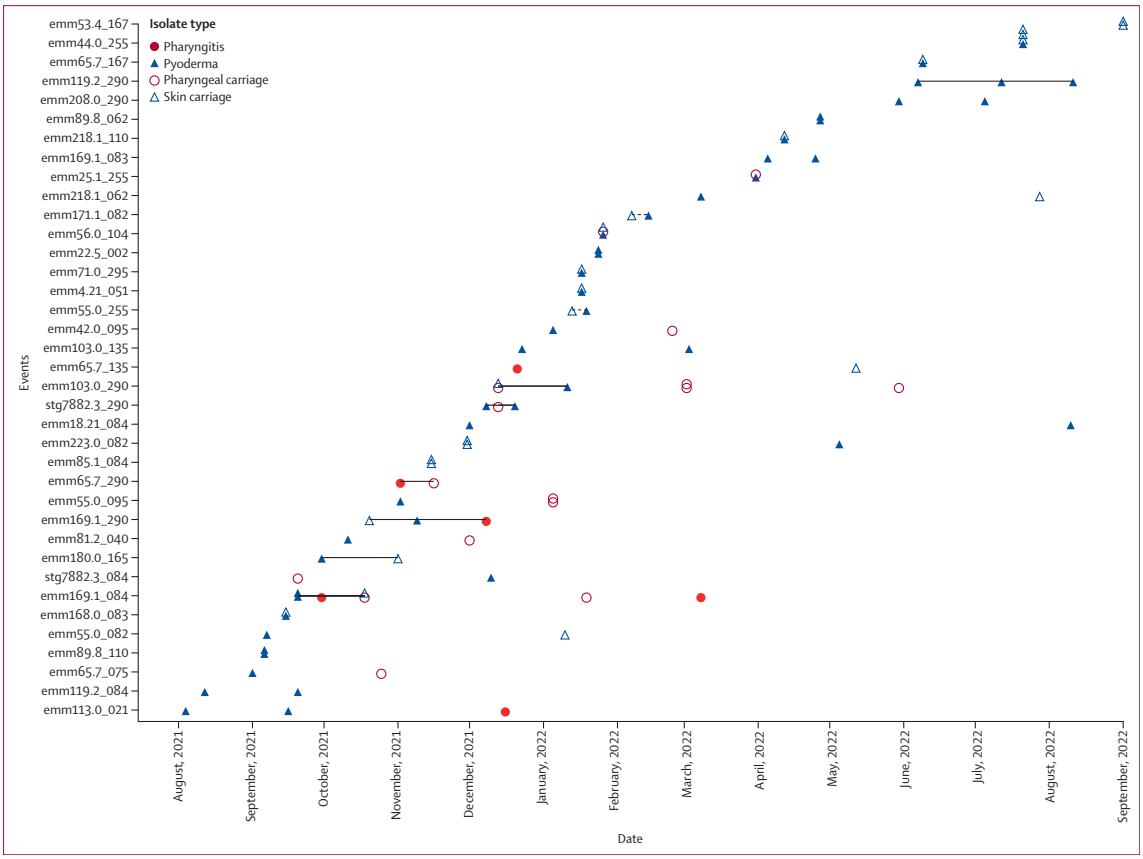

Figure 3: **Household transmission linkage timeline plot of *Streptococcus pyogenes* events across the cohort study period**
Events are grouped by each *emm* type within a household. The y-axis is arranged by date of first isolate, with labels written as *emm* type_household ID. Each point represents an isolate cultured from an individual with pharyngitis, pyoderma, pharyngeal carriage, or skin carriage. Isolates from the same visit are stacked. The solid black lines represent between-visit transmission (3–42 days) linkages of the same *emm* type (note that the lines are stacked in some instances, so not all 18 lines are visible). The dashed black lines represent between-visit event-type changes within the same individual.

untreated in settings at high risk of rheumatic heart disease. Further studies are needed to delineate the impact of treatment of asymptomatic carriage, particularly in LMICs, on transmission and immune responses.

There is an urgent need to identify strategies to reduce *S pyogenes* transmission. Although screening and treating children for *S pyogenes* carriage could potentially reduce transmission, interventions aiming to improve diagnosis and treatment of pyoderma and to increase handwashing with soap could also have a large impact.[28,29] Research is required to understand the impact that such interventions would have on transmission.

Our study has several limitations. First, although we found a considerable degree of presumed transmission with identical *emm* types, *emm* typing does not fully distinguish between *S pyogenes* lineages compared with whole-genome sequencing and pairwise identity at the single nucleotide polymorphism level. Genome sequencing of the isolates collected in this study is planned but these data are not yet available. As such, some presumed transmission events might reflect alternative introductions of separate lineages of the same *emm* type. Second, we actively identified and treated cases of disease and, as a result, the risk of transmission from household infections is probably underestimated. Third, we relied on culture to identify *S pyogenes*. Baseline *S pyogenes* nasopharyngeal colonisation prevalence of children aged 2–4 years in The Gambia using qPCR was 8·1% (95% CI 5·4–11·7) in a previous study[16] compared with 1·9% (95% CI 0·3–7·5) in children younger than 5 years in this study detected by culture. Molecular tests probably would have identified additional *S pyogenes* carriage and disease

events and transmissions. Similarly, a larger cohort would have provided greater confidence around transmission. Fourth, weekly swabbing of clearance time episodes was too infrequent to accurately estimate pharyngeal and skin clearance time and the insensitivity of culture probably led to an underestimated clearance time. Daily swabbing and diagnosis by a molecular method would be required to give a more accurate estimation. Given the short clearance times found for carriage, we probably missed incident carriage events between monthly visits. Fifth, results should be interpreted with caution due to participant absenteeism at monthly visits, the loss of several households to follow-up, and a lack of population demographics for comparison. Finally, clinical reporting of pharyngitis was less than anticipated. We recently conducted a study of health-care-seeking behaviour which suggested that many pharyngitis episodes are not reported.[30] For all of these reasons it is likely that we have underestimated the true burden of *S pyogenes* carriage, disease, and transmission in this setting.

Our study addresses a crucial gap in understanding of the burden of *S pyogenes* in Africa and the importance of pyoderma and asymptomatic carriage in the transmission of *S pyogenes* within households. Further studies to capture the burden of *S pyogenes* and its sequalae more fully in this setting are required and will underpin work towards developing effective vaccines and other interventional tools for the control of *S pyogenes*.

MRCG StrepA Study Group members

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Contributors

EPA, AJK, AK, TIdS, and MMar were responsible for conceptualisation of the study. EPA, AJK, and GdC curated the data. EPA performed the formal analysis. EPA, AJK, and GdC acquired the main funding, supported by AK, TIdS, MMar, CET, BK, and PRS. EPA, AJK, GdC, ES, FEC, MJ, ABi, HC, IC, BS, and MMan were responsible for the investigations. EPA, AJK, GdC, TIdS, MMar, AK, PRS, and CET developed and designed the methodology. EPA, GdC, MJ, and AJK were responsible for project administration. MMar, TIdS, BK, CET, AK, ABo, and PRS supervised the project. EPA, AJK, GdC, MMar, and TIdS were involved in validation. EPA was responsible for visualisation and the writing of the original draft. EPA, GdC, and AJK accessed and verified the underlying data reported in the manuscript. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. EPA, AJK, GdC, TIdS, MMar, CET, BK, PRS, ES, and FEC assisted with reviewing and editing the manuscript. All authors reviewed and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

The study protocol has been published previously.[17] The REDCap data dictionary and informed consent documents used are in the public domain (https://doi.org/10.5281/zenodo.7463052 and https://doi.org/10.5281/zenodo.7501168). All R code used for analysis in this manuscript is publicly available at https://edwinarmitage.github.io/SpyCATS_primary_analysis.html. Requests for access to individual participant data that underlie the results reported in this Article will be considered on formal request and should be directed to edwin.armitage@lshtm.ac.uk.

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5.2 Supplementary appendix

Supplementary Appendix

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Supplementary Methods

Case definitions. Symptomatic pharyngitis was defined as a sore throat (or parental reporting of pharyngitis-like symptoms in children under 5 years old) and evidence of tonsilo-pharyngeal erythema on examination. Symptomatic pyoderma was defined as one or more purulent or crusted skin lesion. *S pyogenes* pharyngitis and pyoderma were defined as symptomatic pharyngitis and pyoderma in the presence of a *S pyogenes* culture-positive swab. Swabs from the last attended visit prior to the event must have been negative or have occurred at least 14 days ago for the episode to be considered a new disease event.

S pyogenes pharyngeal carriage was defined as a culture-positive oropharyngeal swab in participants without symptoms of pharyngitis. Skin carriage was defined as a *S pyogenes*-positive normal skin swab. A carriage acquisition event was defined as new pharyngeal or skin carriage at a monthly visit where there was: no carriage at the two consecutive previous visits; or no carriage at one preceding visit and the last positive had been more than 28 days earlier; or the previous swab was positive but more than 42 days earlier. The 42-day cut-off was chosen to accommodate instances where a participant may have missed a monthly visit where they may have had a negative swab, ensuring that our analysis did not miss potential new carriage events over extended intervals.

Variable definitions. Age groups were defined as under 5 years, 5 to 11 years, 12 to 17 years, and 18 years or over at time of enrolment. Household size was defined as the number of people who reportedly slept in the household the night before a visit. Rainy season was defined as whether the visit occurred during the rainy season months of July to September.

Follow-up time definition. Due to the open cohort design, participants were able to enrol at any monthly visit. In this setting, household composition frequently changes, and many participants missed monthly visits due to travel, work or school commitments. To capture an accurate reflection of follow-up time for each individual we defined follow-up periods according to the following criteria. For calculation of disease incidence follow-up: follow-up time starts from the day of enrolment at a monthly visit and if no monthly visits were missed, continues until the final monthly visit; if a participant ended the study early due a permanent reason such as moving out, travelling without return or death, the follow-up period ends at the midpoint between the last attended monthly visit and the first missed monthly visit; if a participant missed one or more monthly visit (but not more than one in a row) for a temporary reason such as work, school or travel, follow-up time was from enrolment until the final monthly visit (or the midpoint between the last attended monthly visit and the first missed

monthly visit if ended early due to a permanent reason); if a participant missed more than one monthly visit in a row due to temporary reasons their first follow-up period is from enrolment until the midpoint between the last attended and first missed monthly visit, and a second follow-up period will commence from the midpoint between their last missed monthly visit and their next attended monthly visit (the same rules apply for subsequent follow-up periods); if participants arranged an unscheduled visit at a time during a gap in follow-up as previously calculated, an additional 15 days of follow-up time was added (7·5 before and 7·5 days after the unscheduled visit date); in the case of overlapping follow-up periods due to unscheduled visits the timelines were joined.

Examples:

1. Participant X attended MV1, MV2, MV3, missed MV4 to MV6, then attended MV7 and MV8 then at MV9 was reported to have moved out. The follow-up time would start from the date of MV1 until the midpoint between MV3 and MV4, where there would then be a gap until the midpoint between MV6 and MV7, when a second window of follow-up time would continue until the midpoint between MV8 and MV9.
2. Participant Y attended MV0, MV1, missed MV2, attended MV3, then missed MV4 to MV7, but attended MV8 to MV12. The follow-up time would be from MV0 to MV12 with a gap in follow-up between the midpoint between MV3 and MV4, and the midpoint of MV7 and MV8.
3. Participant Z attended MV0 to MV4, then had an UV 12 days after MV4, and another UV 42 days after MV4, but missed all the rest of the monthly visits. The follow-up time would start at MV0 and would normally continue until the midpoint between MV4 and MV5 (~15 days after MV4), however as they had an UV which would add a further 7·5 days of follow-up time afterwards which extends past the midpoint between MV4 and MV5, the follow-up time would end after the 7·5 days after the UV. A second window of follow-up would then start 7·5 days prior to the second UV and end 7·5 days after the second UV.

Statistical analysis. The data from this study were collected into REDCap case report forms for each visit (1). Data were then cleaned and set up into a long dataframe format (one row for each visit identified by participant ID, date and visit type) using a variety of bespoke R functions. These functions facilitated the accurate extraction of relevant data for analysis and defined events according to the criteria established in the main manuscript. For data cleaning, organisation, and event definition, we employed several R packages including *dplyr*, *lubridate*, *tidyr*, *stringr*, and *survival*. These packages collectively aided in data manipulation, date-time data management, dataset transformation, textual data processing, and survival analysis.

The incidence analysis was done using the *popEpi* package's *rate* function. The data were set up for the time-to-event regression using the *tmerge* fuction of the survival package, and the Andersen-Gill Cox regression was done using *coxph* function, including a *Surv* object for the outcome with both start time and stop time, and accounting for both participant ID (for individual clustering allowing for recurrent event inclusion), and household ID (for household clustering) with robust standard errors (2,3).

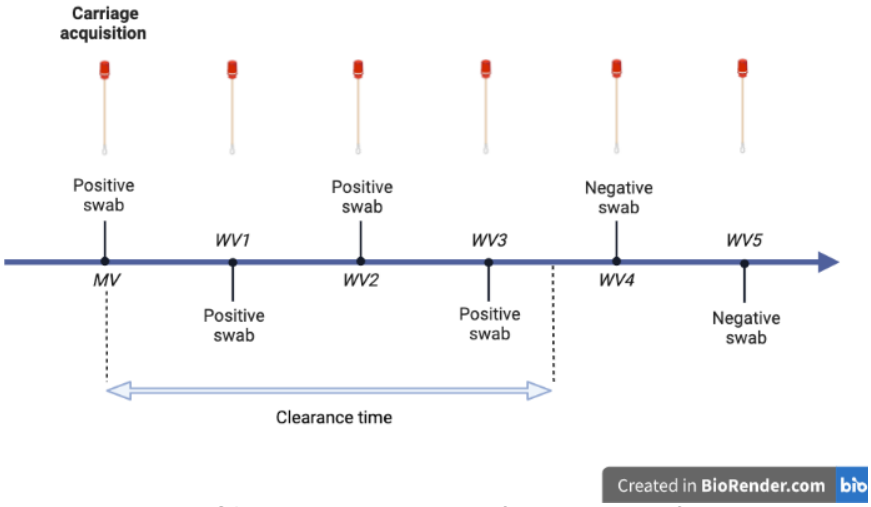
Descriptive analysis and presentation of regression results were managed using the *gtsummary* package. For visual data representation, *ggplot2* was used to create histograms for clearance time analysis and the household transmission plot. Additionally, GraphPad Prism V10·0.3 was used to produce the monthly prevalence plot.

Bacteriology and emm-typing. Swabs from participants were collected and placed into liquid Amies transport medium according to manufacturer's instructions. Swabs were transported to the laboratory in a cold box and plated for culture the same day. Upon arrival at the laboratory, swabs were vortexed in the transport medium, then the swab streaked onto Columbia Blood Agar (CBA) plates (produced by the MRCG microbiology platform, The Gambia). CBA plates were then incubated in a BSL2 laboratory at 37°C 5% CO_2 overnight, then inspected for beta-haemolytic colonies. Single colonies were then picked from the primary plate and replated on CBA purity plates. After overnight incubation at 37°C 5% CO_2, catalase negative colonies underwent latex agglutination testing for group A *Streptococcus* (Prolex™, Pro-Lab, Bromborough, UK). Isolates positive for group A *Streptococcus* by latex testing were assumed to be *S pyogenes*. *S pyogenes* isolates were then stored at -70°C in 17% glycerol.

Isolates were shipped to the Molecular Bacteriology Laboratory in Brussels, Belgium for *emm*-tying. Isolates were reconfirmed as group A *Streptococcus* using latex agglutination (Pastorex™, Bio-Rad, Marnes-la-Coquette, France), and underwent PCR-based *emm*-typing using the updated protocol (4). Sequences were analysed with the Center for Disease Control (CDC) Streptococci Group A Subtyping Request form Blast 2·0 server (available at https://www.cdc.gov/streplab/groupa-strep/emm-background.html)

Supplementary Table S1. Empirical treatment guidelines used for potential *S pyogenes* infections.

Diagnosis	Signs and symptoms	First-line treatment	Age	Dose and course length	Alternative treatment
Pyoderma / Infected scabies	Purulent or crusted skin lesion	Cloxacillin	Under 7 days 7-21 days 21-28 days 1 month-2 years 2-7 years 7-12 years >12 years	25mg/kg bd 5-7 days 25mg/kg tds 5-7 days 25mg/kg qds 5-7 days 62·5mg qds 5-7 days 125mg qds 5-7 days 250mg qds 5-7 days 500mg qds 5-7 days	Azithromycin (all ages: 12 mg/kg up to max 500mg od for 5 days)
Pharyngitis	Sore throat, pharyngeal/tonsillar erythema	Penicillin V	1 month to 1 year 1-5 years 6-11 years >11 years	62·5mg qds 5-7 days 125mg qds 5-7 days 250mg qds 5-7 days 500mg qds 5-7 days	Azithromycin (all ages: 12 mg/kg up to max 500mg od for 5 days)
od = once a day; bd = twice a day; tds = three times a day; qds = four times a day					



Supplementary Figure S1. Visual representation of clearance time following carriage acquisition. Clearance time was defined as the time from carriage acquisition (skin or pharynx) until the midpoint between the date of the last positive swab (for the same *emm*-type) and the date of the first of the two subsequent negative swabs. WVs were commenced when a new carriage event was identified at an MV or IV (weekly intensive visit). MV, monthly visit; WV, weekly visit.

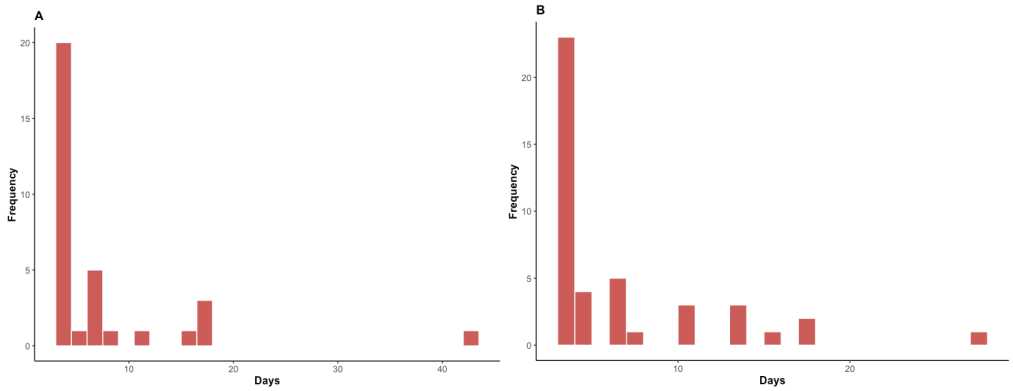
Supplementary Table S2. Cross tabulation of age group and sex.

Sex	Male n (%)	Female n (%)
Age group		
Under 5 years	68 (65)	36 (35)
5-11 years	36 (46)	43 (54)
12-17 years	32 (44)	41 (56)
≥18 years	72 (39)	113 (61)

Supplementary Table S3. Baseline prevalence of *S pyogenes* carriage and disease by sex and age group. Baseline events were events that occurred at the first monthly visit for each participant, whether enrolment or a later monthly visit.

N=442		Pharynx			Skin		
Characteristic	**Participants[1]**	**Event N**	**Prevalence (%)**	**95% CI[2]**	**Event N**	**Prevalence (%)**	**95% CI[2]**
Carriage							
Overall	442	12	2·7	1·4-4·7	1	0·2	0·0-1·3
Sex							
Male	208	6	2·9	1·1-6·2	1	0·5	0·0-2·6
Female	233	6	2·6	1·0-5·5	0	0	0·0-1·6
Age group							
0-4 years	104	2	1·9	0·2-6·8	0	0	0·0-3·5
5-11 years	79	4	5·1	1·4-12·0	1	1·3	0·0-6·9
12-17 years	73	3	4·1	0·9-12·0	0	0	0·0-4·9
≥18 years	185	3	1·6	0·3-4·7	0	0	0·0-2·0
Disease							
Overall	442	1	0·2	0·0-1·3	17	3·8	2·3-6·1
Sex							
Male	208	0	0	0·0-1·8	11	5·3	2·7-9·3
Female	233	1	0·4	0·0-2·4	6	2·6	1·0-5·5
Age group							
0-4 years	104	1	1·0	0·0-5·2	6	5·8	2·1-12·0
5-11 years	79	0	0	0·0-4·6	3	3·8	0·8-11·0
12-17 years	73	0	0	0·0-4·9	7	9·6	3·9-19·0
≥18 years	185	0	0	0·0-2·0	1	0·5	0·0-3·0

[1]Demographic data missing for one participant. [2]Binomial exact confidence intervals



Supplementary Figure S2. Histograms showing the frequency lengths of clearance time episodes in days for (A) *S pyogenes* pharyngeal clearance time episodes, and (B) *S pyogenes* skin clearance time episodes.

Supplementary Table S4. Summary of transmission types for within visit transmission linkages (0-2 days) where direction of transmission is unknown.

Transmission type	Number (%)
Pyoderma – Pyoderma	8 (19·0)
Pyoderma – Skin carriage	10 (23·8)
Pyoderma – Pharyngeal carriage	2 (4·8)
Skin carriage – Skin carriage	12 (28·6)
Skin carriage – Pharyngeal carriage	6 (14·2)
Pharyngeal carriage – Pharyngeal carriage	4 (9·5)
TOTAL	42 (100·0)

Supplementary Table S5. Summary of between-visit (3-42 days) transmissions of the same *emm*-type.

Source site	Recipient site	Number (%)	Median transmission time (days)
Pharyngitis	Pharyngitis	0 (0)	-
Pharyngitis	Pyoderma	0 (0)	-
Pharyngitis	Pharyngeal carriage	2 (11)	16·0
Pharyngitis	Skin carriage	1 (6)	18·0
Pyoderma	Pharyngitis	3 (17)	10·0
Pyoderma	Pyoderma	3 (17)	30·0
Pyoderma	Pharyngeal carriage	2 (11)	28·0
Pyoderma	Skin carriage	3 (17)	28·0
Pharyngeal carriage	Pharyngitis	0 (0)	-
Pharyngeal carriage	Pyoderma	2 (11)	18·0
Pharyngeal carriage	Pharyngeal carriage	0 (0)	-
Pharyngeal carriage	Skin carriage	0 (0)	-
Skin carriage	Pharyngitis	0 (0)	-
Skin carriage	Pyoderma	2 (11)	24·5
Skin carriage	Pharyngeal carriage	0 (0)	-
Skin carriage	Skin carriage	0 (0)	-
	TOTAL	18 (100)	

Supplementary Table S6. Maximum time delay between events of the same *emm*-type within a household.

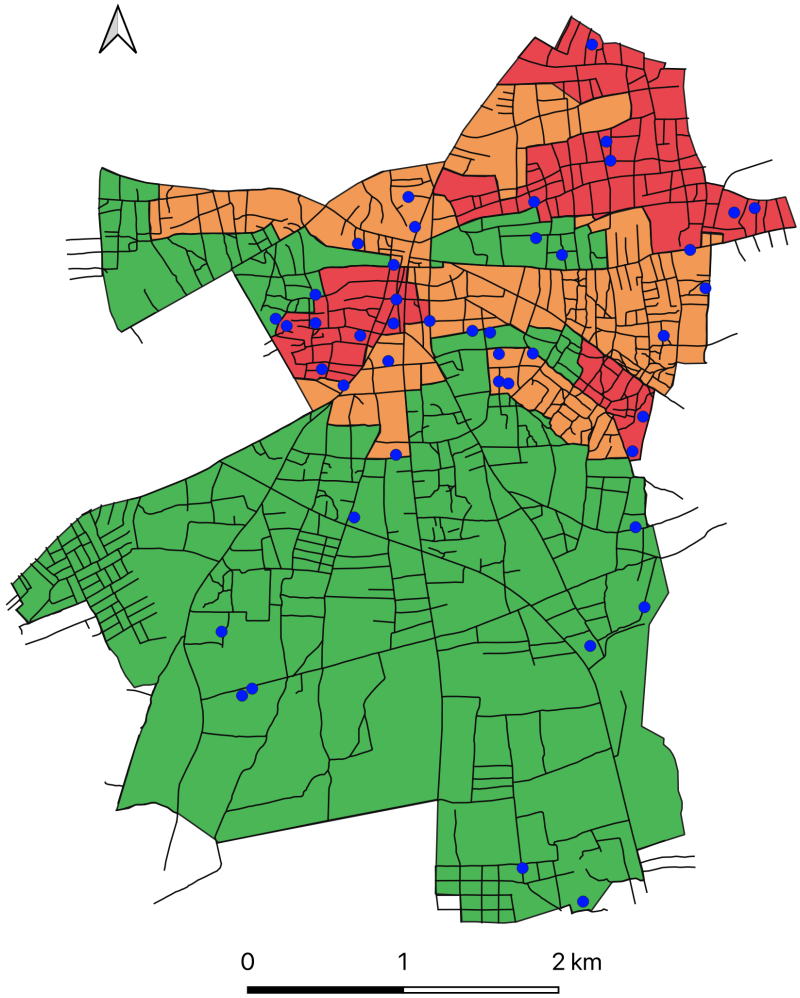
Household	*Emm*-type	Maximum time between events (days)
110	89.8	0
083	168.0	0
084	85.1	0
051	4.21	0
295	71.0	0
002	22.5	0
104	56.0	0
255	25.1	0
110	218.1	0
062	89.8	0
167	65.7	0
255	44.0	0
167	53.4	0
255	55.0	6
082	171.1	7
290	STG7882.3	12
290	65.7	14
083	169.1	20
165	180.0	32
290	208.0	36
084	119.2	39
290	169.1	49
095	42.0	50
040	81.2	51
075	65.7	54
095	55.0	64
290	119.2	65
135	103.0	70
084	STG7882.3	81
082	55.0	125
021	113.0	134
135	65.7	142
062	218.1	142
082	223.0	156
290	103.0	168
084	169.1	169
084	18.21	252

Supplementary Table S7. Instances of event-type changes of the same *emm*-type within one individual.

PID	Household	Site 1	Site 2	*Emm*-type	Transmission window	Translocation time (days)
05110F	051	Pyoderma	Skin carriage	4.21	Within-visit (0-2 days)	N/A
10406C	104	Pyoderma	Pharyngeal carriage	56.0	Within-visit (0-2 days)	N/A
11011B	110	Pyoderma	Skin carriage	218.1	Within-visit (0-2 days)	N/A
25522K	255	Pyoderma	Skin carriage	44.0	Within-visit (0-2 days)	N/A
29504E	295	Pyoderma	Skin carriage	71.0	Within-visit (0-2 days)	N/A
25520H	255	Skin carriage	Pyoderma	55.0	Between-visit (3-42 days)	6
08207K	082	Skin carriage	Pyoderma	171.1	Between-visit (3-42 days)	7
29022J	290	Pyoderma	Pharyngeal carriage	STG7882.3	Between-visit (3-42 days)	5

Supplementary Table S8. Instances of concurrent event-types with different *emm*-types in an individual.

PID	Visit	Site 1	Site 2	*Emm*-type 1	*Emm*-type 2
08434D	MV1	Pyoderma	Pharyngeal carriage	169.1	77.0
09506H	MV4	Pyoderma	Pharyngeal carriage	42.0	55.0
29022J	WV	Pyoderma	Pharyngitis	STG7882.3	169.1



Supplementary Figure S3. Map of the area of Sukuta, The Gambia. GPS locations of households enrolled in the study are shown as blue dots. GIS data was available from the 2013 census. Based on house and building location, the area of Sukuta was divided into high (red), medium (orange) and low (green) housing density regions. Random GPS points were generated, stratified by housing density region. Households nearest to GPS locations were approached for sensitization and consent to participate in the study until the desired sample size was reached.

6 Chapter 6: Optimising the detection of S*treptococcus pyogenes* events in surveillance studies

6.1 Introduction

In the global context of increasing interest in StrepA disease and its consequences, the WHO has recently prioritised StrepA research and published a roadmap aiming to expedite StrepA vaccine development (1-3). It highlights the importance of establishing centres of StrepA research excellence in LMIC, with a view to expanding StrepA surveillance and building sites for future StrepA vaccine testing (3). Establishing protocols for appropriate, sustainable and effective surveillance methodologies for StrepA surveillance programmes is therefore vital. Surveillance will play an important role in tracking disease burden over time, monitoring epidemiological trends and assessing the impact of public health interventions (including vaccines) (4). To this end, the Strep A Vaccine Global Consortium (SAVAC) Global Disease Burden working group has recently published a series of case definitions for StrepA disease aiming to standardise surveillance methodologies globally (5-12). However, the specifics of surveillance study design such as visit frequency and diagnostic methodology are not included, and no data exist comparing such strategies in high-StrepA burden settings. The SpyCATS study generated data to contribute to this field, not just on the StrepA carriage and disease burden and transmission dynamics in The Gambia, but also on relative strengths and weaknesses between different surveillance study methodologies.

A subgroup of households recruited into the SpyCATS study underwent more frequent visits for sampling for carriage event identification, which also allowed for greater identification of StrepA infection events (chapter 4). The median clearance time for a StrepA strain in SpyCATS was just 4 days from first positive for both pharyngeal and skin carriage (chapter 5). This short carriage time contrasts with previous studies from high-income countries, in which certain individuals were found to carry StrepA for long periods of time (13,14). This suggests that the monthly visits within the main SpyCATS cohort likely missed many carriage events between visits. Therefore, weekly visits to detect carriage, as well as to allow for active case finding of StrepA disease events, would likely identify more StrepA events than monthly visits, though how many more is not known.

The primary diagnostic method used for identifying StrepA events in SpyCATS was microbiological culture. The PharynGAS study (chapter 3) highlighted the relative insensitivity of microbiological culture in this setting to identify StrepA events compared to molecular gene-amplification tests which found a more than 3 times higher proportion of participants with StrepA. The clinical significance of this is not yet clear, as the samples with higher bacterial load on qPCR were more likely to be culture positive, indicating that culture positivity may indicate more severe infection, and samples that are culture negative but gene-amplification test-positive may be less clinically relevant. Nonetheless, significantly

higher proportion of individuals with pharyngitis who were StrepA-positive on gene-amplification tests indicates a potentially large underestimation of StrepA infection if relying on culture.

Understanding the impact of more frequent weekly visits for the detection of StrepA carriage and disease over monthly visits, as well as the impact of PCR-based detection of StrepA over culture for symptomatic individuals in the SpyCATS study, is important to know, and has significant implications for deciding on diagnostic methods for surveillance studies. This study presents data from the intensive weekly visit subgroup of SpyCATS, and data from PCR-based diagnosis of clinical pharyngitis and pyoderma episodes within the whole SpyCATS period to investigate whether weekly surveillance visits and molecular StrepA diagnostics would be valuable methodologies for future StrepA surveillance programmes.

6.2 Methodology

6.2.1 Intensive weekly visits

The visit schedule for the SpyCATS study is described in chapter 4. The “Intensified incidence surveillance cohort” section of the paper describes the subgroup of 16 randomly chosen households who underwent weekly visits for six weeks. The households were divided into four strata based on household size quartiles. A random sample of 16 households was identified using StataSE version 17, stratified by household size quartiles. These households were approached for participation, and if they declined, a replacement household was sought by the same method. Six households initially approached declined to participate in the intensive incidence sub-study. The households that agreed to be included were divided into four groups, and each group of four households, in turn, underwent a block of six weeks of weekly visits during the second half of the study period. At the weekly visits, all present and willing household member underwent oropharyngeal (OPS) and normal skin swabbing (NSS) as well as at all other visits, participants were asked about any pharyngitis of pyoderma symptoms. If pyoderma was present an additional wound swab (WS) was collected. The swabs underwent same-day culture as described in the protocol (chapter 4) and the main manuscript (chapter 5).

In the main study incidence analysis, carriage events that were identified at weekly intensive visits were excluded to avoid bias. In this study, StrepA carriage and infection incidence was calculated separately for the intensive visit six weeks for the households included in the sub-study. For the calculation of follow-up time, it was assumed that all participants who were seen at any one weekly visit were under follow-up for the whole five-week period between the first and the sixth weekly visit.

Events were defined as those at which a swab was culture-positive (with symptoms for infections or without for carriage) with a new *emm* type, or with the same *emm* type but after more than 28 days. The 28-day threshold for defining new events was selected based on previous studies assessing StrepA carriage and infection dynamics (15,16). This interval balances the need to differentiate new

acquisitions from persistent carriage while accounting for bacterial clearance and reinfection patterns observed in similar settings. Shorter intervals risk misclassifying persistent carriage as new events, whereas longer intervals may underestimate the frequency of reinfection. A longer threshold of 42 days was required for the main study analysis to include the time period between monthly visits. This approach aligns with methodologies employed in comparable epidemiological studies and provides a pragmatic framework for assessing StrepA transmission dynamics.

6.2.2 Bacteriology and *emm* typing

Swabs collected from participants were placed in liquid Amies transport medium, transported to the laboratory in a cold box, and plated for culture the same day. After the swab was used for plating, the residual Amies media was preserved at -70°C for later DNA extraction and PCR. Swabs were plated on Colombia blood agar (CBA) plates and incubated at 37°C 5% CO_2 overnight. Beta-haemolytic colonies were then picked and plated again for purity on CBA plates. After a further overnight incubation, catalase negative colonies underwent latex agglutination testing (Prolex) for group A *Streptococcus*. Isolates positive for group A latex testing were assumed to be *Streptococcus pyogenes* (StrepA). StrepA isolates were later shipped to the Molecular Bacteriology Laboratory in Brussels, Belgium for *emm* tying. They underwent PCR-based *emm* typing, as previously described (17,18), and the sequences analysed with the Centers for Disease Controls (CDC) Streptococci Group A Subtyping Request form Blast 2.0 server (https://www.cdc.gov/streplab/groupa-strep/emm-background.html).

6.2.3 PCR for disease events

During the SpyCATS study, participants were regularly encouraged to report symptoms of pharyngitis and skin infections to the study team. When the study team was alerted to a possible StrepA infection in this way, an unscheduled visit was arranged to assess the participant. In the case of pharyngitis symptoms, a full clinical assessment of the pharynx and neck was done, the tonsilo-pharynx was examined and swabbed as described in the protocol (chapter 4). At unscheduled visits, an OPS was only taken if there were symptoms of sore throat, as well as evidence of tonsilo-pharyngeal erythema on examination (or evidence of tonsilo-pharyngeal erythema alone for participants under 5 years old, where parents had reported non-specific symptoms). Additionally, at scheduled monthly (and weekly visits for both the intensive visit cohort, and for those undergoing weekly swabbing for clearance time), the study team asked participants about pharyngitis and pyoderma symptoms and examined both participants' pharynx and the skin checking for evidence of infection. Consequently, clinical symptomatic episodes were identified, of which a proportion were positive for StrepA on culture, which were then classified into events based on the event definitions described in the chapter 5 supplementary appendix.

As shown in PharynGAS (chapter 3), culture was less sensitive at detecting StrepA than the two gene-amplification methods used (qPCR for *speB* and ID NOW for *cep5)*. We therefore undertook qPCR for *speB* using the same methods described in chapter 3, on the cryo-preserved liquid Amies from swabs taken at clinical symptomatic episodes identified in SpyCATS (oropharyngeal swabs for pharyngitis and wound swabs for pyoderma). Briefly, DNA was extracted using QIAamp DNA kits (Qiagen), and quantitative PCR performed using Bio-Rad CFX 96 Touch Real-Time PCR detection system with primers and probes to detect the *S. pyogenes*-specific gene *speB* (see chapter 3 for details) (19,20). Samples were run in a single well, and a cycle threshold (Ct) of more than 40 was defined as negative. A Ct less than 36 was considered positive, and Cts between 36 and 40 were repeated and considered positive if amplification was seen and Ct was below 40 for both runs. PCR amplification curves were examined manually to ensure consistency with true target amplification. Additionally, we utilised 200μl of liquid Amies from a subset of pyoderma wound swabs for the ID NOW Strep A 2 (Abbott), rapid isothermal rapid nucleic acid amplification test to compare the sensitivity of ID NOW to PCR for detection of StrepA in pyoderma cases.

6.2.4 Statistical analysis

Analysis was performed in R version 4.3.1. Incidence rates were calculated as events per 1000 person years (pyrs) with 95% CIs, stratified by sex and age group, using the *popEpi* package. The Andersen-Gill extension of the Cox proportional hazards model was used to identify socio-demographic risk factors for StrepA carriage and disease using the *coxph* function of the *survival* package, including a *Surv* object for both start and stop time for each outcome, accounting for both participant ID (for individual clustering allowing for recurrent event inclusion), and household ID (for household clustering) with robust standard errors (21,22). Hazard ratios (HR) were calculated in multivariable models including sex, age group, season, and household size. Age group, sex and household size were added to the model as fixed variables, while season was added as a time-varying covariate. Household secondary attack rate (HSAR) was calculated with 95% CIs as the proportion of individuals swabbed within then transmission windows, following an index event, who were positive for StrepA by culture. Two transmission windows were defined: within-visit linkages were those occurring within 0-2 days; between-visit linkages were those occurring within 3-42 days. P values <0.05 were considered significant.

6.2.5 Ethics

The SpyCATS study including this sub-study and additional sample testing with PCR was approved by the Gambia Government/MRC Joint Ethics Committee and the LSHTM Research Ethics Committee (LEO24005). The study was registered on ClinicalTrials.gov (NCT05117528). Written informed consent was obtained from all adult participants and from the parents or guardians of participants under 18 years old. Children aged 12–17 provided assent. The study adhered to ethical principles outlined in the Declaration of Helsinki and Good Clinical Practice guidelines.

6.3 Results

6.3.1 Weekly intensive visits

A total of 166 participants underwent weekly intensive visits from 16 households (table 6.1). One household missed the final two weekly visits due to travel, so their total follow-up time was reduced to the time between the first and the fourth visits. The total follow-up time recorded was 16.0 person years. The median household size of the 16 households was 7 (IQR: 5.75-11.1, range: 5-37), the same as in the main cohort (IQR 6-10; range 4-37). There were 21 clinical pyoderma episodes identified of which 10 (47.6%) were confirmed as culture-positive StrepA pyoderma events, and of five clinical pharyngitis, none (0%) were StrepA-positive by culture. Five asymptomatic pharyngeal carriage events, and 13 skin carriage events were also identified by culture. Two skin carriage events occurred at the baseline weekly visit so were excluded from the incidence analysis.

Table 6.1 Summary of demographics of participants in the intensive visit cohort

Characteristic	**N = 166** N (%)
Age group	
0-4 years	52 (32%)
5-11 years	33 (20%)
12-17 years	21 (13%)
Over 18 years	59 (36%)
(Missing)	1
Sex	
Male	80 (48%)
Female	85 (52%)
(Missing)	1
Ethnic group	
Mandinka	113 (69.3%)
Wolof	15 (9.2%)
Fula	7 (4.3%)
Jola	13 (8.0%)
Serehule	8 (4.9%)
Serere	7 (4.3%)
(Missing)	3
Median household size (IQR; range)	7 (5.75-11.1;5-37)

6.3.1.1 StrepA carriage and infection incidence by culture

The incidence rates of StrepA carriage and infection events per 1000 person years are presented in Table 6.2. The highest incidence rate was seen in the 5-11-year-old age group at 1252/1000pyrs (95% CI 470-3336) for both skin carriage and pyoderma incidence. No pharyngitis events were seen. Pyoderma was more frequently observed in males (1042/1000pyrs, 95% CI 521-2083) than females (243/1000pyrs, 95% CI 61-970). The overall pharyngeal carriage rate (312/1000pyrs, 95% CI 130-750) was lower than that of skin carriage (687/1000pyrs, 95% CI 380-1240).

Table 6.2. Pharyngeal and skin carriage acquisition and infection incidence rates over the weekly intensive visits period stratified by sex and age group.

		Carriage		Infection		
		Events	Rate* (95% CI)	Symptomatic episodes	Events	Rate* (95% CI)
Pharynx						
Overall		5	312 (130-750)	5	0	0 (0-0)
Sex	Male	2	260 (65-1041)	1	0	0 (0-0)
	Female	3	364 (117-1129)	4	0	0 (0-0)
Age	0-4 years	3	598 (193-1856)	2	0	0 (0-0)
	5-11 years	0	0 (0-0)	1	0	0 (0-0)
	12-17 years	1	491 (69-3485)	1	0	0 (0-0)
	Over 18 years	1	176 (25-1251)	1	0	0 (0-0)
Skin						
Overall		11	687 (380-1240)	21	10	624 (336-1161)
Sex	Male	9	1172 (610-2252)	13	8	1042 (521-2083)
	Female	2	243 (61-970)	8	2	243 (61-970)
Age	0-4 years	6	1197 (538-2664)	12	5	997 (415-2396)
	5-11 years	4	1252 (470-3336)	6	4	1252 (470-3336)
	12-17 years	0	0 (0-0)	1	0	0 (0-0)
	Over 18 years	1	176 (25-1251)	2	1	176 (25-1251)

*Incidence rate per 1000 person years

Table 6.3. Multivariable Cox proportional hazards regression models showing the impact of socio-demographic factors on StrepA carriage acquisition and infection during the weekly intensive visit period.

	Carriage						Infection					
	Pharynx			Skin			Pharynx			Skin		
Characteristic	**Event N**	**HR[1] (95% CI[2])**	**p-value**	**Event N**	**HR[1] (95% CI[2])**	**p-value**	**Event N**	**HR[1] (95% CI[2])**	**p-value**	**Event N**	**HR[1] (95% CI[2])**	**p-value**
Rainy season	5	0.26 (0.08-0.86)	**0.027**	11	1.55 (0.62-3.88)	0.3	0	NA	-	10	2.08 (0.84-5.17)	0.11
Sex	5		0.7	11		0.2	0		-	10		0.066
Male		ref.			ref.			ref.			ref.	
Female		1.52 (0.24-9.40)			0.25 (0.03-1.91)			NA			0.27 (0.07-1.09)	
Age Group	5		**<0.001**	11		**<0.001**	0		-	10		**<0.001**
0-4 years		3.60 (0.44-29.5)			4.99 (1.02-24.5)			NA			4.02 (0.39-41.1)	
5-11 years		NA			6.00 (0.92-39.0)			NA			5.84 (0.53-64.8)	
12-17 years		2.23 (0.18-27.9)			NA			NA			NA	
Over 18 years		ref.			ref.			ref.			ref.	
Household size	5	1.04 (1.01-1.08)	**0.023**	11	1.00 (0.96-1.05)	0.8	0	NA	-	10	1.00 (0.97-1.04)	>0.9

[1]HR = Hazard Ratio, [2]CI = Confidence Interval, ref. = Reference category used. P-values in bold are significant <0.05.

6.3.2 Socio-demographic risk factors for StrepA carriage and infection events

The results of the multivariable Cox proportional hazards regression assessing for socio-demographic risk factors for StrepA carriage and infection events are shown in Table 6.3. Pharyngeal carriage risk was significantly lower during the rainy season (HR 0.26, 95% CI 0.08-0.86, p=0.027) and significantly higher in larger households (HR 1.04, 95% CI 1.01-1.08, p=0.023). Although the HR of 1.04 for household size appears to be a small effect size, this represents a 4% increased risk per additional household member. The reduced risk of pharyngeal carriage during the rainy season should be interpreted with caution as the intensive visit cohort period was not over the whole year so the rainy season is not fully represented in the follow up time. Skin carriage and pyoderma were not significantly affected by season or household size, but risk of both was significantly higher in children compared to adults, as was pharyngeal carriage risk.

6.3.3 qPCR for *speB* at symptomatic pharyngitis and pyoderma episodes

6.3.3.1 Proportion of symptomatic pharyngitis episodes positive by PCR

Throughout the whole SpyCATS study period, participants reported pharyngitis symptoms on 168 occasions. Of those, 93 (55%) were identified at scheduled monthly visits, 50 (30%) were reported to the study team and seen at an unscheduled visit, 23 (14%) were identified at weekly visits for clearance time, and 2 (1%) were identified at intensive weekly visits. On two occasions, both at unscheduled visits, an OPS was not taken, once due to no evidence of tonsilo-pharyngeal erythema, once due to parental refusal.

From the remaining 166 symptomatic pharyngitis episodes, 17 (10.2%) were positive for StrepA by culture (chapter 4). By PCR, an increased proportion of 38 (23.9%) were positive for StrepA.

6.3.3.2 Incidence of PCR-positive pharyngitis in the main SpyCATS study

The incidence rates of PCR-positive pharyngitis across the whole SpyCATS study period are presented in table 6.4. The overall incidence rate was 99/1000pyrs (95% CI 70-142), higher than the rate by culture detection (51/1000pyrs, 95% CI 31-84). By culture, male participants had a higher incidence of StrepA pharyngitis than female participants (58/1000pyrs, 95% CI 29-116 vs 46/1000pyrs, 95% CI 23-92). The same difference was observed for PCR-positive pharyngitis (males: 109/1000pyrs, 95% CI 66-181 vs females: 92/1000pyrs, 95% CI 56-150). Also similar to the culture results, though more marked, the age group with the highest rate of pharyngitis by PCR was the 5-11-year-olds (culture: 120/1000pyrs, 95% CI 57-252 vs PCR: 275/1000pyrs, 95% CI 168-448).

Table 6.4. PCR-positive pharyngitis incidence rates over the whole SpyCATS study period stratified by sex and age group. Baseline events excluded.

		PCR-positive pharyngitis	
		Events	**Rate* (95% CI)**
Overall		31	99 (70-142)
Sex	Male	15	109 (66-181)
	Female	16	92 (56-150)
Age	0-4 years	7	79 (38-166)
	5-11 years	16	275 (168-448)
	12-17 years	3	73 (23-225)
	Over 18 years	5	41 (17-97)

*Incidence rate per 1000 person-years

6.3.3.3 Proportion of symptomatic pyoderma episodes positive by PCR

There were 211 episodes of clinical pyoderma, of which swabs were available for 209. Of the 211 pyoderma episodes, 143 (68%) were identified at monthly scheduled visits, 17 (8%) at weekly visits for clearance time, 11 (5%) at weekly intensive visits, while 40 (19%) were reported and seen at unscheduled visits). 197 samples were available for PCR, of which 148 (75.1%) were positive for StrepA, higher than the proportion of those same 197 that were positive for StrepA by culture (95/197, 48.2%).

6.3.3.4 Incidence of PCR-positive pyoderma in the main SpyCATS study

The incidence rates of PCR-positive pyoderma across the whole SpyCATS study period are presented in table 6.5. PCR-positive pyoderma incidence was higher than culture-positive pyoderma incidence overall (379/1000pyrs, 95% CI 316-454, vs 263/1000pyrs, 95% CI 212-327). As with culture, PCR-positive pyoderma incidence was higher in males (669/1000pyrs, 95% CI 545-821) than females (150/1000pyrs, 95% CI 102-220), and most frequent in children under 5 years old (746/1000pyrs, 95% CI 586-949).

Table 6.5. PCR-positive pyoderma incidence rates over the whole SpyCATS study period stratified by sex and age group. Baseline events excluded.

		PCR-positive pyoderma	
		Events	**Rate* (95% CI)**
Overall		118	379 (316-454)
Sex	Male	92	669 (545-821)
	Female	26	150 (102-220)
Age	0-4 years	66	746 (586-949)
	5-11 years	35	601 (431-837)
	12-17 years	7	170 (81-356)
	Over 18 years	10	81 (44-151)

*Incidence rate per 1000 person-years

6.3.4 Proportion of symptomatic pyoderma swabs positive by ID NOW

A randomly selected sub-group of wound swabs (n=56) underwent StrepA detection using ID NOW. The proportion of swabs tested that were positive was 71.4% (40/56), as compared to 75.9% (41/54) by PCR (figure 6.1).

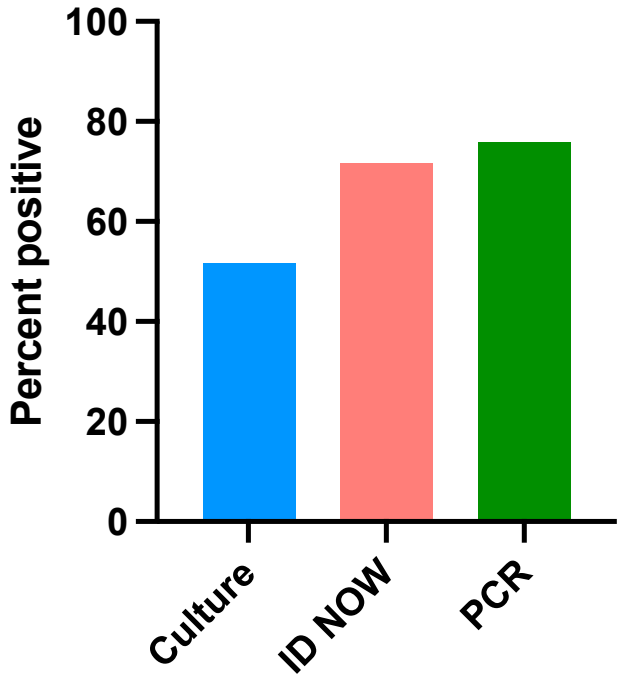


Figure 6.1. Percentage of n=56 wound swabs tested that were positive by ID NOW vs culture and PCR.

6.3.5 PCR-positive disease incidence during the weekly intensive visit period

During the weekly intensive visit cohort period, there were no PCR-positive pharyngitis events except one at baseline (which was therefore excluded). For PCR-positive pyoderma there were 13 events, translating to an overall incidence rate of 812/1000pyrs (95% CI 471-1398), higher than both the main study PCR-positive incidence (379/1000pyrs, 95% CI 316-454), and the weekly intensive visit culture-positive incidence (624/1000pyrs, 95% CI 336-1161). Most events were seen in the 0-4 and 5-11 years age groups.

Table 6.6. Incidence of PCR-positive disease events in the intensive weekly visit cohort of 16 households.

		PCR-positive pyoderma		PCR-positive pharyngitis	
		Events	Rate* (95% CI)	Events	Rate* (95% CI)
Overall		13	812 (471-1398)	0	0 (0-0)
Sex	Male	10	1302 (701-2420)	0	0 (0-0)
	Female	3	364 (117-1129)	0	0 (0-0)
Age	0-4 years	8	1596 (798-3191)	0	0 (0-0)
	5-11 years	4	1252 (470-3336)	0	0 (0-0)
	12-17 years	0	0 (0-0)	0	0 (0-0)

Over 18 years	1	176 (25-1251)	0	0 (0-0)

*Incidence rate per 1000 person-years

6.3.6 Association between qPCR cycle threshold value and culture positivity of pharyngitis and pyoderma swabs

The bacterial load of PCR-positive samples was assessed using the starting quantity of StrepA in copies per millilitre, computed from the standard curves generated by serial dilutions from 10,000,000 to 1 copy per μl. The differences in *ex vivo* bacterial quantity in Amies fluid between culture-positive and culture-negative samples are shown in figure 6.2.

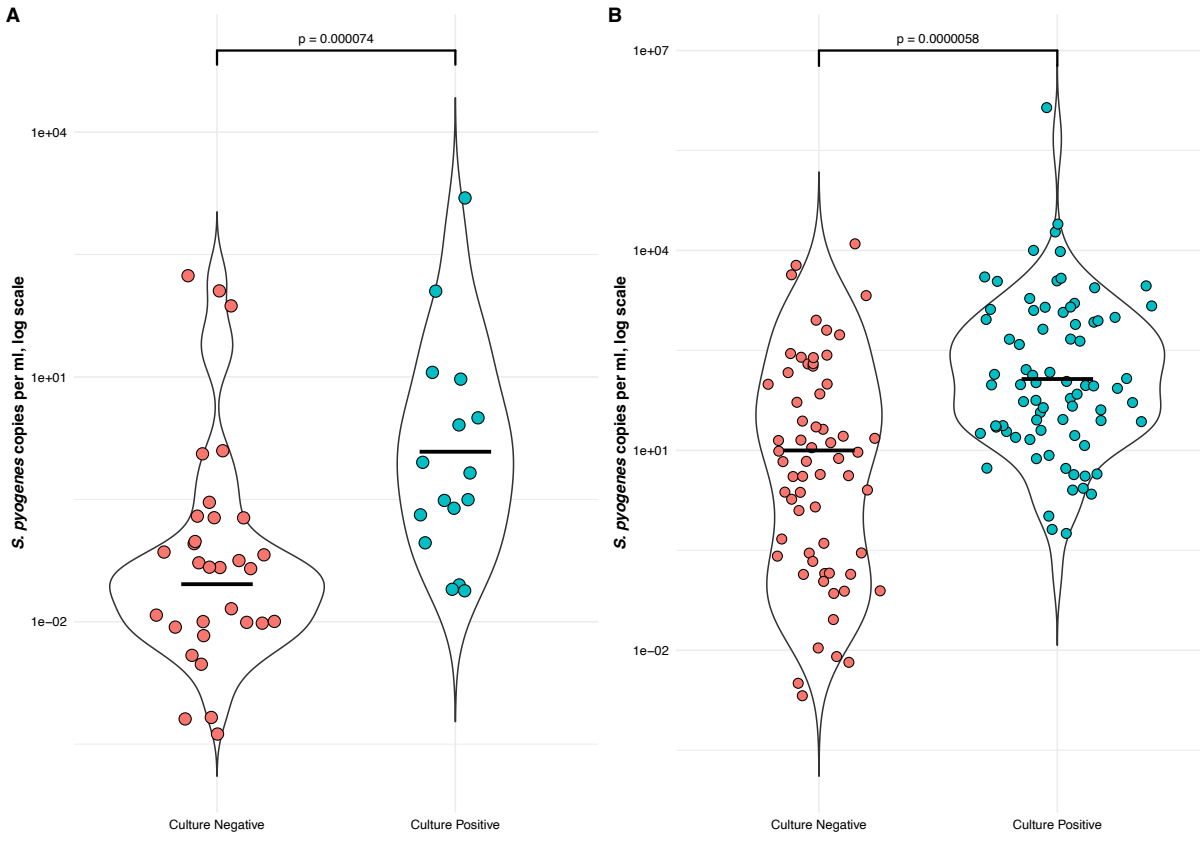


Figure 6.2. Violin plots showing bacterial load detected by quantitative PCR in PCR-positive samples by microbiological culture status for (A) pharyngitis episodes and (B) pyoderma episodes. Samples with Ct value >40 also included to avoid bias. P values calculated by Wilcoxon test with a Benjamini and Hochberg correction for multiple testing.

6.3.7 Household transmission dynamics in the intensive visit cohort

During the intensive visit period, within the 16 households, 29 culture-positive events occurred: 10 pyoderma, 13 skin carriage, and 5 pharyngeal carriage. From the 29 isolates, *emm* types were available for 27 events (one pyoderma and one throat carriage not *emm*-typed). There were 16 distinct *emm*

types detected from the 27 events, translating to a Simpson's diversity index of 0.91 (very high diversity). The *emm* types are listed in table 6.7.

Table 6.7. *Emm* types of isolates from events identified in the intensive visit cohort. NT=no *emm* type available

Household ID	*Emm* type	Event type
082	emm223.0	Pyoderma
083	emm169.1	Pyoderma
084	emm18.21	Pyoderma
084	emm65.7	Pyoderma
093	emm207.0	Pyoderma
108	emm218.1	Pyoderma
147	NT	Pyoderma
290	emm208.0	Pyoderma
290	emm119.2	Pyoderma
290	emm119.2	Pyoderma
290	emm43.7	Pyoderma
040	NT	Pharyngeal Carriage
082	emm68.0	Pharyngeal Carriage
084	emm44.0	Pharyngeal Carriage
290	emm208.0	Pharyngeal Carriage
290	emm103.0	Pharyngeal Carriage
021	emm122.0	Skin Carriage
040	emm171.1	Skin Carriage
040	emm171.1	Skin Carriage
082	emm223.0	Skin Carriage
083	emm162.1	Skin Carriage
084	emm44.0	Skin Carriage
093	emm207.0	Skin Carriage
290	emm119.2	Skin Carriage
290	emm119.2	Skin Carriage
290	emm43.7	Skin Carriage
290	emm119.2	Skin Carriage
290	emm208.0	Skin Carriage
290	emm49.10	Skin Carriage

From 22 index events at which at least one other person was swabbed within the household in the between-visit transmission window (3-42 days), there were 55 epidemiologically linked secondary events (not necessarily of identical *emm* type), of which 13 were *emm*-linked (of identical *emm* type). The proportion of epidemiologically linked events that were emm-linked was 23.6%, compared to 14.1% for the main cohort analysis. The mean household secondary attack rate (HSAR) was calculated for epidemiologically linked events and for *emm* type-linked events, and for each index event type. The overall HSARs for both epidemiologically linked events (11.0) and for *emm* type-linked events (2.9) were higher than for the main SpyCATS cohort (epidemiologically linked events: 4.9; and *emm* type-linked events: 0.7) (table 6.8). In contrast to the main cohort, the index event type with the highest emm type-linked HSAR was skin carriage at 4.1 (compared to pyoderma at 0.81 in the main cohort).

Table 6.8. Mean household secondary attack rate (HSAR) for between-visit transmissions for epidemiologically linked events (any StrepA positive event occurring within 3-42 days) and *emm* type-linked events (StrepA positive event with identical *emm* type occurring within 3-42 days) within the intensive visit cohort period. *Events at which *emm* type was available and at least one household member was swabbed within 3-42 days.

		Between-visit (3-42 days) transmissions			
		Epidemiologically linked events		*Emm* type-linked events	
	Index event N*	Secondary event N	Mean HSAR (95% CIs)	Secondary event N	Mean HSAR (95% CIs)
Overall	22	55	11.0 (6.4-15.6)	13	2.9 (0.9-4.9)
Event					
Pharyngeal carriage	3	14	10.3 (0.0-32.3)	1	1.3 (0.0-6.8)
Skin carriage	12	33	12.9 (6.3-19.6)	9	4.1 (0.9-7.3)
Pharyngitis	0	0	0.0 (0.0-0.0)	0	0.0 (0.0-0.0)
Pyoderma	3	14	8.1 (0.0-18.9)	3	1.5 (0.0-5.3)

Only one index event was linked with a within-visit (0-2 days) event transmission of the same *emm* type. This was when a pharyngeal and skin carriage event occurred at the same visit of identical *emm* type in two household members.

For the 13 *emm*-linked between visit transmission events, the median lag time was 28 days (IQR 7-35). The most common transmission route was skin carriage to pyoderma (5/13, 38%), followed by skin carriage to skin carriage (4/13, 31%). Two pyoderma to skin carriage transmissions were seen (2/13, 15%), one pyoderma to pyoderma (1/13, 8%) and one throat carriage to pyoderma (1/13, 8%).

6.4 Discussion

The findings from the intensive visit cohort and molecular diagnostics data from the SpyCATS study provide novel understandings and insights regarding optimisation of StrepA surveillance methodology. A robust comparison of different swabbing frequencies as well as different StrepA diagnostic methods

within a cohort study has never been done before to our knowledge. These data show that both increased visit frequency and the use of molecular gene-amplification diagnostic methods translate to a marked increased detection rate of StrepA carriage and disease events in this setting. This indicates that there is a substantial undetected burden of StrepA carriage and disease which is impacts transmission and may be clinically and immunologically significant. Consideration of these factors is necessary when designing surveillance programmes and deciding on vaccine trial endpoints.

The intensive visit cohort period detected significantly higher incidences by culture of all event types except for pharyngitis. The small size, short time-period of the intensive visit cohort and relative infrequency of pharyngitis episodes overall likely led to the sample size being insufficient to detect pharyngitis incidence. Active over passive surveillance may also increase detection of pharyngitis as much as pyoderma, as healthcare-seeking for pharyngitis could be better than for pyoderma routinely. However, the substantially higher detection rate of pharyngeal, and particularly skin carriage, compared to the main SpyCATS cohort, underscores the importance of more frequent surveillance visits if attempting to capture comprehensive picture of StrepA transmission within households.

The observed association between household size and risk of StrepA events should be interpreted as a statistical relationship rather than a strictly biological one. The hazard ratio of 1.04 suggests an average 4% increased risk per additional household member, but this does not necessarily imply a uniform incremental risk. The assumption of a linear relationship between household size and StrepA risk is a simplification, as the impact of household size may not be constant at different thresholds. While an increase from four to five household members may not have the same impact as an increase from ten to fifteen, larger households are more likely to have increased contact networks and a greater probability of sustained transmission. Future studies could explore non-linear models to better capture this dynamic. In addition, the relationship between pharyngeal carriage risk and the rainy season should be interpreted with caution due to the sub-study's limitations in accounting for seasonality. Different households were sampled at different times of the year, meaning that some had no data collected during the rainy season while others were sampled exclusively during this period. Since the study did not cover a full annual cycle, the observed lower risk of pharyngeal carriage in the rainy season may be a result of this sampling variation rather than a true seasonal effect. This contrasts with the findings from the Chapter 5 which benefited from more comprehensive year-round surveillance.

The higher proportion of epidemiologically linked events that were *emm*-linked than in the main cohort suggests that e*mm*-linked events were missed due to infrequent sampling, which may have underestimated household transmission in the primary analysis. The predominance of skin carriage in household transmission in the intensive visit cohort, as evidenced by skin carriage having the highest *emm*-linked HSAR and being involved in the most common between-visit transmission routes, suggests an underestimation of skin carriage's importance by less frequent surveillance visits. Although this study did not directly observe transmission from skin carriage to pharyngeal carriage within households, we did observe transmission of StrepA from skin to throat in some cases. This suggests that there may be

a link between skin and pharyngeal carriage, even though we did not find evidence that skin carriage directly led to pharyngitis in either the same individual or others in the household. Further research is required to better elucidate the interplay between these two carriage sites and their respective roles in transmission dynamics.

The higher incidence of pyoderma detected in the intensive visit cohort indicates that disease events may be missed with less frequent visits, despite a symptom reporting system. Active case finding through weekly visits appears to detect more pyoderma, which when treated, would likely decrease onward StrepA transmission within the household. These findings suggest that the role of measures to reduce skin carriage such as hand hygiene, combined with active pyoderma surveillance in StrepA control strategies should be further investigated in settings such as this. Active pyoderma surveillance has potential as a public health tool, particularly when combined with education on antibiotic use for skin infections, wound care, and hygiene interventions. While it remains to be demonstrated whether active pyoderma surveillance can sufficiently suppress transmission to reduce overall disease burden, the role of pyoderma in transmission suggests that such an approach is likely to be beneficial. Further studies evaluating the long-term impact of active surveillance and intervention strategies will be important in defining best practices for StrepA control.
The sub-study's sample size was not powered for precise estimates of StrepA event incidence, its primary aim was to provide greater granularity on the impact of visit frequency and diagnostic method on case detection. Despite the wider confidence intervals around incidence, the findings indicate a consistent increase in detection rates when using more frequent visits. The observed trends suggest that these methodological factors have a meaningful impact on surveillance outcomes, supporting their consideration in future studies.

Regarding the feasibility of frequent sampling, our experience suggests that weekly swabbing was well tolerated by participating families, facilitated by strong relationships with the study team and community engagement. However, the main limiting factor is resource availability, particularly in terms of field and laboratory personnel, as well as consumables such as swabs and reagents. Future studies should carefully weigh the trade-off between increased case detection and logistical constraints, considering the specific research and public health objectives.

The reanalysis of swabs taken from clinically symptomatic pharyngitis and pyoderma episodes throughout the SpyCATS study using gene-amplification diagnostic methods (PCR and ID NOW) with their superior sensitivity over traditional microbiological culture shows that culture-based surveillance may also significantly underestimate StrepA disease. Using a molecular diagnostic method could translate to an approximately 40% higher proportion of pyoderma being StrepA positive, and a more than 3 times higher proportion pharyngitis being StrepA positive. A higher bacterial load did increase the likelihood of detection by culture, which suggests that culture may be adequate for detecting the more "severe" (by bacterial load) cases, although it appears unreliable as many higher bacterial load samples were still culture negative (figure 6.2). Furthermore, the level at which a lower bacterial load,

which is undetectable by culture but detectable by molecular methods, becomes clinically and immunologically significant is unknown; it is not known whether a higher bacterial load is correlated with likelihood of a “rheumatogenic” immune response. It has been shown that asymptomatic pharyngeal colonisation in children aged 2-4 years in The Gambia, detected by PCR, was associated with a rise in antibodies to M1, Mac and SpyCEP antigens, therefore culture-negative, PCR-positive cases may well be able to stimulate an anti-streptococcal immune response (19). It is possible therefore that these events may also contribute to immune priming for rheumatic heart disease risk, something which should be further investigated. Collectively these data raise questions about whether culture-based diagnostics are insufficiently sensitive to detect cases that may be clinically and immunologically significant, especially as we have shown that these cases are important in household transmission. These less symptomatic or asymptomatic carriers, with bacterial loads undetectable by culture, could serve as undetected and untreated reservoirs that may facilitate the spread of the bacteria within populations. The clinical and immunological importance of positive molecular-based tests should be investigated further, and the inclusion of gene-amplification diagnostics should be considered in future StrepA surveillance programmes in order to further elucidate transmission pathways and the impact of interventions on StrepA carriage and disease.

This study’s findings have important implications for the design of future transmission studies and selection of endpoints in future vaccine trials. A key observation is the difference in detection rates of disease between active and passive surveillance, especially of pyoderma, which may be overlooked by patients, compared to symptomatic pharyngitis. For vaccine trial endpoints, it is necessary to attempt to capture all significant events, therefore active surveillance, combined with molecular diagnostics, such as PCR, for both pharyngeal and skin infections should be considered. This approach would improve detection of relevant events, whilst allowing for subsequent analysis using culture data for confirmation. While this level of surveillance may not be feasible for routine implementation, it would be needed for the accurate evaluation of vaccine efficacy. Moreover, the significantly higher carriage detection with frequent visits and PCR underscores their importance in transmission studies, even if this may not be directly relevant to vaccine trials. To comprehensively understand and characterise StrepA transmission dynamics within households or other settings, shorter periods of frequent carriage swabbing (possibly more than weekly) alongside active case detection, molecular diagnostics and *emm*-typing or sequencing will be required.

This study has several limitations which may impact on the interpretation and generalisability of the findings. Firstly, the intensive swabbing period was only 5 weeks, and the cohort size was small. This led to the sub-study being underpowered to detect pharyngitis and resulted in large uncertainties around the incidence rates of the other StrepA events. Secondly, resources were not available to perform PCR on all the asymptomatic carriage swabs from the intensive visit period, but this would likely have revealed an even greater burden of both pharyngeal and skin carriage. Thirdly, the urban location of the study limits the generalisability of the results to rural or other settings. Fourthly, this sub-study was conducted towards the end of the main SpyCATS study periods, where households had been under

active StrepA disease surveillance for at least 6 months prior to the start of the sub-study. All possible StrepA events were treated empirically with antibiotics, therefore by the start of the sub-study, the level of circulating StrepA may have been supressed in the participating households, leading to us detecting a lower incidence of StrepA events than if we had enrolled non-study households. Fifthly, a number of households declined to be involved in the sub-study, possibly due to the intensity of the visits. This may have led to sampling bias where certain households self-excluded. Finally, the study design, focussing on the household as the site of transmission was not able to explore community transmission dynamics, or investigate sites of transmission outside the household which may be important. Nonetheless, the drawbacks of these limitations are unlikely to have overridden the main findings from this analysis of a sub-group of SpyCATS participants undergoing intensive sampling, as many of them would point towards an underestimation rather than an overestimation of incidence. Therefore, this study provides important and relevant findings regarding StrepA carriage and disease burden in The Gambia despite these limitations.

The findings from this study highlight the need for careful consideration of sampling intervals and diagnostic tools in StrepA surveillance programmes and future vaccine trials. Traditional culture methods, infrequent swabbing visits and passive case finding likely miss a large burden of StrepA carriage and disease which may be clinically and immunologically significant as well as playing a critical role in transmission. To accurately capture the true burden of StrepA, as well as the impact of any future interventions, frequent visits, active case finding, and molecular diagnostics should be employed. Additionally, the significantly larger burden and impact of skin carriage in this study than the main SpyCATS results (chapter 5), highlight the need for further investigation into the role of skin hygiene interventions. Future work should include longitudinal studies utilising molecular diagnostics and more frequent visits and active case finding over a longer period of time to measure the true burden of StrepA in a variety of settings including rural ones. The role of skin hygiene should be investigated in observational and interventional studies to further investigate the importance of skin carriage and infection and transmission, and whether this can be interrupted with skin hygiene interventions.

6.5 References

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7 Water access, sanitation and hygiene-related risk factors for S*treptococcus pyogenes* carriage and infection within households in The Gambia

7.1 Introduction

Bacterial skin infections (pyoderma), are a significant global health concern, affecting around 111 million people worldwide at any one time, especially in LMICs and tropical regions (1). StrepA is one of the primary causative pathogens, contributing substantially to global morbidity and mortality. In The Gambia, high prevalence rates of pyoderma in children under 5 have been recorded, with StrepA identified in over half of pyoderma wounds by microbiological culture (2). StrepA is important not only for direct infectious complications but also for its potential to cause severe immune-mediated diseases, such as acute rheumatic fever and rheumatic heart disease (RHD), which cause the majority of StrepA-related mortality, and are particularly prevalent in LMICs (1,3-5). The link between StrepA pharyngitis has been well established (6,7). In contrast, there has been historical debate about the role of StrepA skin infections in the aetiology of RHD (8,9). However, it is now widely believed that repeated exposure to StrepA skin infections in childhood may play a role in priming the immune system to be more likely to react pathologically to a later StrepA infection (10-14). For this reason, prevention of StrepA skin infections is an important component of the WHO StrepA vaccine development roadmap (15-17).

Transmission of StrepA is multifaceted, involving skin-to-skin contact, airborne spread, and environmental reservoirs (18,19). Though studies from the 1970s highlighted the important role of StrepA skin colonisation in household transmission, with skin colonisation often preceding pyoderma in individuals, subsequent research has frequently neglected skin colonisation (20-26). The SpyCATS study identified asymptomatic StrepA carriage as frequent in this setting, with evidence suggesting that it contributes to household transmission dynamics, particularly through skin carriage, which was more commonly linked to subsequent infections than pharyngeal carriage (chapters 5 and 6) (27). Furthermore, similar to recent findings in Australia, we found that StrepA transmission occurs bilaterally between skin and throat, therefore skin carriage and infections likely play a role in spreading infections around high-burden communities, therefore contributing indirectly to RHD risk (28,29). The incidence of skin carriage, as well as its contribution to household transmission, was also greater when utilising weekly surveillance visits as compared to monthly visits (chapter 6). Pyoderma was found to be the most important source of transmission, as well as the most frequently observed StrepA event overall, particularly in young children, and even more so when using molecular diagnostic methods such as PCR or ID NOW. For all these reasons, understanding risk factors for StrepA skin carriage and infection is vital to devising effective StrepA disease and RHD prevention programmes.

Socioeconomic factors, including poor housing, overcrowding, and low socioeconomic status, are established risk factors for StrepA disease (26). Water access, sanitation, and hygiene facilities (WASH) interventions including handwashing have demonstrated efficacy in reducing pyoderma in other settings (30,31). However, there remain key knowledge gaps around the influence of personal hygiene behaviours and WASH on the skin carriage and infection, especially in low-income settings and in particular in Africa.

In this study we utilise SpyCATS data to investigate the impact of personal hygiene behaviours and household-level WASH characteristics, assessed via a nested cross-sectional survey, on clinical pyoderma, StrepA pyoderma and StrepA skin carriage acquisition risk within the SpyCATS cohort.

7.2 Methodology

7.2.1 Study design and participants

The SpyCATS cohort study was conducted between July 2021 and September 2022 enrolling all willing residents living in 44 households in Sukuta, The Gambia (chapter 5). Full study methods results have been described previously (chapters 4 and 5) (27,32). Briefly, households containing at least three members including one child were eligible and households underwent monthly visits (MV0-MV12) with normal skin swabs taken from all participants, and wound swabs taken from participants with evidence of pyoderma. Incident pyoderma events were identified and swabbed at unscheduled visits when required. Data on socio-demographics were collected at each monthly visit.

Additionally, at each monthly visit, two environmental swabs from commonly touched surfaces within the household were taken to investigate household non-human sources of StrepA. A settle plate was also placed inside the household's main living area for the duration of each household visit to detect airborne StrepA presence.

A nested-cross sectional survey design was conducted in the second half of the SpyCATS study to investigate personal hygiene and WASH risk factors to StrepA skin carriage and disease. The survey data were combined with the StrepA event incidence data to investigate associations between StrepA event risk and personal hygiene and WASH characteristics. To coincide with the survey, an additional five environmental swabs were taken from the household at the same visit, and a further one from the main household grey water source, to further investigate environmental sources of StrepA.

The intensively sampled cohort (chapter 6) was excluded from this analysis to avoid bias, as these households underwent an increased number of visits and sampling events compared to the rest of the study population. Their inclusion would have disproportionately influenced carriage and infection

incidence estimates due to their higher surveillance intensity, making direct comparisons across all households less reliable. By excluding this subgroup, the analysis ensures that carriage and infection rates are representative of the standard monthly visit schedule followed in the main study cohort.

7.2.2 Survey design

At MV6-MV9, household-level data were collected on drinking and grey water sources and access, and handwashing and sanitation facilities. Questions were adapted from UNICEF WASH surveys to ensure alignment with established international standards (33). To enhance contextual relevance, the study team, including nurses and field workers, reviewed these questions and provided feedback based on their local experience. This collaborative process led to the inclusion of additional questions pertinent to the local environment. For personal hygiene behaviours, subject areas were selected based on existing hygiene and infection prevention literature (34,35). Response options were developed with input from the field team to reflect local practices accurately. While formal piloting of the data collection tool was not feasible due to constraints in field staff availability, the survey instrument was iteratively refined based on ongoing feedback during the initial phase of data collection. Additional questions were asked about cooking facilities, flooring material and animal presence in the compound. At visits MV11 and MV12 additional individual-level data were collected on personal hygiene behaviours. Parents or guardians were asked to complete the survey for younger children unable to answer for themselves. Question subjects included handwashing and bathing frequency, laundry habits, dental care, and wound care practices. Question wording can be seen in tables 7.2 and 7.3.

There was no strict age cut-off for determining when a parent or guardian should respond on behalf of a child. Instead, a pragmatic approach was taken. Field staff were instructed to ask children first whether they could answer the survey questions themselves. If a child appeared too young to provide reliable responses, or if they expressed uncertainty, the parent or guardian was asked to respond on their behalf. This approach ensured that data were as accurate as possible while maintaining flexibility across different age groups.

7.2.3 Sampling and laboratory procedures

At each visit monthly visit, and intensive weekly visit, a composite normal skin swab (CITOSWAB® flocked nylon fibre swabs) was collected from skin surfaces on the arms, legs and forehead after being moistened with sterile water. Participants identified as having evidence of skin lesions with pus or crusts (pyoderma) underwent a wound swab (Copan Transystem™ 140C). Commonly touched household surfaces for environmental swabbing were selected during the household enrolment visit, following a structured conversation with household members. Study staff identified and recorded two frequently used surfaces per household, such as door handles, chair armrests, or curtain edges, aiming to capture potential inanimate reservoirs of StrepA. While no previous studies in similar settings provided a standardised approach, this method was exploratory and designed to reflect household-specific patterns of surface contact. The selection process was included in the study protocol (chapter 4) to

ensure consistency across households.. A swab (Copan) was taken from each of the two sites at every monthly household visit by moistening the tip with sterile water, then rubbing the swab tip, while slowly twisting it, over up to 20-30cm of the surface.

Clinical and environmental swabs taken were placed in liquid Amies transport medium and transported in a cold box to the laboratory and plated for culture on the same day. Columbia blood agar culture medium was used for primary and purity plates. Beta-haemolytic colonies underwent latex agglutination testing (Prolex™) for group A *Streptococcus* (as previously described in chapters 4, 5 and 6). The remaining liquid Amies from clinical samples was stored at -70°C for later use.

Pre-prepared Colombia blood agar culture plates were used for the settle plates. The settle plate location was also determined at the enrolment visit to be in the main social room of the household, placed at least 1 metre off the floor and 1 metre away from the walls. The settle plate was left exposed to the room air for the duration (at least one hour) of the household visit, and collected at the end, and transported to the laboratory in a cold box. Settle plates were then incubated overnight and treated as the primary culture plate, undergoing subsequent purity plating and latex testing for group A *Streptococcus* as described above.

Stored liquid Amies from wound swabs taken from participants exhibiting pyoderma symptoms underwent qPCR for StrepA as described in chapter 6. DNA was extracted using QIAamp DNA kits (Qiagen), and quantitative PCR performed using Bio-Rad CFX 96 Touch Real-Time PCR detection system with primers and probes to detect the *S. pyogenes*-specific gene *speB*. Samples were run in a single well, and a cycle threshold (Ct) of more than 40 was defined as negative. Ct less than 36 was considered positive, and Cts between 36 and 40 were repeated and considered positive if amplification was seen and Ct was below 40 for both runs.

7.2.4 Event definitions and follow-up time

Outcome measures investigated were clinical pyoderma events, culture-positive skin carriage events, culture-positive pyoderma events and PCR-positive pyoderma events. Carriage and infection events were defined as described in chapter 5. Culture-positive and PCR-positive pyoderma events were when wound swabs taken from a participant exhibiting clinical pyoderma were positive for StrepA by each method. StrepA skin carriage was defined as a culture-positive normal skin swab a monthly visit. New pyoderma events had to be 14 days after the last positive swab, or after a negative swab. Carriage acquisition events were defined as carriage after two consecutive carriage-negative visits, or 28 days after the last carriage-positive visit with one carriage-negative visit in between, or 42 days after the last carriage-positive visits with no visits in between. Baseline events were defined as events occurring at an individual's enrolment visit (whether at MV0 or a later MV) and were excluded from the analysis. Clinical pyoderma episodes were treated empirically with antibiotic (chapter 5).

Follow-up time for the incidence rates were calculated differently for skin carriage and pyoderma incidence to include unscheduled visits for pyoderma incidence (chapter 5).

7.2.5 Statistical methods

Survey data were entered into REDCap by trained nurses (36). Analysis was performed in R version 4.3.1. Baseline carriage and disease events, detected at participants' enrolment monthly visit were excluded from the analyses. For the WASH survey analysis, events that occurred throughout the whole cohort period in all SpyCATS participants living in a household for which WASH data were available were included. For the personal hygiene survey, only events occurring in participants who answered the survey were included. Cox proportional hazards regression models were used to identify socio-demographic risk factors for infection and carriage accounting for both household clustering and individual recurrent events using robust standard errors (Andersen-Gill extension). To account for clustering within households, mixed-effects (frailty) Cox proportional hazards regression models were used for household-level WASH characteristics, with a shared frailty term to account for household-level heterogeneity. This approach was chosen over Andersen-Gill models, which assume independence between recurrent events, as household-level factors likely introduce shared unmeasured confounders influencing infection risk. For personal hygiene characteristics, individual-level Andersen-Gill models with robust standard errors were used, as these exposures are inherently personal rather than shared within households. Each risk factor of interest from the personal hygiene survey was included in adjusted multivariable AG models including sex and age group. Each WASH characteristic was included in adjusted multivariable mixed-effects Cox proportional hazards models including sex, age group, household size, and parental (or highest household) educational level. Household size and highest educational level were included as proxy markers for household wealth, given the absence of a wealth index in the dataset. Age group (defined as under 5 years, 5 to 11 years, 12 to 17 years, and 18 years or over at time of enrolment) and sex were added to the model as fixed variables. These variables were selected as they represent robust socio-demographic determinants of infection risk in the absence of more direct economic indicators..

7.2.6 Ethics

The study was approved by the Gambia Government/MRC Joint Ethics Committee and the LSHTM Research Ethics Committee (LEO24005). This study was included in the original ethics proposal for the SpyCATS study. Written informed consent was obtained from all adult participants and from the parents or guardians of participants under 18 years old. Children aged 12–17 provided assent. The study adhered to ethical principles outlined in the Declaration of Helsinki and Good Clinical Practice guidelines.

7.3 Results

7.3.1 Study participants

442 participants were enrolled and followed up between July 27, 2021 and September 28, 2022 from 44 households. The socio-demographic characteristics of the cohort can be seen in chapter 5. The median age was 15 (IQR) and 53% were female. The median household size was 7 (IQR), though varied for each household throughout the study period. Data from the WASH survey were available for 43 of the 44 households (419 individuals).

Of 442 participants in the SpyCATS cohort, the personal hygiene questionnaire was answered by 231 participants from 36 households. They were 42% male and 36% adults with a median age of 12 years (range 0-85) similar to the main SpyCATS cohort (table 7.1).

Table 7.1 Sociodemographic characteristics of the survey respondents. Data are n (%) unless indicated otherwise. †Median household size for each participating household (n=36) across all monthly visits. *Total cohort was 442 but demographic information was missing for one participant.

	Survey respondents	**Whole SpyCATS cohort**
Characteristic	**n (%)**	**n (%)**
Total	231 (100)	441* (100)
Sex		
Male	98 (42)	208 (47)
Female	133 (58)	233 (53)
Median age, years (range)	12 (0–85)	15 (0-85)
Age group, years		
<5	70 (30)	104 (24)
5–11	49 (21)	79 (18)
12–17	29 (13)	73 (17)
≥18	83 (36)	185 (42)
Ethnic group		
Mandinka	168 (73)	311 (71)
Wolof	17 (7)	30 (7)
Fula	17 (7)	43 (10)
Jola	13 (6)	17 (4)
Serere	9 (4)	12 (3)
Manjago	2 (1)	6 (1)
Serehule	0 (0)	12 (3)
Non-African	3 (1)	3 (<1)
Other	1 (<1)	2 (<1)
Missing	1	5
Median household size, n (range)	7 (4–37)†	7 (4-37)†

7.3.2 Events

Within the 43 households where WASH data were available, there were 172 incident clinical pyoderma events, 117 incident StrepA PCR-positive pyoderma events, 82 incident StrepA culture-positive pyoderma events and 38 incident StrepA skin carriage events throughout the cohort follow-up period.

When considering only the 231 participants who answered the personal hygiene questionnaire, there were 118 incident clinical pyoderma events, 84 incident PCR-positive pyoderma events, 58 incident culture-positive pyoderma events and 29 incident skin carriage events.

7.3.3 WASH and personal hygiene questionnaire findings

In the personal hygiene survey, 106 (46%) of 231 respondents reported washing their hands more than five times per day. Most respondents reported always washing the hands before eating (83%, 168/203), after urinating (68%, 138/203), after defecating (81%, 164/203) and before praying (85%, 171/203). A higher proportion of respondents reported always using soap when washing hands after defecating (74%, 142/191) than after urinating (45%, 88/196), before eating (37%, 74/199) or before praying (25%, 46/183). Only 3% (8/231) reported bathing less frequently than daily, and 82% (187/227) reported always using soap when bathing. Only 34% (78/231) of respondents reported always cleaning cuts or scratches, and only 21% (48/231) reported always covering wounds/skin infections with some kind of dressing. Full responses are shown in table 7.2.

Table 7.2 Participant responses to the personal hygiene questionnaire.

Question	N = 231[1]
How many times do you wash your hands per day?	
< 5 times a day	95 (41%)
Too young to wash alone	29 (13%)
> 5 times a day	106 (46%)
Don't know	1 (<1%)
Do you wash your hands before eating?	
Never	7 (3%)
Sometimes	28 (14%)
Always	168 (83%)
Missing	28
Do you use soap to wash your hands before eating?	
Never	30 (15%)
Sometimes	94 (47%)
Always	74 (37%)
Don't know	1 (1%)
Missing	32
Do you wash your hands after urinating?	
Never	3 (1%)
Sometimes	58 (29%)
Always	138 (68%)
Don't know	4 (2%)
Missing	28
Do you use soap to wash your hands after urinating?	
Never	39 (20%)
Sometimes	68 (35%)
Always	88 (45%)
Don't know	1 (1%)

Question	N = 231[1]
Missing	35
Do you wash your hands after defecating?	
Never	2 (1%)
Sometimes	28 (14%)
Always	164 (81%)
Don't know	9 (4%)
Missing	28
Do you use soap to wash your hands after defecating?	
Never	1 (1%)
Sometimes	47 (25%)
Always	142 (74%)
Don't know	1 (1%)
Missing	40
Do you wash your hands before praying?	
Never	16 (8%)
Sometimes	12 (6%)
Always	171 (85%)
Don't know	2 (1%)
Missing	30
Do you use soap to wash your hands before praying?	
Never	105 (57%)
Sometimes	29 (16%)
Always	46 (25%)
Don't know	3 (2%)
Missing	48
Do you use a spoon or other utensil to eat with?	
Never	42 (18%)
Sometimes	152 (66%)
Always	37 (16%)
How often do you take a bath?	
Less than daily	8 (3%)
At least daily	220 (95%)
Don't know	3 (1%)
Do you use soap when you take a bath?	
Never	1 (0%)
Sometimes	38 (17%)
Always	187 (82%)
Don't know	1 (<1%)
Missing	4
How often do you change your clothes?	
Less than daily	7 (3%)
At least daily	222 (96%)
Don't know	2 (1%)
How often are your clothes ironed after washing?	
Never	5 (2%)
Sometimes	202 (87%)
Always	16 (7%)
Don't know	8 (3%)
How often do you change your bed sheets?	
Never	2 (1%)
Once a week or less	97 (42%)
More than once a week	128 (55%)
Don't know	4 (2%)
How often do you brush your teeth?	
Less than every 2 days	32 (14%)
At least every 2 days	192 (83%)
Don't know	7 (3%)
What do you use to brush your teeth?	
None	8 (3%)
Mother's hand	14 (6%)
Chew stick	28 (12%)
Toothbrush	180 (78%)
Missing	1
Do you use toothpaste to clean your teeth?	
Never brush teeth	8 (3%)
Never	29 (13%)

Question	N = 231[1]
Sometimes	36 (16%)
Always	158 (68%)
When you have a small cut or scratch, do you use something to clean it?	
Never	8 (3%)
Sometimes	135 (58%)
Always	78 (34%)
Don't know	10 (4%)
What do you use to clean cuts or scratches?	
Nothing	8 (4%)
Tomato paste, palm oil or other	21 (10%)
Water	40 (18%)
Saline or soap and water	77 (35%)
Antiseptic	74 (34%)
Missing	11
Do you cover wounds/skin infections with something?	
Never	51 (22%)
Sometimes	124 (54%)
Always	48 (21%)
Don't know	8 (3%)
What do you use to cover wounds?	
Nothing	59 (26%)
Fabric	92 (40%)
Bandage or plaster	72 (31%)
Don't know	8 (3%)
How often do you change the dressing of wounds?	
No dressing	51 (22%)
Less than daily	17 (7%)
Daily	143 (62%)
Don't know	20 (9%)

[1]n (%)

Most participating households (84%, 36/43) had piped or tap water as their main drinking water source, and 44% (19/43) had a tap inside the house. 44% (18/41) of households had a fixed handwashing place in the compound, but only 22% (9/41) had any handwashing place with soap available. Most compounds had flush toilets (61%, 25/41), 52% (22/42) had the toilet located in the yard (not inside the house), and 7% (3/42) of compounds shared toilet facilities with other households. Tiles were the most common indoor flooring material (70%, 30/43), but most (67%, 29/43) compounds had earth or sand as the main outdoor flooring material. Chicken, ducks or other poultry were observed in 56% (24/43) of compounds, while goats seen in 5% (2/43) and sheep in 9% (4/43).

The WASH survey results in table 7.3 align closely with national data for urban areas, where 92.2% of households had access to improved drinking water sources, only slightly higher than the survey's findings. Nationally 31.8% of urban households had a fixed handwashing place available, similar to the 44% we observed, though soap availability was lower than the 61.0% nationally. 61% of households using an improved toilet (flush or pour) is also similar to national urban data at 73.9%, while the proportion of household sharing toilets with other households was lower than the 26.5% nationally (37).

Table 7.3 Responses to WASH questions for participating households.

Question	N = 43[1]
What is the main source of drinking water for the household?	
Borehole	7 (16%)

Question	N = 43[1]
Piped water/tap	36 (84%)
Where is the piped water source?	
No piped water source	7 (16%)
Public tap/standpipe	1 (2%)
Neighbour's tap	8 (19%)
Tap in yard/compound	8 (19%)
Tap inside house	19 (44%)
What is the main source of other non-drinking water for the household?	
Well	2 (5%)
Borehole	8 (19%)
Piped water/tap	33 (77%)
Study team asked to see where household members most often wash their hands	
Not seen, not in compound	11 (27%)
Seen, mobile/temporary handwashing place	12 (29%)
Seen, fixed handwashing place	18 (44%)
Missing	2
Study team observed presence of water at the handwashing place	
Not seen, not in compound	11 (27%)
Water not available	2 (5%)
Water available	28 (68%)
Missing	2
Study team observed presence of soap or other cleaning agent at the handwashing place	
Not seen, not in compound	11 (27%)
Soap not available	21 (51%)
Soap available	9 (22%)
Missing	2
What type of toilet facility do household members usually use?	
Pit latrine	16 (39%)
Flush or pour toilet	25 (61%)
Missing	2
What kind of flush toilet?	
No flush toilet	16 (39%)
Flush to pit latrine	2 (5%)
Flush to septic tank	17 (41%)
Flush to piped sewer system	6 (15%)
Missing	2
What kind of pit latrine?	
Flush toilet	25 (61%)
Pit latrine without slab/open pit	5 (12%)
Pit latrine with slab	6 (15%)
Ventilated improved pit latrine	5 (12%)
Missing	2
Do you share the toilet with other households?	
No	39 (93%)
Yes	3 (7%)
Missing	1
How many other households use the toilet?	
Not shared	39 (93%)
Shared with 1 other household	1 (2%)
Shared with 2 other households	1 (2%)
Shared with 4 other households	1 (2%)
Missing	1
Where is the toilet located?	
In the compound/yard	22 (52%)
Inside the house	20 (48%)
Missing	1
Where is the cooking usually done?	
Outdoors	19 (45%)
In a separate building/kitchen	22 (52%)
Inside the house	1 (2%)
Missing	1
What type of fuel does your household mainly use for cooking?	
Wood	15 (36%)
Charcoal	27 (64%)
Missing	1
What is the main material of the floor of the indoor space?	

Question	N = 43[1]
Earth/sand	1 (2%)
Cement/concrete	2 (5%)
Vinyl/linoleum	8 (19%)
Tiles	30 (70%)
Carpet	2 (5%)
What is the main material of the floor of the outdoor space within the compound?	
Earth/sand	29 (67%)
Cement/concrete	6 (14%)
Tiles	8 (19%)
Any domestic pets (cats and dogs) seen in the compound?	
No	31 (72%)
Yes	12 (28%)
Any domestic goats seen in the compound?	
No	41 (95%)
Yes	2 (5%)
Any domestic sheep seen in the compound?	
No	39 (91%)
Yes	4 (9%)
Any domestic chicken, ducks or other poultry seen in the compound?	
No	19 (44%)
Yes	24 (56%)

[1]n (%)

7.3.4 Socio-demographic risk factors for pyoderma and skin carriage

In multivariable Cox regression models for each outcome, including season, sex, age group and household size, none of the outcomes were associated with either season or household size, though risk of all four outcomes were significantly higher in males than females, and highest in the under 5 years age group (table 7.4).

Table 7.4 Multivariable Cox regression models for each outcome including all four socio-demographic measures.

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)[2]**	**p-value**	**Event N**	**HR (95% CI)[2]**	**p-value**	**Event N**	**HR (95% CI)[2]**	**p-value**	**Event N**	**HR (95% CI)[2]**	**p-value**
Rainy season	172	1.55 (0.60-3.97)	0.36	117	0.87 (0.31-2.44)	0.79	38	0.42 (0.09-1.91)	0.26	82	1.14 (0.34-3.84)	0.83
Sex			**<0.0001**			**<0.0001**			**0.030**			**<0.0001**
Male	122	—		91	—		27	—		63	—	
Female	50	0.44 (0.30-0.65)		26	0.32 (0.18-0.56)		11	0.45 (0.22-0.92)		19	0.34 (0.19-0.61)	
Age Group			**<0.0001**			**<0.0001**			**0.022**			**<0.0001**
Over 18 years	26	—		10	—		1	—		6	—	
0-5 years	94	3.71 (2.25-6.10)		65	5.96 (2.84-12.50)		21	22.69 (3.08-167.21)		46	7.00 (2.78-17.63)	
5-12 years	43	2.92 (1.70-5.01)		35	5.71 (2.80-11.67)		10	18.44 (2.7-126.08)		24	6.60 (2.77-15.74)	
12-18 years	9	0.96 (0.40-2.28)		7	1.91 (0.68-5.34)		6	16.52 (2.58-105.93)		6	2.69 (1.18-6.12)	

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)**[2]	**p-value**	**Event N**	**HR (95% CI)**[2]	**p-value**	**Event N**	**HR (95% CI)**[2]	**p-value**	**Event N**	**HR (95% CI)**[2]	**p-value**
Household size	172	1.01 (1-1.02)	0.25	117	1 (0.99-1.02)	0.59	38	1.00 (0.98-1.01)	0.74	82	1.01 (1.00-1.03)	0.14

[1]Adjusted for age group, sex, season and household size

[2]HR = Hazard Ratio

7.3.5 Impact of reported personal hygiene behaviour on skin carriage and pyoderma

The impact of personal hygiene behaviours on skin carriage and pyoderma risk are presented in table 7.5. Adjusting for sex and age group, reporting washing of hands more than 5 times per day was associated with a reduced risk of StrepA culture-positive pyoderma (HR 0.56, 95% CI 0.32-0.99, p=0.047) and reporting never washing hands after urination was associated with an increased risk of both clinical (HR 1.6, 95% CI 1.01-2.53, p=0.047) and culture-positive pyoderma (HR 2.00, 95% CI 1.09-3.67, p=0.026) compared to always washing hands. Reporting only sometimes washing hands after defecation was also associated with an increased risk of culture-positive pyoderma (HR 2.02, 95% CI 1.01-4.04, p=0.047) compared to always washing hands. Daily changing of clothes was associated with a reduced risk of both culture-positive pyoderma (HR 0.22, 95% CI 0.05-0.96, p=0.044) and skin carriage (HR 0.11, 95% CI 0.04-0.27, p<0.001) compared to less than daily changing. Brushing of teeth more than every two days was associated with a reduced risk of clinical pyoderma (HR 0.62, 95% CI 0.39-0.98), p=0.040) compared to less frequent brushing. Reported cleaning of cuts and scratches with antiseptic was associated with a reduced risk of clinical pyoderma (HR 0.26, 95% CI 0.09-0.75, p=0.012), StrepA PCR-positive pyoderma (HR 0.25 95% CI 0.09-0.65, p=0.005) and culture-positive pyoderma (HR 0.27, 95% CI 0.13-0.55, p<0.001) compared to not cleaning.

Table 7.5 Multivariable Cox regression models for risk of personal hygiene behaviours on each outcome, adjusting for sex and age group.

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Question[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
How many times do you wash your hands per day?												
< 5 times a day	50	—		36	—		31	—		11	—	
Too young to wash alone	36	1.57 (0.93-2.64)	0.089	24	1.42 (0.75-2.71)	0.3	17	1.2 (0.64-2.24)	0.6	8	1.49 (0.56-4.01)	0.4
> 5 times a day	32	0.97 (0.58-1.62)	0.9	24	1.25 (0.68-2.32)	0.5	10	0.56 (0.32-0.99)	**0.047**	10	1.53 (0.66-3.57)	0.3
Do you wash your hands before eating?												
Always	63	—		48	—		38	—		16	—	
Never	2	0.63 (0.18-2.26)	0.5	2	0.96 (0.27-3.44)	>0.9	1	0.62 (0.17-2.24)	0.5	0	NA	NA
Sometimes	18	1.15 (0.69-1.9)	0.6	11	0.88 (0.42-1.82)	0.7	3	0.31 (0.13-0.73)	**0.007**	5	1.38 (0.56-3.4)	0.5

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Question[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Do you use soap to wash your hands before eating?												
Always	21	—		16	—		11	—		6	—	
Never	16	1.73 (0.91-3.28)	0.095	12	1.68 (0.69-4.11)	0.3	8	1.58 (0.66-3.76)	0.3	3	1.06 (0.34-3.34)	>0.9
Sometimes	45	1.24 (0.69-2.23)	0.5	32	1.13 (0.62-2.05)	0.7	22	1.12 (0.6-2.08)	0.7	12	1.19 (0.56-2.53)	0.7
Do you wash your hands after urinating?												
Always	43	—		32	—		21	—		9	—	
Never	4	1.6 (1.01-2.53)	**0.047**	3	1.23 (0.65-2.34)	0.5	3	2.00 (1.09-3.67)	**0.026**	0	NA	NA
Sometimes	36	0.96 (0.57-1.63)	0.9	26	0.74 (0.47-1.17)	0.2	18	0.83 (0.49-1.42)	0.5	12	1.65 (0.79-3.43)	0.2
Do you use soap to wash your hands after urinating?												
Always	26	—		20	—		14	—		7	—	
Never	19	1.38 (0.64-2.97)	0.4	12	1.12 (0.48-2.61)	0.8	7	0.91 (0.33-2.51)	0.9	4	1.01 (0.43-2.41)	>0.9
Sometimes	34	1.27 (0.72-2.24)	0.4	26	1.2 (0.65-2.24)	0.6	18	1.2 (0.59-2.43)	0.6	10	1.41 (0.59-3.4)	0.4
Do you wash your hands after defecating?												
Always	55	—		39	—		22	—		13	—	
Never	1	0.72 (0.29-1.8)	0.5	1	0.89 (0.34-2.32)	0.8	1	1.73 (0.67-4.42)	0.3	0	NA	NA
Sometimes	25	1.2 (0.7-2.06)	0.5	20	1.13 (0.59-2.19)	0.7	18	2.02 (1.01-4.04)	**0.047**	8	1.92 (0.9-4.1)	0.093
Don't know	2	1.29 (0.38-4.45)	0.7	1	1.79 (0.61-5.25)	0.3	1	2.83 (0.18-43.92)	0.5	0	NA	NA
Do you use soap to wash your hands after defecating?												
Always	46	—		32	—		21	—		13	—	
Never	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
Sometimes	34	1.02 (0.6-1.75)	>0.9	27	0.99 (0.53-1.88)	>0.9	19	1.10 (0.53-2.29)	0.8	8	0.82 (0.38-1.78)	0.6
Do you wash your hands before praying?												
Always	61	—		42	—		33	—		14	—	
Never	7	0.96 (0.49-1.88)	0.9	7	1.32 (0.64-2.71)	0.5	4	0.95 (0.51-1.75)	0.9	1	0.63 (0.1-3.98)	0.6
Sometimes	12	1.18 (0.64-2.16)	0.6	9	1.09 (0.48-2.47)	0.8	3	0.48 (0.15-1.58)	0.2	5	2.42 (0.83-7.06)	0.10
Don't know	1	5.44 (1.11-26.59)	**0.037**	1	19.28 (2.9-128.24)	**0.002**	0	NA	NA	0	NA	NA
Do you use soap to wash your hands before praying?												
Always	14	—		9	—		7	—		3	—	
Never	46	1.46 (0.79-2.68)	0.2	33	1.7 (0.8-3.62)	0.2	21	1.31 (0.58-2.96)	0.5	12	1.77 (0.6-5.24)	0.3
Sometimes	10	1.09 (0.38-3.16)	0.9	6	1.1 (0.28-4.34)	0.9	5	1.05 (0.23-4.88)	>0.9	4	2.27 (0.59-8.72)	0.2
Don't know	3	1.57 (0.95-2.58)	0.078	3	2.21 (1.2-4.06)	**0.011**	3	2.93 (1.41-6.08)	**0.004**	0	NA	NA
Do you use a spoon or other utensil to eat with?												
Never	18	—		16	—		10	—		6	—	

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Question[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Sometimes	84	1.12 (0.71-1.75)	0.6	59	0.85 (0.55-1.31)	0.5	41	0.95 (0.58-1.56)	0.9	18	0.75 (0.34-1.68)	0.5
Always	16	1.22 (0.65-2.31)	0.5	9	0.8 (0.41-1.55)	0.5	7	0.99 (0.37-2.65)	>0.9	5	1.21 (0.42-3.44)	0.7
How often do you take a bath?												
Less than daily	4	—		3	—		4	—		0	—	
At least daily	114	1.77 (0.81-3.85)	0.2	81	1.8 (0.93-3.49)	0.081	54	0.89 (0.41-1.94)	0.8	29	NA	NA
Do you use soap when you take a bath?												
Always	81	—		58	—		41	—		22	—	
Never	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
Sometimes	36	1.55 (1.04-2.31)	**0.030**	25	1.48 (0.97-2.26)	0.067	16	1.35 (0.82-2.22)	0.2	7	1.1 (0.53-2.29)	0.8
How often do you change your clothes?												
Less than daily	3	—		2	—		2	—		3	—	
At least daily	114	0.38 (0.11-1.34)	0.13	81	0.29 (0.05-1.72)	0.2	55	0.22 (0.05-0.96)	**0.044**	26	0.11 (0.04-0.27)	**<0.001**
Don't know	1	0.22 (0.06-0.8)	**0.022**	1	0.23 (0.04-1.42)	0.11	1	0.27 (0.06-1.21)	0.087	0	NA	NA
How often are your clothes ironed after washing?												
Always	8	—		4	—		3	—		1	—	
Never	3	0.68 (0.28-1.65)	0.4	3	1.28 (0.4-4.08)	0.7	2	1.09 (0.45-2.64)	0.8	0	NA	NA
Sometimes	100	0.71 (0.36-1.41)	0.3	74	1.01 (0.39-2.62)	>0.9	51	0.91 (0.38-2.19)	0.8	28	1.26 (0.25-6.27)	0.8
Don't know	7	1.07 (0.5-2.29)	0.9	3	0.97 (0.37-2.55)	>0.9	2	0.88 (0.35-2.22)	0.8	0	NA	NA
How often do you change your bed sheets?												
More than once a week	78	—		59	—		39	—		14	—	
Never	2	1.21 (0.36-4.08)	0.8	1	0.72 (0.49-1.07)	0.11	2	1.96 (0.7-5.46)	0.2	1	1.97 (0.34-11.25)	0.4
Once a week or less	37	0.62 (0.39-0.99)	**0.045**	23	0.49 (0.32-0.74)	**<0.001**	16	0.51 (0.3-0.87)	**0.014**	14	1.22 (0.55-2.71)	0.6
Don't know	1	0.33 (0.24-0.43)	**<0.001**	1	0.46 (0.33-0.64)	**<0.001**	1	0.72 (0.51-1.01)	0.056	0	NA	NA
How often do you brush your teeth?												
Less than every 2 days	35	—		27	—		13	—		7	—	
At least every 2 days	78	0.62 (0.39-0.98)	**0.040**	55	0.59 (0.37-0.96)	**0.033**	43	0.97 (0.54-1.73)	>0.9	22	0.84 (0.45-1.58)	0.6
Don't know	5	0.84 (0.61-1.15)	0.3	2	0.46 (0.23-0.91)	**0.027**	2	1 (0.46-2.15)	>0.9	0	NA	NA
What do you use to brush your teeth?												
Toothbrush	83	—		59	—		45	—		22	—	
None	11	1.35 (0.93-1.97)	0.11	8	1.24 (0.75-2.06)	0.4	4	0.86 (0.5-1.48)	0.6	2	1.01 (0.35-2.95)	>0.9
Mother's hand	9	0.66 (0.37-1.2)	0.2	6	0.59 (0.29-1.22)	0.2	4	0.52 (0.24-1.14)	0.10	3	0.89 (0.31-2.55)	0.8
Chew stick	15	1.97 (0.89-4.39)	0.10	11	3.01 (1.7-5.33)	**<0.001**	5	1.74 (0.66-4.57)	0.3	2	1.88 (0.93-3.79)	0.079
Do you use toothpaste to clean your teeth?												
Always	65	—		48	—		37	—		17	—	

	Clinical pyoderma			StrepA PCR-positive pyoderma			StrepA culture-positive pyoderma			StrepA skin carriage		
Question[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Never brush teeth	11	1.49 (0.97-2.28)	0.067	8	1.3 (0.78-2.18)	0.3	4	0.88 (0.52-1.5)	0.6	2	1.08 (0.34-3.39)	0.9
Never	23	1.36 (0.72-2.56)	0.3	16	1.31 (0.63-2.73)	0.5	9	0.93 (0.43-2.01)	0.8	7	1.73 (0.86-3.49)	0.13
Sometimes	19	1.15 (0.76-1.73)	0.5	12	1.02 (0.61-1.7)	>0.9	8	0.9 (0.51-1.59)	0.7	3	0.74 (0.21-2.57)	0.6
When you have a small cut or scratch, do you use something to clean it?												
Always	29	—		22	—		16	—		7	—	
Never	9	2.91 (1.08-7.82)	**0.035**	7	3.14 (1.27-7.8)	**0.014**	4	2.25 (1.07-4.74)	**0.032**	1	0.97 (0.18-5.25)	>0.9
Sometimes	72	1.06 (0.66-1.71)	0.8	52	0.92 (0.57-1.5)	0.7	35	0.85 (0.43-1.7)	0.6	21	1.21 (0.46-3.19)	0.7
Don't know	8	1.77 (1.17-2.68)	**0.007**	3	0.9 (0.58-1.38)	0.6	3	1.3 (0.66-2.54)	0.4	0	NA	NA
What do you use to clean cuts or scratches?												
Nothing	9	—		7	—		4	—		1	—	
Tomato paste, palm oil or other	6	0.28 (0.09-0.86)	**0.026**	6	0.37 (0.13-1.07)	0.066	2	0.23 (0.08-0.68)	**0.008**	1	0.6 (0.05-6.87)	0.7
Water	21	0.45 (0.12-1.68)	0.2	15	0.4 (0.12-1.36)	0.14	11	0.54 (0.2-1.48)	0.2	5	1.32 (0.2-8.67)	0.8
Saline or soap and water	48	0.42 (0.16-1.13)	0.086	33	0.35 (0.15-0.81)	**0.014**	26	0.51 (0.29-0.91)	**0.021**	9	0.96 (0.2-4.54)	>0.9
Antiseptic	25	0.26 (0.09-0.75)	**0.012**	19	0.25 (0.09-0.65)	**0.005**	11	0.27 (0.13-0.55)	**<0.001**	12	1.53 (0.31-7.59)	0.6
Do you cover wounds/skin infections with something?												
Always	20	—		17	—		13	—		8	—	
Never	32	1.64 (0.86-3.11)	0.13	20	1.22 (0.63-2.36)	0.6	16	1.23 (0.65-2.33)	0.5	9	1.07 (0.5-2.3)	0.9
Sometimes	59	1.25 (0.77-2.04)	0.4	45	1.17 (0.7-1.94)	0.6	27	0.89 (0.43-1.83)	0.8	12	0.62 (0.31-1.24)	0.2
Don't know	7	3.04 (1.92-4.81)	**<0.001**	2	1.21 (0.69-2.12)	0.5	2	1.68 (0.8-3.51)	0.2	0	NA	NA
What do you use to cover wounds?												
Nothing	36	—		24	—		18	—		11	—	
Fabric	47	0.89 (0.51-1.56)	0.7	37	1.12 (0.59-2.11)	0.7	26	1.03 (0.58-1.82)	>0.9	9	0.56 (0.23-1.36)	0.2
Bandage or plaster	28	0.72 (0.39-1.34)	0.3	21	0.85 (0.42-1.72)	0.7	12	0.64 (0.34-1.18)	0.2	9	0.75 (0.35-1.62)	0.5
Don't know	7	2.04 (1.05-3.96)	**0.035**	2	1.07 (0.48-2.38)	0.9	2	1.53 (0.77-3.03)	0.2	0	NA	NA
How often do you change the dressing of wounds?												
No dressing	32	—		20	—		16	—		9	—	
Less than daily	2	0.2 (0.05-0.83)	**0.026**	1	0.15 (0.04-0.64)	**0.010**	1	0.2 (0.05-0.76)	**0.018**	3	1.15 (0.38-3.51)	0.8
Daily	71	0.79 (0.46-1.37)	0.4	56	1.02 (0.54-1.93)	>0.9	36	0.83 (0.49-1.39)	0.5	15	0.62 (0.28-1.36)	0.2
Don't know	13	0.92 (0.47-1.82)	0.8	7	0.83 (0.4-1.75)	0.6	5	0.79 (0.41-1.51)	0.5	2	0.57 (0.25-1.3)	0.2

7.3.6 Impact of different WASH characteristics on pyoderma and skin carriage risk

Associations between household WASH characteristics and event risk are shown in table 7.6. Sharing a toilet with another household was associated with an increased risk of clinical pyoderma (HR 1.78, 95% CI 1.04-3.05, p=0.037) and PCR-positive pyoderma (HR 1.99, 95% CI 1.09-3.61, p=0.024). Compared to a pit latrine, a flush or pour toilet was associated with a reduced risk of PCR-positive pyoderma (HR 0.58, , p=0.019). Having a toilet inside the house, compared to in a separate building in the compound, was associated with a reduced risk of clinical pyoderma (HR 0.56, 95% CI 0.39-0.81, p=0.002) and PCR-positive pyoderma (HR 0.51, 95% CI 0.32-0.80, p=0.004). Indoor flooring with tiles, compared to vinyl/linoleum was associated with a reduced risk of clinical pyoderma (HR 0.64, 95% CI 0.46-0.89, p=0.007) and culture-positive pyoderma (HR 0.57, 95% CI 0.35-0.93, p=0.026), as was outdoor tiling compared to no flooring (HR 0.44, 95% CI 0.20-0.97, p=0.042).

Table 7.6. Multivariable mixed-effects (frailty) Cox regression models for risk of different household WASH characteristics on each outcome, including each question separately adjusting for sex, household size, highest household educational level and age group. NB frailty models can fail to calculate confidence intervals when event numbers are low.

	Clinical pyoderma			PCR-positive pyoderma			Culture-positive pyoderma			Skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
What is the main source of drinking water for the household?												
Piped water/Tap	160	—		110	—		77	—		33	—	
Borehole	12	0.62 (0.32 - 1.19)	0.150	7	0.53 (0.23 - 1.19)	0.124	5	0.52 (0.2 - 1.37)	0.200	5	1.24 (0.6 - 2.59)	0.600
Where is the piped water source?												
No piped water source	12	—		7	—		5	—		5	—	
Public tap/standpipe	4	1.79 (NA)	0.369	3	2.24 (NA)	0.284	3	3.37 (1.27 - 8.93)	**0.015**	1	1.16 (0.57 - 2.39)	0.700
Neighbour's tap	21	1.43 (0.66 - 3.08)	0.360	18	2.09 (0.83 - 5.25)	0.117	8	1.33 (0.39 - 4.58)	0.700	7	1.06 (0.43 - 2.62)	0.900
Tap in yard/compound	50	1.46 (NA)	0.323	33	1.52 (NA)	0.374	27	1.95 (0.68 - 5.60)	0.200	9	0.66 (0.27 - 1.62)	0.400
Tap inside house	85	1.74 (0.89 - 3.39)	0.105	56	1.94 (0.84 - 4.49)	0.123	39	2.03 (0.76 - 5.40)	0.200	16	0.81 (0.36 - 1.80)	0.600
What is the main source of other non-drinking water for the household?												
Borehole	14	—		9	—		5	—		6	—	
Well	5	2.34 (0.76 - 7.2)	0.137	3	2.25 (0.56 - 9.1)	0.254	2	2.66 (0.72 - 9.82)	0.140	NA	NA	NA
Piped water/Tap	153	1.59 (0.86 - 2.92)	0.136	105	1.70 (0.81 - 3.54)	0.157	75	2.25 (0.8 - 6.28)	0.120	32	0.80 (0.42 - 1.51)	0.500
Study team asked to see where household members most often wash their hands												
Not seen, not in compound	31	—		20	—		15	—		10	—	
Seen, mobile/temporary handwashing place	30	1.08 (NA)	0.761	22	1.26 (NA)	0.465	13	0.94 (0.45 - 1.95)	0.900	10	1.19 (0.54 - 2.63)	0.700
Seen, fixed handwashing place	109	1.42 (NA)	0.095	73	1.49 (NA)	0.129	53	1.38 (0.74 - 2.56)	0.300	16	0.69 (0.36 - 1.33)	0.300
Study team observed presence of water at the handwashing place												
Not seen, not in compound	31	—		20	—		15	—		10	—	
Water not available	4	0.74 (NA)	0.586	3	1.02 (NA)	0.973	3	1.75 (0.28 - 11.12)	0.600	1	0.88 (0.15 - 5.37)	0.900

	Clinical pyoderma			PCR-positive pyoderma			Culture-positive pyoderma			Skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Water available	135	1.37 (0.91 - 2.04)	0.127	92	1.45 (0.88 - 2.38)	0.144	63	1.26 (0.67 - 2.35)	0.500	25	0.81 (0.44 - 1.50)	0.500
Study team observed presence of soap or other cleaning agent at the handwashing place												
Not seen, not in compound	31	—		20	—		15	—		10	—	
Soap not available	95	1.30 (NA)	0.215	64	1.37 (NA)	0.231	45	1.26 (0.66 - 2.38)	0.500	23	1.06 (0.58 - 1.96)	0.800
Soap available	44	1.40 (0.86 - 2.28)	0.180	31	1.60 (0.88 - 2.91)	0.122	21	1.30 (0.62 - 2.70)	0.500	3	0.30 (0.12 - 0.73)	**0.008**
What type of toilet facility do household members usually use?												
Pit latrine	66	—		51	—		28	—		16	—	
Flush or pour toilet	84	0.78 (NA)	0.261	50	0.58 (NA)	**0.019**	41	0.90 (0.54 - 1.50)	0.700	19	0.74 (0.43 - 1.27)	0.300
What kind of flush toilet?												
No flush toilet	66	—		51	—		28	—		16	—	
Flush to pit latrine	6	0.70 (NA)	0.430	4	0.55 (NA)	0.265	5	1.34 (0.54 - 3.36)	0.500	2	0.96 (0.39 - 2.32)	>0.900
Flush to septic tank	41	0.68 (NA)	0.066	24	0.50 (NA)	**0.009**	18	0.66 (0.38 - 1.17)	0.200	12	0.79 (0.41 - 1.55)	0.500
Flush to piped sewer system	37	1.20 (NA)	0.498	22	0.86 (NA)	0.664	18	1.21 (0.77 - 1.91)	0.400	5	0.59 (0.35 - 0.99)	0.047
What kind of pit latrine?												
Flush toilet	84	—		50	—		41	—		19	—	
Pit latrine without slab/open pit	14	1.21 (NA)	0.570	11	1.68 (NA)	0.146	7	1.21 (0.45 - 3.30)	0.700	4	1.44 (0.65 - 3.20)	0.400
Pit latrine with slab	25	1.27 (NA)	0.426	19	1.72 (NA)	0.079	11	1.16 (0.58 - 2.30)	0.700	5	1.22 (0.42 - 3.52)	0.700
Ventilated improved pit latrine	27	1.32 (NA)	0.324	21	1.77 (NA)	**0.045**	10	1.00 (0.55 - 1.83)	>0.900	7	1.42 (0.73 - 2.77)	0.300
Do you share the toilet with other households?												
No	151	—		101	—		75	—		33	—	
Yes	20	1.78 (1.04 - 3.05)	**0.037**	15	1.99 (1.09 - 3.61)	**0.024**	7	1.27 (0.67 - 2.42)	0.500	4	1.59 (1.15 - 2.19)	**0.005**
How many other households use the toilet?												
Not shared	151	—		101	—		75	—		33	—	
Shared with 1 other household	7	2.51 (NA)	**0.020**	5	2.68 (NA)	**0.036**	3	2.36 (NA)	0.156	1	1.55 (NA)	0.671
Shared with 2 other households	11	2 (NA)	0.047	8	2.13 (NA)	0.065	4	1.04 (NA)	0.936	2	1.4 (NA)	0.664
Shared with 4 other households	2	0.7 (NA)	0.620	2	1.02 (NA)	0.981	NA	0 (NA)	0.998	1	1.3 (NA)	0.802
Where is the toilet located?												
In the compound/yard	131	—		91	—		63	—		24	—	

	Clinical pyoderma			PCR-positive pyoderma			Culture-positive pyoderma			Skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Inside the house	40	0.56 (0.39 - 0.81)	**0.002**	25	0.51 (0.32 - 0.8)	**0.004**	19	0.61 (0.36 - 1.05)	0.073	13	1.02 (0.5 - 2.08)	0.959
Where is the cooking usually done?												
Outdoors	66	—		50	—		31	—		14	—	
In a separate building/kitchen	98	0.66 (NA)	0.062	64	0.57 (NA)	**0.027**	48	0.68 (NA)	0.205	24	0.95 (NA)	0.909
Inside the house	4	1.71 (0.55 - 5.27)	0.351	2	1.05 (0.24 - 4.54)	0.947	2	1.78 (0.41 - 7.65)	0.440	NA	0 (0 - Inf)	0.999
What type of fuel does your household mainly use for cooking?												
Wood	78	—		59	—		44	—		15	—	
Charcoal	90	0.94 (0.62 - 1.44)	0.782	57	0.73 (0.49 - 1.09)	0.125	37	0.73 (0.45 - 1.18)	0.196	23	1.38 (0.68 - 2.82)	0.376
What is the main material of the floor of the indoor space?												
Vinyl/linoleum	68	—		49	—		33	—		14	—	
Earth/sand	4	1.43 (0.5 - 4.09)	0.501	4	1.99 (0.68 - 5.81)	0.209	3	2.25 (1.59 - 3.18)	**<0.001**	NA	NA	NA
Cement/concrete	5	0.48 (0.19 - 1.21)	0.120	2	0.29 (0.07 - 1.2)	0.087	1	0.21 (0.07 - 0.63)	**0.005**	NA	NA	NA
Tiles	93	0.64 (0.46 - 0.89)	**0.007**	61	0.59 (0.4 - 0.87)	**0.007**	45	0.62 (0.41 - 0.94)	**0.023**	23	0.72 (0.4 - 1.32)	0.300
Carpet	4	1.43 (0.5 - 4.09)	0.501	4	1.99 (0.68 - 5.81)	0.209	3	2.25 (1.59 - 3.18)	**<0.001**	NA	NA	NA
What is the main material of the floor of the outdoor space within the compound?												
Earth/sand	122	—		88	—		63	—		26	—	
Cement/concrete	31	0.96 (0.63 - 1.47)	0.856	17	0.73 (0.42 - 1.28)	0.278	12	0.69 (0.36 - 1.33)	0.268	5	0.76 (0.28 - 2.08)	0.597
Tiles	19	0.57 (0.35 - 0.93)	**0.026**	12	0.5 (0.27 - 0.93)	0.029	7	0.44 (0.2 - 0.97)	**0.042**	7	1.09 (0.46 - 2.58)	0.846
Any domestic animals (cats and dogs) seen in the compound?												
No	133	—		91	—		66	—		31	—	
Yes	39	1.18 (0.76 - 1.84)	0.457	26	1.15 (0.68 - 1.93)	0.606	16	1.05 (0.59 - 1.9)	0.860	1.05 (0.59 - 1.9)	0.85 (0.36 - 2)	0.703
Any domestic goats seen in the compound?												
No	149	—		100	—		67	—		36	—	
Yes	23	1.02 (0.46 - 2.3)	0.954	17	1.46 (0.71 - 3)	0.297	15	1.39 (0.68 - 2.83)	0.361	2	0.3 (0.06 - 1.43)	0.132
Any domestic sheep seen in the compound?												
No	159	—		106	—		73	—		36	—	

	Clinical pyoderma			PCR-positive pyoderma			Culture-positive pyoderma			Skin carriage		
Characteristic[1]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]	**Event N**	**HR (95% CI)**[2]	**p-value**[3]
Yes	13	1.07 (0.55 - 2.1)	0.839	11	NA	NA	9	1.82 (0.89 - 3.71)	0.101	2	0.75 (0.18 - 3.17)	0.699
Any domestic chicken, ducks or other poultry seen in the compound?												
No	65	—		48	—		35	—		16	—	
Yes	107	1.3 (0.9 - 1.89)	0.166	69	1.17 (0.75 - 1.82)	0.479	47	1.12 (0.71 - 1.76)	0.622	22	1.15 (0.59 - 2.21)	0.685

[1]Adjusted for age group, sex, season, highest household educational level and household size

[2]HR = Hazard Ratio

[3]Bold p values significant at <0.05

7.3.7 Environmental swabs and settle plates

StrepA was not isolated from any of the environmental swabs of settle plates collected at monthly household visits, nor from any of the additional environmental swabs collected to coincide with the WASH surveys. Primary culture plates from environmental swabs and settle plates were reported to be heavily contaminated with heavy growth making isolation of beta-haemolytic colonies challenging.

7.4 Discussion

This study investigating the impact of personal hygiene behaviours and household-level WASH characteristics on the risk of StrepA pyoderma and skin carriage within a longitudinal study is, to our knowledge, the first of its kind in Africa. The findings provide evidence of an association between improved personal hygiene, wound care and better WASH facilities and a reduced risk of StrepA pyoderma. Specifically, more frequent reported handwashing, particularly after urination and defecation appeared to confer protection against culture-positive StrepA pyoderma. Additionally, a robust association was seen between cleansing of cuts and scratches with antiseptic, and reduced risk of clinical, PCR- and culture-positive pyoderma. Furthermore, various aspects of improved water access and sanitation facilities were associated with decreased risk of pyoderma, though disentangling their impact from socio-economic status is challenging. Nonetheless, these results reveal potential hygiene and WASH-related factors to include in interventions to reduce StrepA pyoderma and skin carriage, that should be further investigated with regard to their utility in public health programmes in African settings.

The diagnostic outcomes chosen for this study (clinical pyoderma, PCR-positive pyoderma, culture-positive pyoderma, and skin carriage) have varying implications for public health interventions. Clinical pyoderma, though a pragmatic diagnosis that doesn't require laboratory facilities, captures infections cause by other pathogens, such as *Staphylococcus aureus*, which broadens its public health relevance, though has reduced specificity for StrepA. Though prevention of any pyoderma caused by any pathogen is worthwhile and may be of benefit in reducing invasive bacterial infection risk, it is less relevant if targeting RHD – the most significant burden of pyoderma-related mortality in children. PCR is more sensitive that culture at detecting StrepA-positive pyoderma, though may be less discerning in detecting the primary causative pathogen. Higher bacterial load by qPCR increases the likelihood of StrepA detection on culture, which could suggest that culture-positive pyoderma cases are the most clinically and immunologically significant with regard to StrepA (figure 6.2, chapter 6). Skin carriage by contrast has been shown to be relevant in transmission of StrepA and as a precursor for pyoderma, its duration is typically short, and therefore its detection by culture likely underestimated. Though culture-positive pyoderma was the outcome associated with the most risk factors, reduction of skin carriage may be

relevant in the mechanism by which pyoderma risk is reduced, even where skin carriage was not significantly associated, given the probably under-detection of skin carriage.

Various risk factors were found to impact skin carriage risk directly in this study including the availability of soap at the handwashing station, sharing of the toilet with other households, and infrequent changing of clothes. These factors all plausibly increase the potential number of indirect contacts that individuals may have with other people and therefore with StrepA through fomites or reduce the potential for clearing StrepA from the skin. Limited access to soap may hinder effective hand and body hygiene, while shared sanitation facilities could lead to increased contact with contaminated surfaces, reducing overall hygiene standards. Similarly, infrequent clothing changes may prolong skin exposure to bacteria, increasing the likelihood of persistent carriage.

Various measures of handwashing were significantly associated with the outcomes, though the effect was not consistent across the different measures. The presence of a handwashing station with soap available may partially be a marker of household wealth, though the reported frequency of handwashing and use of soap after defecation does suggest that improved handwashing facilities and practice could be a potential mechanism by which to reduce StrepA pyoderma and skin carriage. Handwashing has been shown to be effective at reducing pyoderma in other settings, though not for StrepA specifically, and never before in Africa (30,31). High-quality evidence supports daily handwashing with soap for the treatment and prevention of impetigo, with no benefit found for antibacterial soap over regular soap (38). Hand hygiene promotion, education about effective handwashing, and distribution of soap can be cost-effective public health interventions in resource-limited settings such as this. The findings from this study support the notion that measures that improve hand hygiene and access to soap could potentially reduce StrepA carriage and disease and warrant further investigation.

The association between more frequent brushing of the teeth and reduced risk of clinical pyoderma suggests that oral hygiene could be important in skin disease. As the only outcome to be affected was clinical pyoderma however, it may suggest that the impact is on pathogens that cause pyoderma other than StrepA. Furthermore, better dental hygiene and access to dental care products could be associated with wealth, and therefore not the causative mechanism for reduced pyoderma. Despite this though, the findings support the idea that promotion of oral hygiene may have broader health benefits beyond dental health and warrants further consideration within public health initiatives to reduce infectious diseases.

Our results from the WASH survey were broadly in line with national results, and revealed several associations between various WASH characteristics and the risk of StrepA carriage and pyoderma, revealing the complex interplay between environmental factors and skin health. Sharing of toilets with other households, the use of well water for greywater, and the type of sanitation facilities (pit latrines vs. flush toilets) all carry plausible mechanisms for increased exposure to StrepA and opportunities for transmission. Shared toilets increase the likelihood of contact with contaminated surfaces, well water

could be more susceptible to contamination, and pit latrines may be less likely to have handwashing facilities attached, potentially increasing the risk of transmission after use. Additionally, the choice of tiles over vinyl/linoleum indoors or no flooring outside may contribute to a safer living environment by reducing the likelihood of skin abrasions, which can serve as entry points for pathogens. However, it is challenging to disentangle the influence of wealth from these associations. Our study lacked a robust measure of household wealth to adjust for, as data on household income were often unreliable or missing, and household size did not consistently correlate with economic status. Nonetheless, the use of a mixed-effects model for the WASH analysis strengthens the validity of these findings and allowed for better control of wealth as a confounder through adjustment for household size and highest household education level, which tends to correlate with household income (37,39).
Therefore, while our data suggest that certain WASH improvements that reduce exposure to StrepA and reduce opportunities for skin injuries could plausibly reduce StrepA skin carriage and pyoderma, we cannot conclude from these data that improvement of WASH facilities would be cost-effective or effective at controlling StrepA skin carriage and pyoderma. More targeted research into specific WASH interventions would be required to ascertain whether they could be effective independently of other interventions. Future analyses could benefit from mixed-effects models to account for both individual and household-level factors in StrepA transmission. Adding qualitative assessments would offer insights into socio-cultural influences on hygiene and WASH use. A longitudinal mixed-methods study would help assess temporal links between WASH interventions and StrepA risk while capturing community perspectives to inform targeted public health strategies.

In our study, all environmental swabs and settle plates collected in the households were negative for StrepA when cultured. The primary issue identified was the high level of contamination on these samples, which hindered the accurate identification of StrepA through conventional culture methods. This extensive contamination suggests the presence of numerous pathogens on commonly touched surfaces and within the households studied, suggesting that environmental transmission of not only StrepA but other infectious agents as well could be occurring. Previous research has demonstrated that StrepA can survive on surfaces for extended periods, especially when in biofilm form, enhancing its potential for fomite transmission (40). Additionally, studies have shown that environmental contamination with StrepA is common, and contact with contaminated objects can contribute to the dissemination of the bacterium (41). Although no StrepA was detected via culture, the stored liquid Amies transport medium from the environmental swabs may reveal StrepA presence by PCR on these surfaces. Further work is required to perform this analysis, and could include direct *emm* typing through PCR to see if the *emm* types isolated from participants at the time can be detected on environmental surfaces from the same period.

This study has several limitations, most importantly that the sample size of participants answering the personal hygiene survey was too small to provide adequate power to robustly show associations between the risk factors and pyoderma and StrepA skin carriage risk. The nested nature of the cross-sectional survey was pragmatic but limited the number of participants available to answer at monthly

visits towards the end of the longitudinal study. Hygiene and WASH characteristics are also subjects which may be subject to significant social stigma, therefore responses may reflect how the respondent would like to be perceived, rather than the reality of their hygiene practices. This opens the responses, in particular to the hygiene survey, to social desirability bias. This is commonly observed in infection prevention and control research, where healthcare workers' self-reported compliance does not always match observed practices (42). Additionally, responses could be open to recall bias as participants were asked to recall behaviour in the past, and to report "usual" or "normal" behaviour, which may be overestimated in participant's memories. Certain participants declined to answer particular questions in the survey, or answered "Don't know", which could also introduce some selection bias for those questions, if participants were uncomfortable answering them due to worrying about the perception of them if they answered truthfully. Furthermore, the responses for the age groups of participants most at risk of pyoderma and carriage, were more likely to be open to bias than for adults. In the case of under 5 year olds, because a parent or guardian would have answered the questions for them, thereby leading to a risk of social desirability and recall biases, and for children aged 5-11, they were often at school during monthly household visits, leading to a probable under-sampling of this age group, on top of the potential for reporting bias in those who did answer from that age group. Another significant limitation was the lack of a robust and reliable measure of household wealth from the households with which to adjust the findings, particularly from the household WASH survey responses. Had there been such a measure, the WASH responses associated with outcome risk due to being a proxy measure for wealth rather than being due to the WASH characteristic directly could have been identified. A further limitation of the WASH survey analysis is that the questions were for the household level, which would have reduced the power in relation to individual outcomes. Though the inclusion of the three different definitions of pyoderma as outcomes provides valuable information about the impact of the risk factors on clinical vs culture- vs PCR-positive pyoderma, the reliance on culture for the definition of skin carriage likely underestimated the importance of hygiene on carriage. The improved sensitivity of PCR for StrepA detection would be particularly relevant for skin carriage diagnosis, where other skin pathogens and commensals could contaminate and mask the presence of StrepA by culture. Use of PCR for StrepA detection from the environmental swabs would also be valuable. Addressing these limitations in future studies, as well as the limitation inherent in the SpyCATS study design (see chapter 4-5), could enhance the robustness of our findings. Additionally, future work could exercise a careful selection of research questions focussing on only those found here to be significant, to reduce the impact of multiple hypothesis testing and thereby draw stronger conclusions about their potential utility in public health strategies. Nonetheless, as data on the impact of hygiene and WASH on StrepA skin carriage and infection is sorely lacking from Africa, despite these limitations, these findings provide valuable data to point towards possible interventions and areas for more robust research to be targeted.

Overall, this study suggests that strategies to improve both access to WASH facilities as well as personal hygiene practices could be useful in preventing clinical and StrepA pyoderma, upstream contributors to acute rheumatic fever and rheumatic heart disease. Key findings indicate that wound care in particular, as well as more frequent handwashing with soap and frequent changing of clothes

are associated with a reduced risk of pyoderma and skin carriage, highlighting their potential for inclusion in public health interventions. While the WASH-related findings hint at the impact of environmental factors on skin health, the associations found must be interpreted with caution. Nonetheless, these results imply that additional research such as a longitudinal mixed-methods study assessing the impact of enhanced WASH access and hygiene education on StrepA events, potentially including piloting of community-based interventions, could be useful.

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8 Chapter 8: Discussion

8.1 Summary of PhD findings

The aims of this PhD were met through the two studies conducted and have been presented in this thesis. Described here is the brief overview of each PhD aim, how they were met, and the key findings reported.

Aim 1. To investigate the incidence and seasonality of StrepA pyoderma and pharyngitis, and the proportion of clinical infections caused by StrepA in The Gambia.

This aim was addressed in chapters 3, 5, and 6 through both the PharynGAS and SpyCATS studies.

Incidence

Incidence data from longitudinal studies of StrepA events have never been reported from an African setting before. The incidence of StrepA infections recorded in the SpyCATS study is detailed in chapter 5. This provides important baseline surveillance data on the incidence rates of StrepA pyoderma and pharyngitis in The Gambia in different socio-demographic groups. The robust incidence rates reported show a substantial burden of StrepA infections, with pyoderma in children under 5 years old having the highest incidence rate. This supports findings from other high-RHD settings where pyoderma is the more frequently observed manifestation of StrepA, suggesting that the importance of skin infections in RHD may have been historically understated, and that younger children must be a target of prevention and interventions (1-4). However, limitations in the design of the main SpyCATS study, namely the use of culture for event detection, and the use of monthly surveillance visits lead us to believe that the headline incidence figures for StrepA infections from SpyCATS may be underestimated. Additional data from the SpyCATS intensive weekly visit sub-group and the PCR testing of clinical infection swabs, as reported in chapter 6, confirmed that the overall incidence was higher when using more frequent sampling and sensitive detection methods like PCR. These data offer an indication of what the true incidence rates could be in this high-burden setting.

Seasonality

Despite the findings from our previous study, SpyDERM, which suggested an increased risk of StrepA pyoderma during the rainy season, and other previous work in The Gambia and elsewhere, this finding was not replicated in SpyCATS (1,5,6). However, regression analysis revealed that the risk of StrepA pharyngitis was significantly higher during the rainy season. Seasonal variation in StrepA infections has been observed elsewhere, and a recent study has found that humidity plays an important role in StrepA airborne viability, potentially affecting transmission (1,7-10). It is possible that the intensive screening for StrepA infections in SpyCATS suppressed pyoderma incidence and transmission towards the end of the study, when the rainy season occurred, which may have masked a seasonal increase in StrepA pyoderma. Given the strong association previously observed, the suggests that active pyoderma

surveillance in settings such as this could be an effective public health intervention to reduce pyoderma burden. This also suggests that we may have underestimated the pyoderma burden in The Gambia. Nonetheless, the seasonal association with StrepA pharyngitis highlights the importance of considering environmental and climate factors in the epidemiology of StrepA infections and when planning intervention and vaccination campaigns in future.

Proportion of clinical infections caused by StrepA

The proportion of clinical infections caused by StrepA was reported in PharynGAS, the main SpyCATS cohort, and the intensive weekly swabbing SpyCATS cohort (chapters 3, 5, and 6). These chapters collectively provided a detailed breakdown of how StrepA contributes to the overall burden of clinical infections in the study population. The findings consistently indicated that StrepA was a major aetiological agent in both pyoderma and pharyngitis, and that a higher proportion of StrepA is detected for both pharyngitis and pyoderma when using molecular gene-amplification based diagnostic techniques such as PCR or ID NOW. The proportion of pharyngitis caused by StrepA was lower than data from other LMICs by culture (9.8% in PharynGAS [chapter 3] compared to 17.6% in a global meta-analysis), and was lower in the SpyCATS study, where participants were under active surveillance, than in PharynGAS, where participants were under passive surveillance (11). This was also seen in the meta-analysis of StrepA pharyngitis and may be due to more severe cases being both more likely to be caused by StrepA, and to present to a clinical setting (11-13). Active surveillance may be more likely to pick up less severe pharyngitis episodes, a higher proportion of which could be viral in origin.

Meta-analyses of StrepA pharyngitis and carriage show variations between high- and low-income settings, with lower reported prevalence in low-income countries (11,14,15). Studies in Africa estimate a pooled StrepA pharyngitis prevalence of around 21%, with regional variation while high-income settings report higher rates, particularly in passive surveillance studies (11,16). Differences in healthcare-seeking behaviour and diagnostic methods may contribute to these discrepancies. Passive case detection in high-income settings identifies more StrepA cases, whereas active surveillance studies tend to report lower rates, suggesting under-detection in settings where healthcare utilisation is lower. The SpyCATS findings align with these estimates but likely underestimate the true rate due to surveillance frequency, reliance on culture-based detection and the use of active surveillance.

These findings have implications for understanding immune responses and RHD risk and for trial design. In settings with frequent StrepA exposure, even asymptomatic carriage may play a role in immune priming, so culture-negative, PCR-positive pharyngitis is likely stimulating an immune response as well. Therefore, when considering outcomes for future intervention and vaccine trials, molecular detection methods should be included to ensure that StrepA events that may be contributing to RHD are not missed.

Aim 2. To investigate the monthly prevalence, incidence, persistence and seasonality of asymptomatic StrepA skin and pharyngeal carriage in The Gambia.

This aim was addressed in Chapters 5 and 6 through SpyCATS.

Monthly prevalence and incidence

Chapter 5 provides data on the monthly prevalence and incidence of asymptomatic StrepA skin and pharyngeal carriage. Monthly visits conducted throughout the year of the SpyCATS study allowed for continuous monitoring to assess for patterns of StrepA carriage over time. The findings showed that asymptomatic carriage prevalence was low (1.4-2.8% mean monthly prevalence), but present throughout the year. Incidence in the main SpyCATS cohort of pharyngeal (120/1000pyrs, 95% CI 87-166) and skin carriage (124/1000pyrs, 95% CI 90-170) were similar. The equivalence between skin and pharyngeal carriage was surprising as most historical research interest has been in pharyngeal carriage, and skin carriage is rarely thought to be clinically significant, though it has not been investigated as much (17). In the intensive weekly swabbing cohort, the estimated incidence of both forms of carriage were higher, but skin carriage markedly so (687/1000pyrs, 95% CI 380-1240), giving a higher incidence than for the pharynx (312/1000pyrs, 95% CI 130-750). Furthermore, skin carriage was much higher in males than females (1172/1000pyrs vs 243/1000pyrs), and very high in both the 0-4 and 5-11-year-old age groups (0-4 yrs: 1197/1000pyrs and 5-11 yrs: 1252/1000pyrs), those age groups most at risk of StrepA disease, possibly indicating a previously underappreciated importance of skin carriage in precipitating disease.

The prevalence and incidence estimates in this study are robust, with relatively narrow confidence intervals, reflecting the strength of the surveillance approach. This is the first study in Africa to provide high-resolution estimates of StrepA carriage, offering essential baseline data for future epidemiological studies and vaccine trials. The mean monthly prevalence of asymptomatic carriage was low but persistent, while incidence rates varied significantly with sampling frequency. These findings contrast with data from high-income settings, where higher pharyngeal carriage prevalence is often reported, but are consistent with other studies from low-resource settings that show lower detection rates when relying on culture-based methods (1,18-23). Molecular diagnostics consistently detect a higher proportion of carriage than culture, and their use in future studies could provide a more accurate assessment of StrepA epidemiology.

Persistence of carriage

Within SpyCATS, weekly swabbing after initial carriage acquisition was used to estimate the persistence of pharyngeal and skin carriage episodes. These results are presented in chapter 5. Interestingly, the study found no significant difference in the persistence between skin and throat carriage (both 4 days). Moreover, unlike findings from other studies, prolonged pharyngeal carriage was not observed in this cohort as has been observed elsewhere, suggesting potential differences in the dynamics of StrepA pharyngeal carriage in this population (21,22). Previous data on carriage duration in LMIC do not exist, but studies from HIC have found school-aged children carrying a single *emm* type for an average of 10 weeks (between 3 and 34 weeks) (22). The short carriage persistence we observed suggests that weekly swabbing may not have been sufficiently frequent to capture the

true persistence time, compounded by the fact that PCR-based detection in this sub-study would improve the reliability of the results. However, there may be selective pressure on StrepA in HIC to develop more prolonged carriage compared to LMIC where multiple *emm* types are circulating at a time and transmission may be more frequent.

Seasonality

While the graphical presentation of monthly prevalence across the study did not show a clear seasonal pattern, regression analysis revealed a significant association between pharyngeal carriage risk and the rainy season. This seasonal increase in risk during the rainy months highlights the possible influence of environmental and behavioural factors on StrepA transmission dynamics.

Aim 3. To investigate the relationship between StrepA carriage and infection and to determine socio-demographic and hygiene-related risk factors for StrepA carriage and infection within households.

This aim was addressed in chapters 3, 5, 6 and 7.

Relationship between carriage and infection

To investigate how StrepA asymptomatic carriage and infections interact within individuals, data on carriage and infection events from SpyCATS, combined with *emm* types from isolates, were used. We looked for instances where pharyngeal carriage of a particular *emm* type progressed to pharyngitis in an individual, but we did not observe this, nor did we observe pharyngeal carriage following pharyngitis (chapter 5). This, combined with the short persistence of pharyngeal carriage, suggests that unlike other pathogens where carriage is a prerequisite for infection, for StrepA in the pharynx in this setting, carriage and infection appear to manifest as similar syndromes but on different ends of a symptomatic spectrum. Additionally, we know from previous data from The Gambia that asymptomatic carriage can result in an anti-streptococcal antibody response (20).

In contrast, skin carriage was observed occurring concurrently with both pharyngeal carriage and pyoderma of the same *emm* type in an individual. Twice, skin carriage progressed to pyoderma after 3 days in individuals, and once pyoderma progressed to pharyngeal carriage in an individual (chapter 5). These data suggest for the first time in Africa that skin carriage plays and important role in the development of StrepA infections in individuals, suggesting that reducing StrepA skin carriage, for example through hygiene measures, could be effective in reducing StrepA infections. This evidence of skin carriage contributing to StrepA transmission, particularly in children, supports historical data from Native American communities in the USA where skin carriage was shown to be important in transmission and propagation (17,24). While recent studies from Australia have demonstrated bidirectional throat-to-skin transmission within households, there has been limited modern investigation of skin carriage as a reservoir for transmission (2,25,26). The high incidence of skin carriage observed in SpyCATS, particularly among young children, suggests that skin may be an overlooked niche for

StrepA persistence and spread. This contrasts with studies in high-income settings, where pharyngeal carriage is more commonly studied and assumed to be an important route of transmission (21). If skin carriage is a significant driver of transmission, interventions aimed at reducing StrepA infections should incorporate decolonisation regimes, hygiene and wound care strategies alongside active surveillance of infections.

Socio-demographic and hygiene-related risk factors

The impact of different socio-demographic risk factors was assessed using StrepA carriage and infection events as outcomes. This was done for the both the main SpyCATS cohort (chapter 5) and the intensive visit cohort (chapter 6), as well as for the PharynGAS study (chapter 3). The results were consistent across the different groups within SpyCATS with pharyngitis and pharyngeal carriage risk being highest during the rainy season, in the 5-11-year-old age group and higher in larger households. The elevated risk of pharyngitis and pharyngeal carriage in children aged 5-11 years is consistent with global data indicating that school-aged children are particularly susceptible to StrepA pharyngitis (27,28). In HIC the most frequently observed manifestation of StrepA is scarlet fever which is peaks in children aged 3-9 (8,29-31). Scarlet fever is highly seasonal with peaks typically seen in late winter and early spring (8,29). Though scarlet fever is not observed in The Gambia, the pharyngitis pattern reflects what is seen in scarlet fever in HIC. Both skin carriage and pyoderma risk were not associated with household size or season but were higher in males than females and highest in the 0-5-year-old age group, closely followed by 6-11-year-olds. This also reflects findings from elsewhere confirming that boys under 5 are the highest risk (32-38). Pharyngitis risk in the PharynGAS cohort, however, was higher in the 12-15-year-old age group than in 5-11-year-old age group, and there was no observed impact of household size. The slight difference in age group may be explained by the passive nature of case identification leading to a bias towards more severe cases, which may be more prevalent in the higher aged children (11).

The nested cross-sectional surveys of personal hygiene behaviour and household WASH characteristics described in chapter 7 investigated the impact of these potential risk factors on the risk of StrepA pyoderma and skin carriage in the SpyCATS study. Key findings included that more frequent handwashing, particularly after urination and defecation, and better wound care practices significantly reduced the risk of culture-positive StrepA pyoderma. Hand hygiene is recognised as key in preventing the spread of StrepA infections in healthcare settings, as it effectively removes pathogens from the skin, thereby reducing transmission risk, though its impact in community settings in Africa was not previously shown (39-42). Proper wound care is equally critical in mitigating infection risks. Studies into StrepA outbreaks have found that educating people on hand hygiene and appropriate personal protective equipment use, along with offering wound care training, significantly reduced infection rates (43-45). Our findings, combined with those elsewhere suggest that hand hygiene and wound care could be effective in reducing StrepA infection burden in Africa. Interventions including these elements should be piloted and assessed for acceptability and feasibility in this setting.

Improved water access and sanitation facilities were also associated with decreased risk of pyoderma, although the impact is difficult to separate from socio-economic status. There are questions around the cost-effectiveness and efficacy of WASH interventions in low-resource settings, so stronger associations would be needed to justify their use in settings such as The Gambia to prevent StrepA infections (40,46-48).

Aim 4. To evaluate different clinical scoring systems and point-of-care tests for the diagnosis of StrepA pharyngitis in Gambian children.

This aim was addressed in chapter 3 through the PharynGAS study.

Clinical decision rules

Studies indicates that no single symptom or combination of symptoms can definitively diagnose StrepA pharyngitis, and reviews have highlighted that CENTOR, among others, have limited positive predictive value, ranging between 35% to 50% for correctly predicting GAS pharyngitis (49). Nonetheless they are an attractive proposition for use in low-resource settings due to their low cost. The PharynGAS study assessed the diagnostic accuracy of five clinical decision rules (CDRs) for diagnosing StrepA pharyngitis against both culture and PCR. It found that none of the CDRs performed well enough on their own to be reliably used in the Gambian context. The best-performing CDR, the Smeesters score, had an area under the curve (AUC) of 0.643 when compared against PCR, indicating moderate accuracy. The CDRs, particularly those developed for high-income settings, CENTOR, Modified McIsaac's and FeverPAIN, had lower AUCs, indicating their limited usefulness in this setting. However, combining a CDR into a diagnostic algorithm with a point-of-care test like ID NOW could be a feasible way to reduce the need for using a point-of-care test on everyone, whilst maintaining an adequate sensitivity. This approach has been proposed elsewhere to improve diagnostic precision. A study evaluating the impact of point-of-care molecular testing for StrepA pharyngitis found that integrating such tests into clinical practice could enhance diagnostic accuracy and reduce unnecessary antibiotic prescriptions (50-52). This approach could be explored further in this setting alongside cost-effectiveness analyses to optimise diagnostic accuracy and resource use.

Point-of-care tests

The study assessed the diagnostic accuracy of two rapid diagnostic tests: the SD Bioline lateral flow rapid antigen detection test (LFT) and the ID NOW rapid nucleic acid gene-amplification test against both culture and PCR. The ID NOW test showed high sensitivity (94.6%) and specificity (87.6%) against PCR. These results are in line with others including from low-resource settings that molecular testing can provide a high sensitivity rapid test (53-59) In contrast, the LFT had lower sensitivity (55.7%) and specificity (80.0%) against PCR. Despite the high accuracy of ID NOW and PCR, their cost and laboratory requirements would make them currently unfeasible for widespread use in healthcare centres around The Gambia. The study highlights the need for most cost-effective gene-amplification

diagnostics or novel, highly sensitive and affordable tests. In high-RHD burden settings, the high sensitivity of diagnostic tests is important to avoid false negatives and prevent the progression to acute rheumatic fever and RHD.

Aim 5. To investigate transmission within households using data on the time of infection and carriage acquisition and *emm* type of isolates.

This aim was addressed in chapters 5 and 6, in both the main SpyCATS cohort and an intensive visit cohort.

In the main SpyCATS cohort (chapter 5), analysis of household transmission found bidirectional transmission between the pharynx and skin, confirming recent findings from Australia, and indicating that the StrepA *emm* types do not necessarily exhibit tissue tropism for pharynx or skin as previously thought (2,60,61). However, most StrepA infection events appeared to originate from outside the household, highlighting the significance of community transmission. Other studies have demonstrated that community-level transmission plays a significant role in the spread of StrepA infections. For instance, whole genome sequencing in remote communities has revealed extensive transmission networks, highlighting the importance of community interactions (2,62,63). These studies emphasise that while household transmission contributes to the burden of StrepA infections, community-based transmission is also important, meaning that public health interventions that extend beyond the household are necessary. The intensive visit cohort (chapter 6) provided higher resolution data through frequent sampling and found that skin carriage was the predominant source of transmission and had the largest household secondary attack rate. Whether pyoderma can contribute to RHD directly is debated, but this finding emphasises the importance of prevention of StrepA skin carriage and infections in the controlling StrepA transmission, even if pyoderma does not directly lead to RHD (3,61,64,65).

Historically, certain *emm* types such as 3, 5, 6, 14, 18, 19, and 29 were thought to be associated with a higher incidence of ARF (66). However, this concept is now substantially outdated. Modern sequencing of StrepA has revealed substantial genetic diversity within *emm* types, and evidence suggests that ARF can be caused by a far broader range of *emm* types than previously recognised. A systematic review identified at least 73 different *emm* types associated with ARF, with the traditionally defined rheumatogenic types accounting for only a small proportion of cases (67). Moreover, genetic analysis has shown that the proposed "rheumatogenic motifs" are present in only a minority of ARF-associated strains, undermining their predictive value. These findings indicate that host factors, environmental conditions, and strain diversity play a greater role in ARF pathogenesis than was previously assumed (68). Therefore it is unsurprising that we did not find a strong association with the *emm* types and classically “rheumatogenic” ones, with only 3, 6, and 18 being identified.

Aim 6. To compare surveillance study methodologies and diagnostic methods for StrepA to inform and optimise surveillance study design for the future.

This aim was addressed in chapter 6 by comparing the effectiveness of different surveillance methodologies and diagnostic methods within SpyCATS.

Comparison of surveillance methodologies

The incorporation of the intensive visit cohort into the SpyCATS study allowed for the evaluation of the impact of different visit frequencies on the detection of StrepA carriage and infection. The main SpyCATS cohort, which underwent monthly visits for carriage swabbing and active case finding for infection, was compared to the intensive visit cohort which underwent weekly visits for 6 weeks. The findings revealed that weekly visits resulted in a higher detection rate of StrepA events, particularly for skin carriage and pyoderma. Monthly visits likely missed many short-duration carriage events, given the median clearance time for a StrepA carriage was found to be only 4 days. This highlights the importance of more frequent surveillance visits to more fully capture the true burden of StrepA carriage and infection.

Diagnostic methods

We were also able to compare microbiological culture with PCR-based diagnosis for StrepA infections. We showed that, as shown in PharynGAS, PCR was significantly more sensitive, detecting a higher proportion of StrepA-positive pharyngitis and pyoderma cases than culture, and therefore higher incidences over the follow-up period. While culture identified 10.2% of symptomatic pharyngitis episodes and 45.6% of symptomatic pyoderma episodes, PCR identified 25.9% and 77.8%, respectively. This suggests that reliance on culture alone may lead to substantial underestimation of the true burden of StrepA disease, though the importance of PCR-positive, culture-negative events both clinically and immunologically needs to be further investigated.

Optimisation of surveillance studies

The findings indicate that less frequent surveillance visits and reliance on culture-based diagnostics may miss a significant proportion of StrepA carriage and infection events. More frequent visits and the use of molecular diagnostics, such as PCR, can provide a more accurate assessment of StrepA transmission and disease burden. Though the choice of which surveillance methodology to use will depend on the outcomes of interest and the context, this study provides novel data to justify such decisions and allow estimation of true disease burden in situations where only culture and less frequent visits were possible. Future surveillance programs should consider these findings to enhance the detection of StrepA carriage and disease where required and to increase the power of such studies given the higher detection rates.

8.2 Limitations

The studies presenting in this thesis had several limitations that should be considered when interpreting the findings.

Biases

Firstly, in the PharynGAS study there was potential for selection bias due to the use of convenience sampling and the selection of participants from a health centre outpatient setting, likely excluding individuals less inclined to seek medical care. This approach may have led to an overrepresentation of more severe cases, resulting in a higher prevalence of StrepA infections. The nature of the data collection also left the study open to the possibility of recall bias and social desirability bias in the reliance on parental reporting of clinical history and socio-demographics. Household selection for SpyCATS also potentially introduced selection bias as many households approached declined to participate in the study, which may have introduced a systematic bias.

Study site

The studies were both conducted in an urban area, limiting the generalisability of the findings to rural settings where healthcare access, WASH conditions, socio-demographics and disease dynamics might differ. Rural areas may experience different transmission dynamics due to greater household crowding, limited access to medical care, and variations in hygiene practices. Future studies should include both urban and rural sites to improve generalisability.

Time period of the studies

The studies were conducted over a fixed time period, and although data were collected across all seasons, it is possible that year-to-year variations in environmental or social conditions influenced the results. Longer-term surveillance would allow for a more comprehensive assessment of seasonal and temporal trends in StrepA infections and carriage, particularly in relation to climate variability.

Sample size

The relatively small sample sizes for both studies, particularly in the intensive visit cohort and the nested cross-sectional surveys in SpyCATS, limits the power and precision of some findings. While the main SpyCATS cohort provided robust epidemiological data, the smaller sub-cohorts had reduced statistical power, potentially affecting the precision of estimates for risk factors and transmission dynamics. Cohort studies by their nature are resource intensive. Given the limited budget, the study achieved a substantial sample size, allowing for meaningful epidemiological results. The scale of data collection, particularly the longitudinal follow-up and intensive visit cohort, represents a significant achievement within the available resources. Nonetheless, larger future studies could further refine these findings, enhance statistical power and allow for more ac subgroup analyses.

Culture-based diagnostics

The original PharynGAS study design used culture-based diagnosis as the reference standard, which is relatively insensitive in this setting. We also used a non-selective culture medium, which could have allowed for overgrowth of oropharyngeal commensal bacterial, which may be more significant in this setting, contributing to a lower StrepA detection than in other settings. Given that relying on culture as

the reference standard would have limited the interpretation of the study results, we chose to include PCR diagnosis as a secondary reference standard to improve the robustness of the results. By doing this we overcame a potential limitation of the study design.

SpyCATS also relied on culture for event detection in the main cohort that may have led to under-detection of asymptomatic carriage and clinical infections, especially those with low bacterial loads. To overcome this in part, PCR testing was conducted on clinical infection samples, though not on asymptomatic carriage samples. The PCR sub-study showed significantly higher detection rates, underscoring the need for more sensitive diagnostic techniques in epidemiological studies of StrepA. PCR testing of all asymptomatic carriage samples from the study, including with PCR-based *emm* typing could greatly improve the transmission analysis as well, which as limited by the culture-based diagnosis and reliance on *emm* typing only from available isolates. Whole genome sequencing of the isolates would also improve the sensitivity of the analysis over *emm* typing allowing for separation of distinct lineages of the same *emm* type.

Visit frequency

The SpyCATS study, while robust in design, used monthly surveillance visits in the main cohort which likely missed many short-duration carriage events, leading to an underestimation of the true incidence and prevalence of StrepA carriage. The intensive visit sub-study, which employed weekly swabbing, provided higher resolution data but was limited to a smaller subset of the overall cohort, thus reducing statistical power. However, this sub-study highlighted the potential for underestimation in the main cohort and suggested that more frequent sampling is necessary to capture the full burden of StrepA carriage.

Lack of qualitative data

The studies relied on quantitative data collection methods, limiting insights into behavioural and contextual factors influencing hygiene practices and healthcare-seeking behaviour. A qualitative component, such as interviews or focus groups, could have provided a richer understanding of the barriers to WASH interventions, perceptions of infection risk, and adherence to hygiene recommendations, thereby informing more effective public health strategies. However, utilising the SpyCATS staff and participants, a nested qualitative study was performed by an MSc student into health seeking behaviour and perceptions of sore throat, which I supervised and has been published, though it was not included in this thesis (13).

Linked findings to ARF or RHD

Although StrepA infections are the precursor to ARF and RHD, which are the main component of StrepA-related disease burden in Africa, these studies did not include direct clinical follow-up to assess progression to these complications. While findings highlight risk factors for infection and carriage, further research is needed to establish causal links between these infections and long-term RHD

outcomes. Prospective cohort studies, integrating echocardiographic screening, would help clarify the relationship between early StrepA infections and the development of RHD in high-burden settings.

Wealth as confounder

In the investigation of the various hygiene and WASH-related risk factors, the main limitations were the small sample size and the lack of a robust measure of wealth to adjust for, limiting the reliability of the associations found. Due to the lack of inclusion of collection of reported household wealth, and other wealth-related data being incomplete and unreliable, the only data that could be used was educational background. It was impossible to extrapolate from reported individual income, which was collected, due to complex family structures and missing data. Additionally, many participants declined to answer questions about wealth or income due to social norms. Future studies should use multiple methods to better capture both income and asset wealth to adequately control for these components.

Suppression of events

The findings related to the seasonality of StrepA infections, particularly the discrepancy between pyoderma and pharyngitis seasonality, suggest that the study design may have influenced the observed patterns. Intensive screening and treating of participants for StrepA infections throughout the study period may have suppressed circulating StrepA towards the end of the study, thus reducing carriage and infection incidence, potentially masking true seasonal variations. While a limitation of the study design, this also hints at the possible efficacy of active screening and treating as an intervention to reduce StrepA.

Survey design and data collection

Another important limitation of this study was in the design of the data collection forms and survey structure. The monthly visit format was extensive, incorporating social mixing data, clinical assessments, past medical history, and socio-demographics. Given limited staff and budget constraints, efforts were made to reduce the burden by spreading certain questions, such as socio-demographic data, across multiple visits. However, this approach proved suboptimal due to inconsistent participant availability, resulting in incomplete datasets for some individuals. Additionally, the social mixing questionnaires were lengthy, and their frequent administration led to fatigue among both participants and field staff, potentially compromising data reliability. Furthermore, certain measurements, such as baseline blood pressure and some vital signs, were likely unnecessary and added to the time burden of household visits. These challenges highlight the need for streamlined, well-prioritised data collection forms that ensure essential information is gathered at the most reliable time points, minimising loss to follow-up and optimising data quality. Future studies should carefully balance comprehensiveness with feasibility, particularly in resource-limited settings.

Summary

Overall, while the PharynGAS and SpyCATS studies provide valuable insights into the epidemiology of StrepA in The Gambia, the limitations in study design, diagnostic methods, and surveillance frequency

should be considered when interpreting the results. Future research should aim to address these limitations by incorporating more frequent sampling, using more sensitive diagnostic methods, and controlling for a wider range of environmental and socio-demographic factors.

8.3 Conclusions

This thesis highlights several key findings regarding the epidemiology, transmission, and management of StrepA in The Gambia.

Primarily, the project demonstrates a substantial burden of StrepA disease, particularly when employing active surveillance, frequent visits, and molecular diagnostic methods. The incidence of StrepA infections is highest in the age groups most at risk for RHD, underscoring the critical need for better control and reduction strategies for these infections.

While household transmission of StrepA was observed on several occasions, the majority of infections appeared to originate from outside the household, suggesting a dominant role for community-based transmission. This aligns with findings from high-income settings, where school and daycare environments are major drivers of StrepA spread. In this setting, high rates of carriage and infection outside the home may reflect extensive social mixing, overcrowding, and limited access to hygiene facilities. These findings underscore the need for interventions beyond household-level prevention, incorporating broader community-based strategies such as school hygiene programmes and improved WASH infrastructure in public spaces..

The results highlight the importance of skin carriage and pyoderma in transmission, which have previously been underappreciated. The bidirectional transmission between the pharynx and skin further indicates the need for interventions to target not just pharyngitis but also skin carriage and pyoderma. This could include promoting hygiene practices, such as handwashing, improving wound care, and prompt treatment of pyoderma. Given our findings, the traditional model of primary healthcare-based management of StrepA infections may be insufficient in this setting. Effective strategies to reduce StrepA disease must incorporate community-based interventions, including hygiene education, behaviour change, and public health programmes, alongside vaccine development that targets both pyoderma and pharyngitis.

However, healthcare-based diagnosis and treatment of StrepA infections remains essential, especially given the high burden of RHD in The Gambia. There is a pressing need for affordable, sensitive, and scalable diagnostic tools suitable for use in LMICs, where reliance on clinical diagnosis alone risks both overtreatment with antibiotics and missed cases of StrepA infection. Molecular testing remains prohibitively expensive and impractical for routine use, necessitating the development of low-cost, rapid diagnostic tests with sufficient accuracy to guide clinical decision-making. Investment in such

diagnostics should be a public health priority, given their potential to enable targeted antibiotic use, improve surveillance efforts, and support vaccine trials aimed at reducing StrepA disease burden..

Implementation of StrepA surveillance and design of vaccine trials will soon become a priority across Africa, and our findings suggest that chosen methodologies should incorporate frequent sampling and molecular diagnostic methods to capture the full extent of StrepA disease and carriage, which plays a crucial role in transmission and can stimulate immune responses. Further research is needed to explore the clinical importance of these findings and the extent to which reduction of transmission and disease can translate into a reduction in RHD.

Vaccines targeting StrepA are in development, yet their impact on carriage, in addition to disease prevention, remains uncertain. While some candidates aim to reduce symptomatic infections, whether they will also reduce asymptomatic carriage, requires further investigation and assessment in early trials. Given that skin carriage was frequently observed in this study, effective vaccines would ideally reduce both pharyngeal and skin carriage to curb StrepA spread and indirectly reduce invasive disease, though vaccines are unlikely to have an effect on skin carriage, so alternative or adjunctive interventions may be necessary such as decolonisation regimes. Vaccine design must also account for the extensive genetic diversity of StrepA in LMICs, ensuring broad *emm* type and cluster coverage. Future vaccine trials and epidemiological studies should integrate both clinical and asymptomatic carriage detection to fully capture StrepA transmission dynamics. This study also highlights the importance of frequent surveillance using molecular diagnostics to provide accurate estimates of carriage and infection. Vaccine impact assessments should consider not only reductions in symptomatic disease but also effects on carriage and transmission, as persistent carriage may continue to drive infection cycles even if disease incidence is reduced. These considerations are critical for designing effective vaccination strategies in high-StrepA and RHD burden settings.

In summary, this thesis underscores the importance of carefully designed surveillance systems to monitor StrepA carriage and disease. Comprehensive, community-based public health interventions are required alongside healthcare and vaccine strategies to effectively control StrepA infections and reduce the burden of related diseases in African settings. This study's analysis of household transmission of StrepA is unprecedented in Africa, highlighting the critical role of skin carriage and pyoderma. These findings have significant public health implications, suggesting that interventions aimed at reducing skin carriage and infections and improving hygiene practices could substantially impact the overall burden of StrepA and, consequently, RHD.

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9 Chapter 9: Future studies

The work and findings from this PhD are already leading to much additional research and future studies on StrepA and related issues The Gambia.

9.1 Additional work leading on from SpyCATS and PharynGAS

The immunology samples collected in SpyCATS are being used for work by Dr Alex Keeley and Dr Gabrielle de Crombrugghe for their PhDs and will utilise and build upon the epidemiology data presented here. This work will involve the use of the serum, dried blood spots and salivary samples to assess IgG and IgA responses to a wide range of conserved and non-conserved anti-streptococcal antigens in response to StrepA carriage and infection events (1). This will provide valuable insights into the immune responses associated with naturally occurring StrepA events in high-disease burden, high-diversity settings such as Gambia. This work is vital for better understanding immunity to StrepA to contribute to vaccine development work and in the development of assays for correlates of protection to measure the impact of vaccines in future studies.

Whole genome sequencing of StrepA isolates from SpyCATS is already completed, and the analysis is currently underway at the University of Sheffield. This molecular epidemiology and lineage analysis will enhance our understanding of the genetic diversity and transmission dynamics of StrepA in The Gambia. A more in-depth analysis of the *emm* type diversity and molecular epidemiology based on the *emm* typing data by Dr de Crombrugghe is also underway and is already in the final stages. Additionally, isolates from SpyCATS that were Groups C and G streptococci (*Streptococcus dysgalactiae* subsp. *equisimilis;* SDSE) will provide similar epidemiological analysis and understanding to this presented here for StrepA. The SDSE isolates have also been *emm* typed and sequenced, which will provide a platform to explore horizonal gene transmission between the species in this setting and their interaction with StrepA and RHD. The epidemiological analysis is in its final stages, as is the *emm* type analysis.

Another area of further investigation leading on from PharynGAS is the development of a novel multiplex PCR assay to simultaneously detect SDSE, StrepA, and *Fusobacterium necrophorum*, another common cause of bacterial pharyngitis. Samples from the PharynGAS study will be utilised for this assay, which is in the calibration stage and will be transferred to The Gambia in 2024, with the aim of further investigating causes of pharyngitis in this setting. In future it may be possible to extend this assay to detect the presence of SDSE expressing Group A carbohydrate, something already identified in The Gambia (2,3).

We plan to also perform PCR testing for StrepA and *emm* typing on all asymptomatic samples from SpyCATS, which will significantly enhance the transmission analysis giving much higher resolution and granularity. We have applied for funding for this, but it is not yet secured. This work would additionally include developing a novel agent-based mathematical model of household transmission in this setting using the data from SpyCATS. Modelling of StrepA transmission with this level of data including skin and pharyngeal carriage has never been done before. It would also be able to utilise the social mixing data captured in SpyCATS to calibrate the model.

SpyCATS baseline serum samples will also be used for a genome-wide association study to identify host genetic markers associated with RHD susceptibility and StrepA carriage dynamics. This analysis, conducted in collaboration with genetic epidemiologists, aims to determine whether genetic predisposition influences individual susceptibility to StrepA infection and disease progression, thereby informing targeted prevention strategies.

9.2 Future research studies

In addition to this further work with existing samples already described, we have also already secured funding for several further studies in The Gambia. The first of these is the iSpySchool project, part of the iSpy-LIFE global network consortium funded by the Leducq Foundation. This school-based study aims to capture transmission events in a school setting, that were missed by SpyCATS study design. It will also employ cell-based immunology techniques for the first time in Africa to examine immune responses to naturally occurring StrepA infections. I am the local PI of this project and am in the process of setting up the project to start in September 2024. Additionally, Dr Ed Monk has secured a Wellcome Trust clinical PhD fellowship to carry out a project embedded within this study, focusing on classroom transmission of StrepA. This study will address key research questions around T-cell responses to naturally occurring StrepA infections, dominant transmission routes in school classes, social mixing patterns and behaviour in schools, whilst exploring some novel immunological techniques for StrepA as well.

Secondly, The Gambia has been selected as one of four sites for the SAVAC 2.0 surveillance studies on StrepA. SAVAC 2.0 is hosted by the International Vaccines Institute in Seoul, funded by the Leducq Foundation, Open Philanthropy and Wellcome Trust with the aim of establishing surveillance studies and building capacity for future StrepA vaccine trials in four sites around the world. This project would include harmonised surveillance studies in the four sites, based, in part, on SpyCATS protocols and SAVAC case definitions. In The Gambia it will include rural and hospital-based surveillance and cost-of-illness studies, aiming to establish long-term surveillance infrastructure. These studies will support the development of The Gambia as a vaccine trial site for when StrepA vaccines progress through the development pipeline. Some of the key research questions that this work will address include establishing a baseline for rural community-based StrepA events, and for hospital presentations of iGAS

and ARF/RHD, in order to provide data to adequately power any future StrepA intervention or vaccine trials in The Gambia, providing case fatality rates for severe StrepA infecitons, providing data on APSGN in The Gambia for the first time, and providing data on antibiotic useage and hospital management. Furthermore, the studies designed will provide valuable information on other pathogens, such as *Staphylococcus aureus,* another major cause of morbidity and mortality in Africa.

Future research should adopt a multidisciplinary approach to bridge the gap between epidemiological findings and effective public health interventions. Qualitative studies could provide insights into healthcare-seeking behaviour, hygiene practices, and perceptions of WASH interventions, while implementation science and health systems research would support the integration of these findings into sustainable programmes. Expanding surveillance beyond urban areas and incorporating real-time diagnostic tools, such as affordable molecular or rapid antigen tests, could improve disease detection and case management, particularly in low-resource settings.

Further studies should also evaluate the impact of StrepA vaccines on both disease and carriage to inform immunisation strategies. While reducing symptomatic infections is critical, understanding whether vaccination decreases asymptomatic transmission will be essential for long-term control. By integrating molecular epidemiology, immunology, and public health implementation research, future work should aim to develop scalable interventions to reduce the burden of StrepA and RHD in high-risk settings.

9.3 References

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