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Original Article

The prevalence of laboratory-confirmed *Pneumocystis jirovecii* in HIV-infected adults in Africa: A systematic review and meta-analysis

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Abstract

Background: The epidemiology of *Pneumocystis jirovecii*, known to colonize the respiratory tract and cause a life-threatening HIV-associated pneumonia (PCP), is poorly described in Africa. We conducted a systematic review to evaluate *P. jirovecii* prevalence in African HIV-positive adults with or without respiratory symptoms. Methods: We searched Medline, Embase, Cochrane library, Africa-Wide, and Web of Science for studies employing PCR and/or microscopy for *P. jirovecii* detection in respiratory samples from HIV-positive adults in Africa between 1995 and 2020. Prevalence with respiratory symptoms was pooled using random-effect meta-analysis, and stratified by laboratory method, sample tested, study setting, CD4 count, and trimethoprim/sulfamethoxazole prophylaxis. Colonization prevalence in asymptomatic adults and in adults with non-PCP respiratory disease was described, and quantitative PCR (qPCR) thresholds to distinguish colonization from microscopy-confirmed PCP reviewed. Results: Thirty-two studies were included, with 27 studies (87%) at high risk of selection bias. *P. jirovecii* was detected in 19% [95% confidence interval (CI): 12–27%] of 3583 symptomatic and in 9% [95% CI: 0–45%] of 140 asymptomatic adults. Among symptomatic adults, prevalence was 22% [95% CI: 12–35%] by PCR and 15% [95% CI: 9–23%] by microscopy. Seven percent of 435 symptomatic adults had PCR-detected *Pneumocystis* colonization without evidence of PCP [95% CI: 5–10%, four studies]. One study established a qPCR cutoff of 78 copies/5μl of DNA in 305 induced sputum samples to distinguish *Pneumocystis* colonization from microscopy-confirmed PCP. Conclusion: Despite widened access to HIV services, *P. jirovecii* remains common in Africa. Prevalence estimates and qPCR-based definitions of colonization are limited, and overall quality of studies is low.

Key words: HIV, *Pneumocystis jirovecii*, Africa.

Introduction

Pneumocystis pneumonia (PCP) is a life-threatening opportunistic infection caused by the fungus *Pneumocystis jirovecii*. *Pneumocystis* has a worldwide distribution, with human infections reported from almost all regions of the world.[1,2] After airborne exposure, both immunocompromised and immunocompetent individuals may temporarily harbor *Pneumocystis* cysts or trophozoites, which colonize the respiratory tract in the absence of clinical and radiological features of PCP. Depending on the host immune status, colonizing organisms may be cleared, persist at low burdens, or progress to cause clinical pneumonia.[3–6] In HIV-positive individuals, PCP usually occurs with advanced immune deficiency (CD4 count ≤ 200 cells/μl)[7] and carries an estimated case fatality of 19% in sub-Saharan Africa.[8]

A systematic review that examined the burden of clinically suspected or laboratory-confirmed PCP in sub-Saharan Africa from 1995 to 2015 reported a pooled PCP prevalence of 19% among HIV-positive adults presenting with respiratory disease.[8] However, significant heterogeneity exists in reported PCP rates (ranging from 1[9] to 77%[10]), reflecting differences in the populations studied and difficulties associated with both clinical and laboratory PCP diagnosis. Interpretation of typically non-specific clinical and radiological signs is challenging, and diagnostic difficulties are compounded by the potential for colonization, frequent co-infection with other respiratory pathogens, and poor access to sensitive, albeit costly and invasive, diagnostic tools.[8] Given the poor specificity of clinical definitions of PCP, there is a need to establish more robust prevalence estimates, focusing on laboratory-confirmed (microscopy or polymerase chain reaction [PCR] proven) *P. jirovecii* in respiratory samples from HIV-positive adults with respiratory disease in Africa.

Since highly sensitive PCR testing may detect scanty organisms in respiratory samples from individuals colonized with *P. jirovecii* in the absence of PCP, interpreting a positive PCR from an individual with non-specific respiratory signs may be challenging for clinicians. A non-quantified positive PCR result cannot, in isolation, distinguish between a colonizing or clinically significant *P. jirovecii* organism burden—only for the latter of which high dose trimethoprim/sulfamethoxazole or other PCP-targeted treatment would be appropriate. In light of this, several studies have investigated quantitative PCR (qPCR) cycle thresholds (C_T), or fungal load cutoffs, which may then be used to distinguish between the typically low-burden colonization state and the high-burden infected (PCP) state in immunocompromised patients.[11–17] Previous thresholds (ranging from 27 to 39 cycles),[11,13,14,18] generally explored in non-African settings, have been developed to correspond to robust definitions of microscopy-confirmed *Pneumocystis* disease and are specific to the respiratory specimen analyzed, the population studied, and the laboratory PCR technique (including choice of *P. jirovecii* target gene) employed.[19]

Pneumocystis colonization has two further significant implications. First, it enables person-to-person transmission and allows the fungus to circulate in the community, threatening severe disease when encountered by HIV-positive persons or other individuals with depleted immunity.[4,20] Second, fungal reservoirs that accumulate in individuals with immune defects have been documented to evolve into PCP.[3,5,6] Current knowledge on the epidemiology of *Pneumocystis* colonization in HIV-positive adults is largely shaped by studies in Europe and North America, with a paucity of data from Africa.[4] Given these implications, and as PCR-based diagnostics become increasingly available, there is a need to establish African prevalence estimates of *Pneumocystis* colonization, as well as to explore African qPCR colonization thresholds that can improve the interpretation of, and therapeutic decisions based on, positive PCR assays.

To address these gaps, we conducted a systematic review and meta-analysis with the primary aim to determine the prevalence of laboratory-detected *P. jirovecii* in African HIV-positive adults with respiratory symptoms, and to contrast this with the rates at which *P. jirovecii* is harbored in HIV-positive adults without respiratory complaints.

Methods

Objectives

Our primary objective was to determine the prevalence of laboratory-detected *P. jirovecii* (using any PCR or microscopy technique) in HIV-positive adults (≥13 years of age) in Africa (1) with respiratory symptoms and (2) without respiratory symptoms. As secondary objectives, we evaluated (1) quantitative *Pneumocystis* PCR fungal burden thresholds, established in African laboratories, that attempt to differentiate between PCR-detected *Pneumocystis* colonization and confirmed PCP (with laboratory detection of *Pneumocystis* plus a compatible clinical syndrome) in HIV-positive adults in Africa, and (2) the proportion of HIV-positive adults presenting with respiratory symptoms with PCR-detected *P. jirovecii* who are *Pneumocystis* colonized without other supportive clinical, radiological and laboratory features to confirm PCP.

Study inclusion

Observational studies or randomized controlled trials meeting eligibility criteria, outlined in Table 1, published in peer-reviewed journals and enrolling at least 10 participants after January 1, 1995, were included. This date was chosen to reflect *P. jirovecii* prevalence after wider availability of PCR diagnostics in Africa. No language restriction was applied. Studies enrolling mixed pediatric, adult, HIV-negative and HIV-positive participants, without reporting disaggregated data in HIV-positive adults, were excluded. Definitions of *Pneumocystis* colonization and PCP applied in the selection of and interpretation of studies are outlined in Table 2.

Table 1. Study eligibility criteria.

Population	HIV-positive adults (≥13 years of age) in Africa, with or without respiratory symptoms.
Intervention	Laboratory investigation (any PCR or microscopy staining method) for *Pneumocystis jirovecii*, on any respiratory sample (oral wash, sputum, endotracheal aspirate, bronchoalveolar lavage, or biopsy) in at least 10% of enrolled cohort
Comparator	Nil
Outcomes	Proportion of HIV-positive adults, with or without respiratory symptoms, with detectable *Pneumocystis jirovecii* in those undergoing laboratory investigation (primary objective)
	OR
	Quantitative PCR fungal burden thresholds that differentiate between *Pneumocystis* colonization and confirmed PCP (laboratory detection of *Pneumocystis* plus compatible clinical syndrome) (secondary objective)
	OR
	Proportion of symptomatic HIV-positive adults undergoing laboratory investigation and colonized with *Pneumocystis jirovecii* (without evidence of laboratory-confirmed PCP) (secondary objective)
Timing	Enrolment after January 1, 1995

PCP – pneumocystis pneumonia; PCR – polymerase chain reaction.

Table 2. Definitions of Pneumocystis colonization and PCP applied in the selection and interpretation of studies.

Primary objectives	
Pneumocystis colonization (asymptomatic adults)	Laboratory-detected *Pneumocystis jirovecii* in the absence of respiratory symptoms
Secondary objectives	
Pneumocystis colonization (symptomatic adults) [3,4]	PCR-detected *Pneumocystis jirovecii* and:
	1. Negative microscopy with clinical improvement in the absence of PCP-specific treatment,
	2. Negative microscopy and without supportive clinical or radiological features of PCP (as per study clinician and blinded radiologist assessment), or
	3. Organism burden below a predefined (laboratory as well as population specific) African qPCR colonization threshold i.e., previously developed in a laboratory from samples obtained from a particular study group, then later reapplied, within that laboratory and replicating the established method, to individuals from the same community or target population
PCP[3,4,51]	1. Microscopy detection of *Pneumocystis jirovecii*, with supportive clinical or radiological features (as per study clinician and blinded radiologist assessment) and/or clinical improvement with PCP-specific treatment or
	2. PCR-detected *Pneumocystis jirovecii* in symptomatic adults with organism burden exceeding a predefined (laboratory and population-specific) African qPCR colonization threshold.

PCP – pneumocystis pneumonia; qPCR – quantitative polymerase chain reaction.

Literature search strategy

A search was conducted on July 10, 2018, then updated on July 11, 2019 and May 19, 2020, in Medline, Embase, Cochrane library, Africa-Wide, Web of Science, ClinicalTrials.gov and PRISMA databases. Our search strategy, limited to published literature from 1995 to present, incorporated four key components (*Pneumocystis*, respiratory infection, HIV, and Africa). Full search terms are included in Supplementary File S1 (Table S3).

Record management and data collection

Records from the primary search were entered into Mendeley Reference Management Software Version 1.19.4 (https://www.mendeley.com/) and duplicates removed. Titles and abstracts were screened against the study eligibility criteria (Table 1) with

review of the full texts of potentially eligible articles for inclusion, followed by extraction of variables of interest onto a Microsoft Excel spreadsheet by N.K.W., verified by E.B., D.S.L., and M.W.T. Study authors were contacted if data of interest was missing or unclear. J.N.J. was consulted for review of any conflict regarding study inclusion or data discrepancies. Reference lists of included studies were searched to identify additional eligible studies. Included studies (all observational in design) were assessed using an adapted Newcastle–Ottawa scoring tool,[21] with judgment of attrition and selection bias using the Cochrane Risk of Bias guidelines (see Supplementary File S2).[22]

Data analysis

Pneumocystis jirovecii prevalence proportions were pooled using the random effects meta-analysis, after stabilizing for variance using the Freeman–Tukey double arcsine transformation.
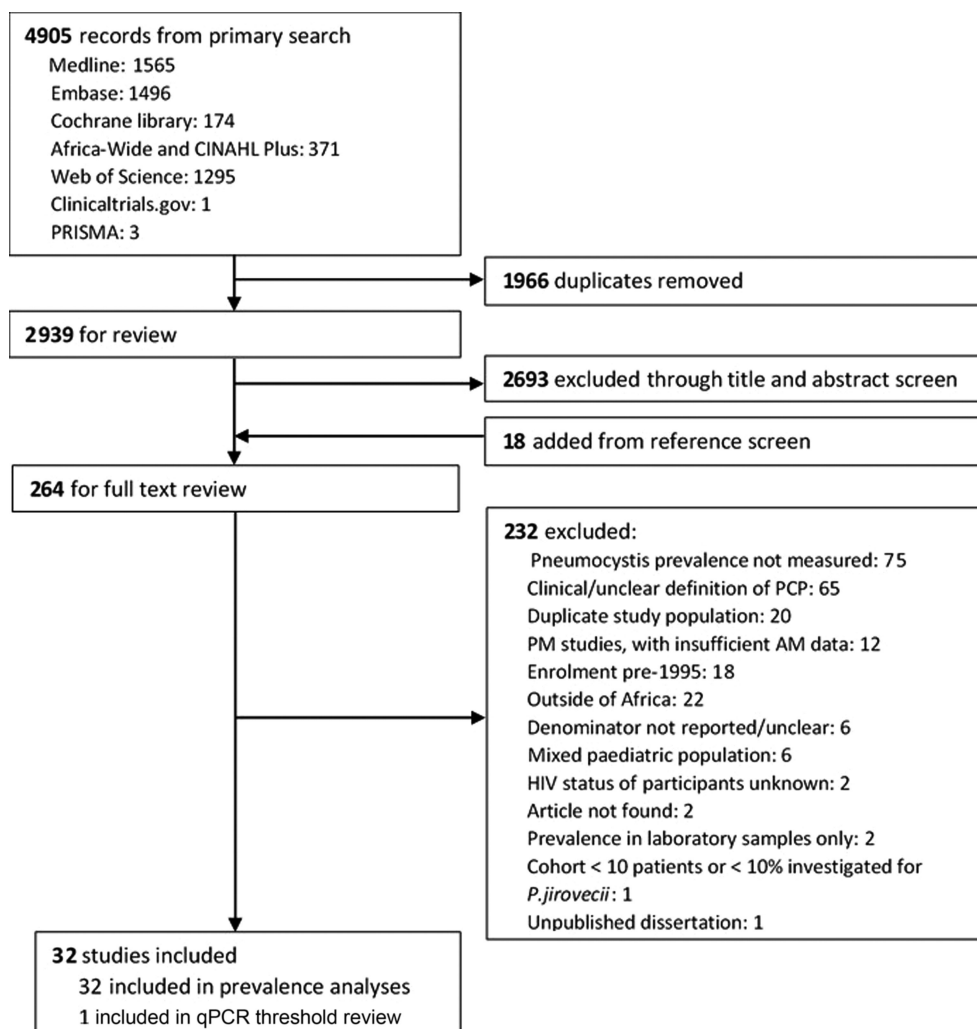
Figure 1. PRISMA diagram. AM – antemortem, CINAHL – cumulative index of nursing and allied health, HIV – human immunodeficiency virus, PCP – pneumocystis pneumonia, *P. jirovecii* – *Pneumocystis jirovecii*, PM – post-mortem, PRISMA – preferred reporting items for systematic reviews and meta-analysis.

Heterogeneity was quantified using the I^2 statistic. We performed additional stratified analyses by variables known to influence reported prevalence in symptomatic adults, including: time period of evaluation (1995–2005, the pre-ART era in most African countries, versus 2006–2020), patient setting (inpatient versus outpatient), median CD4 count (< or $\geq$ 100 cells/μl) and trimethoprim/sulfamethoxazole exposure (< or $\geq$ 50%) among investigated adults, laboratory method (PCR versus microscopy) and type of respiratory sample tested. We presented pooled estimates with 95% confidence intervals in forest plots and summary tables (in text and in Supplementary Files S3, 4 and 5). Analyses were conducted in R Studio using *metaprop* in the *meta* package. Due to the paucity of data, descriptive analyses of *P. jirovecii* prevalence in adults without respiratory symptoms, qPCR thresholds to distinguish colonization from PCP, and prevalence of *Pneumocystis* colonization among symptomatic HIV-positive adults with non-PCP respiratory disease, were conducted.

Results

Characteristics of included studies

Figure 1 outlines the flow of records from the primary database search through to study inclusion. Two hundred and sixty-four full text articles were reviewed, and 32 studies were included. Details of included studies are summarized in Supplementary File S3, Table S2.

In the 32 included studies from 15 African countries, 3723 HIV-positive adults were investigated in total for *P. jirovecii*, 140 of whom did not report any respiratory complaint. Twenty-six percent of participants ($n = 1177$, 13 studies) were on ART with 38% ($n = 956$, nine studies) taking trimethoprim/sulfamethoxazole prophylaxis. Restricted to patients evaluated after 2005, 45% were on ART ($n = 655$, six studies) and 52% taking trimethoprim/sulfamethoxazole prophylaxis ($n = 673$, five studies). Median CD4 count ranged from 58 to 342 cells/μl ($n = 1855$, 15 studies).

All included studies were observational. Using an adapted Newcastle–Ottawa score,[21] 19 studies (59%) were assessed to be poor quality (see detailed assessment of quality and risk of bias for each included study Supplementary File S4, Figure S1 and Table S3). Twenty-seven studies (87%) were at high risk of selection bias – conducting investigations for *P. jirovecii* on highly selected cohorts, often after exclusion of smear-positive pulmonary tuberculosis (*n* = 13 studies) and/or after poor clinical response to antibiotic treatment (*n* = 6 studies) or only in targeted subgroups with suggestive clinical or radiological features of PCP (*n* = 8 studies). Studies that utilized bronchoscopy only as a diagnostic tool (*n* = 12 studies) excluded severely ill or hypoxic participants; in other studies, adults with suspected PCP but with advanced disease may have been physically unable to provide a sputum or other respiratory sample, possibly further under-representing the true *P. jirovecii* prevalence.

Prevalence of *Pneumocystis jirovecii* in HIV-positive adults with respiratory symptoms

Prevalence estimates were derived using data from 32 distinct populations (counted as separate studies). One study conducted independent cross-sectional surveys in Senegal and Central African Republic, and prevalence estimates from these two regions were input separately into the meta-analysis model.[23] Two studies reported sequential prevalence data derived from the same investigated cohort in Uganda, and were included as one combined prevalence estimate.[24,25] The pooled prevalence of *P. jirovecii* detected on any respiratory specimen in adults with respiratory symptoms was 19% [95% confidence interval (CI) 12–27%, see Supplementary File S5, Figure S2]. A high level of heterogeneity was observed (I^2 = 97%, *p* < 0.01). Stratified by laboratory testing method, prevalence of *P. jirovecii* reported in studies conducting PCR testing on any respiratory sample was 22% [2244 participants, 95% CI: 12–35%, *n* = 17 studies]; comparatively, prevalence in studies utilizing microscopy was 15% [2659 participants, 95% CI: 9–23%, *n* = 25 studies] (Figure 2).

Subanalysis by time-period did not reveal evidence for a marked decline in reported prevalence of *P. jirovecii* among HIV-positive adults with respiratory symptoms, with a prevalence of 21% in 1995–2005 [*n* = 1425 participants, 95% CI: 12–31%, 15 studies] and 18% in 2006–2020 [*n* = 2158 participants, 95% CI: 9–30%, 17 studies] (see Supplementary File S5, Figure S3). A higher prevalence was reported from 17 studies exclusively enrolling inpatients [24%, 95% CI: 12–38%, *n* = 1753 participants] compared to six studies enrolling outpatients [14%, 95% CI: 4–28%, *n* = 898 participants] (Supplementary File S5, Figure S4).

In 15 studies reporting median CD4 count among investigated adults, *Pneumocystis* prevalence did not differ between studies in which median CD4 count was < or ≥ 100 cells/μl (see Supplementary File S5, Figure S5). In studies in which <50%

of the investigated adults had reported exposure to trimethoprim/sulfamethoxazole prophylaxis, prevalence was 18% [95% CI: 4–38%, seven studies, *n* = 659 participants], versus a prevalence of 13% [95% CI: 7–21%, *n* = 307 participants] in two studies in which more than 50% of adults had prior exposure (see Supplementary File S5, Figure S6).

Pneumocystis jirovecii prevalence by respiratory sample tested (employing PCR and/or microscopy) is outlined in Table S4 (see Supplementary File S5; see also Figure S7 for forest plot). The highest prevalence was reported in studies testing induced sputum [23%, eight studies, *n* = 1062, 95% CI: 6–46%] with a similar prevalence in BAL specimens [21%, 14 studies, *n* = 1098, 95% CI: 13–30]. Further restricting analysis to prevalence estimates from five studies (*n* = 769 participants) conducting PCR on induced sputum yielded a pooled prevalence of 27% [95% CI: 5–57%]; in comparison, prevalence across five studies (*n* = 509 participants) that used PCR testing on BAL was 24% [95% CI: 9–44%] (see Supplementary File S5, Figure S8).

Prevalence of Pneumocystis colonization in HIV-positive adults without respiratory symptoms

Three small studies reported the prevalence of *P. jirovecii* in HIV-positive adults without respiratory symptoms and were all conducted alongside investigation of symptomatic HIV-positive adults. Studies in Tanzania,[26] Guinea-Bissau,[27] and Cameroon[28] reported 0% (0/8), 1.8% (2/111), 42.9% (9/21) of participants, free of any respiratory complaint, to be colonized with *P. jirovecii* respectively [pooled prevalence of 9%, 95% CI: 0–45%, see Supplementary File S5, Figure S9]. All studies employed PCR testing in either outpatient or community settings - the first two on oral wash and the third Cameroon study on induced sputum. The same type of respiratory specimen was analyzed from symptomatic and asymptomatic participants within each study. The aims of the three studies, rationale for testing asymptomatic adults for *Pneumocystis* colonization and comparison of the PCR techniques employed are outlined in Supplementary File S3 (Table S2).

Out of 11 colonized participants across these three studies, fungal load was only quantified in two participants from Guinea-Bissau, with fungal loads of 524 copies/μl and 3 copies/μl (CD4 count 23 and 18 cells/μl, respectively). Little disaggregated data were available on the asymptomatic cohorts from Cameroon (involving 21 HIV-positive outpatients) and Tanzania (eight matched community controls included in a study of *P. jirovecii* prevalence among inpatients with pulmonary tuberculosis).

qPCR thresholds to distinguish between Pneumocystis colonization and PCP

One laboratory-based study, through review of 305 induced sputum samples from an inpatient South African cohort with clinically suspected PCP, evaluated a qPCR fungal load that may
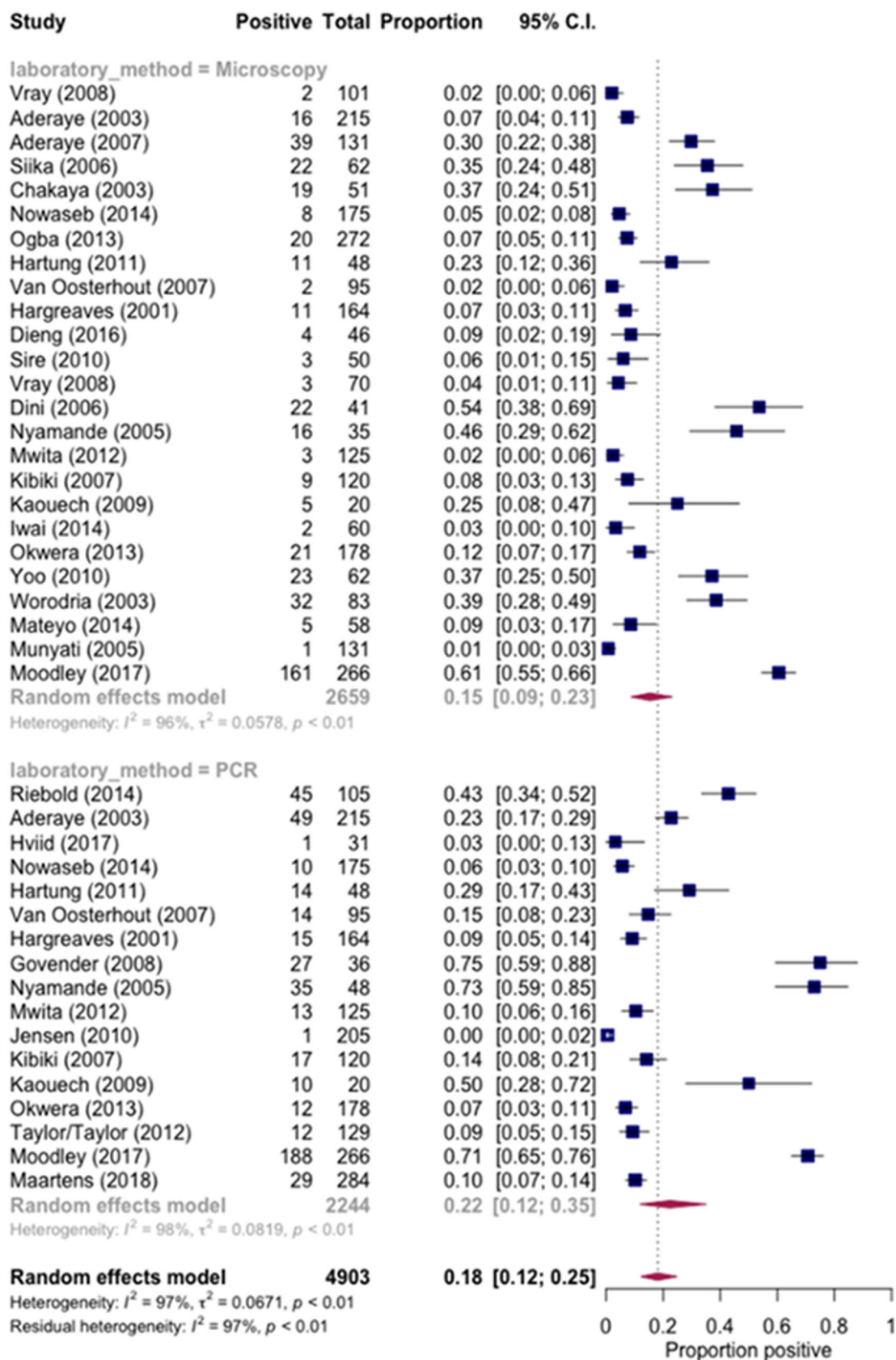
Figure 2. Pooled prevalence of *Pneumocystis jirovecii* in symptomatic HIV-positive adults, stratified by laboratory testing method (PCR versus microscopy). PCR – polymerase chain reaction.

Table 3. Details of studies examining *Pneumocystis* colonization in symptomatic adults.

Study	Proportion *Pneumocystis* colonized (%)[†]	Criteria used, alongside negative microscopy, to exclude PCP in *Pneumocystis* colonized adults	Outcome in *Pneumocystis* colonized adults
Expectorated sputum testing			
Van Oosterhout (2007)[29]	9/95 (9.5)	Clinical recovery in the absence of PCP treatment (minimum 4 weeks' follow-up)	1 death (11% mortality rate) after 23 weeks follow-up.
Aderaye (2003)[38]	10/96 (10.4)[‡]	Physician assessment at baseline and 2–3 day follow-up, with blinded CXR review by chest physician and two independent radiologists	Not reported
BAL testing			
Taylor (2012)[25]	7/124 (5.6)	Standardized clinical assessment by study investigator with blinded CXR review by radiologist[§]	Significantly increased mortality in colonized versus non-colonized adults (71 versus 25%) over 2-month follow-up.
Kibiki (2007)[30]	6/120 (5)[¶]	Physician assessment with blinded CXR review by radiologist	Not reported

[†]Colonized cases (PCR positive, microscopy negative, without supportive clinical and radiological features of PCP) among symptomatic HIV-positive adults investigated for *Pneumocystis jirovecii*. A separate study exploring a qPCR threshold to distinguish between colonization and PCP reported 16% of 305 samples to yield a fungal burden below the colonization threshold of 78 copies/5μl of DNA; the number of *Pneumocystis* colonized adults (and not samples) was not reported and hence not included in this table.[19]
[‡]Reported in a sub-group of 96 *Mycobacterium tuberculosis* culture-positive HIV-positive adults investigated for *P. jirovecii*. The 10 patients with positive PCR and negative microscopy had neither clinical or radiological suspicion of PCP and were diagnosed, based on CXR, with pulmonary tuberculosis ($n = 6$), other pneumonia ($n = 2$), and two patients had normal CXRs. [§]Conducted as part of a broader study examining the causes of HIV-associated opportunistic pneumonias in Uganda[52] - details of clinical and radiological features in colonized adults, or possible exposure to trimethoprim-sulfamethoxazole for treatment for another infection, not specifically reported.
[¶]Eight adults had negative microscopy and positive PCR, but two had clinical features warranting introduction of trimethoprim-sulfamethoxazole by the attending physician and were excluded from our analysis. AFB – acid fast bacilli; BAL – bronchoalveolar lavage; CXR – chest X-ray, PCP – pneumocystis pneumonia.

be used to distinguish between *Pneumocystis* colonization and IFA-confirmed PCP.[19] Copies of *P. jirovecii* DNA (with qPCR primers targeting the well-conserved mitochondrial large subunit ribosomal RNA locus) that correlated with PCP (IFA-positive cases) versus colonization (IFA-negative cases) were investigated. On receiver operating characteristic analysis, a qPCR cutoff of 78 copies/5μl of DNA (C_T 38.2) was found to correctly classify 92% of all IFA results. Notably, although enrolled participants were clinically reviewed, this study group did not comment on the participants' radiological features; a subset of the PCR-positive and IFA-negative cases may have had radiological changes in keeping with PCP. This limits the accuracy of the established C_T to distinguish true *Pneumocystis* colonization from PCP.

Pneumocystis colonization in HIV-positive adults with non-PCP respiratory disease

Across four studies investigating 435 adults with respiratory symptoms, 7% of individuals [95% CI: 5–10%] had PCR-detected *P. jirovecii*, and in the absence of positive microscopy and other clinical and/or radiological features to support a diagnosis of PCP, were deemed to be colonized (see Supplementary File S5, Figure S10). Details of these studies are outlined in

Table 3. Significantly, outcomes in colonized participants were only reported in two studies.[25,29] Possible exposure to high-dose trimethoprim/sulfamethoxazole (or other PCP-active) treatment given for another infection, as well as transparent description of clinical and radiological features that lead to the exclusion of PCP in PCR-positive cases, were not clearly reported across all studies, limiting the certainty with which PCP can be excluded in these patients.

Median CD4 count was reported in two of the investigated cohorts (65 cells/μl[30] and 88 cells/μl[25]), with ART and trimethoprim/sulfamethoxazole exposure only reported in the latter group.[25] Fungal load in colonized versus non-colonized adults was not explored in the above four studies. One group reported a significantly lower mean C_T value in nine individuals with both microscopy and PCR-detected *P. jirovecii*, compared to mean C_T in eight individuals positive on PCR only (two of whom had suggestive clinical features of PCP, hence not meeting strict criteria for colonization).[30]

Discussion

Across 32 distinct African HIV-positive populations undergoing respiratory specimen testing, we found a pooled *P. jirovecii* prevalence of 19% in adults with respiratory symptoms and 9%

in adults without any respiratory complaint. Using strict laboratory criteria to confirm a microbiological diagnosis rather than highly variable and non-specific clinical definitions of PCP, this review confirms that *P. jirovecii* remains a significant respiratory pathogen in HIV-positive adults in Africa presenting with respiratory disease, despite expanded access to ART as well as trimethoprim/sulfamethoxazole prophylaxis. These two interventions are essential for reducing the incidence of PCP;[31–33] in this review, we observed an increase in ART use (from 5 to 45%) and trimethoprim/sulfamethoxazole use (from 5 to 52%) among adults investigated for PCP in 1995–2005 and 2006–2020. However, *P. jirovecii* prevalence in symptomatic adults remained relatively constant at 21% in 1995–2005 and 18% in 2006–2020. Although our study does not provide any data regarding the overall number of PCP cases over this time, it is concerning that the prevalence of *P. jirovecii* has not markedly declined in HIV-positive individuals presenting with respiratory symptoms in Africa. PCP typically develops in the setting of advanced HIV (CD4 count < 200 cells/μl),[7] and the minimal observed change in *P. jirovecii* prevalence over time may be in part explained by the documented persistently high burden of advanced HIV among adults presenting to African healthcare settings in the post-ART era.[34–37]

With increasing use of highly sensitive PCR testing in African settings, prevalence estimates of *Pneumocystis* colonization, as well as quantitative PCR thresholds that distinguish colonization from microscopy-confirmed PCP, are needed to guide therapeutic decisions and enhance the clinical utility of these emerging diagnostics. In this review, limited data from three very small studies in Africa reported between 0 and 49%[26–28] of asymptomatic HIV-positive adults to be colonized with *P. jirovecii*. Differences in the type of respiratory sample analyzed (induced sputum versus oral wash), PCR technique used, and degree of control for amplicon contamination, may have contributed to the marked differences in yields observed across the studies. Further details, including CD4 data, ART, and trimethoprim/sulfamethoxazole prophylaxis exposure were also not comprehensively reported within the three sub-groups, restricting further analysis. The small number of asymptomatic adults studied (140 in total) limits the ability to compare the prevalence of asymptomatic *Pneumocystis* colonization with the prevalence of *P. jirovecii* derived from the 3583 symptomatic adults studied in our review. Non-African estimates of asymptomatic colonization are similarly limited; one early UK study reported 16% of asymptomatic HIV-positive men to be colonized on PCR testing of induced sputum, with rates inversely proportional to CD4 count.[5]

Four African studies in symptomatic adults, that defined colonization as a positive *Pneumocystis* PCR and negative microscopy with either (1) clinical recovery in the absence of PCP-specific treatment or (2) absence of other clinical and radiological features of PCP, reported 5–10% of adults to be col-

onized.[25,29,30,38] In non-African studies using these same definitions, *Pneumocystis* colonization has been reported in 13[6] and 19%[39] of HIV-positive adults presenting with respiratory disease. Hence, isolated use of PCR to confirm PCP in HIV-positive adults with non-specific clinical features, without microscopy validation or application of a valid qPCR threshold, risks inappropriate and potentially deleterious treatment of colonized adults with high-dose trimethoprim/sulfamethoxazole, steroids or other PCP-specific treatment.

The use of quantitative PCR thresholds may be used to guide therapeutic decisions by indicating which adults, among those who are PCR-positive, have sufficiently high (PCP-associated) fungal burdens that warrant PCP treatment. In comparison to the fungal burden cutoff (C_T of 38.2) identified above in a South African laboratory,[19] three non-African studies have reported widely varying C_T value cutoffs of greater than 27[11], 35[14] and 39[13] to indicate *Pneumocystis* colonization rather than PCP. Although the African and mentioned non-African studies all amplified a fragment of the mitochondrial large subunit (MtLSU) rRNA gene in their PCR assays, these cutoffs still carry limitations, since they are derived from laboratory-specific microscopy and qPCR techniques and require caution when applied in other settings. Furthermore, while IFA is regarded as the gold standard for PCP diagnosis in many texts[40,41] and significantly higher qPCR fungal loads have shown to correlate with microscopy positivity,[30,42] limited evidence suggests colonized adults may have small numbers of IFA-detectable *Pneumocystis* organisms in respiratory secretions.[43,44]

Other studies in Africa have used less stringent definitions to delineate *Pneumocystis* colonization from PCP in individuals with respiratory symptoms. A Malawian group used a qPCR C_T of >35 cycles to infer colonization[45] – this cutoff was developed in European populations with a low representation of HIV-positive adults,[15,18] who typically harbor higher fungal loads than other immunosuppressed groups.[3,4] A Cameroon study utilized a two-step (conventional followed by nested) PCR technique to delineate high from low fungal burdens, and reported 43% of adults to be colonized.[28] Last, a recent laboratory-based study, defining colonization as detectable *P. jirovecii* DNA with negative IFA microscopy, reported 24% of 712 symptomatic individuals to harbor colonizing organisms.[42] These definitions are subject to error without a critical review of clinical and radiological features or therapeutic outcome in the absence of PCP treatment.

Furthermore, without a true gold standard to exclude PCP in symptomatic colonized adults, it may be argued that the very low fungal loads detected through PCR testing may have represented early, evolving PCP, rather than colonization. Two of the above African prospective cohort studies reported substantially high mortality rates in *Pneumocystis* colonized adults,[25,29] with one study reporting a significantly increased mortality in colonized compared to non-colonized participants.[25] Whether

this mortality risk reflects either a failure to appropriately initiate PCP-specific treatment in participants misdiagnosed as being *Pneumocystis* colonized, or points toward colonization as a risk factor for subsequent *Pneumocystis* disease, are questions not yet answered in current African literature. A UK study that examined the genotypic evolution of colonizing strains of *P. jirovecii* before and after episodes of HIV-associated PCP found no genotypic correlation between colonizing strains and those implicated in prior episodes of PCP, although in the two individuals examined who had evidence of colonization prior to developing PCP, the type of *P. jirovecii* observed during the subclinical infection was the same as that causing the clinical disease.[6] Other genotypic studies have reported both repeated isolation of the same *P. jirovecii* strain across recurrent episodes of PCP within the same individual, as well as detection of new strains in subsequent PCP episodes in other individuals.[46–48] Recent studies have demonstrated heterogeneous *P. jirovecii* genotypes in respiratory samples from individuals with PCP[49,50] suggesting PCP may represent a failure of the immune system to contain a rapidly growing, and diverse, population of both newly acquired and reactivated latent strains. Arguably therefore, patients who are identified to be colonized through PCR testing, but are felt to not have other suggestive features of PCP, should receive at minimum effective trimethoprim/sulfamethoxazole prophylaxis to reduce or eliminate this fungal load.

This review has several limitations. First, prevalence data were derived and pooled from studies of largely poor quality, with significant selection bias identified in 87% of studies. Pursuing select investigation for the fungus in only AFB-smear-negative individuals, those with non-response to antibiotics or with clinically suggestive PCP (68% of all studies) may misrepresent true *P. jirovecii* prevalence in adults with respiratory symptoms. Further, 39% of included studies conducted BAL only testing, and often excluded hypoxic participants most at risk of being infected with *P. jirovecii*. Our review was not designed to evaluate the performance of various laboratory tests for isolation of *P. jirovecii*, but the heterogeneous prevalence reports across included studies is likely also reflective of differences in laboratory methods employed (including type of microscopy stain used, experience of microscopist(s), use of conventional versus real-time PCR, and selected PCR gene target). Second, due to missing or unreported data, some intended sub-analysis, such as prevalence of *P. jirovecii* stratified by plasma HIV-1 viral load, or meaningful analysis of laboratory prevalence by CD4 strata (only available for 47% of studies) could not be completed. Last, most studies did not report the specific clinical and radiological criteria that were used, alongside negative microscopy, to exclude PCP in individuals thought to be *Pneumocystis* colonized. This limits the ability to make comparisons and draw generalizable conclusions from studies that have examined colonization prevalence in symptomatic adults.

Pneumocystis jirovecii is a commonly isolated pathogen in HIV-positive patients with respiratory symptoms in Africa. In the context of *Pneumocystis* colonization, accurate interpretation of a positive PCR result requires consideration of fungal load, microscopy findings as well as the patient's clinical and radiological features. Further studies in African populations are required to better quantify the burden of colonization in both symptomatic and asymptomatic HIV-positive adults, and to develop more widely applicable qPCR thresholds that can guide therapeutic decision making.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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