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

Running title: *Pneumocystis jirovecii* prevalence in Africa

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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 *Pneumocystis 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 *Pneumocystis jirovecii* detection in respiratory samples from HIV-positive adults in Africa between 1995-2020. 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. *Pneumocystis 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. Amongst 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 cut-off 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, *Pneumocystis jirovecii* remains common in Africa. Prevalence estimates and qPCR-based definitions of colonization are limited, and overall quality of studies low.

Word count: 250 words

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 harbour *Pneumocystis* cysts or trophozoites, which colonize the respiratory tract in the absence of clinical and radiological features of PCP. Depending on 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 – 2015 reported a pooled PCP prevalence of 19% amongst 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) *Pneumocystis 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 *Pneumocystis 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 *Pneumocystis 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 cut-offs, 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- 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 analysed, the population studied, and the laboratory PCR technique (including choice of *Pneumocystis jirovecii* target gene) employed.[19]

Pneumocystis colonization has two further significant implications. Firstly, it enables person-to-person transmission and allows the fungus to circulate in the community,

143 threatening severe disease when encountered by HIV-positive persons or other
144 individuals with depleted immunity.[4,20] Secondly, fungal reservoirs that accumulate in
145 individuals with immune defects have been documented to evolve into PCP.[3,5,6] Current
146 knowledge on the epidemiology of *Pneumocystis* colonization in HIV-positive adults is
147 largely shaped by studies in Europe and North America, with a paucity of data from
148 Africa.[4] Given these implications, and as PCR-based diagnostics become increasingly
149 available, there is a need to establish African prevalence estimates of *Pneumocystis*
150 colonization, as well as to explore African qPCR colonization thresholds that can improve
151 the interpretation of, and therapeutic decisions based on, positive PCR assays.
152
153 To address these gaps, we conducted a systematic review and meta-analysis with the
154 primary aim to determine the prevalence of laboratory-detected *Pneumocystis jirovecii* in
155 African HIV-positive adults with respiratory symptoms, and to contrast this with the rates
156 at which *Pneumocystis jirovecii* is harboured in HIV-positive adults without respiratory
157 complaints.
158

159 ## Materials and Methods

160

161 ### Objectives

162 Our primary objective was to determine the prevalence of laboratory-detected
163 *Pneumocystis jirovecii* (using any PCR or microscopy technique) in HIV-positive adults
164 (≥ 13 years of age) in Africa (1) with respiratory symptoms and (2) without respiratory
165 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 *Pneumocystis jirovecii* who are *Pneumocystis* colonized without other supportive clinical, radiological and laboratory features to confirm PCP.

Study inclusion

Observational studies or randomised controlled trials meeting eligibility criteria, outlined in Table 1, published in peer-reviewed journals and enrolling at least 10 participants after 1 January 1995, were included. This date was chosen to reflect *Pneumocystis jirovecii* prevalence after wider availability of PCR diagnostics in Africa. No language restriction was applied. Studies enrolling mixed paediatric, 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.

Literature search strategy

A search was conducted on 10 July 2018, then updated on 11 July 2019 and 19 May 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 – present, incorporated four key components (*Pneumocystis*,

respiratory infection, HIV and Africa). Full search terms are included in Supplementary file 1 (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 NKW, verified by EB, DSL and MWT. Study authors were contacted if data of interest was missing or unclear. JNJ 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 judgement of attrition and selection bias using the Cochrane Risk of Bias guidelines (see Supplementary file 2).[22]

Data analysis

Pneumocystis jirovecii prevalence proportions were pooled using random effects meta-analysis, after stabilizing for variance using the Freeman-Tukey double arcsine transformation. 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 (less than or ≥ 100cells/μL) and trimethoprim/sulfamethoxazole exposure (less than or ≥ 50%) amongst 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 3, 4 and 5). Analyses were conducted in R Studio using *metaprop* in the *meta* package. Due to the paucity of data, descriptive analyses of *Pneumocystis jirovecii* prevalence in adults without respiratory symptoms, qPCR thresholds to distinguish colonization from PCP, and prevalence of *Pneumocystis* colonization amongst 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. 247 full text articles were reviewed, and 32 studies included. Details of included studies are summarised in Supplementary file 3, Table S2.

In the 32 included studies from 15 African countries, 3723 HIV-positive adults were investigated in total for *Pneumocystis 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 4, Figure S1 and Table S3). Twenty-seven studies (87%) were at high risk of selection bias – conducting investigations for *Pneumocystis 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 = six studies) or only in targeted sub-groups with suggestive clinical or radiological features of PCP (n = eight studies). Studies that utilised 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 *Pneumocystis 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 *Pneumocystis jirovecii* detected on any respiratory specimen in adults with respiratory symptoms was 19% (95% confidence interval (CI) 12% – 27%, see Supplementary file 5, Figure S2). A high level of heterogeneity was observed (I^2 = 97%, p < 0.01). Stratified by laboratory testing method, prevalence of *Pneumocystis 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 utilising microscopy was 15% (2659 participants, 95%CI 9% - 23%, n = 25 studies) (Figure 2).

Sub-analysis by time-period did not reveal evidence for a marked decline in reported prevalence of *Pneumocystis 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 5, 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 5, Figure S4).

In 15 studies reporting median CD4 count amongst investigated adults, *Pneumocystis* prevalence did not differ between studies in which median CD4 count was less than or ≥ 100cells/μL (see Supplementary file 5, Figure S5). In studies in which less than 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 5, Figure S6).

Pneumocystis jirovecii prevalence by respiratory sample tested (employing PCR and/or microscopy) is outlined in Table S4 (see Supplementary file 5; 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 5, Figure S8).

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

Three small studies reported the prevalence of *Pneumocystis 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 *Pneumocystis jirovecii* respectively (pooled prevalence of 9%, 95% CI 0% – 45%, see Supplementary file 5, 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 analysed 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 3 (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 cells/μL and 18 cells/μL respectively). Little disaggregated data was available on the asymptomatic cohorts from Cameroon (involving 21 HIV-positive outpatients) and Tanzania (eight matched community controls included in a study of *Pneumocystis jirovecii* prevalence amongst 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 be used to distinguish between *Pneumocystis* colonization and IFA-confirmed PCP.[19] Copies of *Pneumocystis 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 cut-off of 78 copies/5μL of DNA (C_T 38.2) was found to correctly classifying 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* colonisation 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 *Pneumocystis 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 5, 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 *Pneumocystis 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 *Pneumocystis 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 *Pneumocystis 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%) amongst adults investigated for PCP in 1995 – 2005 and 2006 – 2020. However, *Pneumocystis 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 *Pneumocystis 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),[34] and the minimal observed change in *Pneumocystis jirovecii* prevalence over time may be in part explained by the documented persistently

high burden of advanced HIV amongst adults presenting to African healthcare settings in the post-ART era.[35–38]

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 *Pneumocystis 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* colonisation with the prevalence of *Pneumocystis 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 colonized.[25,29,30,39] In non-African studies using these same definitions, *Pneumocystis* colonization has been reported in 13%[6] and 19%[40] 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, amongst those who are PCR-positive, have sufficiently high (PCP-associated) fungal burdens that warrant PCP treatment. In comparison to the fungal burden cut-off (C_T of 38.2) identified above in a South African laboratory,[19] three non-African studies have reported widely varying C_T value cut-offs 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 cut-offs still carry limitations, since they are derived from laboratory-specific microscopy and qPCR techniques and require caution when applied in other settings. Furthermore, whilst IFA is regarded as the gold standard for PCP diagnosis in many texts[41,42] and significantly higher qPCR fungal loads have shown to correlate with microscopy-positivity,[30,43] limited evidence suggests

colonized adults may have small numbers of IFA-detectable *Pneumocystis* organisms in respiratory secretions.[44,45]

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 greater than 35 cycles to infer colonization[46] – this cut-off was developed in European populations with a low representation of HIV-positive adults,[15,18] who typically harbour higher fungal loads than other immunosuppressed groups.[3,4] A Cameroon study utilised 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] Lastly, a recent laboratory-based study, defining colonization as detectable *Pneumocystis jirovecii* DNA with negative IFA microscopy, reported 24% of 712 symptomatic individuals to harbour colonizing organisms.[43] Without a critical review of clinical and radiological features, nor therapeutic outcome in the absence of PCP treatment, these definitions are subject to error.

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 represented early, evolving PCP, rather than colonization. Two of the above African prospective cohort studies reported substantially high mortality rates in *Pneumocystis* colonized adults,[24,29] 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 towards 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 colonising strains of *Pneumocystis jirovecii* before and after episodes of HIV-associated PCP found no genotypic correlation between colonising strains and those implicated in prior episodes of PCP, although in the two individuals examined who had evidence of colonisation prior to developing PCP, the type of *Pneumocystis 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 *Pneumocystis 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.[47–49] Recent studies have demonstrated heterogenous *Pneumocystis jirovecii* genotypes in respiratory samples from individuals with PCP[50,51] 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. Firstly, prevalence data was 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 *Pneumocystis 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 *Pneumocystis jirovecii*. Our review was not designed to evaluate the performance of various laboratory tests for isolation of *Pneumocystis jirovecii*, but the heterogenous 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). Secondly, due to missing or unreported data, some intended sub-analysis, such as prevalence of *Pneumocystis 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. Lastly, 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 make comparisons and draw generalizable conclusions from studies that have examined colonization prevalence in symptomatic adults.

Conclusions

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.

Word count: 3964 words

Acknowledgements

The authors thank the London School of Hygiene and Tropical Medicine (LSHTM) library service, consulted on 8 July 2018, who provided input on the search strategy for this review. No funding was received for the review. JNJ receives support from the U.K. National Institute for Health Research using Official Development Assistance (ODA) funding through a Global Health Professorship (RP-2017-08-ST2-012). MWT received salary support outside of this submitted work through the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under Award Number F32AI140511. The content is solely the responsibility of the authors (JNJ and MWT) and does not necessarily represent the official view of the NIH, NHS, the NIHR or the Department of Health and Social Care.

Disclosures of potential conflicts of interest

All authors have no competing interests to declare.

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Figure/Table legends

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

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 1. Study eligibility criteria

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

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

Tables

Table 3. 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 1 January 1995

PCP – Pneumocystis pneumonia, PCR – polymerase chain reaction

728 **Table 4. Definitions of *Pneumocystis* colonization and PCP applied in the**

729 **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, or 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 [3,4,52]	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.

730 PCP – Pneumocystis pneumonia qPCR – quantitative polymerase chain reaction

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

749 **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)[39]	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)	Standardised 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) amongst 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 *Pneumocystis 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[53] - 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