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## ABSTRACT

**Introduction** Cryptococcal meningitis is a neglected disease and an AIDS-defining illness, responsible for 15% of all AIDS-related deaths globally. In 2014, the estimated number of incident cryptococcal meningitis cases was 223 100, with 73% of them occurring in Africa. Currently available data on the prevalence, incidence, aetiologies and mortality of cryptococcal meningitis across Africa are sparse and of limited quality. We propose to conduct the first systematic review to summarise the epidemiological data available on cryptococcal meningitis and its aetiological causes in Africa.

**Methods and analysis** We will search PubMed, MEDLINE, Excerpta Medica Database, ISI Web of Science, Africa Index Medicus, Cumulative Index to Nursing and Allied Health for studies on cryptococcal meningitis published between 1st January 1950 and 31st December 2017, involving adults and/or children residing in Africa. After study selection, full text paper acquisition and data extraction, we will use validated tools and checklists to assess the quality of reporting and risk of bias for each study. Heterogeneity across studies will be assessed using the  $\chi^2$  test on Cochrane's Q statistic and a random effect meta-analysis will be used to estimate the overall prevalence, incidence density and mortality of cryptococcal meningitis across studies with similar characteristics. This protocol is prepared and presented in accordance with the 2015 Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols guidelines. Reporting of the results will be compliant with the Meta-Analysis Of Observational Studies in Epidemiology (MOOSE) guidelines.

**Ethics and dissemination** There is no requirement for ethical approval since we will be using data from published studies. The final report will be published in a peer-reviewed journal and further presented at conferences. This study is expected to provide useful contextual estimates needed to inform treatment policies on the African continent and assess the impact of diagnostic and prevention strategies on the burden of cryptococcal meningitis in the post antiretroviral therapy era.

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## INTRODUCTION

The invasive fungal disease cryptococcal meningitis (CM) is caused by members of the genus *Cryptococcus* which belongs to the

## Strength and limitation of this study

- To the best of our knowledge, this will be the first systematic review summarising data on the epidemiology of cryptococcal meningitis in Africa.
- We will use state-of-the-art statistical tools to pool prevalence, incidence and mortality data, and this will ensure the reliability and the validity of our results.
- Potential limitations of this review could be the small number of eligible studies due to the poor quality of data and the high heterogeneity across studies in terms of diagnostic procedures and standard of care. This will impact our overall estimates of the disease burden and its aetiologies and might ultimately prevent us from performing a meta-analysis.

phylum Basidiomycota.<sup>1–3</sup> The genus *Cryptococcus*, a polyphyletic fungi, has been known for over a century and now comprises at least 100 species described, with only a few known to cause diseases.<sup>4–6</sup> The first cases of cryptococcosis in humans and animals were reported in the late 19<sup>th</sup> century but their cumulative incidence increased in the 1900s.<sup>7–9</sup> Cases of cryptococcosis started increasing in Africa in the period 1940–1960 and it is thought that this was a result of the emergence of HIV/AIDS in the Congo River basin.<sup>10</sup> However, no evidence is available from the literature, laboratory or clinical reports to prove this assumption.<sup>9</sup> The HIV/AIDS epidemic raised the profile of the genus *Cryptococcus* from being an obscure yeast pathogen to becoming one of the most important fungal cause of morbidity and mortality worldwide.<sup>11–15</sup>

CM is a neglected disease and is known to occur in individuals with immunosuppression as well as in apparently immunocompetent subjects. In 2014, it was estimated that globally 278 000 people had cryptococcal antigenaemia per annum, with 73% of the 223 100 incident CM cases occurring in sub-Saharan Africa (sSA).<sup>11–15</sup> CM is responsible for 15% of all AIDS-related deaths globally and its

mortality is thought to be higher in sSA.<sup>15</sup> The majority of CM cases in sSA are caused by *Cryptococcus neoformans sensu stricto* (genotype AFLP1/VNI), although cases due to other species are on the rise.<sup>2,16</sup>

Currently available data on the prevalence, incidence and mortality of CM across Africa are sparse and of limited quality.<sup>12,13,17</sup> The majority of discrepancies in African countries are undoubtedly attributable to the shortage of resources for surveillance and clinical studies.<sup>12</sup> More accurate estimates are needed to inform treatment policies on the continent and assess the impact of diagnostic and prevention strategies on the burden of CM in the postantiretroviral therapy (ART) era. Here, we present a protocol for the systematic review of the epidemiology and the aetiologies of CM in Africa. This protocol is prepared and presented according to the 2015 Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols guidelines.<sup>18</sup>

### General objective

The objective of this study is to summarise data on the epidemiology and aetiologies of CM in Africa.

### Specific objectives

This review intends to:

- Estimate the prevalence of CM in Africa and summarise the changes in incidence density over time using data of studies published between 1950 and 2017.
- Estimate the mortality of patients diagnosed with CM in Africa using data from studies published between 1950 and 2017.
- Determine the relative contribution of various *Cryptococcus* species to the burden of CM in Africa.

## METHODS

### Search strategy for the identification of relevant studies

A comprehensive literature search will be performed in PubMed, MEDLINE database, the Excerpta Medica Database, the ISI Web of Science (Science Citation Index), the Africa Index Medicus and the Cumulative Index to Nursing and Allied Health to identify publications meeting the inclusion criteria. The literature search strategy is summarised in table 1. Following the search in databases, we will screen the reference lists of eligible articles and relevant reviews as well as conference proceedings in order to identify additional sources of information. Finally, principal investigators of ongoing cohort studies and clinical trials will be contacted to request interim data relevant to our objectives, and a co-authorship will be offered as counterpart if they extract and supply the data. Search results will be compiled using the citation management software EndNote V.X6.0.1. The anticipated start date for this review is the 15th November 2017 and ending on the 30th June 2018.

### Criteria for considering studies for the review

#### Inclusion criteria

We will include all the observational studies and clinical trials conducted between the 1st January 1950 and 31st December 2017, involving adults and/or children residing in Africa, and reporting the prevalence, the incidence and the mortality of CM or enough data to compute these estimates. Published data of studies will be considered without language restriction. For CM diagnosis, there should be at least one positive test on the cerebrospinal fluid (CSF) regardless of the blood sample result.

**Table 1** Search strategy

Search	Search terms and combinations
1	“ <i>Cryptococcus</i> ” OR “ <i>Cryptococcal</i> ” OR “ <i>Cryptococciosis</i> ” OR “ <i>Cryptococcus neoformans</i> variety <i>neoformans</i> ” OR “ <i>Cryptococcus neoformans</i> variety <i>grubii</i> ” OR “ <i>Cryptococcus neoformans</i> variety <i>gattii</i> ” OR “ <i>Cryptococcus neoformans</i> ” OR “ <i>Cryptococcus gattii</i> ” OR “ <i>Cryptococcus deneoformans</i> ” OR “ <i>Cryptococcus bacillisporus</i> ” OR “ <i>Cryptococcus deuterogattii</i> ” OR “ <i>Cryptococcus tetragattii</i> ” OR “ <i>Cryptococcus decagattii</i> ” OR “ <i>C. neoformans</i> var. <i>neoformans</i> ” OR “ <i>C. neoformans</i> var. <i>grubii</i> ” OR “ <i>C. neoformans</i> var. <i>gattii</i> ” OR “ <i>C. neoformans</i> ” OR “ <i>C. gattii</i> ” OR “ <i>C. deneoformans</i> ” OR “ <i>C. bacillisporus</i> ” OR “ <i>C. deuterogattii</i> ” OR “ <i>C. tetragattii</i> ” OR “ <i>C. decagattii</i> ”
2	“Meningitis”
3	“Mortality” OR “Death” OR “Fatality” OR “Prevalence” OR “Incidence” OR “Outcome” OR “Epidemiology” OR “Burden”
4	((“Africa” OR “Africa”*) OR “Algeria” OR “Angola” OR “Benin” OR “Botswana” OR “Burkina Faso” OR “Burundi” OR “Cape Verde” OR “Cameroon” OR “Central African Republic” OR “Chad” OR “Comoros” OR “Democratic Republic of Congo” OR “Congo” OR “Ivory Coast” OR “Djibouti” OR “Egypt” OR “Equatorial Guinea” OR “Eritrea” OR “Ethiopia” OR “Gabon” OR “Gambia” OR “Ghana” OR “Guinea” OR “Guinea-Bissau” OR “Kenya” OR “Lesotho” OR “Liberia” OR “Libya” OR “Madagascar” OR “Malawi” OR “Mali” OR “Mauritania” OR “Mauritius” OR “Morocco” OR “Mozambique” OR “Namibia” OR “Niger” OR “Nigeria” OR “Rwanda” OR “Sao Tome and Principe” OR “Senegal” OR “Seychelles” OR “Sierra Leone” OR “Somalia” OR “South Africa” OR “South Sudan” OR “Sudan” OR “Swaziland” OR “Tanzania” OR “Togo” OR “Tunisia” OR “Uganda” OR “Zambia” OR “Zimbabwe”) NOT (“Aspergillus” OR “pig”* OR “Papua”))
5	#1 AND #2 AND #3 AND #4
6	Filters: Publication date from 1950/01/01 to 2017/12/31; Humans

## Exclusion criteria

- i. Studies conducted outside the African continent.
- ii. Studies with small sample size (less than 30 participants), letters, commentaries, narrative reviews and editorials.
- iii. Studies lacking primary data or with incomplete methods description.
- iv. Duplicates (for studies leading to more than one publication, only the most comprehensive report including the largest sample size will be considered).
- v. Studies not involving human participants (if any).
- vi. Studies in which the diagnostic criteria for CM are unclear or not scientifically acceptable (with no report/record of a test performed on the CSF sample).

## Selection of studies for inclusion in the review

Titles and abstracts of studies identified by our search strategy will be screened independently for their eligibility by two members of the research team (TKN and JKT). Full texts of articles deemed eligible will be retrieved and further assessed for inclusion by the same investigators. A screening guide will be developed to ensure consistency of the screening method applied by both assessors. Any disagreement will be resolved by discussion and consensus. If the latter is not reached, arbitration will be sought from a third member of the team (FH). The inter-rater agreement for the selection of studies will be assessed using a non-weighted Cohen's kappa statistic.<sup>19 20</sup>

## Appraisal of the quality of reporting and the risk of bias

The quality of reporting of the studies included will be assessed using either the Strengthening the Reporting of Observational Studies in Epidemiology or the Consolidated Standards of Reporting Trials checklist depending on the nature of the study (observational study or clinical trial).<sup>21 22</sup>

The 10-item risk of bias tool for prevalence studies developed by Hoy *et al*,<sup>23</sup> will be used to assess the methodological quality and the risk of bias for all the studies included using the full text publications. Risk of bias and quality of reporting scores will be presented in a table and inter-rater agreement will be assessed using a weighted Cohen's kappa statistic.<sup>24 25</sup>

## Data extraction

A standardised data extraction sheet will be used to collect information on:

- Study identification: first author name, year of publication, year of participants' inclusion, country, type of publication, language of publication (full text).
- Study characteristics: study design (cross-sectional, cohort, case-control, clinical trial), setting (hospital, population), period of recruitment, sample size, mean or median age, age range, proportions of male participants, proportion of patients on anti-fungal prophylaxis (if any), proportion of HIV-negative participants (if any), proportion of HIV-positive patients under ART (if any), number of patients with

CM, diagnostic criteria for CM (India ink staining, culture, genotyping, detection of cryptococcal antigen in blood, detection of cryptococcal antigen in the CSF, lateral flow assay in whole blood, plasma or serum), treatment protocol used in patients diagnosed with CM (drug, route of administration, duration of treatment), duration of follow-up for cohort studies.

- Epidemiological estimates: prevalence, incidence and mortality of CM. Whenever these estimates are not readily available or computable using primary data in the publication, the corresponding author will be contacted to request the missing information.
- Genotypic results (proportion of cases caused by each *Cryptococcus* species).

## Data analysis and reporting

Data will be analysed using the metaprop command provided with the software STATA (V.13, StataCorp).<sup>26</sup> Heterogeneity will be evaluated by the  $\chi^2$  test on Cochrane's Q statistic<sup>27</sup> and quantified using  $I^2$  values, assuming that  $I^2$  values of 25%, 50% and 75%, represent low, medium and high heterogeneity, respectively.<sup>28</sup> Study-specific estimates will be determined from the point estimate and the appropriate denominators, assuming a binomial distribution. Then, the study-specific estimates will be pooled through a random effects meta-analysis to obtain an overall summary estimate of the prevalence, incidence and mortality of CM across studies, after stabilising the variance of individual studies using the Freeman-Tukey double arcsine transformation.<sup>29 30</sup> Where substantial heterogeneity will be detected, a subgroup analysis will be performed to detect its possible sources. Specific subgroup analyses by period of recruitment or by proportion of patients under ART will also be performed to explore how specific public health interventions influence the prevalence, the incidence density and the mortality of CM over time. Visual analysis of funnel plot and Egger's test will be done to detect small study effect.<sup>31</sup> All tests will be two-sided, and statistical significance will be defined as  $p<0.05$ .

The results of this systematic review will be reported according to the Meta-Analysis Of Observational Studies in Epidemiology (MOOSE) guidelines.<sup>32</sup> The study selection process will be summarised using a flow diagram. Reasons for study exclusion will be described. Quantitative data will be presented in summary tables and forest plots where appropriate.

## Ethics and dissemination

This systematic review will be based on published data and will therefore not require a specific ethics clearance. The results will be published in peer-reviewed journals and further presented at conferences. They will also be submitted to relevant health authorities. The review will be updated regularly at 5-year interval as new publications become available.



## Patient and public involvement

This systematic review will use published scientific data and will not involve patients or members of the public.

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**Competing interests** None declared.

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