Diagnostic capacity for invasive fungal infections in advanced HIV disease in Africa: a continent-wide survey

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Summary

Background Fungal infections are common causes of death and morbidity in those with advanced HIV infection. Data on access to diagnostic tests in Africa are scarce. We aimed to evaluate the diagnostic capacity for invasive fungal infections in advanced HIV disease in Africa.

Methods We did a continent-wide survey by collecting data from 48 of 49 target countries across Africa with a population of more than 1 million; for Lesotho, only information on the provision of cryptococcal antigen testing was obtained. This survey covered 99·65% of the African population. We did the survey in six stages: first, questionnaire development, adaptation, and improvement; second, questionnaire completion by in-country respondents; third, questionnaire review and data analysis followed by video conference calls with respondents; fourth, external validation from public or private sources; fifth, country validation by video conference with senior figures in the Ministry of Health; and sixth, through five regional webinars led by the Africa Centres for Disease Control and Prevention with individual country profiles exchanged by email. Data was compiled and visualised using the Quantum Geographic Information System software and Natural Earth vectors to design maps showing access.

Findings Data were collected between Oct 1, 2020, and Oct 31, 2022 in the 48 target countries. We found that cryptococcal antigen testing is frequently accessible to 358·39 million (25·5%) people in 14 African countries. Over 1031·49 million (73·3%) of 1·4 billion African people have access to a lumbar puncture. India ink microscopy is frequently accessible to 471·03 million (33·5%) people in 23 African countries. About 1041·62 million (74·0%) and 1105·11 million (78·5%) people in Africa do not have access to histoplasmosis and *Pneumocystis* pneumonia diagnostics in either private or public facilities, respectively. Fungal culture is available in 41 countries covering a population of 1·289 billion (94%) people in Africa. MRI is routinely accessible to 453·59 million (32·2%) people in Africa and occasionally to 390·58 million (27·8%) people. There was a moderate correlation between antiretroviral therapy usage and external expenditure on HIV care (R^2=0·42) but almost none between external expenditure and AIDS death rate (R^2=0·18), when analysed for 40 African countries.

Interpretation This survey highlights the enormous challenges in the diagnosis of HIV-associated *Pneumocystis* pneumonia, cryptococcal disease, histoplasmosis, and other fungal infections in Africa. Urgent political and global health leadership could improve the diagnosis of fungal infections in Africa, reducing avoidable deaths.

Funding Global Action For Fungal Infections.

Introduction

By the end of 2020, an estimated 37·7 million people worldwide were living with HIV, the majority of whom lived in Africa.[1] Moreover, the African region has only 1·3 billion of the world's 7·9 billion people, but accounts for 67% of the global HIV-infected population and 60% of new HIV infections.[1] Despite these sobering statistics on the HIV epidemic in Africa, the world has made progress in the HIV response. As of 2020, 84% of people living with HIV around the world knew their status, 87% of whom were receiving antiretroviral therapy (ART).[1,2] 90% of those receiving ART were virally suppressed, accounting for 66% of all those with HIV.[1,2]

However, unlike the progress made in global AIDS prevention and control, countries in northern Africa, west Africa, and central Africa are still lagging behind in achieving the 95–95–95 global UNAIDS targets, with the unintended consequence of increasing burden of advanced HIV disease in the African region.[2,3] WHO defines advanced HIV disease as a CD4 cell count of less than 200 cells per µL or a WHO stage 3 or 4 event in an adult, adolescent, or child who is older than 5 years,[4] and it is this population which is at high risk of opportunistic infections, including the major killers cryptococcal meningitis, tuberculosis, *Pneumocystis* pneumonia, disseminated histoplasmosis, and unspecified sepsis.

Lancet Infect Dis 2022

Published Online December 21, 2022 https://doi.org/10.1016/S1473-3099(22)00656-9

See Online/Comment https://doi.org/10.1016/S1473-3099(22)00682-X

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Research in context

Evidence before this study

Despite excellent national and regional data on people living with HIV in Africa, there are many gaps in information related to fungal infections in HIV-infected people. WHO has listed several diagnostic tests for fungal infections as essential. Comprehensive and detailed information on fungal infections relies upon diagnostic tools that are lacking in many African settings. We searched PubMed from inception to Aug 26, 2022, using the search terms "diagnostic capacity of fungal infections AND HIV AND Africa", and identified only 20 articles. No language restrictions were applied. The information contained in all these articles focused on describing individual fungal diseases. To our knowledge, there is no comprehensive dataset for diagnostic capacity of invasive fungal infections in advanced HIV in Africa.

Added value of this study

To our knowledge, this is the first continent-wide survey to assess the capacity of African countries to diagnose invasive fungal infections in HIV. Supporting diagnostic interventions such as CD4 cell count is essential as it is accessible to only 68% of the African population of 1·4 billion people. Only 358·39 million (25·5%) people in Africa had routine access to cryptococcal antigen tests. More than 74·0% and 78·5% of the African population do not have access to diagnostic testing for histoplasmosis and *Pneumocystis* pneumonia in the public sector, respectively. However, 41 African countries have some facilities for fungal culture, covering 1·29 billion (94·3%) people, but this is slower and insensitive. MRI as an ancillary diagnostic tool was frequently accessible to 453·59 million (32·2%) people.

Implications of all the available evidence

Despite the impressive uptake of antiretroviral therapy in most African countries, advanced HIV disease is still a frequent presentation. Most of these countries are poorly equipped to handle the diagnosis of potentially fatal fungal opportunistic infections in HIV. Our study has identified major gaps in diagnostic capacity, despite WHO listing these tests as essential. The findings can be used to develop actions for improvement of fungal diagnosis in Africa, including the use of data to prioritise clinician awareness, initiate surveillance, and curricula development for training health-care workers.

In many African countries more than 30% of patients have advanced HIV disease when they first seek care, leading to considerable mortality from fungal infections, including cryptococcal meningitis, *Pneumocystis* pneumonia (PCP), disseminated histoplasmosis, and other opportunistic fungal and non-fungal infections.[5–10] Compared with HIV-related cancers, which account for only 7% of HIV-related morbidity, 90% of HIV-related morbidity and mortality are caused by opportunistic infections.[11] In some African countries, it is estimated that 50% of deaths related to fungal infections occur in patients with advanced HIV disease.[12]

A large proportion of patients with opportunistic infections and advanced HIV disease are never reported mainly due to lack of diagnostic and human resource capacity. Apart from South Africa, surveillance systems for fungal infections in Africa are scarce.[13,14]

In the past decade, rapid diagnostic methods for fungal infections have been developed and put on the market. For example, the cryptococcal antigen lateral flow assay has excellent performance (>99% sensitivity and specificity compared with culture), is easy and quick to perform, and costs less than US$5. However, using these low-cost, highly sensitive, and specific tests in low-income and middle-income countries remains a challenge.

Serious fungal infections in advanced HIV disease are often subtle in their presentation and can mimic other conditions such as tuberculosis, bacterial sepsis, or pneumonia.[3–8] Multiple predisposing factors such as tuberculosis, poverty, cancer, asthma, and diabetes contribute to ill health and worse outcomes among people with invasive fungal diseases.[15,16] Implementation of WHO recommendations to provide a package of screening, treatment, and prevention of fungal infections for patients with advanced HIV disease improves outcomes.[17,18] In Uganda, for example, blood screening for cryptococcal diseases is associated with reduced mortality.[19]

Our continent-wide survey sought to identify gaps in the diagnosis of invasive fungal infections and barriers to implementation in Africa.

Methods

Study design and sampling technique

We did a continent-wide survey by collecting data from 48 of 49 target countries across Africa, all with a population of more than 1 million; for Lesotho, only information on the provision of cryptococcal antigen testing was obtained. The questionnaire was completed by respondents affiliated with 72 health facilities in the surveyed countries. The stakeholders participating varied but included heads of public health programmes, ministry of health officials, hospital administrators, heads of laboratories, and senior clinicians. Among the respondents were physicians, pharmacists, laboratory scientists, and nurses. The respondents provided services in public health programmes or public and private hospitals.

We collected information from all African countries with a population of more than 1 million. Cabo Verde, Comoros, Djibouti, São Tomé and Príncipe, Seychelles, Western Sahara, and the two Spanish enclaves of Ceuta and Melilla were not included in this study as their

population was less than 1 million. We applied a snowball sampling technique to disseminate the questionnaire, starting with Global Action For Fungal Infections (GAFFI) ambassadors and existing networks of contacts. Respondents were encouraged to reach out to colleagues in areas where they did not have first-hand knowledge. In some countries, few clinical or laboratory professionals were available and willing to complete the questionnaire. To ensure thorough coverage, additional responses were sought from different centres in larger countries.

The survey was done in six stages: first, questionnaire development, and later adaptation and improvement; second, questionnaire completion by in-country respondents; third, questionnaire review and data analysis by the GAFFI team and then video conference calls with respondents; fourth, external validation from public or private sources; fifth, country validation via video conference call with stakeholders in the health sector in each country; and sixth, five regional webinars lead by Africa Centres for Disease Control and Prevention (Africa CDC) with email follow-up of individual country summaries of diagnostic capacity to seek feedback and reassurance from additional senior country leaders.

No individual patient data was requested or obtained and so ethical consent was unnecessary.

Questionnaire development

The questionnaire consisted of seven sections. Part 1 of the questionnaire covered the respondents and their facility. Part 2 of the questionnaire covered the WHO-recommended list of essential fungal diagnostics used for fungal disease diagnosis, including India ink microscopy cryptococcal antigen test, *Pneumocystis* PCR test, and *Histoplasma* antigen test, selected based on the third WHO Model List of Essential in Vitro Diagnostics as shown in appendix (p 2).[20] Diagnostic availability was classified by type of facility providing the diagnostic test and the frequency of use, in different strata of the health system. Qualitative comments were also collected when diagnostic tests were not regularly performed due to lack of awareness, cost, or lack of trained personnel. Additionally, inquiry was made to determine if patients always paid for diagnostic tests and procedures or if this was covered by insurance, the national or regional government, or a non-governmental organisation. Detailed questions were asked about the CD4 count policy in part 3 of the questionnaire: which patients have CD4 counts taken and which assay is used? If the policy about CD4 counts had recently changed, this was also noted in the survey. Part 4 of the questionnaire covered essential clinical procedures to collect samples (such as lumbar puncture) and radiological imaging. These were selected because they pertain to diagnosing invasive, chronic, and allergic fungal disease. Several respondents were also able to provide approximate costs of different diagnostics or procedures, if they were done, usually based on private charges. Additional open questions captured other information, variation across the country, or nuance with respect to fungal diagnostics used. The questionnaire used for this study is available in appendix (pp 3–10).

Categorisation of test frequency and location

In terms of the frequency of testing, we use the terms regularly, often, or frequently (or routinely in some facilities) to indicate a good diagnostic service. The term occasionally refers to either intermittent provision of tests, infrequent clinical requests, or patient inability to pay or a combination. We found numerous countries with capacity to test, but with rare use of the test, although one or more facilities could provide it, referred to as rarely. For many tests, there was no capability at all in that country, in either the public or private sector.

We collected data separately on public and private sector provision. Public sector health-care facilities were categorised as specialist or university centres, district hospitals (including regional referral hospitals), or community health centres.

Questionnaire clarification and external validation

After receipt of a completed questionnaire, online meetings were organised to provide clarification, as well as qualitative data and narrative. In some cases, the questionnaire was filled in during these meetings. Translators were used when necessary. Companies with point-of-care diagnostic assays (cryptococcal antigen, *Aspergillus* antigen, *Aspergillus* antibody, and *Histoplasma* antigen tests) were also contacted to check for regular sales in countries reporting regular use of a given test.

Data compilation and display and country summaries

Data was compiled and visualised using the Quantum Geographic Information System (QGIS) software (version 31.01.1) and Natural Earth vectors (version 40.0) to design maps showing each diagnostic's coverage across the continent. QGIS is a software system that allows geographical data visualisation, questioning, analysis, and interpretation and has been used to study some public health events in the past.[21]

See **Online** for appendix

One-page profiles were also created for each country, summarising the data collected alongside basic information about the country, demographic data, key health indicators relevant to fungal disease, and its health system. This information included country-specific data on: HIV (provided by UNAIDS); tuberculosis (WHO); population (2021), age structure, and area (Central Intelligence Agency world factbook); and health system and number of hospitals (WHO).[1,22–24]

For the **one-page profiles** see https://gaffi.org/africa-diagnostic-reports

In-country validation

Collected data and country profiles were distributed to relevant local stakeholders and experts, with the purpose of verifying data and correcting inaccuracies. Online validation meetings were held with individual country

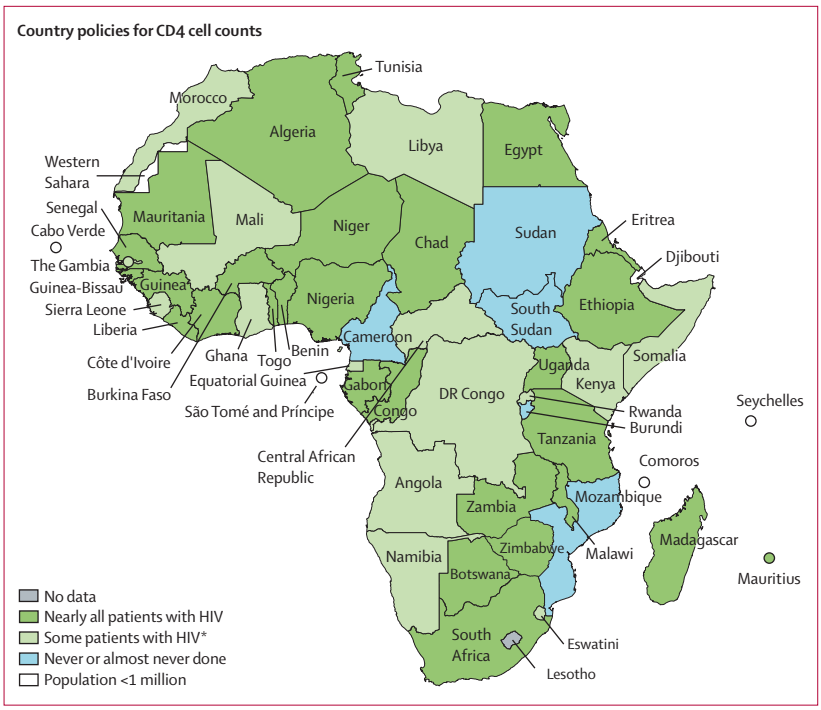

Figure 1: **Indications for measuring CD4 cell counts in Africa**
Criteria for CD4 cell counts included only: new patients; those with a high HIV viral load; and those who are ill, admitted to hospital, or both. Countries with a population of less than 1 million were not included.

stakeholders including representatives of the ministry of health and the national laboratory services, as well as the initial questionnaire respondent(s). A second round of validation led by Africa CDC combined a mixture of online dialogues with each of the five regions of Africa and additional review of the individual country profiles. In this report, only the procedures and diagnostics most pertinent to advanced HIV disease are presented.

Statistical analysis

Most of the data are represented as simple percentages and are qualitative. External HIV expenditure (adjusted) donated by The Global Fund to Fight AIDS, Tuberculosis and Malaria and the US President's Emergency Plan for AIDS Relief (PEPFAR) in 2018 was taken from Granich and colleagues.[25] Using data for all 40 countries in their report,[25] univariate regression was used to correlate expenditure with ART coverage and deaths attributable to HIV per 1000 HIV-infected patients per country.

Role of the funding source

GAFFI executed this survey and its analysis and reporting from internal resources only.

Results

We did a continent-wide survey between Oct 1, 2020, and Oct 31, 2022. 33 (45·8%) of 72 respondents reported data for the whole country (33 countries), while 39 (54·2%) respondents from 15 countries reported data for their institution or region, with multiple responses from Egypt, Nigeria, Tanzania, DR Congo, Cameroon, Guinea-Bissau, Angola, Gabon, Mauritania, Tanzania, Equatorial Guinea, Somalia, and South Africa. Validation meetings with each country allowed others to input and confirm diagnostic availability with three to 25 attendees per country. The additional validation webinars hosted by Africa CDC involved 191 participants from 43 different countries. We sought information separately from two states in Somalia (Somaliland and Puntland). The population of the 48 included countries in 2021 was 1407·39 million.

Determination of the CD4 count allows stratification of patients by risk of multiple opportunistic infections, not including tuberculosis. CD4 counts were listed as an essential diagnostic by WHO in 2018.[20] 29 African countries, covering a population of 962·84 million (64·8%), have CD4 cell counts available in public health facilities for almost all people living with HIV (figure 1). In 14 countries, including central African countries (Central African Republic, DR Congo, and Equatorial Guinea), southern African countries (Angola, Eswatini, and Namibia), west African countries (Gambia, Mali, and Sierra Leone), east African countries (Kenya, Rwanda, and Somalia) and north African countries (Morocco and Libya), CD4 cell counts were done in only new patients or those patients treated with ART drugs with a high viral load and in those who are ill, admitted to hospital, or both. CD4 counts are rarely or never done in Burundi, Cameroon, Mozambique, South Sudan, and Sudan; population of approximately 133·22 million (9·5%); 2·91 million patients with HIV.

Cryptococcal disease in AIDS is usually disseminated when patients who are symptomatic seek care, with antigen detectable in blood and cerebrospinal fluid (CSF).[26] If cultured, these samples will usually grow *Cryptococcus* spp. CSF microscopy is best done with India ink staining but is less sensitive than both antigen detection and culture.[27]

In many African countries, there was some diagnostic capacity to perform India ink staining on CSF or cryptococcal antigen testing (figures 2, 3). Covering a population of 358·39 million (25·5%) of the African population, 14 African countries provide cryptococcal antigen testing in public hospitals on a frequent basis and an additional nine countries have testing occasionally available (population 389·35 million [27·7%]). In Cameroon and Mozambique, regular testing is available in private health facilities and occasionally in Angola and the state of Puntland in Somalia, but not in public services. Many of the countries with capacity to frequently perform cryptococcal antigen testing were in the southern African region (Botswana, Eswatini, Malawi, Mauritius, Namibia, South Africa, Zambia, and Zimbabwe) and east African region (Kenya, Mozambique, Rwanda, Tanzania, and Uganda). Many countries in

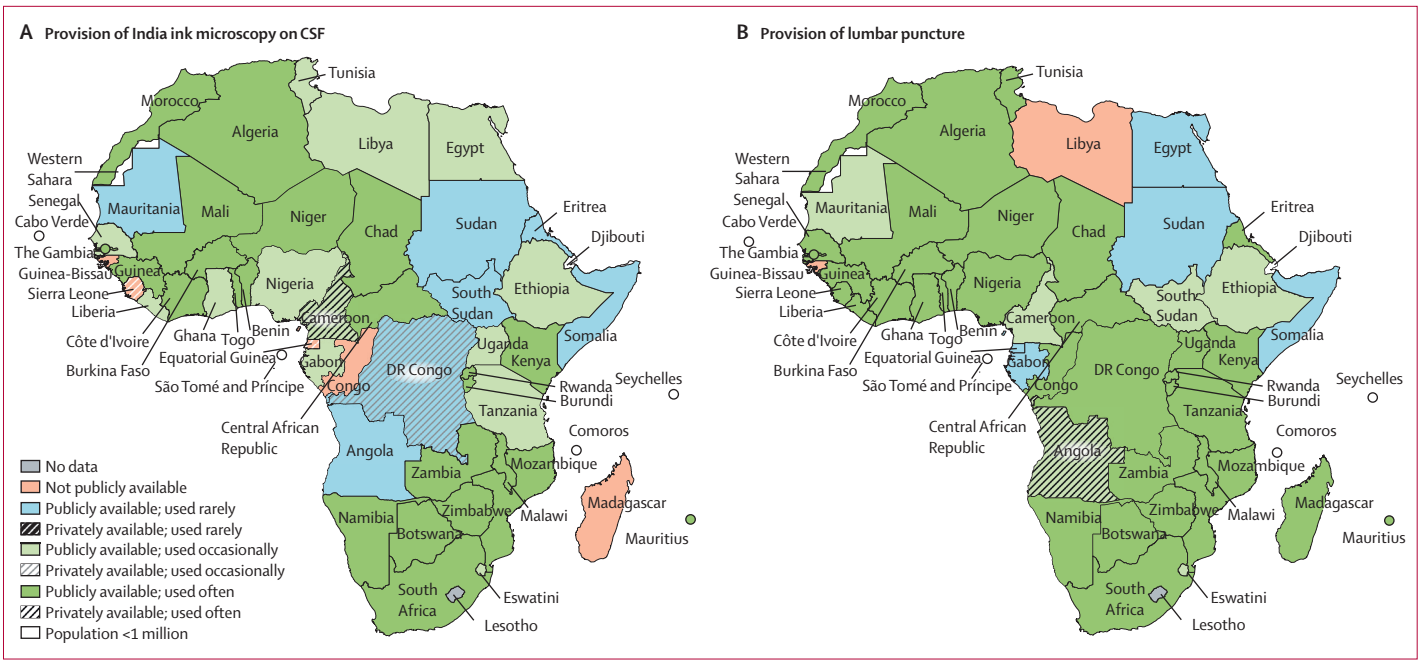

Figure 2: **Diagnostic capacity for cerebrospinal fluid (using India ink microscopy) and cryptococcal diseases using lumbar puncture in Africa**
Countries with a population of less than 1 million were not included.

these regions lack the capacity to frequently provide diagnostic services for cryptococcal disease (figure 2). Some countries in western Africa (Côte d'Ivoire, Niger, Nigeria, Senegal, and Sierra Leone) can provide occasional cryptococcal antigen diagnosis in public hospitals. Angola and Burundi in the southern African region can provide occasional diagnostic services for cryptococcal disease in both public and private facilities. In total, however, 613·10 million (43·6%) people in 22 African countries had no access to cryptococcal antigen testing in either public or private health services, including in Algeria, Benin, Cameroon, Central African Republic, Chad, DR Congo, Egypt, Equatorial Guinea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Libya, Mali, Mauritania, Somalia, South Sudan, Sudan, and Togo.

Many countries in Africa undertake lumbar puncture as an ancillary investigation to the diagnosis of CNS infections (figure 2). Over 1031·49 million (73·3%) African people in 35 countries frequently have access to a lumbar puncture procedure, at least in teaching hospitals. Notwithstanding, 21·55 million (1·5%) people in Guinea-Bissau, Libya, and Somalia (apart from the states of Puntland and Somaliland) do not have any access to lumbar puncture. Lumbar punctures are rarely done in Egypt, Sudan, the state of Somaliland, Gabon, and Equatorial Guinea (figure 2). In Angola, Benin, Cameroon, Republic of the Congo, DR Congo, Egypt, Equatorial Guinea, Liberia, Mali, Mauritania, Nigeria, Sierra Leone, Somalia, the state of Somaliland, Sudan, Tanzania, and Tunisia, only teaching hospitals offer lumbar punctures. In contrast, lumbar punctures in community settings are regularly or occasionally done in Algeria, Burkina Faso, Chad, The Gambia, Niger, and Togo.

India ink microscopy was used as a diagnostic tool for cryptococcal meningitis in many African countries as shown in figure 2. It is less sensitive than cryptococcal antigen, but still very useful, if cryptococcal antigen is not available. 23 countries covering a population of 471·03 million (33·5%) African people can perform India ink staining frequently and another 13 occasionally (664·78 million [47·2%]). Nonetheless, a large population of Africa (271·58 million [19·3%]) had only rare or no access to diagnostic services using India ink staining.

Many countries were only able to diagnose cryptococcal disease using India ink staining including in Ghana, The Gambia, Guinea, Mali, and Togo in west Africa; Cameroon, Central African Republic, Chad, DR Congo, Equatorial Guinea, and Gabon in central Africa; Algeria and Egypt in north Africa; and Ethiopia, Kenya, and Mozambique in east Africa. Guinea-Bissau, Equatorial Guinea, and Somalia (with the exception of the state of Puntland) had no capacity to diagnose fungal infections using either India ink stain or cryptococcal antigen tests.

The fastest and most sensitive assay for disseminated histoplasmosis in AIDS is the detection of urinary antigens; this assay was listed as an essential diagnostic by WHO in 2019.[20] *Histoplasma* antigen is not regularly available in the public sector in Africa, but is in the private sector in Eswatini, Kenya, Mozambique, the state of Somaliland, and South Africa. Occasionally, Burundi, Mozambique, Niger, and the state of Somaliland provide

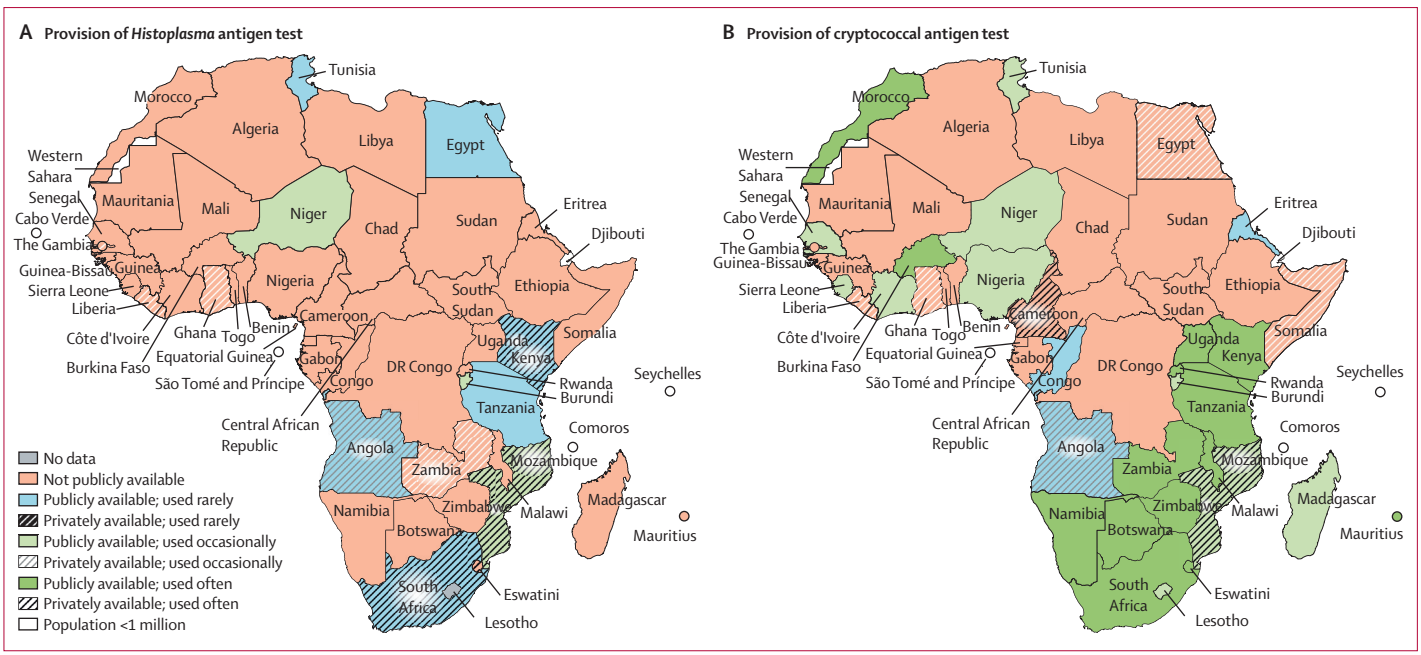

***Figure 3:* *Histoplasma* and cryptococcal antigen national testing capability in Africa**
Countries with a population of less than 1 million were not included.

testing for the diagnosis of *Histoplasma* infections in public health facilities (figure 3A). Additionally, both Burundi and Niger can occasionally perform *Histoplasma* antigen tests in private hospitals. Egypt, Tunisia, Kenya, Tanzania, Angola, and South Africa rarely perform *Histoplasma* antigen assays in their public health institutions. 40 African countries with a population of 1041·62 million (74·0% of the African population) have no access to histoplasmosis diagnostic services in either private or public facilities.

Pneumocystis jirovecii can be visualised with specialised microscopy on respiratory samples or with PCR. In babies and small children, nasopharyngeal aspiration is the only realistic sample type, and only PCR can be used for diagnosis.[28] As shown in figure 4, diagnosis of PCP using PCR is regularly done in public health facilities in south African countries (South Africa) and Burundi and Madagascar in east Africa. In private hospitals, it is also frequently available in Kenya and South Africa. Côte d'Ivoire, Eswatini, Tunisia, and Morocco have occasional access to *Pneumocystis* PCR in public facilities and Burundi and Eswatini in private hospitals. Some west African countries (Guinea, Guinea-Bissau, and Liberia), north African countries (Egypt), and Botswana and Zimbabwe (send out test) in South Africa rarely perform PCR for *Pneumocystis* in their public or private facilities. In total, 1105·11 million (78·5%) African people do not have access to the optimal diagnostic for PCP in either the public or private sectors.

Fungal culture is a cornerstone diagnostic method for many fungal diseases, especially those for which there is no rapid diagnostic. Here, we report fungal culture from all specimens except blood culture, which is reported separately. 41 African countries have facilities for fungal culture, covering a population of 1·29 billion (94·3%) African people. Of these, 22 countries provide frequent diagnostic fungal culture in their public health sector, whereas 13 countries provide fungal culture services in their private health institutions (notably Angola and Kenya only in private centres). Eight countries—Equatorial Guinea, Guinea, Liberia, Libya, Sierra Leone, Somalia, South Sudan, and Zambia—have no facilities for fungal culture in both private and public facilities, representing 79·68 million (5·7%) people in Africa.

Figure 5 provides details on the availability of MRI as an ancillary diagnostic tool for infections complicating AIDS in Africa. In AIDS, an MRI scan of the brain is a superior investigation to a CT scan, particularly for documenting and distinguishing cerebral toxoplasmosis, cytomegalovirus encephalitis, lymphoma, cryptococcoma, tuberculoma, and progressive multifocal leukoencephalopathy.[29] MRI as an ancillary diagnostic tool is often accessible to 453·59 million (32·2%) people in Africa in 15 countries, occasionally accessible to 390·58 million (27·8%) people, and rarely accessible to 337·33 million (24·0%). Nonetheless, 12 countries—in west Africa (Burkina Faso, Guinea-Bissau, and Sierra Leone); central Africa (Central African Republic, Equatorial Guinea, and DR Congo); east Africa (Somalia and South Sudan); southern Africa (Eswatini, Namibia, and Zimbabwe); and Libya in north Africa—have no MRI facilities in the public health sector. 15 countries—in west Africa (Gambia, Guinea, Guinea-Bissau, Mali, and Senegal); central Africa (Cameroon, Central African

Republic, Chad, Congo Republic, and Gabon); east Africa (Eritrea, South Sudan, and Uganda); and southern Africa (Angola and Malawi)—do not offer MRI diagnostic services in their private facilities (figure 5).

We found a moderate correlation between ART usage and external expenditure on HIV care (R^2=0·42), but almost none between external expenditure and AIDS death rate (R^2=0·18), when analysed for 40 sub-Saharan countries (figure 6).

Discussion

To our knowledge, this is the first Africa-wide survey to assess the ability of countries to diagnose invasive fungal infections. We identified some strengths and gaps in the region in diagnosing these infections. Our findings have several clinical and public health implications for the prevention and control of fungal infections in Africa. Provision of fungal diagnostics for people living with HIV, in parallel with tuberculosis diagnosis, saves lives.[17–19]

Substantial funding for sub-Saharan African HIV and tuberculosis care comes through The Global Fund and PEPFAR. Some is supported by domestic funding, especially in South Africa. Contributions from The Global Fund and PEPFAR by country, as well as domestic funding, have recently been assessed in terms of their impact on deaths in 2018 and other parameters of programmatic success.[25] There was almost no correlation between external expenditure and AIDS death rate (R^2=0·18), when analysed for 40 sub-Saharan countries. The many possible reasons for this substantial difference include domestic spending; hospital capability in terms of caring for sick patients with AIDS (diagnostics, drug availability, monitoring for drug toxicity, quality of care, etc); distance between patients, homes, and hospitals; and prevailing attitudes and awareness of opportunistic infections in AIDS. What is clear is that the emphasis on ART roll-out is somewhat impactful, but this expenditure is not adequately addressing mortality from AIDS.

It is encouraging that most countries in Africa can diagnose cryptococcal disease using India ink staining or cryptococcal antigen testing. Recent updated estimates indicate that 19% of AIDS deaths are attributable to cryptococcal meningitis and concentrated in Africa.[9] However, we have identified major gaps in the diagnosis of cryptococcal diseases in Africa. The coverage of antigen testing to diagnose cryptococcal disease is low, with less than 30% of the African population having access to routine cryptococcal antigen testing. Cryptococcal antigen can be detected in serum before meningitis occurs and recent work indicates that such early detection reduces overall mortality in Africa.[19] Many of the countries' ability to diagnose cryptococcal disease is hinged on the use of India ink CSF staining after lumbar puncture, which has a lower sensitivity of 86% (*vs* CSF culture) in expert hands or even lower than 43% in the early stage of the disease when the burden of the fungal antigen is low.[26,27] To circumvent this limitation in detecting cryptococcal disease, lateral flow cryptococcal antigen testing was commercialised in 2012. The sensitivity and specificity of cryptococcal antigen tests in

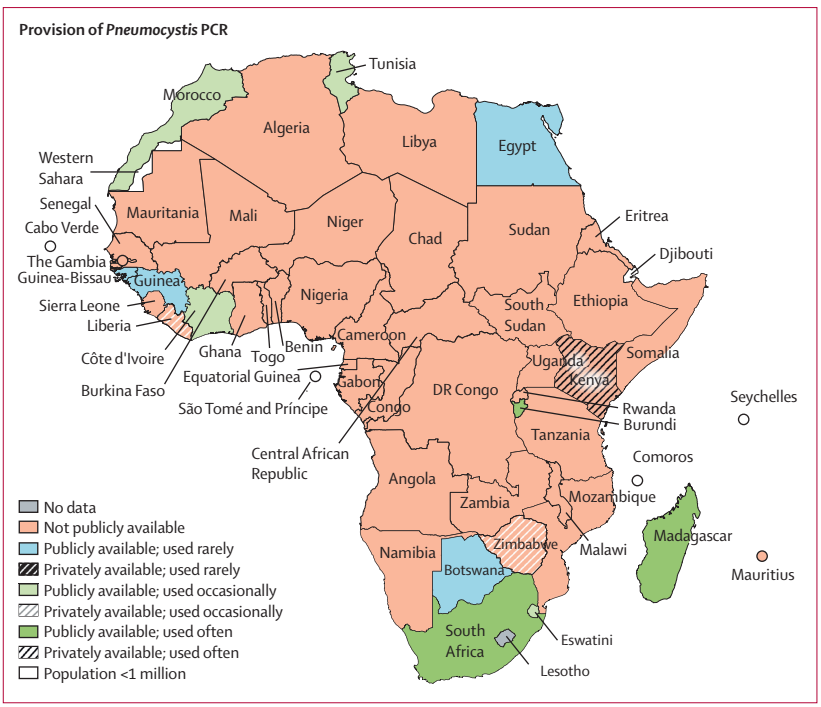

Figure 4: **Diagnostic capacity of *Pneumocystis* detection using PCR in Africa**
Countries with a population of less than 1 million were not included.

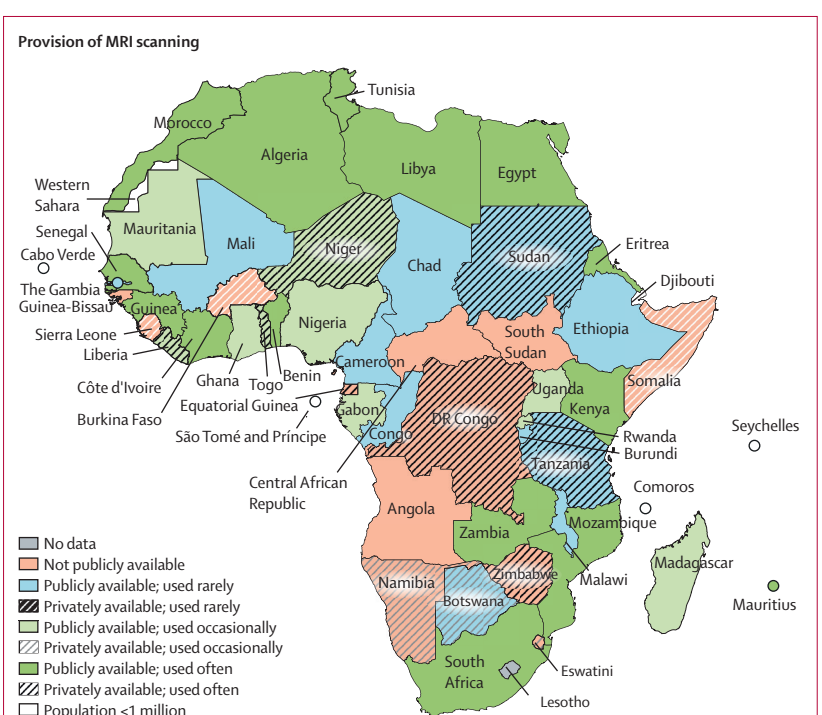

Figure 5: **Capacity to perform MRI scans in Africa**
Countries with a population of less than 1 million were not included.

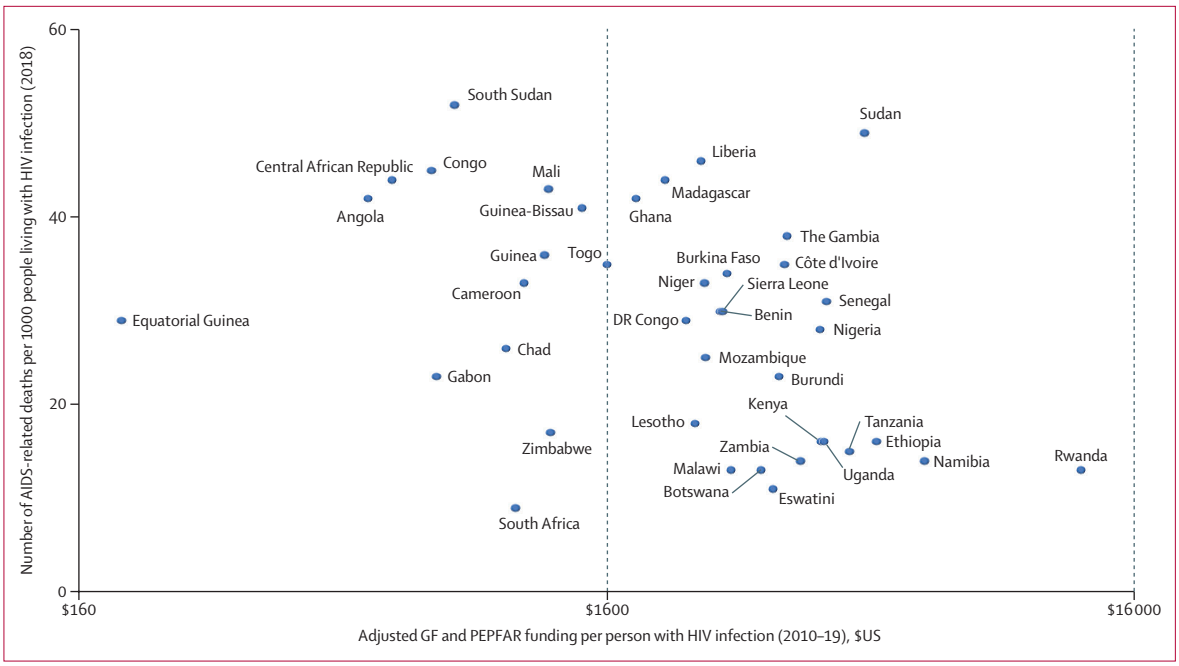

Figure 6: **External HIV funding per person with HIV infection (2010–19) and number of AIDS-related deaths per 1000 people living with HIV infection (2018; R^2=0·18)**
GF=The Global Fund to Fight AIDS, Tuberculosis and Malaria. PEPFAR=US President's Emergency Plan for AIDS Relief.

CSF samples are 99·3% and 99·1%, respectively.[26] The low use of cryptococcal antigen testing in many African countries is not reflective of the 2018 recommendations by WHO to provide tests for cryptococcal disease using antigen testing to all HIV patients with CD4 counts less than 100 per µL,[30,31] and now less than 200 per µL.[32] Recent efforts in several eastern and southern African countries to address the lack of diagnosis and treatment for cryptococcosis by UNITAID working with many local non-governmental organisations and the Clinton Health Access Initiative have improved the situation, but much remains to be done. As many cryptococcal infections in Africa remain undiagnosed and thus untreated, the gap in the diagnosis of cryptococcal disease can be bridged through advocacy to African governments, and their donor partners such as The Global Fund and PEPFAR to address the challenges of sustainable access to cryptococcal antigen lateral flow assays. With greater capacity in the southern and eastern African regions, the focus of advocacy for increased access to the diagnostic tools for cryptococcal antigen should take due consideration of the uneven distribution of diagnostic capacity in western and central Africa.

Both the diagnosis of cryptococcal meningitis, and its therapeutic management, requires lumbar puncture. Additionally, patients with cryptococcal antigenaemia identified through screening programmes require a lumbar puncture to exclude subclinical meningitis. A lumbar puncture is a straightforward procedure with many indications including the diagnosis of tuberculous and fungal meningitis.[33] In our survey, 73·3% of the African population had access to routine lumbar puncture services. However, this was often only in teaching hospitals, and not regional or local hospitals.

Stratifying infection risk in adults with HIV infection requires a rapid CD4 cell count assay, which this survey shows is accessible to only 69% of the African population. We found less accessibility in countries in the west and central African region that already have existing challenges in achieving the UNAIDS 90–90–90 global targets.[3,4] We add our voices to global advocacy to maintain funding for baseline CD4 cell counts as it provides strategic information to guide clinical and public health decision making and understanding of the HIV epidemic in Africa.[34]

We identified major gaps in the continent's ability to detect PCP and histoplasmosis in many African countries, despite their major cause of morbidity and mortality in the African region and elsewhere.[18,35] *Histoplasma capsulatum* and *Pneumocystis* have emerged as potential global fungal pathogens of public health importance due to challenges in providing ART to those with HIV, including treatment failure and late presentation to HIV care.[36] In this survey, more than 74·0% and 78·5% of the African population do not have access to diagnostic services for histoplasmosis and PCP, respectively. This is an age-old problem as Oladele and colleagues[37] reported few data on histoplasmosis in the African region between 1952 and 2017. A recent study in Ghana found twice as many cases of disseminated histoplasmosis as cryptococcal meningitis,[10] and

substantially more data from Nigeria also show a substantial problem with histoplasmosis in AIDS, dwarfing tuberculosis in some places, notably in those with very low CD4 counts.[38] Rapid diagnosis of histoplasmosis reduces deaths from AIDS and avoids inappropriate anti-tuberculous therapy.[18] In the UNAIDS 2020 Global AIDS update, nearly 100 000 infants died from AIDS[1] and many died from pneumonia, which could be undiagnosed PCP.[39] Work by Morrow and colleagues[28] in South Africa has shown how *Pneumocystis* PCR can greatly increase diagnostic yield in infants and young children, using nasopharyngeal specimens.[28] In older children and adults, sputum can be used for diagnosis with PCR. Some patients diagnosed with smear or Xpert negative tuberculosis have PCP.[39] Although co-trimoxazole prophylaxis is regularly used, it is ineffective for established PCP in newly presenting patients with advanced HIV disease. Prophylaxis has a failure rate of 5% over 1 year in those with CD4 counts of less than 200 cells per µL, contrasting with 19% if prophylaxis has never been given.[40]

The lack of diagnostic tools to detect these infections in Africa leads to under-reporting and a lack of comprehensive data necessary to prioritise clinician awareness, initiate surveillance, and design systems for training health-care workers and allocating the resources needed to diagnose and treat patients. Although the Pan American Health Organization and WHO guidelines for diagnosing and managing histoplasmosis among people living with HIV[41] might yield a dividend in improving the diagnosis of histoplasmosis in Africa, international agencies and governments need to be much more proactive in delivering diagnostic tests into health systems if AIDS mortality is to substantially fall, as shown nationally in Guatemala.[17,18]

41 countries covering 94% of the African population have the capacity to perform fungal cultures alongside bacterial culture in microbiology laboratories. Whether this high percentage in fact reflects regular usage to detect fungal infections in the continent is not so clear, as many culture facilities are only in teaching, specialist, and private hospitals, and not in regional hospitals or clinics. However, the main gap is the limited capacity for fungal culture in previously described "fragile" countries including Guinea, Libya, Sierra Leone, Somalia, and South Sudan.[42] As this might reflect the general picture of poor health systems in these countries, a logical strategy would be to support them to strengthen their overall health systems and thereby improve the prevention and control of fungal infections and other diseases.

Access to ancillary investigations and clinical procedures such as imaging and lumbar puncture for the diagnosis of invasive fungal infections is essential for the promotion of universal health coverage. A laboratory diagnosis of meningitis in adults is very important given that both tuberculosis and fungal meningitis are unresponsive to empirical anti-bacterial treatment. Unlike the good coverage of lumbar puncture, we observed that MRI as a diagnostic tool is reported to be often available for only 33·2% of the African population. The gap in diagnostic provision might be due to the high cost of the MRI devices or lack of radiology professionals and reflects the clinical realities of poor imaging services in many African countries. As similar gaps in the diagnostic capacity for invasive fungal infections and other conditions have been previously identified in some African countries, advocacy is needed to encourage African governments to increase health budgetary allocation to at least 15% in line with the 2001 Abuja declaration.[43–46]

Although this Africa-wide survey provides the first evidence of the success and challenges of invasive fungal diagnosis in patients with advanced HIV disease in Africa, it has some limitations. This survey used information reported by selected respondents in each country and although we tried to confirm their responses by several methods, we could have misclassified the country-level availability of diagnostic assays in some instances. Additionally, our study used a snowball sampling technique, which is prone to bias due to the small and unrepresentative sample size. We did not survey countries with populations of less than 1 million or receive any data from Lesotho.

Although this survey has found some encouraging data on the diagnostic capacity of fungal infections in some African countries and regions, there are enormous challenges in the diagnosis of PCP, cryptococcal diseases, histoplasmosis, and other HIV-associated fungal infections in the continent. We need to advocate to the political and global health leadership and mobilise resources to improve and sustain the capacity of African countries to detect fungal infections.

Contributors

The surveys were done by EO and ROSP, and designed by EO, ROSP, and DWD. Data analysis was done by ROSP and DWD. SL and PSK wrote the paper, with support from DWD. JNJ, AJH, NLEM, BKO, SI, SMH, SK, JS, ATA; NPG, CIMM, and KG all contributed their country data to the survey or arranged the validation of the data and commented on article drafts. ROSP and DWD validated all the data. EO, ROSP, and DWD had full access to all the data in the study, and each author has had opportunities to view and correct their country data and comment on the data presented. EO, ROSP, and DWD took final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

A comprehensive report of the results of this survey was presented at the International Conference of Public Health in Africa and the final report is available on the GAFFI website. For data-related enquiries please contact the corresponding author.

Acknowledgments

We are indebted to the many respondents to the survey, which has been published by the Africa Centres for Disease Control and Prevention and GAFFI on Dec 12, 2022. Other results from the survey will be published in medical specialty journals, by the survey contributors who are not authors on this paper.

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