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Prevalence of Group A β -Hemolytic Streptococcal Carriage in Children in Africa: A Systematic Review

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DISSERTATION in PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE MASTERS OF PUBLIC HEALTH
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF CAPE TOWN

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September 2015

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Acknowledgements

I would like to express my sincere gratitude to my supervisor, Dr Mark Engel, for his continuous support throughout my thesis as well as personally. For his patience, accessibility, motivation, and immense knowledge. Not only did I gain immeasurable knowledge, you also managed to transfer your passion of the subject. I could not have imagined having a better supervisor for my thesis. My co-supervisor, Ms Leila Abdullahi, I am grateful, for your patience and assistance, especially at beginning of my thesis, it was a rough and patchy journey, but you managed to steer me in the right direction.

Thank you, Dr Collect Dandara, who initially, gave me the opportunity to join his research team as intern. Then continued to mentor, motivate and encourage me throughout the public health program. Without his precious support, it would have been difficult to graduate in a timely manner.

To my fellow co-reviewer, Mr Dylan Barth, thank you for availing yourself to answer my questions, and offer insightful tips on surviving the thesis process.

To my family and friends, I greatly appreciate your spiritual support and providing me with a break from the academic life, I found sanity in those silly moments. Last but not the least, to my mother, Malefaso Moloi, there are not enough I can use to express my gratitude.

In conclusion, I recognize that this research would not have been possible without the University of Cape Town, department of Medicine, for welcoming me into their team, and the School of Public Health for giving me the opportunity to be part of the public health program.

Abstract

This MPH dissertation undertakes a systematic review of the prevalence of Group A β -Hemolytic *Streptococcal* Carriage in Children in Africa.

Part A is the research protocol, which outlines the background and the process of this research. In this research, I aimed to explore the prevalence of Group A β -Hemolytic *Streptococcal* Carriage in children aged 5- 15 years, residing in African countries. The review also attempted to investigate the *emm* type distribution of GAS carriage isolates. This study made use of the Hoy prevalence quality assessment criteria and the metaprop to aggregate the pooled prevalence.

Part B elaborates on the background and highlights the importance of this research by examining the existing theoretical and empirical literature relevant to the topic. This literature review aimed to establish the possibility of GAS carriage infection and transmission to the carrier and close contact. Thus, it also describes the importance of understanding the epidemiology of GAS carriage and the *emm* type distribution of carrier isolates in light of the M-protein base vaccine.

Part C presents the entire research project in a format suitable for Plos One journal submission. The background of this research project is summarized and the results are presented and discussed.

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PART A: PROTOCOL

Definitions

Antigens	Any foreign substance that induces an immune response in the body.
Asymptomatic	A condition that does not show any clinical signs or symptoms
β-Hemolytic	When a bacterium Completely destructs red blood cells to release haemoglobin, it is classified as β -Hemolytic. In a blood median this will appear transparent and light yellow.
Glomerulonephritis	Acute inflammation of the kidney, typically caused by an immune response.
Lysis	Destruction of red blood cells with the release of haemoglobin
Necrotizing fasciitis	Commonly known as flesh-eating disease
Sequelae	A condition that is the consequence of a previous disease or injury.
Serotype	Distinct variations within a species of bacteria or viruses
Serology	The scientific study of serum and other bodily fluids
Serum	A blood component that is made of protein factors such antibodies, antigens, electrolytes, hormones and any exogenous substances. However it does not include white and red blood cells and clotting protein.
Symptomatic	A sign or symptom of a disease

1. Background

Group A β -Hemolytic *Streptococcus*, commonly known as Group A *Streptococcus* (GAS) is a bacteria that causes a range of human infections. The most common infection attributable to GAS is pharyngitis, in children between 5 and 15 years of age. The global estimate of pharyngitis is 600 million cases annually, with over 550 million of these occurring in less developed countries [1]. The greatest global burden of GAS is due to Rheumatic Heart Diseases (RHD), a sequelae disease of pharyngitis. Globally, RHD causes 349,000 deaths annually from 15 million incidents; of 2.4 million affected children residing in developing countries, 1 million live in Sub-Saharan Africa [1].

GAS is diagnosed through a rapid antigen detection test and/or a laboratory culture. In the laboratory, the bacteria are classified into different hemolytic groups according to their reaction with the blood medium. Those that completely lyse blood cells are classified as β -Hemolytic *Streptococcus* [2]. These can be classified into different serological groups using the Lancefield grouping system, a characterization based on the carbohydrate composition of bacterial antigens found on their cell walls [3]. Amongst these, Group A (*Streptococcus pyogenes*) is found [3]. GAS can be further classified into distinct pathogenic strains based on its virulence factor, M-protein. Briefly, the M-protein, consists of two polypeptide chains extending from the cell wall, comprises of four blocks of amino acid residues [4]. The variability within the N-terminal region of the M-protein allows for differentiating GAS strains (i.e. M-typing) using specific M-typing antisera [5]. The M-protein is encoded by the *emm* gene and is the major target for the immune system [6]. Recent advances in molecular biology have resulted in DNA sequence-based methods (emm-typing) making it possible to differentiate the more than 200 variations in the *emm* sequence [7] ,(CDC Protocol for emm typing available at http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm).

GAS can infect children, and be stored in the upper respiratory tract and other anatomic sites asymptotically [8]. This subgroup of children is referred to as GAS carriers, which are

defined as children who have a positive throat culture with no GAS symptoms. An alternate definition refers to children who remain throat culture-positive after appropriate treatment for GAS pharyngitis [9]. GAS carriers are thought not to require any or further antimicrobial treatment, as they are at little to no risk of GAS infections, complications and transmission [8]. However, several studies have revealed that GAS carriage isolates can be infectious and transmittable [9,10]. Cockerill and colleagues showed that, a single clone of GAS that was responsible for an outbreak of invasive disease in Minnesota, was identical to one found in throat cultures of healthy children within the same community [11]. Also, a follow-up study of a family of three siblings, showed that these siblings were transmitting the same serotypes amongst themselves as patients when they had different episodes of GAS infections and also as carriers [12]. More worrying, penicillin, has been shown not to efficiently eradicate all cases of GAS carriage. Some researchers report penicillin resistance to be as high as 100% in all GAS carriage isolates [13]. GAS carriage is hypothesised to avoid some host defences and some antibiotic eradication due to its ability to get internalized into epithelia cells [14,15].

Thus, it is of interest to understand the background prevalence of GAS carriage in communities. This may aid in identifying risk factors associated with carriage in school-aged children and influence the planning and evaluation of management programmes in the screening of pharyngeal carriers [16]. Secondly, it may reduce unnecessary diagnostic testing [16]. Finally, knowledge of background prevalence will be useful in the light of recent advances towards a streptococcal vaccine [17].

To date, GAS carriage epidemiological information has been published in two separate systematic reviews [16,18]. A review by Shaikh and colleagues on the prevalence of Group A Streptococcus, reported a pooled GAS carriage prevalence of 12% (95% CI: 9%, 14%) in healthy children aged 5 – 17 years, residing in low income, middle and high-income

countries[16]. However, this meta-analysis had several limitations, including, one database was searched, studies were not vigorously assessed for quality and none of the studies were from African countries. In addition, a review by Steer and colleagues on the global distribution of emm-types, reported a high emm type diversity globally [18]. However, this review had only three studies from African.

We therefore undertake to perform a systematic review to determine the prevalence of asymptomatic Group A *Streptococcus* carriage in children aged 5 – 15 years, residing in African countries. Having studies from African countries is essential given the high mortality rate from invasive GAS disease and ARF disease [1]. In addition, Africa has been reported to have a high emm type diversity and also have a high replacement rate of emm types over time [18]. We sought to identify and quantify data on *emm* typing, the information from which could be translated into improved diagnostics and new targets for therapeutic drugs and vaccines [19].

2. Objectives

To describe the burden of asymptomatic GAS carriage in school children and GAS *emm* subtypes isolated from asymptomatic GAS carriers in African countries. This review will serve to update the findings of existing reviews on GAS carriage published in 2010 [16] and GAS carriage emm types published in 2009 [18].

3. Review question

What is the burden of pharyngeal GAS carriage in healthy persons residing in North and Sub-Saharan African countries?

4. Methods

This study will employ rigorous methods drawn from the scientific techniques and guidelines offered by the Cochrane Collaboration [20].

4.1. Criteria for considering studies for this review

4.1.1. Types of studies

Systematic reviews are key elements in evidence-based health care. Randomised control trials are considered to be the gold standard for evidence. However, the burden of disease in a population is less amenable to experimental design. Thus population-based, observational studies are more suitable for this review.

The following designs will be included:

- Cross sectional study
- Longitudinal study

4.1.2. Types of participants

Participants for this study will include healthy children between the ages 5-15 years, who live in any of the African countries.

4.1.3. Types of diagnoses method

GAS carriage will be defined as individuals who have no clinical signs and symptoms, but whose throat isolates test positive for GAS bacteria as diagnosed through a microbiological laboratory throat culture or rapid antigen detection test. Serotypes of GAS will be those characterised through emm typing which is based on sequence analysis of PCR products of the N-terminal hyper-variable region of the M protein gene [21]. This technique is proven to be comparable with the gold standard M-typing technique [22].

4.1.4. Types of outcome measures

The primary outcome is the prevalence of GAS Carriage in children, residing in African countries. The secondary outcome is the molecular characterisation of GAS isolates from asymptomatic children.

4.1.5. Search methods for the identification of Studies

A comprehensive search strategy will be developed to search electronic databases. The search strategies will incorporate both free term text that are controlled to suit specific databases individually and Medical Subject Headings(MeSH) adapted to suit each individual database(table 1).For specificity and sensitivity the search strategy will include an “African filter comprising country names from the continent as well as truncated terms such as ‘north* Africa’ to ensure that records indexed using regional, rather than country-specific, terms are also retrieved. African country names are included in English and languages relevant to the country, for example, ‘Ivory Coast’ and ‘Cote d’Ivoire’.Where country names have changed over time both names are included, for example, ‘Democratic Republic of Congo ’and ‘Zaire” [23].

Table 1: Search strategy

Subject	Search Terms
Group A β-hemolytic Streptococci	Pharyngitis Pharyngitis [Mesh] Sore throat Strep* Group A Streptococcus Streptococcus pyogenes [Mesh]
Carriage	Asymp* Asymptomatic Diseases Asymptomatic Diseases [Mesh] Asymptomatic Infections Asymptomatic Infections [Mesh] Carri* Carriage Carrier State Carrier State [Mesh]
Children	Adolescen* Adolescent [Mesh] Child* Child [Mesh] Infant Infant [Mesh] Pediatric* Pediatric Paediatric* Paediatric Pediatrics [Mesh]
Africa	africa[tw] OR africa'[tw] OR africa's[tw] OR africa1[tw] OR africa2[tw] OR africaans[tw] OR african collaborations[tw] OR africae[tw] OR africae australis[tw] OR african hiv[tw] OR african aid[tw] OR african aid's[tw] OR african in[tw] OR african e[tw] OR african e's[tw] OR african es[tw] OR african s[tw] OR african al[tw] OR african m[tw] OR african um[tw] OR african[tw] OR african'[tw] OR african"[tw] OR african's[tw] OR african 1[tw] OR african 2[tw] OR africana[tw] OR africanae[tw] OR african alleles[tw] OR african american[tw] OR african an[tw] OR african ane[tw] OR african anes[tw] OR african asian[tw] OR african strongylus[tw] OR african alotropis[tw] OR african der[tw] OR african ders[tw] OR african e[tw] OR african endemic[tw] OR african ene[tw] OR african enes[tw] OR african ensis[tw] OR african environment[tw] OR african er[tw] OR african es[tw] OR african i[tw] OR african ised[tw] OR africanism[tw] OR africanist[tw] OR africanists[tw] OR africanity[tw] OR africanium[tw] OR africanizada[tw] OR africanization[tw] OR africanization'[tw] OR africanize[tw] OR africanized[tw] OR africanized'[tw] OR africanizing[tw] OR african journal[tw] OR african num[tw] OR africano[tw] OR africanoides[tw] OR african ol[tw] OR africanos[tw] OR africanoside[tw] OR african patients[tw] OR african piper[tw] OR africans[tw] OR africans'[tw] OR african ton[tw] OR african trinevitermes[tw] OR african triol[tw] OR african um[tw] OR african um'[tw] OR african umsp[tw] OR african umt[tw] OR african us[tw] OR african us'[tw] OR african usgen[tw] OR african z[tw] OR african z[tw] OR african care[tw] OR african rice[tw] OR african s[tw] OR african sia[tw] OR african iva[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR Burundi[tw] OR Cameroon[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR Djibouti[tw] OR Egypt[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR Jamahiriya[tw] OR Jamahiriya[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libya[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayotte[tw] OR Morocco[tw] OR Mozambique[tw] OR Mozambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR Senegal[tw] OR Seychelles[tw] OR Somalia[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw]

4.1.5.1. Electronic searches

The following electronic databases will be searched: PubMed, EbscoHost, Scopus, The American Society for Microbiology, and proceedings from XIX Lancefield International Symposium on *Streptococci* and *Streptococcal* disease. Google Scholar will be searched for grey literature. The search will not be restricted to publication dates or language (However, abstracts must be clearly written in English for study to be considered). Published and unpublished data will be considered for inclusion.

4.1.5.2. Bibliographic databases

The reference lists of all potentially identified papers will be hand searched for possible studies. Also authors and experts in the field will be contacted for published and unpublished data.

4.2. Selection of studies for inclusion

Initially, two reviewers will independently evaluate the titles and abstracts of the search results, to include or excluded studies, or mark the studies as pending if they do not meet the inclusion or exclusion criteria (Table 2). Thereafter, the two reviewers will compare their lists, any contradictory judgments or 'pending' will be temporarily 'included'. Next, full texts of all the potentially included studies will be obtain. The list for inclusion will be finalised following an assessment of the full texts of articles, by applying the predefined inclusion and exclusion criteria. Disagreements in reviewer's selections will be resolved at a meeting between the reviewers and an arbitrator, where necessary. A table of all included studies will be included in the final review, and the reasons for exclusion of studies will also be documented.

Table 2: Inclusion and Exclusion Criteria

Studies will be included if they follow the following criterion:

1. Observational studies such as population based study, cross-sectional and longitudinal study that report on the prevalence of GAS carriage.
2. Population including children aged 5 – 15 years,
3. GAS positive throat isolates, diagnosed through a throat culture or a rapid antigen detection test. Diagnoses should be accompanied by no clinical signs/symptoms.
4. Studies that have a population residing in Africa.

Studies will be excluded if they follow the following criterion

1. Report on participant who, have clinical signs and symptoms.
2. Studies that do not include children in the ages 5 – 15 years.
3. Publications lacking primary data and/or explicit methods descriptions.
4. In the event of duplicate material. The most complete recent version of the data will be used.

5. Data extraction and management

Using a standardised data extraction form (Appendix 1), two reviewers will independently extract data. Contradictions will be resolved through discussion until agreement is reached.

The following descriptive and outcome data will be extracted from each study:

- Study identification: authors, year of publication and possible references.
- Eligibility criteria for inclusion and exclusion into the review.
- Study characteristics: study design, study participants, season of participation, country, study setting, and number enrolled into the study.
- Diagnostic test employed: Throat culture, RADT, PCR.

- Details of the outcome measure: prevalence, prevalence of GAS carriage, molecular characterization of the M-protein of GAS
- Risk of bias analysis: internal and external validity.
- Notes: study general comments.

6. Data synthesis and analysis

Quantitative data synthesis will include two steps namely, the identification of data sources and documenting numerators and denominators that will be used for prevalence calculations and secondly, the application of the Freeman-Tukey double arcsine transformation to stabilise the variance of study-specific prevalence. This will serve to minimize the influence from studies with extremely small, or extremely large prevalence estimates before pooling data using the random-effects meta-analysis [24]. Data will be stratified by different variables such as region, study design, sample size and seasonality so as to assess if these have an affect on the overall prevalence. If the data cannot be aggregated for statistical pooling due to limited data and / or substantial heterogeneity, the findings will be presented in a narrative form.

6.1. Assessment of heterogeneity

Initially, heterogeneity will be assessed with the Cochran's χ^2 test using a 10% level of significance cut-off, and the I^2 statistic, where values of 25%, 50% and 75% reflect low, medium and high heterogeneity, respectively [25]. Confidence intervals around heterogeneity estimates will be calculated using the *heterogi* command in Stata.

6.2. Exploration of heterogeneity

We will not discriminate against ethnicities, socioeconomic and educational backgrounds. However, where heterogeneity is statistically significant, we will conduct a sensitivity analysis to explore potential sources of heterogeneity, using different variables that are alleged to contribute to heterogeneity.

6.3. Missing data

Missing data for each study will be described and discussed as to the extent to which it could alter the results. Where deemed necessary, the studies' authors will be contacted to request missing information.

7. Quality appraisal of included studies

We will apply the quality assessment tool for evaluating prevalence studies as suggested by Hoy and colleagues and modified by Werfalli and colleagues to allow for a composite score that assist with relative comparison between the studies, thereby reducing reviewers' subjectivity [26,27] (Appendix 2). Briefly, Werfalli and colleagues added a quantitative scoring system to the risk of bias table, allocating four points for internal validity score and six points for external validity. The scoring system tool categorizes high risk studies as those with an overall score of 0-5 points, moderate risk as 6-8 and low risk > 8 points [27].

A full assessment of bias will inform the heterogeneity of selection of studies for a pooled analysis. Any disagreements will be resolved by discussion between the two reviewers. If further disagreement continues a third author will be consulted to resolve persistent inconsistencies.

8. Funding

No funding is expected for this review.

9. Ethics

Systematic review draws on publicly available data, and therefore do not require formal ethical review. However, we will seek ethical clearance from the University of Cape Town, Health Research Committee before proceeding with the review.

10. Dissemination

It is expected that this work would contribute to the knowledge base of the prevalence of pharyngeal GAS carriage in Africa, thus potentially influencing policy and practice in this area. The study will be disseminated by peer-review publication and conference presentation.

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12. Appendix

Appendix 1: Data extraction form	
Review Title:	The Prevalence of Group A β -hemolytic Hemolytic Streptococci Carriage in children in Africa: Systematic Review
Study ID (e.g Moloi 2015)	

General information

Date form completed (dd/mm/yyyy)	
Name of person extracting data	
Reference citation (e.g. Medline)	
Study author contact details	
Publication type (e.g. full report, abstract)	
Bibliography hand search, References for possible eligible studies	
Notes:	

Study Eligibility

Study Characteristics	Eligibility criteria	Eligibility criteria met?
Types of study	Observational study reporting on Prevalence.	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Participants	Including children of ages 5-15 years	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Diagnosis	Have throat swab/RADT	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Setting	Study took place in an African country	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Outcome measure	Primary outcome: Prevalence of asymptomatic Group A <i>streptococcus</i>	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
	Secondary outcomes: Typing of asymptomatic Group A <i>streptococcus</i>	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Results	Prevalence	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
INCLUDE <input type="checkbox"/> EXCLUDE <input type="checkbox"/> PENDING <input type="checkbox"/>		
Reason for exclusion/ pending?		
Notes:		

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

Characteristics of the Study

	Descriptions as stated in report/paper
Aim of study	
Study Design	
Start date	
End date	
Seasons of participation	
Diagnosis criteria (RADT, Throat swab)	
No of Diagnosis/tests on individual child	
Informed consent obtained	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Ethical approval needed/ obtained for study	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Notes:	

Participants

	Descriptions as stated in report/paper
Country	
Population description	
Setting (location, social context)	
Inclusion criteria	
Exclusion criteria	
Site of recruitment of participants (e.g. clinic, schools)	
Selection methods (e.g. randomized)	
Recruited sample size	
Age(s)	
Gender	
Ethnicity	
Notes:	

Outcome

Primary outcome: Prevalence of Asymptomatic/Carriage of GAS

Total # recruited	Withdrawals and exclusions	Total sample size analysed	No of asymptomatic GAS cases analysed	<u>Numerator</u> Denominator	Crude Prevalence
Note:					

Risk Factors

Risk Factors	Total sample size analysed per factor	No of asymptomatic GAS cases analysed per factor	<u>Numerator</u> Denominator	Stratified Prevalence
Gender				
Household Number (crowding)				
Seasons				
Notes:				

Secondary Outcome: Distribution of Asymptomatic/Carriage GAS, M-Protein gene (emm) types.

No of asymptomatic GAS cases analysed	No of asymptomatic GAS cases typed	<u>Numerator</u> Denominator	Crude Prevalence (%)	Emm types and their Frequency
Note:				

Discussion

	Descriptions as stated in report/paper
Key conclusions of study authors	
Study limits as reported by authors	
Recommendations	
Notes:	

Other Information

	Descriptions as stated in report/paper
Study funding sources (Including role of funders)	
Possible conflicts of interest (For study authors)	
Missing data	
Correspondence required for further study information (from whom, what and when)	
Notes:	

Appendix 2: Risk of Bias for Prevalence Studies	
1. Was the study's target population a close representation of the national population in relation to relevant variables?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
2. Was the sampling frame a true or close representation of the target population?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
3. Was some form of random selection used to select the sample, or was a census undertaken?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
4. Was the likelihood of nonresponse bias minimal?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
5. Were data collected directly from the subjects (as opposed to a proxy)?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
6. Was an acceptable case definition used in the study?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
7. Was the study instrument that measured the parameter of interest shown to have validity and reliability?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
8. Was the same mode of data collection used for all subjects?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
9. Was the length of the shortest prevalence period for the parameter of interest appropriate?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
Notes:	

PART B: LITERATURE REVIEW

Summary and Conclusions of the Literature Review

Group A *Streptococcus* (GAS) is a bacteria that is common in children between the ages 5 - 15 years, where it can cause common infections such as pharyngitis which can be diagnosed through a rapid antigen detection test or a cultured throat swab. Furthermore, the bacteria can be differentiated into distinct strains through its M-protein using technics such as the polymerase chain reaction. Treatment shortens pharyngitis symptoms and, more importantly, reduces the likelihood of sequelae such as acute rheumatic fever (ARF) and rheumatic heart (RHD). However, GAS can get stored in various anatomic regions asymptotically (referred to as GAS carriage) which is not easily treatable with penicillin. Literature has demonstrated that asymptomatic isolates of GAS have the potential to be infectious and transmittable to the carrier and close contacts. Hence there is a need to understand the epidemiology of GAS carriage.

Two systematic reviews on GAS carriage have been published. In 2010, Shaikh et al published a review that reported a pooled prevalence of 12% for GAS Carriage, in low-, middle- and high-income countries. In 2009, Steer et al published a review that reported a high diversity in the global distribution of *emm* types. Amongst other limitations, both reviews had limited data from African countries.

A systematic review that focuses primarily on African data is needed, given that ARF and RHD have been reported to be the highest in African countries. Additionally, it has been found that Africa has the highest *emm* type diversity and higher replacement rate of *emm* types. This makes it hard for the current M-protein based vaccine to have significant coverage in the African population. We therefore, uptake to conduct a systematic review on children aged 5 – 15 years who reside in African countries. This review will update previously published reviews. It is anticipated that the results will inform clinical practice and policy.

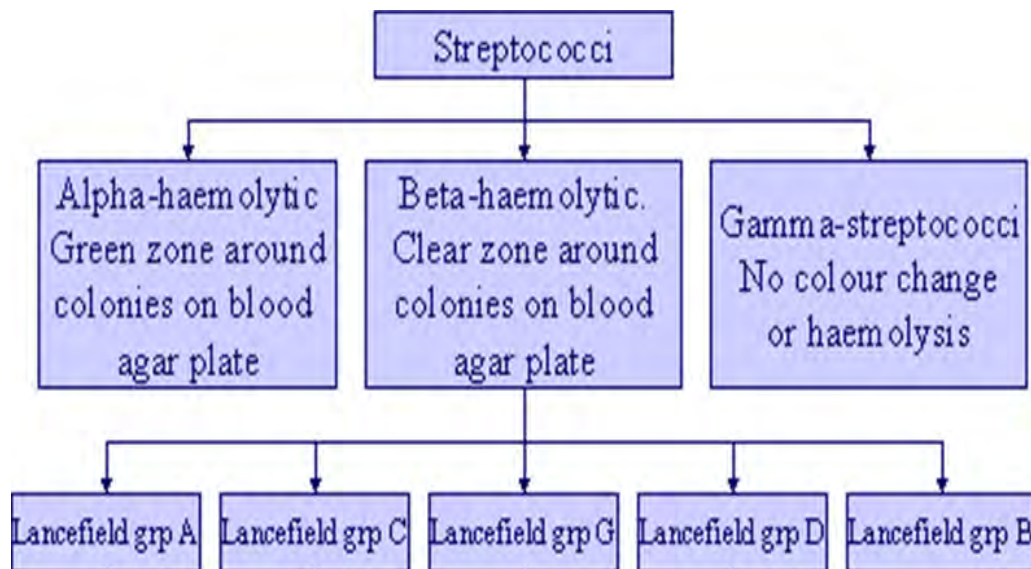
1. THE *STREPTOCOCCUS PYOGENES* (GROUP A *STREPTOCOCCUS*) ORGANISM

Figure 1: *Streptococcus pyogenes*



Gram positive cocci are spherical in shape and retain the blue colour of the gram stain [1]

Streptococcus pyogenes, commonly known as Group A *streptococcus* (GAS) is a bacterium occurring in pairs or in chains (Figure 1). Firstly, it is classified based on its Gram stain reactivity, whereby Gram-positivity is shown as a blue colour; thus GAS are Gram-positive. Secondly, classification is based on its hemolytic characteristics in blood agar. In a blood medium GAS secretes an exotoxin enzyme called streptolysin, which, causes a complete rupture of the red blood cell walls. Complete haemolysis of the agar around the streptococcal colonies presents a pattern referred to as β -Hemolysis. Thirdly, β -Hemolytic *streptococcus* are classified into different serological groups using the Lancefield grouping system, a characterization based on the carbohydrate composition of bacterial antigens found on their cell walls [2]. There are many groups, however only a few are known to cause human infections, amongst which is Group A (*Streptococcus pyogenes*) [2] (figure 2).

Figure 2: Group Classification of *Streptococci* [3]

GAS can be classified into distinct pathogenic strains based on its virulence factor, M-Protein. Briefly, the M-Protein, consists of two polypeptide chains extending from the cell wall, comprises of four blocks of amino acid residues [4]. The variability within the N-terminal region of the M-Protein allows for differentiating GAS strains (i.e. M-typing) using specific M-typing antisera [5]. The M-Protein is encoded by the *emm* gene and is the major target for the immune system [6]. Recent advances in molecular biology have resulted in DNA sequence-based methods (*emm*-typing) making it possible to differentiate the more than 200 variations in the *emm* sequence [5], Centre for Diseases Control Protocol for *emm* typing is available at http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm).

1.1. Identification of Group A *Streptococcus*

Group A *Streptococcus* can be diagnosed through a throat culture of a throat swab isolate or by a rapid antigen detection test (RADT). To increase test accuracy, it is advised that the throat swab isolates be obtained only from the posterior of the pharyngeal wall or the surface of the tonsils. Additionally, both these sites should not be touched before they are sampled. It should be noted that a patient might present with a false-negative results if the throat swab is done shortly after a course of antibiotics [7].

1.1.1. Throat culture

Figure 3: Laboratory Identification of β -Hemolytic *streptococcus*



S. pyogenes cultivated on CAP agar (sheep blood agar with collision + aztreonam). Cultivation 24 hours in anaerobic atmosphere, 5% CO₂. [8]

Throat culture is the gold standard diagnostic test. If it is prepared appropriately, it has up to a 95% sensitivity for the detection of GAS in the pharynx [7]. If the plate gives negative GAS results it is advisable to incubate the plate for an extra night at room temperature to allow for additional growth [7] (figure 3).

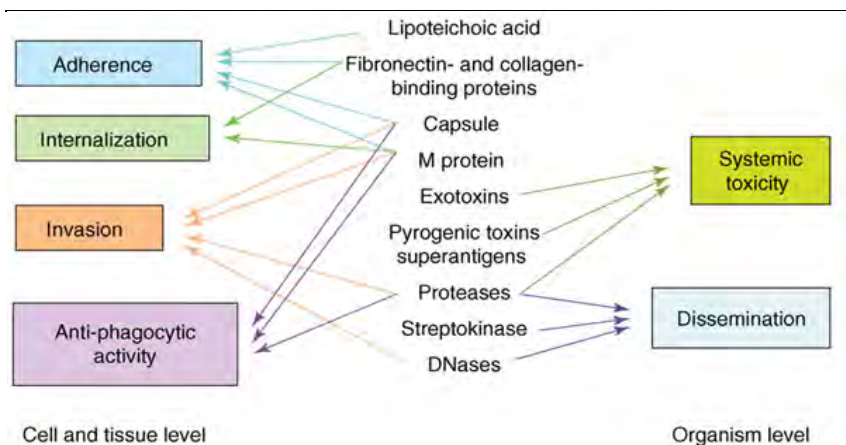
1.1.2. Rapid Antigen Detection Test

The Rapid Antigen Detection Test (RADT) can identify GAS directly from the throat swabs within minutes in a clinician's office. RADT have a specificity of 95%, as such, positive results can be trusted to start a patient on a course of antibiotics. However, RADT, sensitivity can be lower than 80%; therefore, a negative result needs to be confirmed with a throat culture [7].

1.2. Group A Streptococcus Mechanism

GAS has a complex and sophisticated regulatory mechanism that has a number of virulence factors interacting with the host at many levels (Figure 4). These virulence factors enable it to escape host defences, allowing it to successfully colonize and thrive.

Figure 4. Group A *Streptococcus* virulence factors[9]



At the host's cellular and tissue level, these GAS factors contribute to the pathogenicity of GAS through different mechanisms. C5a peptidase virulence factor limits recruitment of the body's first line of defence against toxins [10]. Factors such as M-Proteins and hyaluronic

acid capsule, mimic human tissue, this enables GAS to escape being engulfed by the human defence system. Also, the M-Protein alongside virulence factors such as F-Proteins, fimbrial proteins and lipoteichoic acid, enables GAS to adhere to epithelial cells. Lastly the M-Protein with capsules, the DNases, streptokinase hyaluronidase and SpeB enable GAS to invade tissue and internalize the host [10]. As a result, all these factors work together to mediate adherence to host cells, by avoiding phagocytosis, fostering invasion and internalization [9]. Once the GAS bacterium reaches the host's organism level, it releases, invasion and exotoxins virulence factors such as streptokinase, streptodornase, hyaluronidase and streptolysins, to facilitate dissemination throughout the host and induce systemic toxicity [9]. Notably most of these virulence factors are known to work at several stages of infection.

1.3. Group A *Streptococcus* associated risk factors.

1.3.1. Social economic status

GAS prevalence rates have been associated with low social economic status. Studies, conducted in school environment and homes, found GAS rates to be significantly higher in children coming from environments characterized by lower socioeconomic status and higher household density [11,12]. However, other studies found GAS pharyngitis and carriage not to be associated with the number of household members or social economic statuses [13,14].

1.3.2. Season

GAS prevalence among healthy individuals and patients with sore throats showed contracting seasonal fluctuations. A study, that compared healthy individuals and those with sore throat during the late summer months, found that in summer months the prevalence of GAS was higher among healthy children than children with sore throats [15], while other studies have

found no significant difference in GAS rates when comparing healthy individuals, seasonally [33].

1.3.3. Gender

Studies have found that GAS bacteria sequelae diseases such as Rheumatic Fever and Rheumatic Heart are commonly found in females when compared with males [17]. These results are supported by a study conducted in Tunisia, that found a higher GAS carrier rate in girls compared with boys [18]. However, some have found no significant difference between genders [11,14].

2. CLINICAL MANIFESTATIONS OF GROUP A *STREPTOCOCCUS*

Group A *Streptococcus* exclusively infects humans [20]. The pathogen mainly infects children aged 5 – 15 years. The oropharynx is the primary site for entrance into the human host [9]. GAS' complex and sophisticated regulatory mechanisms enable GAS to be stored in host's upper respiratory tract and other anatomic sites asymptotically [9]. Alternatively, it causes symptomatic infections that manifest into a range of clinical infections [20] (Table 1).

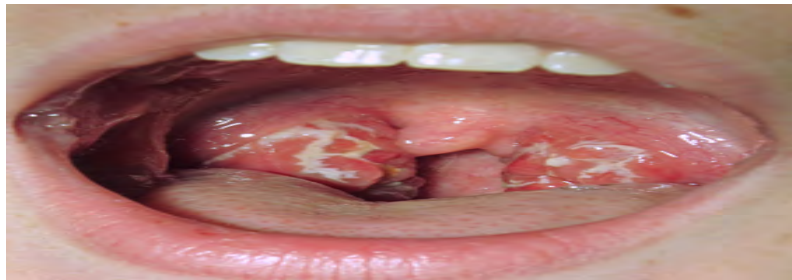
TABLE 1: Clinical symptoms and epidemiology of the major Group A *Streptococcus* infections [20].

Disease	Sign(s) and/or symptom(s)	Estimated global Incidence	Associated M-type
Superficial			
Pharyngitis	Sore throat, malaise, fever	>600 million/yr	1, 3, 5, 6, 12, 14, 17, 19, 24
Scarlet fever	Deep red rash, "strawberry tongue," exudative pharyngitis	111 million	
Impetigo	Skin pustules that mature into honey-coloured scabs	>471,000/yr	33, 41, 42, 52, 53, 70
Sequelae			
Acute rheumatic fever	Polyarthritis, carditis, rapid and jerky movement, rash, subcutaneous nodules	>471,000/yr	1, 3, 5, 6, 11, 12, 14, 17, 18, 19, 24, 27, 29, 30, 32, 41
Rheumatic heart disease	Mitral and/or aortic regurgitation with possible stenosis over time	15.6 – 19.6 million	
Acute Post-streptococcal Glomerulonephritis	Edema, hypertension, urinary sediment abnormalities, complement deficiency	>470,000/yr	1, 4, 12, 49, 55, 57, 60
Invasive			
Bacteremia	High fever, nausea, vomiting		28
Puerperal sepsis	Fever, chills, abdominal pain in a pregnant or early postpartum woman		

Cellulitis	Acute, tender, erythematous, and swollen area of skin		
Necrotizing fasciitis	Fever, exquisitely tender skin lesions, vomiting, diarrhoea, toxæmia, tissue destruction	660,000 cases and 160,000 deaths/yr	1, 3, 28
Streptococcal Toxic Shock Syndrome	High fever, rapid-onset hypotension, accelerated	(all invasive diseases)	1, 3
Syndrome	Multisystem failure		

2.1. Group A Streptococcus Pharyngitis

Figure 5: Pharyngitis Physical examination



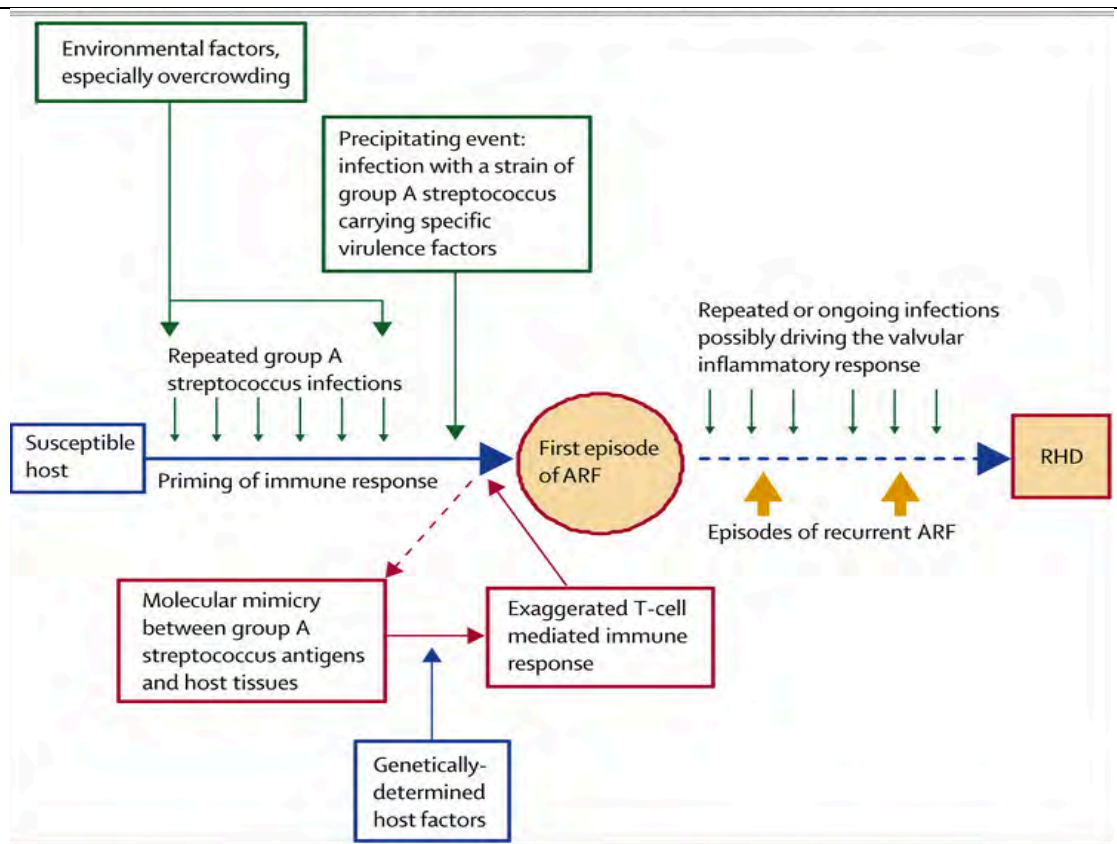
Tonsil pharyngeal erythema, with or without exudates, and tender, enlarged anterior cervical lymph nodes are present. Other findings may include a beefy, red, swollen uvula; petechiae on the palate and a scarlatiniform rash. [7].

In children, GAS infection mainly presents itself clinically as pharyngitis. However, there is a diversity of bacterial and viral pathogens that can also cause pharyngitis. As a result, most viral and bacterial GAS pharyngitis signs and symptoms largely overlap (figure 5). Thus, clinicians should use clinical score cards along side diagnoses tools to carefully evaluate patient suspected to have GAS pharyngitis. This will enable them to appropriately treat GAS infections, while avoiding the development of antimicrobial resistance and resource wastage [21].

2.2. Acute Rheumatic fever and Rheumatic heart disease

In the event that a GAS infection is not treated, it can contribute to the development of Acute Rheumatic Fever (ARF) and Rheumatic Heart Disease (RHD).

Figure 6: Pathogenetic pathway for ARF and RHD[17]



The mechanism above (Figure 6) demonstrates the pathogenetic pathway for ARF and RHD. The pathway shows that low social economic statuses, aids the transmission and infection of GAS because of overcrowding and bad ventilation. Furthermore, low social economic statuses can contribute to poor nutrition and inadequate health services, thus weakening the immune system to be susceptible to GAS infection [17]. If GAS pharyngitis is left untreated the host will make antibodies to fight the GAS bacteria. In some events these antibodies are believed to cross-react with antigens in the cardiac muscle, synovial tissue, or neuronal tissue leading

to a systematic disease that affects peri-arteriolar connective tissues [22]. This systematic disease is called Acute Rheumatic fever. Patients diagnosis with ARF, require continuous prophylaxis; a monthly injection of penicillin G benzathine. Treating ARF reduces acute inflammation, decreases fever and toxicity, prevent episodes of recurrent ARF [23,24].

Untreated or repeated episodes of AFR can lead to repeated inflammations with fibrous repair, which, in turn can lead to rheumatic heart disease (RHD). RHD is diagnosed where there is permanent changes in the heart valves, such as leaflet thickening, commissural fusion, and shortening and thickening of the tendinous cords, resulting in valve stenosis or regurgitation [23]. RHD treatment requires prolonged periods of antibiotics and occasionally valve repair or replacement surgery [23,24]. Primary prevention of RHD focuses on the prevention of ARF, which, in turn, requires adequate and timely treatment of GAS pharyngitis. However, approximately two-thirds of patients who develop ARF, experience streptococcal infections that is either subclinical or too mild to be brought to medical attention. As a result, not all children with GAS pharyngitis receive antibiotic therapy at the time of infection [12,17,23].

2.3. Treatment of Group A Streptococcus Pharyngitis

GAS pharyngitis is a self-limiting disease, with most of the signs and symptoms spontaneously resolving within 3 to 4 days, even without antibiotic treatment. However, appropriate and adequate treatment is suggested for individuals diagnosed with GAS. Treatment will alleviate acute pharyngitis with the possibility of preventing the development of ARF, RHD and/or invasive infections. Treatment will also decrease the transmission of GAS to close contacts.

The penicillin family is used as the first line of treatment for GAS pharyngitis. Penicillin is chosen because of its efficacy, it has a formulation that releases the antibiotic gradually. Thus, bactericidal levels against GAS are provided for as long as 28 days. Secondly, there have been no reports of GAS pharyngitis resistance due to penicillin. Thirdly penicillin has a narrow spectrum while lastly, the drug is affordable in different economic situations [25]. Adults usually get penicillin V, while a single intramuscular dose of benzathine, penicillin G is recommended for children [7]. Where preferred, amoxicillin is prescribed as oral penicillin therapy [7]. Patients who are allergic to the penicillin family are given macrolides, as they are proven to be as effective as penicillin. Alternatively, they are given cephalosporin depending on their reaction with b-lactam antibiotics. The route of administration, dosage and duration of all the individual antibiotics is explained in table 2.

Table 2: Antimicrobial Therapy Options to eradicate GAS pharyngitis in Children [20].

Drugs	Class of antimicrobial	Route of Administration	Children Dosage	Duration
Penicillin V*	Penicillin	Oral	250 mg two to three times per day	10 days
			250 mg three to four or 500 mg two times per day (Adolescents)	
Amoxicillin	Penicillin (broad spectrum)	Oral	Mild to moderate GAS pharyngitis: 12.25 mg per kg two times per day Or 10 mg per kg three times per day	10 days
			Severe GAS pharyngitis: 12.25 mg per kg two times per day Or 13.3 mg per kg two times per day	

Penicillin G benzathine (Bicillin L-A)	Penicillin	Intramuscular	< 60 lb (27 kg); 6.0×10^5 units.	One dose
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Treatment for patients with penicillin allergy

Erythromycin Ethyl succinate	Macrolide	Oral	Children: 30 to 50 mg per kg per day in two to four divided doses.	10 days
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Erythromycin estolate	Macrolide	Oral	Children: 20 to 40 mg per kg per day in two to four divided doses.	10 days
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Cefadroxil*	Cephalosporin (First generation)	Oral	Children: 30 mg per kg per day in two divided doses.	10 days
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Cephalexin (Keflex)	Cephalosporin (First generation)	Oral	Children: 25 to 50 mg per kg per day in two to four divided doses.	10 days
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*Veeids and Duricef brands no longer available in the United states.

2.3.1. Treatment Failures.

In some instances, people continue to have a positive throat isolate, despite adequate and appropriate GAS bacteria treatment. These individuals can be grouped into false treatment failures or true treatment failures. False treatment failures are those who are cured of one specific GAS strain, but immediately acquired a different strain. True treatment failures are those who cannot eradicate a specific GAS strain despite appropriate treatment [26]. There are contradictory hypothesis that explain the cause of true treatment failures. Most, authors regard those who experience bacteriologic treatment as GAS carriers. In a study conducted by Martin and colleagues, it was found that 50% of the *emm* types identified from the first episode of GAS carriage were also found in recurrent episodes of GAS carriage, independent of preceding antibiotic usage and the period between incidents [26].

3. Asymptomatic Children: Group A *Streptococcus* Carriage

Group A *Streptococcus* carriers, are defined as, individuals who have a GAS positive throat isolate that is not associated with clinical signs and/or symptoms [26]. There are many hypotheses surrounding the causation of GAS carriage. One of the hypotheses proposes that GAS is caused by a mutation that leads to a deletion in the virulence regulatory genes of the bacteria; this deletion leads to strains that down-regulates the virulence of GAS [27]. In support of this hypothesis, a 4-year longitudinal study by Martin and colleagues, showed that about 40% of school children were not infected with GAS bacteria each school year, even though they attended the same school as other children who were GAS carriers. Additionally, more than 50% of those who did not acquire the infected in the first year, they remained uninfected in the subsequent year [26]. These results lead to the notion that, GAS carriage is not a danger to the carrier, their family and community, because it is in a state that does not manifest clinical infection, nor can it be disseminated. Thus, it is believed that GAS carriage does not need to be treated.

However, this premise is contradicted by a body of evidence, which, claim that some individuals are not infected with asymptomatic GAS strains, because they either, have protection against that specific infection based on mucosal immunity to the specific portion of the M-Protein, or they have systemic immunity to the conserved portion of the M-Protein, or lastly, because they produce peptides with antibacterial properties [26]. This means that GAS carriage has the potential to be infectious and transmittable.

3.1. Group A *Streptococcus* carrier Epidemiology

In 2010, Shaikh and colleagues, published a systematic review, reporting the pooled prevalence of GAS carriage to be 12% (95% CI: 9% to 14%) in healthy children aged 5 – 17 years residing in low-, middle- and high-income countries [28]. However, this meta-analysis had several limitations, including that only one database was searched, studies were not vigorously assessed for quality and none of the studies were from African countries.

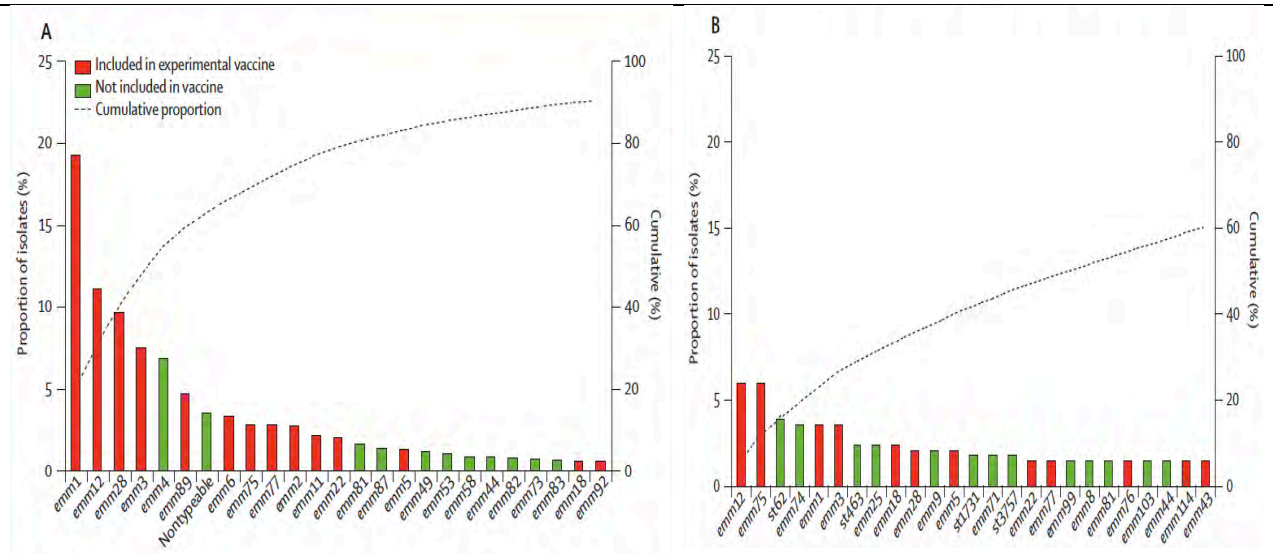
Having a systematic review that includes studies from Africa is essential because developing countries such as Africa have the highest burden of GAS, which can lead to RHD. It has been found that “ninety-five percent of the disease burden from RHD is in low and middle income countries, where it continues to have a significant impact on the health of children and young adults [12]. The latest statistics show that, “there are 2.4 million affected children between 5 and 14 years of age in developing countries, 1 million of whom live in Sub-Saharan Africa, making the continent the major ARF/RHD hotspot” [12]. Thus, much work needs to be done in the African continent to find content specific GAS carriage solutions, especially given the fact that there are differences in the different *emm* types circulating globally, with the highest diversity found in African countries [29].

Group A Streptococcus carrier Emm Types.

Four important factors need to be emphasized:

Firstly, it has been found that *emm* types are distributed unevenly at a global level, whereby the *emm* types which ranked first in high-income countries, ranked fifth in Africa [29]. Distributions differences are also found at country level; for example, three major cities in Ethiopia all had different major *emm* types. Furthermore, out of the 43 *emm* types found in all three cities, only 7 of them overlapped in their distribution among those cities with five of those overlaps occurring in only two of the cities [30].

Secondly, high diversity of circulating *emm* types has been demonstrated. The highest diversity was found in low-income countries when compared with high-income countries [29]. A review by Steer and colleagues found that 90.3% of all isolates from high-income countries were from among 25 *emm* types. While in Africa, 26 *emm* types only accounted for 62.5% of all isolates, and another 65 *emm* types contributed the remaining 37.5% of isolates [29] (figure 7).

Figure 7: Most common *emm* types as proportions [29]

A: high-income countries B: Africa.

Thirdly, *emm* types are shown to change over time. A study conducted in Ethiopia in 2006, isolated two new *emm* types from throat swabs which, a year earlier were only associated with skin isolates [30,31]. In relation to this, is the discovery of new *emm* types: in 2005 in Ethiopia, two new *emm* types were discovered while, a year later, the same population in a different study, had 13 new subtypes belonging to children who are GAS carriers [30,31].

Lastly, *emm* types are reported to get replaced over time. Studies have shown that an individual can acquire a different *emm* type on the next occasion of infection. A sudden appearance and rapid disappearance of some serotypes was reported in a two years' prospective study that took GAS carriage isolates of school children at weekly and biweekly intervals [32]. These results are similar to another study that found 23% of the children studied, acquired a different *emm* type with a different episode of GAS carriage [26]. Similar results are witnessed at a population level: a study conducted in Ethiopia found *emm* 3.19 to be the predominant subtype, accounting for 23% of all isolates. However a year earlier, *emm* 12 was deemed the predominant type, accounting for 23% of all isolates in Abdisa Abba

[30,31]. The change in *emm* type over years is emphasized by the fact that only 15% of the *emm* types that were in circulation in Abdissa Abba in 1990 are found in 2006 in the same city [30]. However, this dramatic replacement of *emm* types over time is only found in low-income countries. Whereas in high income countries, seven of the ten most common *emm* types found in circulation in 1990–99 were also among the ten most common during 2000–09 [29].

3.2. Group A *Streptococcus* carrier: Infection

Some, GAS carriage *emm* types have been found to be associated with clinical symptoms. It has been reported that 5% of children who are GAS carriers, have a serologic response to infection with GAS [33]. Furthermore, a study conducted in Minnesota, reported that a single clone of GAS, which, was responsible for an outbreak of invasive disease was identical to a clone that was found in throat cultures of healthy children within the same community [26,34]. Martin and colleagues, hypothesize that GAS carrier are likely to have a serological response when they acquire a new *emm* type.

3.3. Group A *Streptococcus* carrier: Transmission

A strong body of evidence has demonstrated that GAS carriers can acquire a new carriage state. In a four year prospective study, it was found that, ten out of eleven children were likely to become carriers again, after they had spontaneously cleared one carrier state [26]. The same reinfection pattern was shown in another longitudinal study that included 124 healthy school children. This study found that 23.4% of those children had positive isolates in more than 50% of the examinations [35]. Additionally, it has been

reported that GAS carriers not only maintain the organism, but that they can also disseminate it from person to person within families, closed communities and schools. A ten months follow up study, showed that three siblings were transmitting the same serotypes amongst themselves as patients when they had different episodes of GAS infections, and also as carriers [36]. These results are similar to those found in a randomized open clinical trial where new re-infection strains recovered from children with acute pharyngotonsillitis were the same as those found from healthy carriers who were living in close contact [37]. Furthermore, an increase rate and spread of GAS carriage has been noted in places such as schools and orphanages where children tend to be in close contact with each other for prolonged periods [38]. These results are supported by another longitudinal study that demonstrated that a GAS carriage strain that started in one classroom, quickly spread to other classrooms and was present throughout the study period [35].

The possibility that carrier children can acquire new *emm* strains that are not down regulated and have the possibility to cause GAS infections and be transmitted, leads to the notion that GAS carrier children should be considered for antibiotic treatment.

3.4. Group A Streptococcus Carrier: Treatment

In practice, it is difficult to differentiate between a person who has acute pharyngotonsillitis as a result of GAS infection, and a GAS carrier who has acute pharyngotonsillitis due to a viral infection [26]. To establish that the infection is caused by GAS, it is “theoretically necessary to demonstrate the host’s response to the infection in the form of antibody production”, however, this might not occur for two to three weeks [26]. In terms of preventing the development of acute rheumatic fever, antibiotic treatment cannot be delayed for more than nine days after the onset of GAS pharyngitis [25]. As such, most clinician are advised to treat all children suspected of GAS infection with antibiotics [26].

However, penicillin has been proven not be effective as the first line of treatment for GAS carriage, with some studies reporting, penicillin resistance as high as 100% in all the GAS carriage isolates [39]. Researchers believe that penicillin is not effective in eradicating GAS carriage, because the drug is unable to enter epithelial cells, in which GAS carriage strains are hypothesized to be internalized [27]. Studies have found that GAS carriage isolates have a significantly higher number of genes encoding proteins that are linked to internalization. Internalization of GAS helps to avoid some host defences and some antibiotic eradication; thus GAS carriage strains continue to persist even after adequate antibiotics [7,9,40]. As such, some authors believe that studies, which, reported treatment failure for GAS eradicating, the population was likely contaminated with carrier strains [7]. Thus, another definition of GAS carrier refers to children who still have a GAS positive throat culture isolate after a full course of adequate and appropriate antibiotic treated to eradicate the GAS pathogen [26]. Given the difficulty of treating GAS carriage, the ideal management strategy for GAS carriage would be a suitable vaccine.

4. M-Protein based vaccine

Amongst others, Group A streptococcal vaccine development is focused on the M-Protein, because of its thermal stability, anti-phagocytic properties and the capacity to evoke antibodies with the greatest bactericidal activity[41]. Within the M-Protein vaccines, currently under development, is the 30-valent M-protein vaccine targeting the N-terminal regions [42][43]. An earlier 26-valent M-Protein vaccine is hypothesized to protect against 90% of the *emm* types isolates that cause invasive GAS infections in the United States and Europe [30]. However, the same vaccine is hypothesized to protect a lower percentage of GAS invasive diseases in African countries given, for example, that 59% of the 17 most frequently isolated *emm* types in Ethiopia are not included in this vaccine [30]. This is because each strain in the vaccine will only produce antibodies related to a specific strain [44]. Thus, a larger number of *emm* types need to be included in the vaccine to obtain a coverage of similar proportion of isolates to that found in United States and Europe [30].

The 30-valent vaccine is hypothesized to produce bactericidal antibodies against more GAS subtypes than the 26-valent vaccine, including the production of antibodies against 24 of the 40 non vaccine GAS strains [42]. However this vaccine is still to undergo intensive clinical trial, to insure that it will not induce autoimmunity. Due to the fact that GAS has an alpha helical coiled coil structure similar to human myosin and tropomyosin. Thus M-Protein vaccines have the possibility to induce autoimmunity [44].

If a vaccine can prevent GAS infection, it will prevent all the clinical manifestations of GAS infection, subsequently preventing disability from ARF or death from toxic shock syndrome [41]. Additionally, a vaccine will prevent invasive GAS disease, as they are a frequent cause of sepsis in children and adults and have a high-case fatality rate and are a leading cause of

maternal death in some regions [45]. These dire complications and the huge economic burden lend support to the urgent need of a vaccine. However, currently a GAS vaccine does not seem possible especially for developing countries [44]. As dominant strains in Africa, have the capacity to mutate fast, leading to rapid replacement of *emm* types, in addition there is a high discovery of new *emm* types within the African population. This means that even if researchers were to include all the necessary *emm* types to cover all the currently circulating *emm* types in Africa, the vaccine might still be ineffective because of the possibility of new emerging strains that can be introduced into the vaccinated population and also colonize the naïve [32]. This is further complicated by the fact the rate of replacement with new *emm* types is not known and needs further study [32].

Thus, it is conceivably necessary to understand the epidemiology of GAS carriage, as it may serve to assist clinician and public health specialist in accurately identify GAS carrier, so they can be treated accordingly, to avoid GAS infections, the misuse of antibiotics and unnecessary health cost [21].

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PART C: JOURNAL MANUSCRIPT

Prevalence of Group A β -Hemolytic Streptococcal Carriage in Children in Africa: A Systematic Review

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List of Abbreviations

ARF	Acute rheumatic fever
GAS	Group A <i>Streptococcus</i>
PCR	Polymerase Chain Reaction
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
RHD	Rheumatic heart disease
RADT	Rapid Antigen Detection Test

Prevalence of Group A β -Hemolytic *Streptococcal* Carriage in Children in Africa: A Systematic Review

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Abstract

Background: Asymptomatic children can be a major reservoir of pharyngeal Group A *Streptococcus* (GAS) with reported figures ranging from <10% to >20% in developing countries. There is a need to document GAS carriage in school children, which, together with the molecular characterisation (M-typing) of strains harboured in the pharynx of carriers, will help to ascertain the extent to which disease strains are prevalent amongst carriers. This background information would serve to assist healthcare providers in diagnosing symptomatic GAS pharyngitis, as well as could potentially contribute to the development of a GAS vaccine. Currently, data on GAS carriage prevalence and M-type distribution in African countries are largely scant. We therefore undertook to perform a systematic review to determine the prevalence and type distribution of asymptomatic *streptococcus* carriage in children aged 5-15years, residing in African countries.

Methods: We conducted a comprehensive literature search among a number of databases, using an African search filter to identify GAS prevalence studies that report on children between the ages of 5 – 15 years who reside in African countries. Electronic searches were complemented by a hand search performed on reference lists of potentially included studies. The search was not limited by year of publication and language. Two evaluators independently reviewed, rated, and abstracted data from each article. Prevalence estimates were pooled in a meta-analysis and stratified according to region and study design using Stata®. Specifically, we applied the random-effects *metaprop* routine to aggregate prevalence estimates and account for between study variability in calculating the overall pooled estimates and 95% CI for GAS carriage prevalence.

Results: Of the 1666 articles retrieved, 17 studies incorporating cross-sectional and longitudinal study designs met with the inclusion criteria. The pooled prevalence of GAS carriage was 8% (95% confidence interval (CI), 6 % to 11%) with pooled prevalence for

cross-sectional studies was 8% (95% CI, 6% to 11%) and longitudinal studies, 9% (95% CI, 1% to 26%). Regional pooled rates were similar across East, Central and Southern Africa of between 7% and 8% while countries within Northern Africa had a pooled estimate of 14% (95% CI, 3% to 30%). Western Africa had the lowest pooled estimate of 2% (95% CI, 1% to 2%). Only one study reported data on molecular characterisation of which 11/13 emm-types were included amongst the putative 30-valent vaccine currently under development.

Conclusion: This study revealed a pooled GAS carriage estimate of 8% among African school children. Regional differences in carriages were largely minimal except for West and North Africa with prevalence's of 2% and 14% respectively. There is a dearth of data on molecular strain information, thus emphasizing the need for further studies.

Keywords: Streptococcus, Group A Streptococcus, Carriage, Carrier state, Asymptomatic, Children, Africa

1. Background

Group A β -Hemolytic *Streptococcus*, commonly known as Group A *Streptococcus* (GAS), is one of the causes major infections worldwide, the most common being pharyngitis in children between 5 and 15 years of age [1]. The global estimate of pharyngitis is 600 million cases annually, with over 550 million of these occurring in less developed countries [2]. Of particular concern, repeated inappropriately treated episodes of GAS pharyngitis may result in autoimmune diseases such as acute post-streptococcal glomerulonephritis and acute rheumatic fever (ARF), the latter of which may develop into rheumatic heart disease (RHD). Globally, RHD causes 349,000 deaths annually, 15 million incidents and 2.4 million are children in developing countries [2].

Not all children who are infected by GAS manifest clinical signs and symptoms. In some instances GAS can be stored asymptotically in the upper respiratory tract of children referred to as GAS carriers [3]. Researchers hypothesize that asymptomatic carriage of GAS maybe be a consequence of these four items below as suggested by Tart et al (2007) (i) mutations in the pathogen that down-regulate virulence, (ii) a productive immune response by the host that constrains pathogen proliferation, (iii) internalization into host cells, or (iv) some combination thereof [4]. As a result GAS carriers' strains are thought not to manifest into clinical infection, nor can they be disseminated [4]. However, this hypothesis is contradicted by a strong body of evidence that shows a possibility of GAS carriage infection and transmission associated with clinical symptoms [3,5]. Cockerill and colleagues confirmed that a single clone of GAS responsible for an outbreak of invasive disease in Minnesota, was identical to one found in throat cultures of healthy children within the same community [6]. In a ten-month follow up study, on a family of three siblings, it was found that these siblings were transmitting the same serotypes amongst themselves as patients when they had different episodes of GAS infections and also as carriers [7].

Penicillin has shown success as the first line treatment of GAS infections. However, it is not effective as the first line treatment for eradicating GAS carriage. Some researchers had study results that show penicillin resistance to be as high as 100% in all the GAS carriage isolates [8]. GAS carriage is hypothesised to avoid some host defences and some antibiotic eradication due to its ability of being internalized into epithelia cells [9]. These results are emphasized by Sela and colleagues study which, observed that GAS strains with higher internalization efficiencies, managed to escape antibiotic eradication as supposed to those stains with less internalization efficiencies [10]. This leads to the second GAS carriage definition; which is, children who still have a GAS positive throat culture isolate after a full course of adequate and appropriate antibiotic treated to eradicate the GAS pathogen [3].

Thus, it is of interest to understand the background prevalence of GAS carriage in communities, since this may aid in identifying risk factors associated with carriage in school-aged children and influence the planning and evaluation of management programmes in the screening of pharyngeal carriers [11]. Secondly, unnecessary diagnostic testing in children may be reduced when taking into account likelihood ratios. Finally, knowledge of background prevalence will be useful in the light of recent advances towards a *streptococcal* vaccine [12] given that identification of the DNA sequencing pattern of the 5' hyper variable region of the cell-surface M-protein (so called *emm* typing) may be able to inform vaccine development [13]; thus, assessing the impact of vaccination and monitoring serotype changes may also be facilitated.

Shaikh and Colleagues previously published a systematic review on the prevalence of *Streptococcal* Carriage in Children: they reported a pooled prevalence of GAS carriage of 12% (95% confidence interval (CI), 9% to 14%) in healthy children who were younger than 18 years, residing in low, middle, and high income countries [11]. However, this meta-analysis had a number of limitations including a narrow search strategy, failure to incorporate a quality assessment tool specific for evaluating prevalence studies and, not adjusting for

influences from studies with extremely small or large prevalence estimates on the overall estimate in the meta-analysis [14]. Furthermore, despite being considered as a comprehensive review, the authors failed to include studies on GAS carriage from African countries.

Steer and colleagues published a systematic review on the global distribution of *emm* types in terms of potential coverage by the 26-valent vaccine [12,15]. The review included only three studies from Africa, yielding potential vaccine coverage of 43.7% in the African population. Furthermore, African countries had a high diversity of circulating *emm* types and a high replacement rate of *emm* types over time when compared to those from high-income countries. [15].

We therefore, undertook to perform a systematic review to determine the prevalence and type distribution of asymptomatic *streptococcus* carriage in children aged 5-15 years, residing in African countries. In addition, we wish to describe the *emm* type distribution amongst GAS carrier isolates, where possible. A systematic review that includes studies with a population from African countries is essential, given the high burden of acute and complications caused by GAS infections in Africa [2].

Objective of this review

The objective of this review is to provide a systematic review of studies reporting the prevalence of GAS carriage in North and Sub-Saharan African countries. This review is intended to update the findings of two previous reviews on GAS carriage and global *emm* type distribution [11,15].

Review question

This systematic review will be guided by the following research question: What is the burden of pharyngeal GAS carriage in healthy persons residing in North and Sub-Saharan African countries? We also determined to conduct a sub-analysis of the distribution of GAS *emm* subtypes isolated from GAS carriers in North and Sub-Saharan African countries.

2. Methods

This study employed rigorous methods drawn from the scientific techniques and guidelines offered by the Cochrane Collaboration [16]. This review protocol has been published in the PROSPERO International Prospective Register of systematic reviews (<http://www.crd.york.ac.uk/PROSPERO>), registration number CRD 42015019589.

2.1. Criteria for considering studies for this review

2.1.1. Types of studies

Studies were potentially eligible for inclusion in this review if they satisfy all of the following criteria: Observational studies such as population based study, cross-sectional and longitudinal study that report on the prevalence of GAS carriage. The studies had to have an African population of children aged 5 – 15 years.

Studies were excluded from this review if they were:

1. Studies that do not include a representative sample of children aged 5 – 15 years, from an African country.
2. Studies that do not report on the prevalence of pharyngeal GAS carriage.
3. Publications lacking primary data and/or explicit methods descriptions.
4. Duplicate publications of the same material. When the study has been published in more than one journal/conference, the most complete recent version will be used.

2.1.2. Types of participants

Participants were children aged 5 - 15 years, who have no sign and symptoms of GAS. The participants reside in geographic regions confined to African continent countries in North and Sub-Saharan Africa.

2.1.3. Types of Diagnoses method

A GAS carrier was defined as a participant who does not present any clinical signs and symptoms for disease, who tests positive for GAS bacteria through a positive Rapid Antigen Detection Test (RADT) or positive laboratory throat culture. Serotypes of GAS are diagnosed through *emm* typing which is based on sequence analysis of polymerase chain reaction (PCR) products of the N-terminal hyper-variable region of the M protein gene [13]. This technique is proven to be concurrent with the gold standard M-typing technique [17]. *emm* Typing is simple to perform and has allowed for the detection of several previously unknown GAS sub-types from different geographic regions [17].

2.1.4. Types of outcome measures

The primary outcome of this study was prevalence of pharyngeal GAS carriage in children aged 5- 15 years, residing in Africa.

The secondary outcome was the molecular characterization of GAS isolates from asymptomatic children. For the purpose of our review will only consider studies that report on the subtypes of M protein (*emm*).

2.1.5. Search methods for the identification of Studies

We developed a comprehensive search strategy that incorporated a combination of free term text items including carriage, asymptomatic, etc; and Medical Subject Headings (MESH) such as Group A *Streptococcus* and *Streptococcus pyogenes*. In addition, to maximise the likelihood of finding articles from Africa, we applied an African search filter described previously by Pienaar and colleagues[18]. The complete strategy is shown in Appendix 1. All search results from individual databases, reference searches and unpublished articles were imported and managed with Mendeley referencing software (Copyright © 2009-2013 Mendeley Ltd).

2.1.5.1 Electronic searches

The search strategy was conducted independently by two reviewers (HM; DB) among the following electronic databases: EbscoHost, PubMed and Scopus. In addition, we searched the American Society for Microbiology (<http://www.asm.org/>) websites for additional sources. Furthermore, we searched the proceedings from the XIX Lancefield International Symposium on *Streptococci* and *Streptococcal* disease (<http://www.lancefield2014.com>). Lastly, searches in Google Scholar complemented the searches, including articles among the grey literature. The search strategy was appropriately modified to suit the vocabulary of individual database(s). Publication dates and language restriction were not applied to all searches. Articles published in English or other languages (provided full abstracts have been written in clear English) were eligible for inclusion. Both published and unpublished studies were considered for inclusion.

2.1.5.2. Bibliographic databases

We scanned the reference lists of all potential articles retrieved from electronic searches for additional articles. Also, relevant authors and experts in the field were contacted for unpublished and published work. Lastly, a manual Internet search was performed at the end of the systematic search, conducted on the 9 of July 2015.

2.2. Selection of studies for inclusion

Initially HM and DB independently reviewed the titles and abstracts of the full list of potential articles. The predefined inclusion criteria (Table 1) were applied, after which articles were either excluded, included or marked, as 'pending' if the reviewer was unsure about the inclusion of the study. Thereafter, the reviewers compared their list of included studies, resolving differences by discussion where necessary. A third reviewer (ME) acted as arbitrator.

Table 1: Inclusion Criteria

Characteristic	Criterion
Types of study	Observational study reporting on Prevalence.
Participants	Including children of ages 5-15 years.
Diagnoses	Diagnoses tests for Prevalence Determined by throat culture, Rapid Antigen Detection Test (RADT) Diagnosis test for <i>emm</i> Types Polymerase Chain Reaction (PCR) tests.
Setting	Study must take place in an African country.
Outcome measure	Primary outcome: Prevalence of Group A <i>streptococcus</i> carriage. Secondary outcomes: Molecular characterization of the M-protein, of asymptomatic Group A <i>streptococcus</i> , isolates.
Results	Prevalence.

2.3. Data extraction and management

Using a standardised data extraction form (Appendix 2). Two reviewers (HM; DB) independently extracted data. Contradictions were resolved through discussion until agreement was reached.

The following descriptive and outcome data were extracted from each study:

- Study identification: authors, year of publication and possible references.
- Eligibility criteria for inclusion and exclusion into the review.
- Study characteristics: study design, study participants, country, study setting, season(s) of participation, and the number of children enrolled into the study.
- Diagnostic test employed: Throat culture, RADT, PCR.
- Details of the outcome measure: prevalence of GAS carriage, molecular characterization of the M-protein of GAS.
- Risk of bias analysis: internal and external validity.
- Notes: study general comments.

2.4. Data synthesis

2.4.1. Prevalence of asymptomatic Group A *Streptococcus*

To calculate the unadjusted prevalence estimates of *streptococcus* carriage of children within the age groups of 5 –15 years, we first had to calculate/double check the numerators and denominators of each study using crude numerators and denominators as reported by the individual studies.

For each study the reviewer independently recalculated the reported prevalence to confirm the numerators and denominators. For five studies the reviewers confirmed the individual numerators and denominators as they were reported by the authors [19–23]. However, some

studies had to be adjusted to obtain numerators and denominators that are relevant to our study population.

Calculations readjustments were made, firstly, where the sample populations included adults and children[24], only extracted data pertinent to our target age group. Where data for our target age were reported as part of a wider age range [22,23], we included all the data within that category, provided the age did not exceed 25 years, the upper end of age-at-risk for rheumatic fever [25]. Secondly, where sample populations included both symptomatic GAS children and asymptomatic GAS children [26,27], we only extracted information from healthy children / children without any signs or symptoms of disease. Thirdly, for studies that reported on more than asymptomatic GAS prevalence [24,27–29], we did not extract information for other outcomes such as Group C *Streptococcus*, Group G *Streptococcus*, impetigo and other β -hemolytic *streptococcus* infections. Fourthly, for prevalence of GAS carriage calculations, the whole asymptomatic GAS sample population was taken as the denominator, instead of only calculating it from the β -hemolytic *streptococcus* sample. Lastly, the earliest full set of results were used where studies reported follow-up data of children over a periods of months [30] and years [31], this was to avoid an over and under estimation of GAS carriage, that would arise by taking an average of all follow up data of an individual study. Appendix 4 details studies' individual recalculations.

Stata® (version 13.1) was used to calculate unadjusted prevalence measures, together with standard error. The Freeman-Tukey double arcsine transformation *metaprop* model was used to calculate the combined prevalence estimate across the included studies [32]. The Freeman-Turkey stabilises the variance of study-specific prevalence, minimizing the influence from studies with extremely small prevalence or extremely large prevalence estimates [32].

2.4.2. Subgroup Analysis

The aggregated prevalence was stratified by region in order to confirm earlier reports of regional differences in GAS [15]. Hence, we wanted to assess similarities and difference within the Africa continent. Our hypothesis is that GAS carriage will differ regionally because of settings and climatic differences.

Given our hypothesis that GAS carriage would not be statistically different across study designs, we also evaluated prevalence according to study design in order to assess methodological influences on overall estimates. Furthermore, we performed a sub-group analysis of adequate sample size, we assumed a prevalence of ten percent based on estimates reported in two recent large studies by Abdissa et al and Braitto et al of ten and nine percent respectively [19,33]; together with a precision of two percent, an estimated sample size of 796 is deemed to be adequate [34]. Sub-analysis of GAS colonization risk factors; gender, crowding, and seasonality could not be performed due to data limitations; thus these factors are presented as qualitative data.

2.4.3. Molecular characterisation of asymptomatic Group A

***Streptococcus* isolates**

A meta-analysis of the pooled effect was not possible for secondary objective results due to the insufficiency of data. Therefore, the secondary outcome is presented as qualitative report of *emm* typing data.

2.4.4. Assessment of heterogeneity

Heterogeneity between studies was determined to assess the extent of variation between studies included in the meta-analysis. Heterogeneity was assessed by inspecting the extent of overlap within the forest plots, through the Cochran's Chi-square test (using 10% level of significance), and the I-square statistic (where 50% or higher values indicate substantial heterogeneity) [35]. Confidence intervals around heterogeneity estimates were calculated

using the *heterogi* command in Stata. Where heterogeneity was statistically significant, we conducted a sensitivity analyses to determine the potential sources of heterogeneity.

2.4.5. Exploration of heterogeneity

Sensitivity analyses were performed to assess the influence of various study characteristics such as the quality of the study, age of participants and season(s) of participation.

2.4.6. Missing data

Missing data for each study were described and considered in terms of their impact on the results. None of the missing data was relevant to warrant contacting the corresponding authors to request the missing information.

2.5. Quality appraisal of included studies

Risk of bias, was independently evaluated by two reviewers (HM; DB). Any disagreement was resolved by discussion, with arbitration by a third reviewer (ME) where necessary. Included studies were evaluated for risk of bias related to internal validity, external validity and generalizability of the study results. An assessment of risk of bias informed the evaluation of heterogeneity in the pooled analysis. We employed the quality assessment tool for evaluating prevalence studies as suggested by Hoy and colleagues and adapted by Werfalli and colleagues which, allows for a composite score to assist with relative comparison between the studies, thereby reducing reviewers' subjectivity [36,37]. Briefly, Werfalli and colleagues added a quantitative scoring system to the Risk of Bias table (Table 2) allocating four points for external validity score and six points for internal validity. The scoring system tool categorizes high risk studies as those with an overall score of 0-5 points,

moderate risk as 6-8 and low risk > 8 points (Table 3).

Table 2: Quality assessment criteria for prevalent studies [36]

Items	Quality Score
External Validity	
1. Was the study's target population a close representation of the national population in relation to relevant variables?	(1 point)
2. Was the sampling frame a true or close representation of the target population?	(1 point)
3. Was some form of random selection used to select the sample, OR was a census undertaken?	(1 point)
4. Was the likelihood of nonresponse bias minimal?	(1 point)
	Total (4 points)
Internal Validity	
1. Were data collected directly from the subjects (as opposed to a proxy)?	(1 point)
2. Was an acceptable case definition used in the study?	(1 point)
3. Was the study instrument that measured the parameter of interest shown to have validity and reliability?	(1 point)
4. Was the same mode of data collection used for all subjects?	(1 point)
5. Was the length of the shortest prevalence period for the parameter of interest appropriate?	(1 point)
6. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	(1 point)
	Total (6 points)

Table 3: Risk of assessment [37]

Overall score	Quality
0-5 points	Low Risk: Further research is very unlikely to change our confidence in the estimate
6-8 points	Moderate Risk: Further research is likely to have an important impact on our confidence in the estimate and may change the estimate.
>8 points	High Risk: Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate.

2.5. Funding

We did not receive any funding for this study.

2.6. Ethics

Systematic reviews draw on publicly available data and do not directly involve human nor animal subjects; therefore they do not require formal ethical review [38]. The study protocol was reviewed by supervisors with expertise in systematic review methods and submitted to the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee for approval.

2.7. Dissemination

It is expected that this work would contribute to the knowledge base of the prevalence of pharyngeal GAS carriage in Africa, thus potentially influencing policy and practice in this area. The study will be disseminated by peer-review publication and conference presentation.

3. Results

3.1. Study flow and description of studies

The literature search results are reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Statement [39].

Figure 1 details the search results. We retrieved 1,646 records from electronic database searches which, together with a further 20 references identified through hand-searching of relevant reference lists, resulted in 1,666 articles for consideration. After removing duplicates, 681 references were assessed for eligibility. Of these, 649 articles were excluded on the basis of title and abstract (all titles and abstracts were in the English language) leaving 32 articles requiring further evaluation as full-text articles. Finally, 17 studies met our inclusion criteria (Table 4). Studies excluded are detailed with reasons in Table 5.

3.1.1. Characteristics of included studies

Included studies comprised sixteen peer-reviewed journal articles [19–24,26–31,33,40–42] and one thesis [43]. From these studies, 14 were cross-sectional studies (n=23,549), while three were longitudinal studies (n=6,026). The study populations ranged from minimum age of 2 months to a maximum age of 25 years of age. GAS was diagnosed by microbiological culture of throat swab specimens in all studies, except [27] which utilised a rapid strep diagnostic method as a means of identifying GAS isolates. Studies were conducted in schools (10 studies, [19–21,28–31,33,42,43]) clinics / outpatient departments (4 studies, [22,26,27,40]) homes (1 study [24]). Two studies did not state the setting of their sample population [23,41]

All studies were conducted within a range of African regions: Southern Africa (6 studies, [22,23,28,30,40,43]) Northern Africa (4 studies, [20,21,26,31]) Eastern Africa (4 studies [19,27,29,33]) Western Africa (2 studies, [41,42]) and Central Africa (1 study, [24]). By country, most of the studies were done in South Africa (n=5), followed by Tunisia (n=3), Ethiopia (n=2), Nigeria (n=2), and lastly, Egypt (n=1), Gabon (n=1), Kenya (n=1), Tanzania (n=1), Zambia (n=1). The data included studies that were conducted in both urban and rural areas or exclusively in urban areas; no studies were conducted exclusively in a rural area.

Amongst those included, five studies reported additionally on molecular characterization of GAS isolates [26,29,31,40,43]. However, only one study was included in the summary of molecular characterisation [43]: The study by Engel, employed PCR-based methods as described previously [13]. The remaining studies were excluded for the following reasons: reported only on GAS pharyngitis isolates (n=1) [26]; reported on a mixture of molecular typing of *s. pyogenes* and *s. pneumonia* (n=1) [40]; wrong typing method - T-typed GAS isolates (n=2) [29,31].

Figure 1: PRISMA Flow Diagram

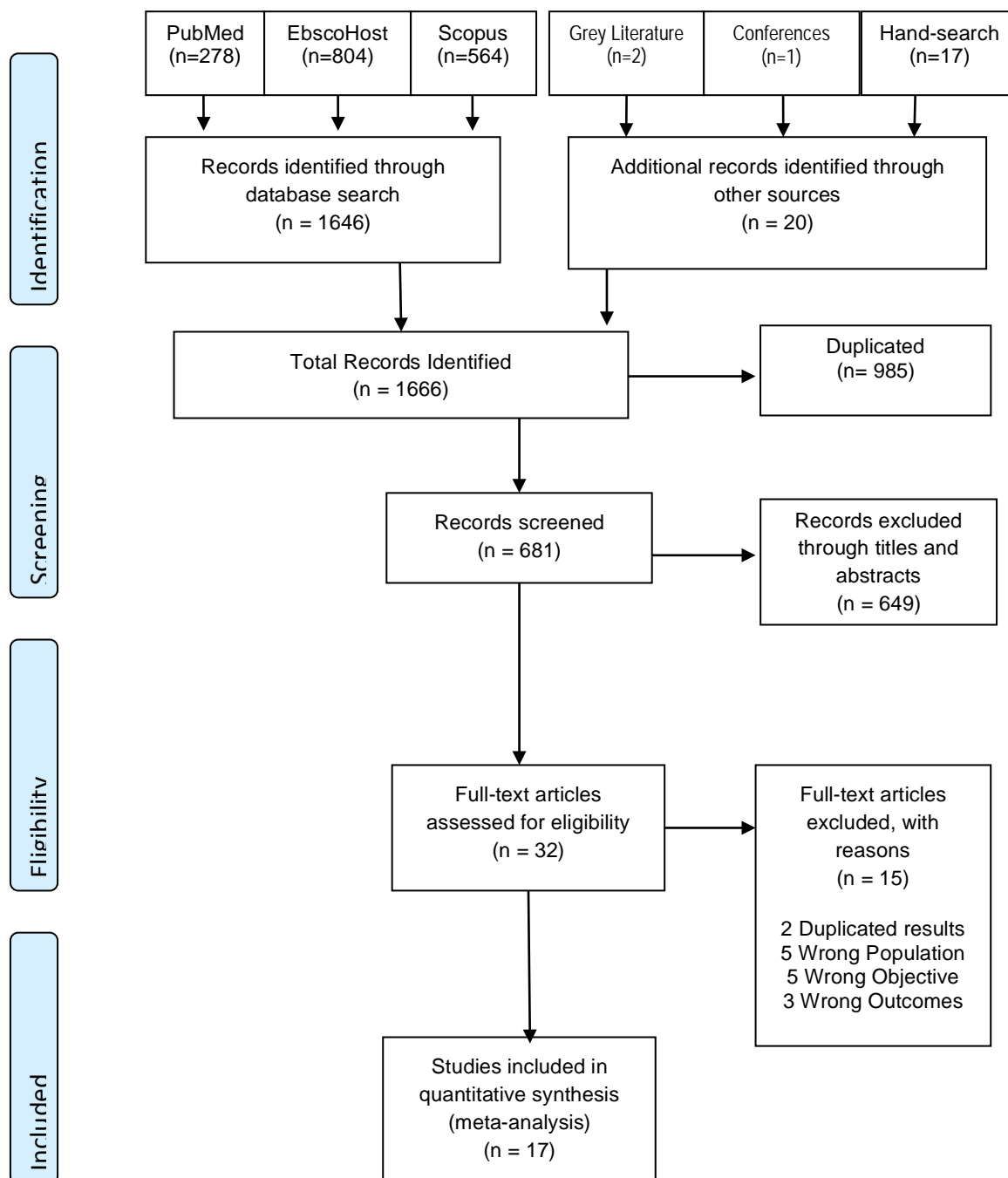


Table 4: Summary of Included studies

Study ID	Region	Country	Study Design	Season of participation	Setting (local, social context)	Inclusion and Population description	Site of recruitment	Selection Methods	Age(s)
Abdissa 2011.	Eastern Africa.	Ethiopia.	Cross Sectional.	Hot rainy and cold dry seasons.	Gondar and Dire-Dawa are located 750 km north and 540 km south east of Addis Ababa.	Healthy School children living in three major cities of Ethiopia.	Schools.	Computer generated random numbers.	6 - 14 years.
Bélard 2014.	Middle Africa.	Gabon.	Cross Sectional.	One rainy season.	Dense rainforest and sparsely populated. Infectious diseases contribute considerably to the total morbidity and mortality burden.	Rural and urban residence within the province of Moyen-Ogooué.	Participants' homes.	Convenience sampling.	2months – 18 years.
Braito 2004.	Eastern Africa.	Tanzania.	Cross Sectional.	Dry season.	Pemba island is densely populated and divided into four districts that differ from each other in some geographical and economical characteristics.	Healthy children living in all the Pemba Districts.	Schools.	Random selection.	7 – 10 years.
El Kholya 1973.	Northern Africa.	Egypt.	Longitudinal	All.	Qalyub district is 20 km North of Cairo.	Primary school children.	Primary schools.	Not stated.	6 - 12 years.
Engel 2012.	Southern Africa.	South Africa.	Cross Sectional.	All.	Peri-urban area of Cape Town.	Asymptomatic children of school-going age.	Schools.	Convenience sampling.	6 – 18 years.
Frederiksen 1988.	Southern Africa.	Zambia.	Cross Sectional.	Not stated.	Two urban areas, a railway clinic and three rural areas in the Choma district. Choma District is situated in the tropical zone of the	Healthy infants and children. Mostly attending the under-five	Clinics.	Not stated.	Infants and under 10 years old.

Gaumer 1972.	Northern Africa.	Tunisia.	Cross Sectional.	Spring.	Urban.	Children from kinder- garden, primary and high school.	Schools.	Not stated.	3 – 17 years.
Khemiri 1982.	Northern Africa.	Tunisia.	Cross Sectional.	All.	Government schools having children from both urban and rural.	School children.	Schools.	Not stated.	6 - 12 years.
Lawal 1990.	Western Africa.	Nigeria.	Cross Sectional.	Not stated.	School located in the zones 1-7 zones of Lagos Nigeria.	Healthy schools children.	Schools.	Not stated.	7 - 12 years.
McLaren 1975.	Southern Africa.	South Africa.	Cross Sectional.	Not Stated.	Soweto lies to the South-Western boundary of Johannesburg. The population is mainly black people from different ethnic groups. Most of the population is unskilled workers, but a small middle class also exists.	Black children attending crèches and schools of Soweto.	Crèches, primary school, higher primary.	Randomized	2 – 18 years.
Mzoughi 2004.	Northern Africa.	Tunisia.	Cross Sectional.	All.	Populous district around Sousse.	Healthy paediatric patients who were attending outpatient clinics for vaccination.	Farhat Hached Hospital and Centre de Protection Maternelle et Infantile.	Not stated.	2 – 8 years.
Ogunbi 1974.	Western Africa.	Nigeria.	Longitudinal.	Dry and Wet seasons.	Urban.	Normal school children.	Not stated.	Not stated.	6 year olds.
O'Meara 2015.	Eastern Africa	Kenya	Cross Sectional	Not Stated.	Webuye District Hospital is located in the peri-urban centre of Bungoma East district. Most families engage in farming and small- scale animal husbandry.	Afebrile children from the eye clinic, follow up fractures, or children accompanying parents for other reason.	Webuye District Hospital (Outpatient).	Not stated.	1 – 12 years.

Ransome 1983.	Southern Africa.	South Africa.	Cross Sectional.	All.	Low social economic classes in Johannesburg and its environs. Approximately 30 pupils per classroom, playgrounds were moderately crowded.	Asymptomatic school children.	Schools.	Not stated.	7 - 9 years.
Tewodros 1992.	Eastern Africa.	Ethiopia.	Longitudinal.	All.	Ethio-Swedish Children's teaching hospital, located in the center of Addis Ababa.	School children without any signs of upper respiratory tract.	Three public elementary schools located near of the Ethio-Swedish Children's Hospital.	Randomized.	Below the age of 12 years
Van Staden 1982.	Southern Africa.	South Africa.	Cross Sectional.	Not States.	Traditional black community.	Separated tribes living in a community with minimum services and Western influence.	Not Stated.	Not Stated.	5 - 25 years.
Van Zyl 1981.	Southern Africa.	South Africa.	Cross Sectional.	Not stated.	Urban Area, Pretoria.	Black and Whites living in Pretoria.	Outpatient Government Hospital and Private hospital.	Not stated.	5 - 25 years.

Table 5: Summary of excluded studies

Author	Title	Reason for exclusion
Duplicated Results		
Abdissa 2005.	Characterization of Group A <i>Streptococci</i> Isolated from Throat of Healthy School Children in Ethiopia.	Thesis results were published as a peer reviewed article title: "Throat carriage rate and antimicrobial susceptibility pattern of group A <i>Streptococci</i> (GAS) in healthy Ethiopian school children"
Engel 2012.	A Study of Determinants and Prevalence of Rheumatic Heart Disease in Cape Town.	The abstract is from an unpublished thesis title: "A Study of Determinants and Prevalence of Rheumatic Heart Disease in Cape Town". The thesis was used as it has more data.
Wrong Population		
Ditchburn 1995.	Rate of carriage of Group A hemolytic <i>streptococci</i> .	The study population was from Britain.
Gunnarsson 1997.	The prevalence of β -Hemolytic <i>streptococci</i> in throat specimens from healthy children and adults: Implications for the clinical value of throat cultures.	The study population was from Sweden.
Hoffman 1985.	The throat carrier rate of Group A and other β -Hemolytic <i>streptococci</i> among patients in general practice.	The study population was from the United States.
Manandhar 2013.	Study on the Prevalence of β -Hemolytic <i>Streptococcus</i> Among School Children.	The study population was from the Nepal.
Noble 1974.	Carriage of <i>Staphylococcus aureus</i> and β -Hemolytic <i>streptococci</i> in relation to race.	The study population is not clearly specified.
Wrong Objective		
Abdissa 2006.	High diversity of Group A <i>streptococcal emm</i> types among healthy schoolchildren in Ethiopia.	The objective of this study was to study the <i>emm</i> types of GAS. The study did not report on the prevalence of GAS carriage.
Engel 2013.	Clinical and epidemiological aspects of <i>streptococcus pyogenes</i> pharyngitis and carriage in Africa: <i>streptococcus pyogenes</i> in Africa.	The primary objective of this review was not the prevalence of asymptomatic GAS.
Engel 2014.	Group A <i>Streptococcal emm</i> type prevalence among symptomatic children in Cape Town and potential vaccine coverage.	The objective of this study was to study the <i>emm</i> types of GAS. The study did not report on the prevalence of GAS Carriage.

Tesfaw 2015.	Prevalence of group A β -hemolytic Streptococcus among children with pharyngitis in Jimma town, Southwest Ethiopia.	The objective of the study was to determine the prevalence of GAS among children with pharyngitis. However, it did not report on the prevalence of GAS carriage.
Tewodros 2005.	Distribution of presumptive pathogenicity factors among beta-hemolytic streptococci isolated from Ethiopia.	The objective of the study was to study the distribution of binding structures of Asymptomatic GAS. However, the study did not report on the prevalence of GAS carriage.
Wrong Outcome		
Feikin 2012.	Etiology and Incidence of viral and bacterial acute respiratory illness among older children and adults in rural western Kenya, 2007-2010.	There were no GAS isolate in the reported results.
Owubu 2013.	Streptococcal throat carriage in a population of nursery and primary school pupils in Benin City, Nigeria.	There were no GAS isolate in the reported results.
Sadoh 2007.	Streptococcal Throat Isolates In School Children In An Urban Centre In Nigeria - Are There Other Rheumatogenic Strains?	There were no GAS isolate in the reported results.

3.2. Assessment of risk of bias in included studies

Risk of bias was assessed according to the Hoy criteria as modified by Werfalli and colleagues [36,37]. Five studies had a low risk of bias [19,21,24,29,43], while the rest of the studies had a moderate risk of bias. No study was deemed as having a high risk of bias (Table 6, Figure 2).

Most of the studies (n=11) had a representative target population in terms of age for inclusion into the systematic review. However, age ranges in six studies were not fully in line with the 5 – 15 year age category for GAS infection. Three studies had a limited age between 2 - 10 years [26,30,44]. While two studies compromised of infants and children under 12 years [29,40]. The remaining study included a narrow age range: only included 6 year olds [41].

All studies had a close representation of the target population in terms of geographical location given that all the participants in the selected studies were recruited from African countries. In terms of sampling frame, as discussed above (*3.1.1 Characteristics of Included studies*), study settings included schools, clinics or outpatient departments, place of residence and two studies did not provide study-setting detail.

Three studies had some form of population randomization; including 2 convenience samples [24,43] and computer-generated random numbers [19]. Three studies did not state the form of randomization used [28,29,33]. Three studies sampled participants within a single season only [20,24,33], and were thus scored as having bias in terms of length of sampling.

The nature of the study methods requires a direct throat swabs from the study participant, as such all the studies collected data directly from the subject as opposed to a proxy. Collecting data directly also reduced no non-response bias in all the studies. Thus all studies scored favourably for these domains.

Most studies defined their recruited population as “healthy Children” or children without any signs of

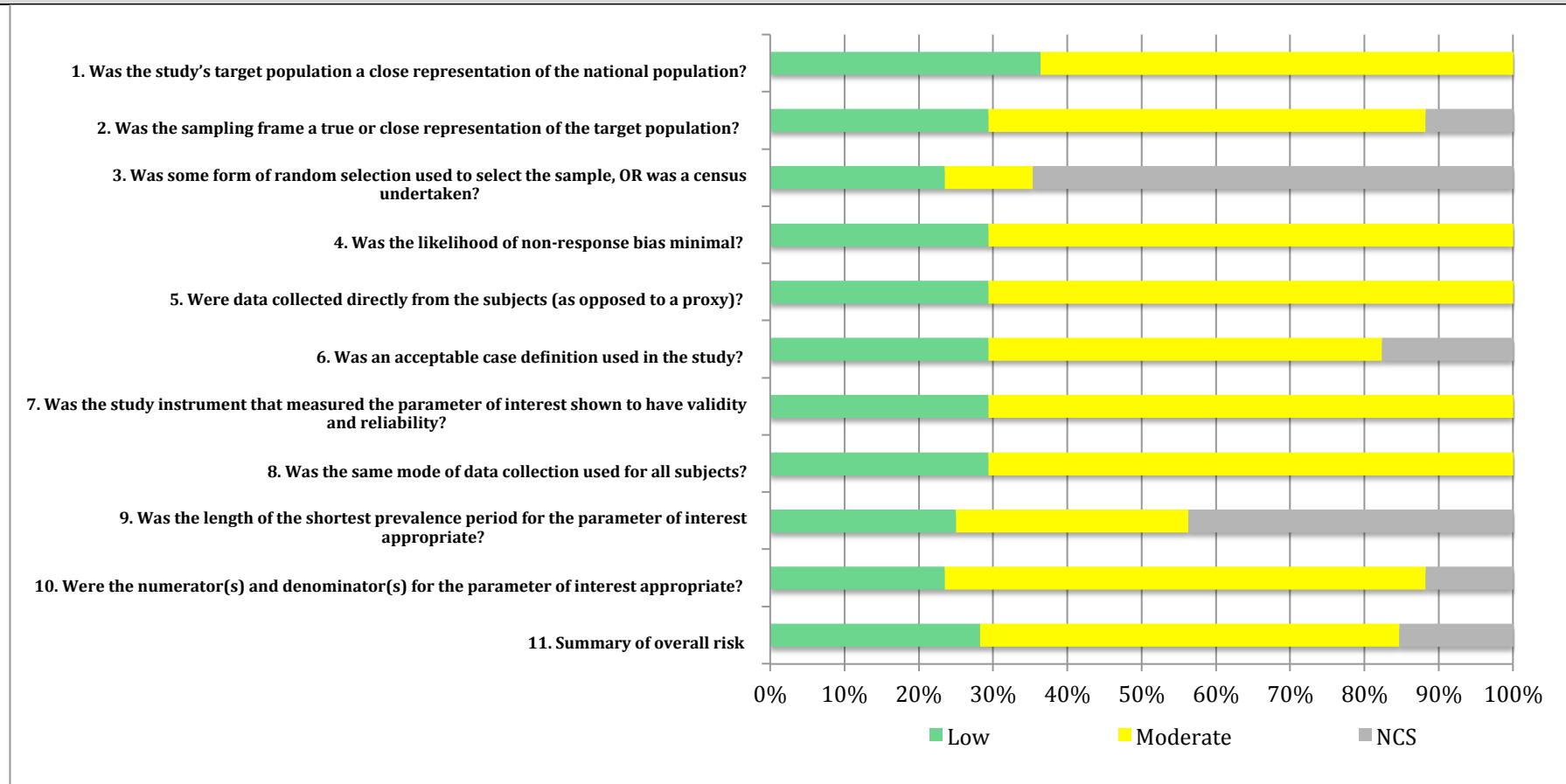
upper respiratory tract infection. However, three studies did not clearly define their participants [23,28,31]. Almost all the studies (n=16) used Microbiological throat swabs, which have been proven to be the gold standard in diagnosing GAS, while one study used a RADT [27], which has been proven to be less sensitive when compared to a throat culture [45]. For each individual study, the same mode of data collection was used for all subjects for each specific study.

Table 6: Summary of risk of bias evaluation

Study ID	Internal Risk of Bias				External Risk of Bias						Quality Score	Risk of Bias
	Risk Of Bias 1	Risk Of Bias 2	Risk Of Bias 3	Risk Of Bias 4	Risk Of Bias 5	Risk Of Bias 6	Risk Of Bias 7	Risk Of Bias 8	Risk Of Bias 9	Risk Of Bias 10		
Abdissa 2011	1	1	1	1	1	1	1	1	Unclear	1	9	Low
Bélard 2014	1	1	1	1	1	1	1	1	1	Unclear	9	Low
Braito 2004	0	1	1	1	1	1	1	1	Unclear	1	8	Moderate
El Kholya 1973	1	1	Unclear	1	1	Unclear	1	1	1	1	8	Moderate
Engel 2012	1	1	1	1	1	1	1	1	1	1	10	Low
Frederiksen 1988	0	1	Unclear	1	1	1	1	1	1	1	8	Moderate
Gaumer 1972	1	1	Unclear	1	1	1	1	1	Unclear	1	8	Moderate
Khemiri 1982	1	1	Unclear	1	1	1	1	1	1	1	9	Low
Lawal 1990	1	1	Unclear	1	1	1	1	1	Unclear	1	8	Moderate
McLaren 1975	1	1	1	1	1	Unclear	1	1	Unclear	1	8	Moderate
Mzoughi 2004	0	1	Unclear	1	1	1	1	1	1	1	8	Moderate
O'Meara 2015	1	1	Unclear	1	1	1	1	1	Unclear	Unclear	7	Moderate
Ogunbi 1974	0	Unclear	Unclear	1	1	1	1	1	1	1	7	Moderate
Ransome 1983	0	1	Unclear	1	1	1	1	1	1	1	8	Moderate
Tewodros 1992	0	1	1	1	1	1	1	1	1	1	9	Low
Van Staden 1982	1	Unclear	Unclear	1	1	Unclear	1	1	Unclear	1	6	Moderate
Van Zyl 1981	1	1	Unclear	1	1	1	1	1	Unclear	1	8	Moderate

Quality score: 0-5 high risk of bias; 6-8 moderate risk of bias; >8 Low risk of bias

Figure 2: Risk of Bias Chart.



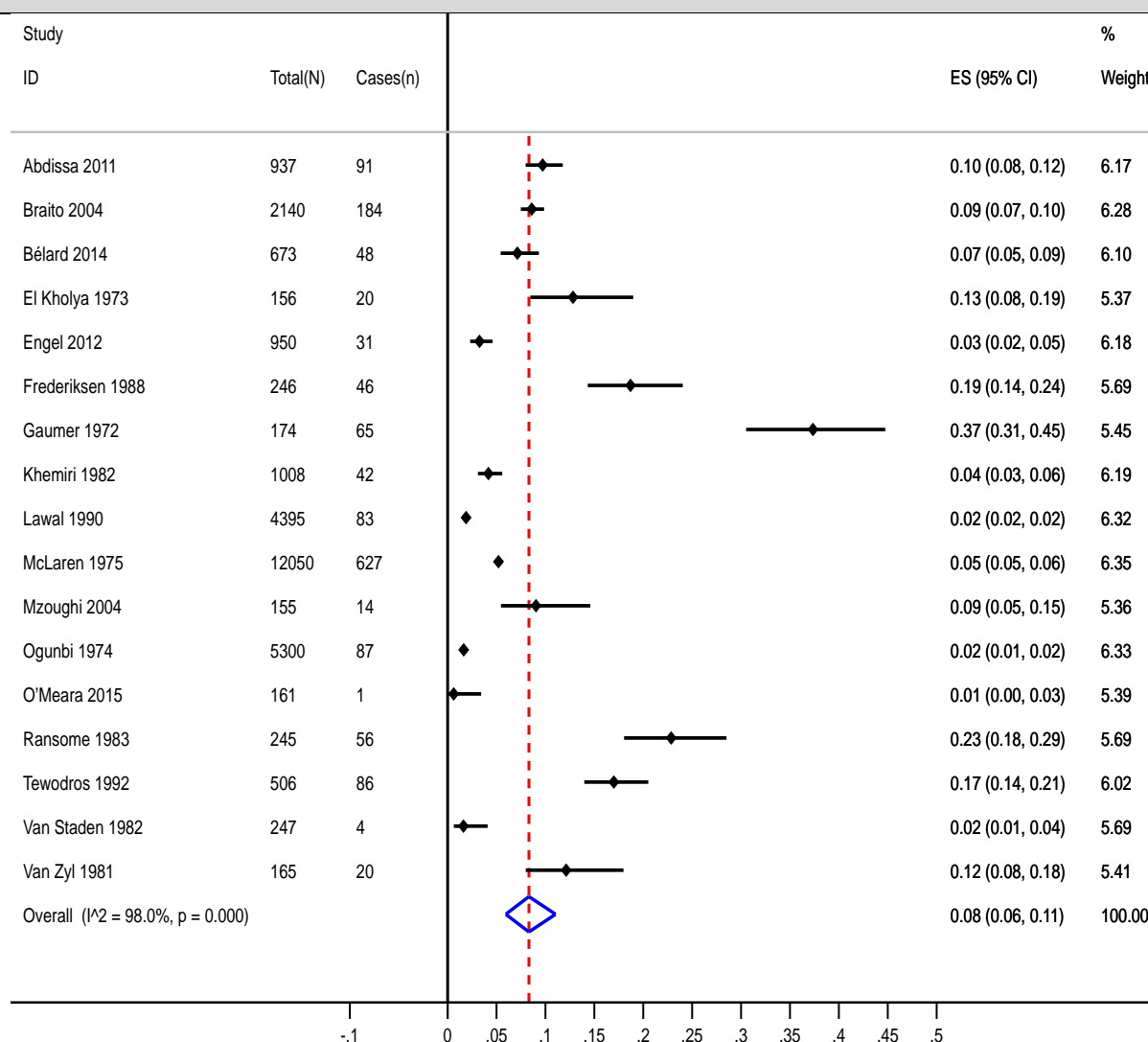
NCS, not clearly stated.

3.3. Quantitative Data Synthesis.

3.3.1. Prevalence of asymptomatic Group A *Streptococcus*.

This review found an overall prevalence of 8% (95% Confidence Interval (CI): 6% to 11%) for *streptococcus* carriage of children within the age groups of 5 –15 years residing in African countries; test for heterogeneity, $I^2 = 97.8\%$ $p < 0.0001$; 17 studies, $n = 29,508$ (Figure 3).

Figure 3: Overall Prevalence: *Streptococcus* carriage in African children.



ES, effect size; CI, confidence interval.

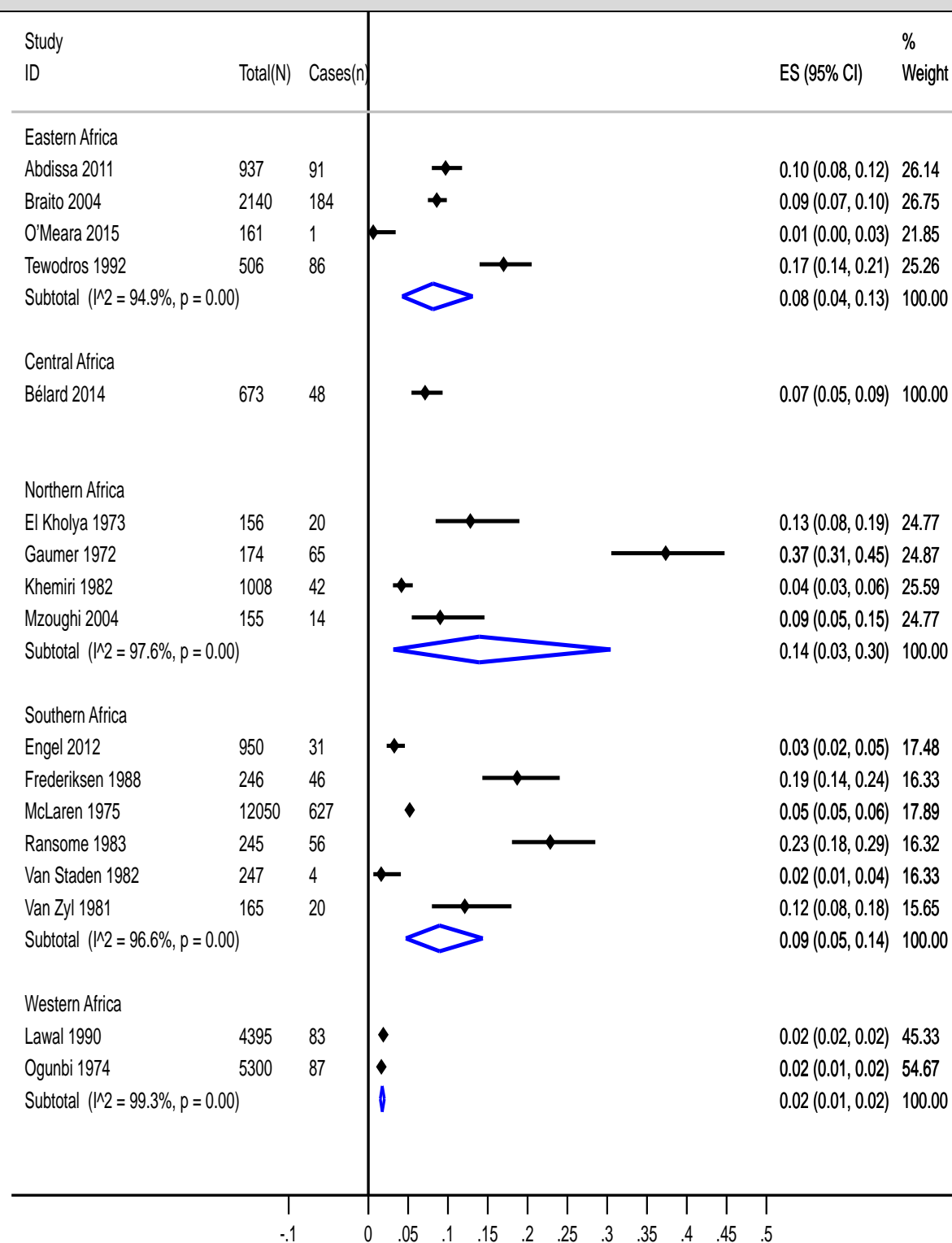
3.3.2. Prevalence of asymptomatic Group A *Streptococcus* according to region.

Figure 4 shows that the regional pooled rates ranged between 2% to 14% across the five regions of the African continent. Pooled rates were similar across Eastern, Central and Southern Africa, ranging between 7% and 8% (n=11 studies). In contrast, countries within the Northern Africa region (n=4 studies) had a pooled estimate of 14% (95% CI, 3% to 30%), while in Western Africa (n=2 studies), a lower pooled estimate of 2% (95% CI, 1% to 2%) was observed. The 95% CIs of the pooled estimates between Western Africa and Eastern, Central and Southern Africa combined, did not overlap, thus indicating a statistically significant difference.

3.3.3. Prevalence of asymptomatic Group A *Streptococcus* according to study design.

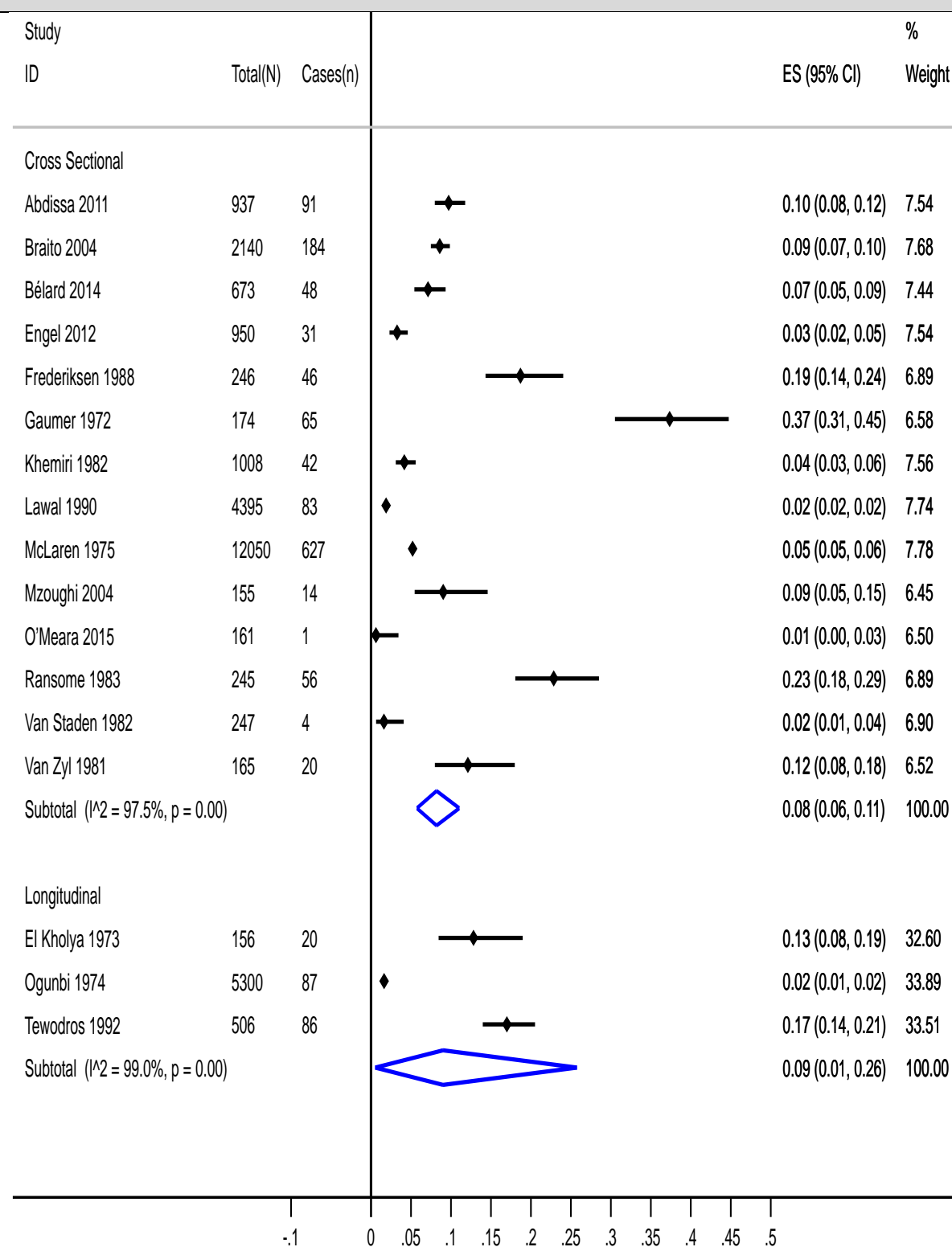
Data for estimating the prevalence of GAS carriage by cross-sectional and longitudinal study design were provided by 14 and 3 studies, respectively (Figure 5). There was no difference between the pooled prevalence estimates for cross-sectional studies 8% (95% CI, 6% to 11%) and longitudinal studies, 9% (95% CI, 1% to 26%); test for heterogeneity between sub-groups, $p=0.90$.

Figure 4: Prevalence stratified by Region: *Streptococcus* carriage in African children.



ES, effect size; CI, confidence interval.

Figure 5: Prevalence stratified by Study Design: *Streptococcus* carriage in African children.



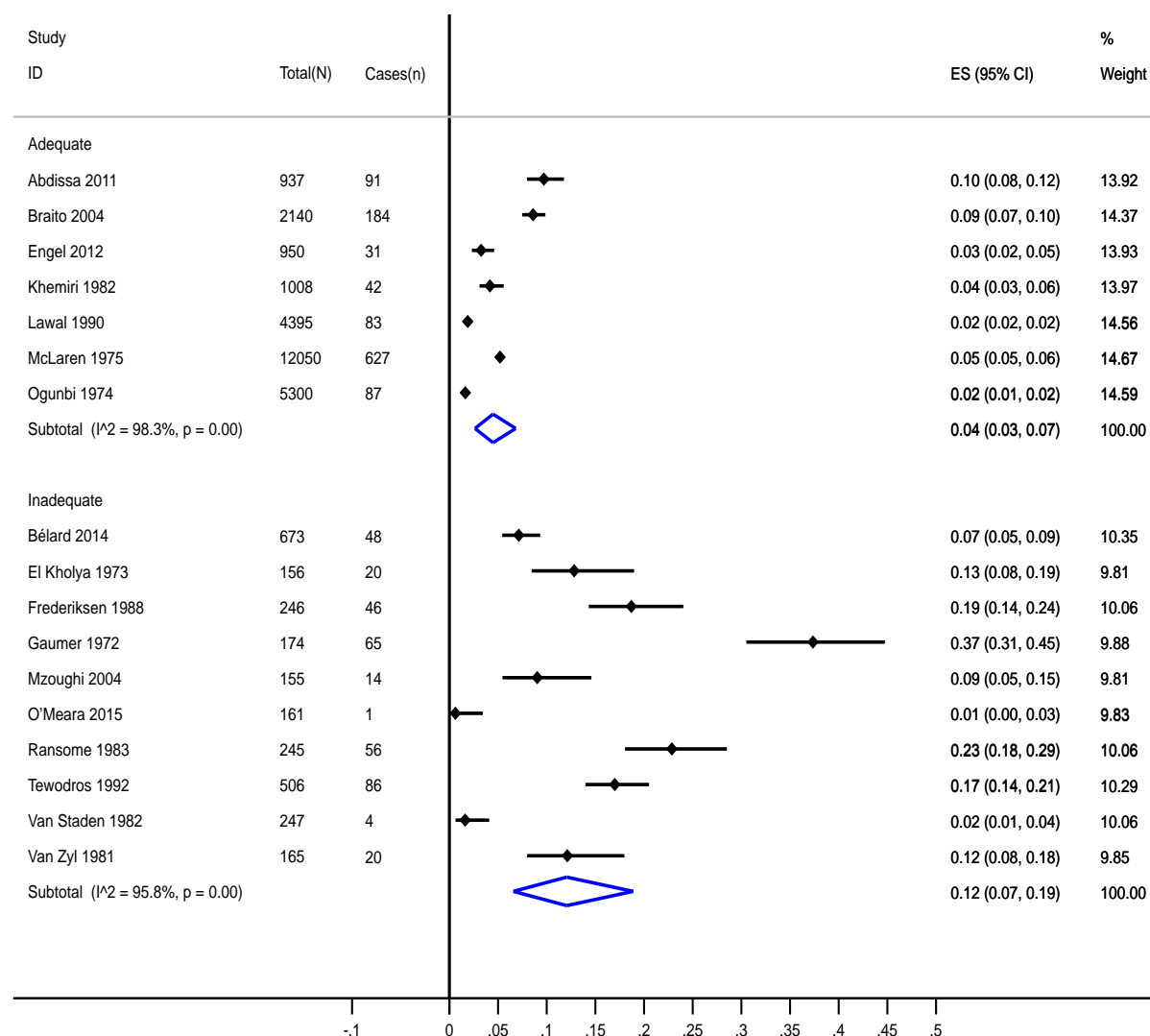
ES, effect size; CI, confidence interval

3.3.4. Prevalence of asymptomatic Group A Streptococcus according to sample

size.

Seven studies had sample sizes of 796 or greater that together, gave a combined prevalence of 4% (95% CI, 3% to 7%) (Figure 6). This contrasted with the combined estimate of 12% (95% CI, 7% to 19%) rendered by inadequately powered studies (n=10 studies). This difference was not statistically significant (test for heterogeneity between sub-groups, p=0.1).

Figure 6: Sample size of included studies.



ES, effect size; CI, confidence interval

3.3.5. Risk factors for Group A *Streptococcus* colonization.

A limited number of studies reported on risk factors having a possible association with GAS colonization in children. However, due to a lack of reporting of the breakdown of prevalence according to possible risk factors, it was not possible to conduct a pooled meta-analysis of the association of prevalence estimates with risk factors, and thus, we discuss the findings in a narrative below.

Overcrowding (n=3 studies)

Two studies, conducted amongst household residences, found the household number not to be an associated risk factor for GAS carriage [24,30]. Another study, conducted in a school environment, found GAS rates to be significantly higher in children coming from suburbs characterised by lower socioeconomic conditions, including a higher household density [43].

Gender (n= 4 studies)

In three studies, no significant difference was found between gender of participants [19,24,43]. While one study reported a higher GAS carrier rate in girls compared with boys [20].

Seasonality (n=5 studies)

Seasonality showed contrasting associations as regards peak GAS carriage. Two studies reported higher GAS carrier rates during the winter months [26,31]. While, two others, found a peak prevalence during summer months [28,30]. The remaining study reported no seasonal variations in carriage rates [43].

3.3.6. Molecular characterization of asymptomatic Group A *Streptococcus* isolates.

Table 6 shows molecular characterisation of asymptomatic GAS isolates. Data from the two studies presenting molecular data could not be pooled in a meta-analysis, given the differences in the methods employed within the studies. Engel et al observed 20 different *emm* types among 25 isolates typed with the PCR technique. The *emm* subtype frequencies (in decreasing order of prevalence) were 12% for *emm* 4, 8% for each of *emm* subtypes 1, 2, 12, 58.2, 82, 89 and 4% for *emm* subtypes 6.4, 9.2, 48,75, 92, 118.1.

Study ID	Typing method	Total GAS carriage isolates	GAS carriage isolates Typed	Subtype	Frequency (%)
Engel 2012	PCR	31	25	<i>emm</i> 4	12
				<i>emm</i> 1	8
				<i>emm</i> 2	8
				<i>emm</i> 12	8
				<i>emm</i> 58.2	8
				<i>emm</i> 82	8
				<i>emm</i> 89	8
				<i>emm</i> 6.4	4
				<i>emm</i> 9.2	4
				<i>emm</i> 48	4
				<i>emm</i> 75	4
				<i>emm</i> 92	4
				<i>emm</i> 118.1	4

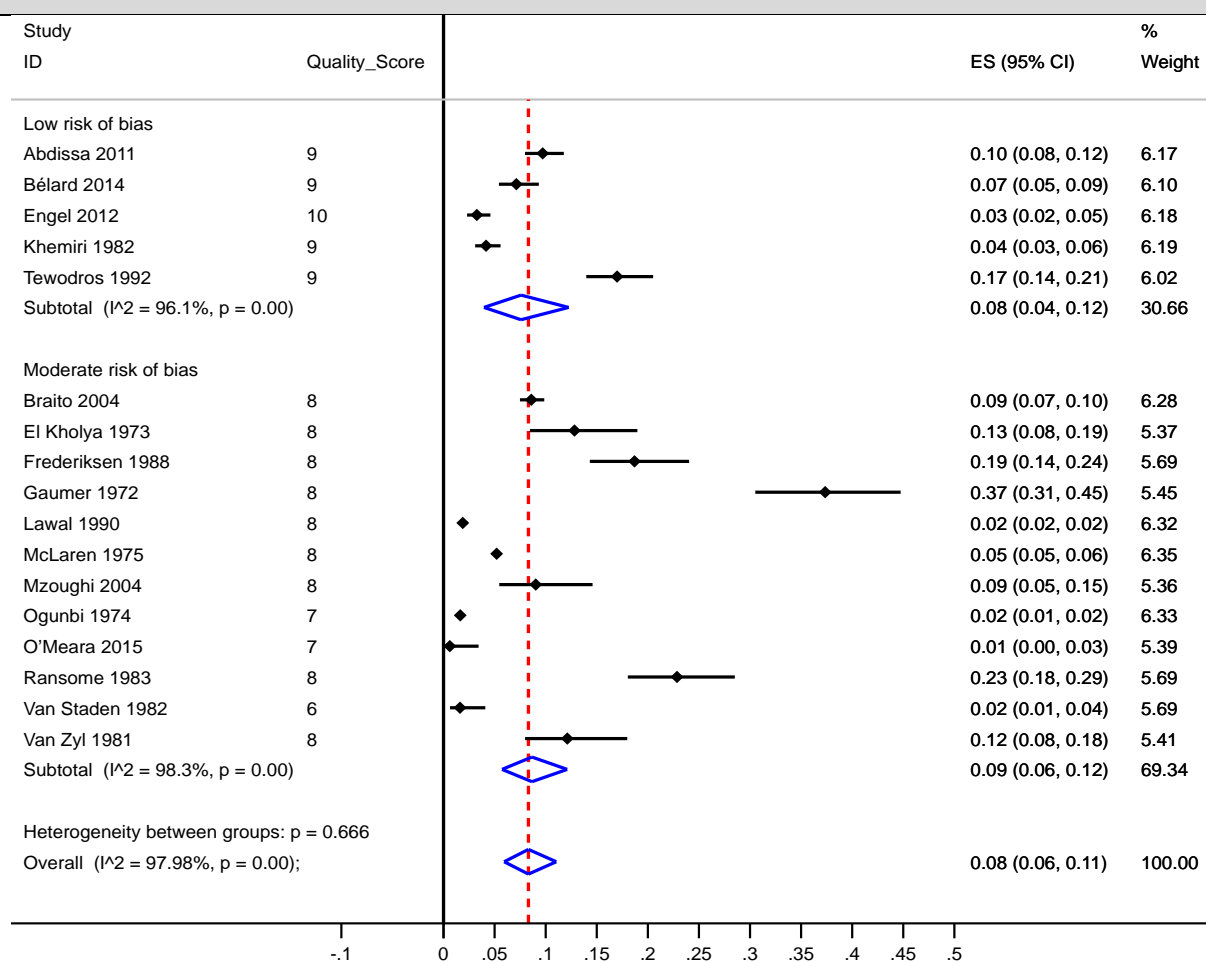
GAS, Group A *Streptococcus*; *PCR*, polymerase chain reaction.

3.3.7. Investigating Heterogeneity

Quality of Study

Given the considerable heterogeneity, $I^2 = 98\%$ (95% CI, 98% to 99%), we conducted sensitivity analyses with respect to study quality and age-ranges of participants. When considering quality assessment (figure 7), studies considered as having a low risk of bias studies (n=5 studies) had a pooled prevalence of 8% (95% CI, 4% to 12%) while studies with moderate risk of bias (n=12 studies) had a pooled prevalence of 9% (95% CI, 6% to 12%); this difference was not statistically significant (test for heterogeneity between sub-groups, $p = 0.67$).

Figure 7: Effect of risk of bias quality score.



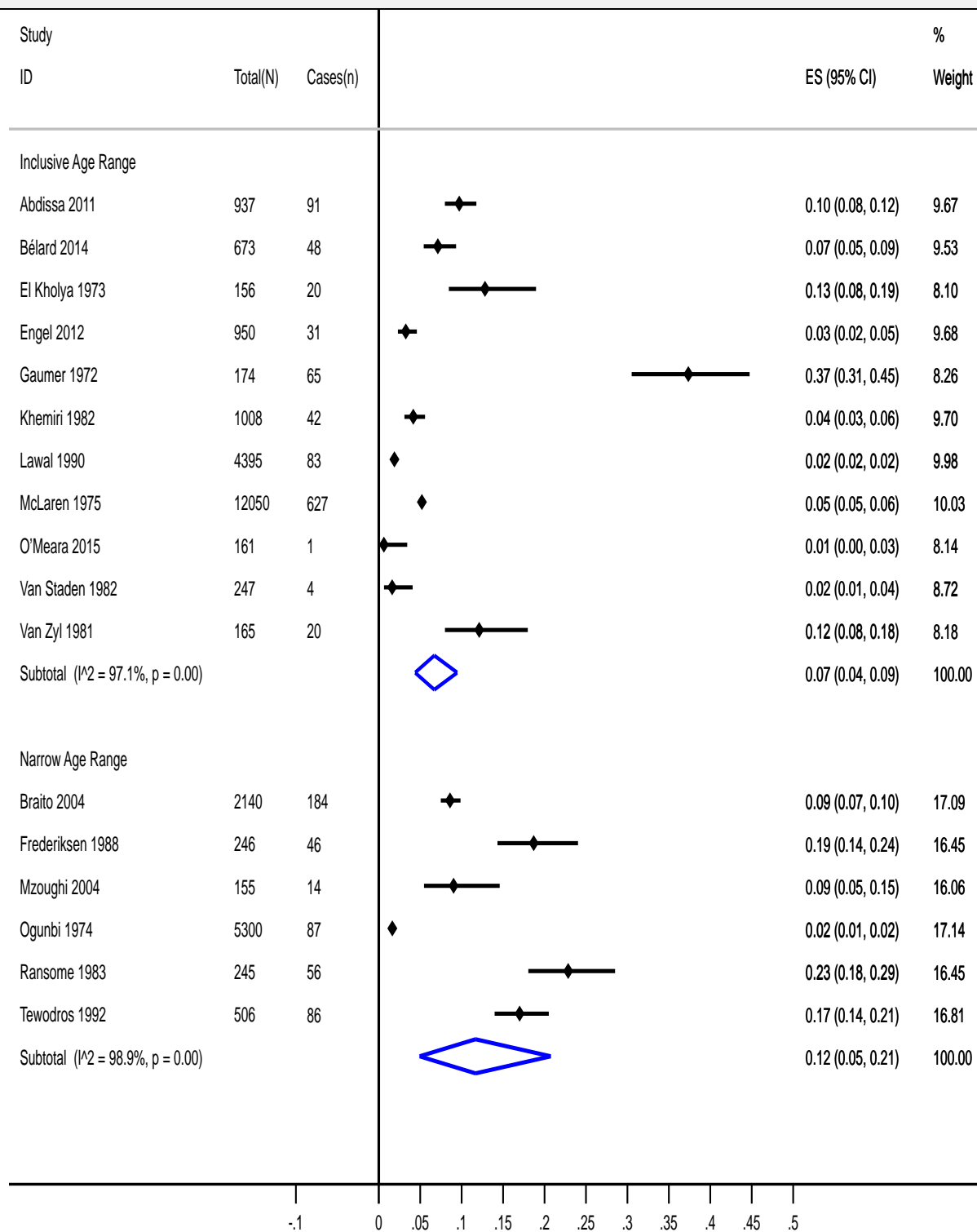
ES, effect size; CI, confidence interval. Low risk of bias >8, Moderate risk of bias 6-8.

Age-range of included participants

Six studies had a limited age-range amongst their participants, [26,29,30,33,40,41] where the age ranges were not inclusive of the whole age range stipulated in the inclusion criteria; thus, we tested the potential contribution to heterogeneity within our meta-analysis, of studies having incomplete age-ranges of participants (Figure 8). The sensitivity analysis revealed a pooled prevalence estimate of 7% (95% CI, 4% to 9%) within studies that had a complete age range and 12% (95% CI, 5% to 21%) in studies having only some of the age ranges within our inclusion criteria. This difference was not statistically significant (test for heterogeneity between sub-groups, $p=0.21$).

There were not enough data to analyse the possible influence of seasonality on the heterogeneity.

Figure 8: Age Range of Included of participants.



ES, effect size; CI, confidence interval

5. Discussion

This systematic review presents the first comprehensive synthesis of the prevalence of *Streptococcus pyogenes* or Group A *Streptococcus* (GAS) in children aged five to twenty-five years residing in Africa, and reveals two important findings: 1) there is a relatively low rate of carriage of GAS in asymptomatic school children residing in Africa and, 2) there are regional differences across the African continent as regards the rate of GAS carriage with the western and northern African regions having rates of GAS carriage that are lower and higher respectively than those of East, Central and Southern African countries, which demonstrated similar rates of carriage.

The pooled GAS carriage estimate of 8% (95% CI, 6% to 11%) among African school children was lower than the 12% previously reported in a systematic review comprising studies in low, middle and high income countries [11]. The low pooled estimate of 2% (95% CI, 1% to 2%) in western Africa contrasted significantly with pooled prevalence rates in East (7%), Central (8%) and Southern Africa (9%). Countries within Northern Africa, on the other hand, had a noticeably higher pooled estimate of 14% (95% CI, 3% to 30%). The data from this review had contradicting results for risk factors, such as gender, seasons and crowding thought to be associated with GAS colonization.

In comparison with single studies among school children in other low income countries, our pooled rate of GAS carriage was similar to the 8.4% found in Chennai, India [46], but less than the 10% and 15 % observed in Nepal and Iraq, respectively [47,48]. Our results were robust regarding study design, with cross-sectional studies and longitudinal studies having similar rates of 7% and 9% respectively, which are in agreement with the overall prevalence. Additionally, taking into account an assessment of the quality of included studies, revealed

similar rates with 8% for low risk of bias studies and 9% for studies with a moderate risk of bias.

We further considered the impact of sample size on the overall prevalence estimate using $n=796$ as the cut-off, based on recommended parameters [34]. Adequately powered studies, rendered a pooled prevalence estimate of only four percent GAS carriage ($n=7$ studies), considerably lower than that the combined 12% estimate amongst smaller inadequately-powered studies. Given that, studies of pharyngitis report GAS prevalence of in excess of 20% [49], our findings emphasize the association between GAS and pharyngitis, given the low background rate of GAS carriage found within our review.

Vaccination has been shown to have an impact on herd immunity, following the reduction of carriage of meningococcal serogroup C conjugate vaccination in the UK and Netherlands [50]. Thus, we considered the potential impact that one of the putative vaccines currently under development, may have on carriage rates in Africa. Data regarding molecular characterization of isolates from GAS carriers were provided for by a single study [43] revealing that 85% of the *emm* types found in Southern African children would be included amongst the putative 30-valent vaccine currently under development [12]. By comparison, potential vaccine coverage by an earlier putative 26-valent GAS vaccine, would only have been 45% among isolates from Ethiopia [51]. Furthermore, the narrow spectrum of *emm* types reported in the studies in our review must be taken into account in the development of putative vaccines, although there is evidence for cross-opsonization activity in recent in-vitro studies [52].

5. 2. Strengths and limitations of this review

One of the main strengths of this review is the comprehensive search of multiple databases using an African search filter for the first time. We systematically and vigorously assessed all the data available with no language exclusions, using the most recently published standard quality assessment tools for prevalence studies. Furthermore, for the first time in GAS prevalence reviews, double arc-sine transformation was used to stabilise the variance of primary studies before pooling, thus limiting the impact of studies with either small or large prevalence on the overall pooled estimates overall, as well as across major subgroups.

Significant heterogeneity in the prevalence estimates is regarded as a limitation to this systematic review. Attempts to completely explain the heterogeneity was unsuccessful in terms of subgroup analyses. It is difficult to make comparisons between populations unless every effort is made to use a standard epidemiological and laboratory efforts in each survey. Thus, caution must be applied in trying to draw too many conclusions about differences in prevalence rates that are in the same range. Also, four articles were translated into English [20–23] which might have led to information loss, thus reducing the quality of data extracted. Unfortunately, only one of the prevalence studies provided molecular typing data, thus not allowing for a detailed analysis of our secondary objective regarding *emm* types in Africa.

5.3. Authors Conclusions

3.2.1. Implications for clinical practice and research

The findings of this review provide important baseline information for healthcare workers regarding the carriage of GAS among asymptomatic children in Africa. Given the low rate of GAS carriage, it is highly likely that GAS isolated during pharyngitis are pathogenic, thus providing support to recommendations to treat all cases of pharyngitis with penicillin, even in the absence of culture [53]. Longitudinal studies with routine swabbing of GAS carriers, is necessary to further elucidate the role, if any, of GAS carriage in pharyngitis. Furthermore, regional differences in GAS carriage rates and various associated risk factors, needs to be explored. Lastly, the limited data on molecular strain information emphasize the need for further molecular studies, given the *emm* type characteristics diversity present in the single study included in this review, especially in light of a putative GAS vaccine. There is a need for the correct characterization of the various strains of GAS *emm* types, which have not been defined in many parts of Africa including South Africa, as they will contribute to development efforts, thus ensuring that the putative vaccine is appropriate for the populations at risk in these high-burden area.

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8. Appendix

Appendix 1: Search strategy	
Subject	Search Terms
Group A β -hemolytic <i>Streptococci</i>	Pharyngitis Pharyngitis [Mesh] Sore throat Strep* Group A Streptococcus Streptococcus pyogenes [Mesh]
Carriage	Asymp* Asymptomatic Diseases Asymptomatic Diseases [Mesh] Asymptomatic Infections Asymptomatic Infections [Mesh] Carri* Carriage Carrier State Carrier State [Mesh]
Children	Adolescen* Adolescent [Mesh] Child* Child [Mesh] Infant Infant [Mesh] Pediatric* Pediatric Paediatric* Paediatric Pediatrics [Mesh]

Africa	<p>africa[tw] OR africa'[tw] OR africa's[tw] OR africa1[tw] OR africa2[tw] OR africaans[tw] OR african collaborations[tw] OR africae[tw] OR africae australis[tw] OR africahiv[tw] OR africaid[tw] OR africaid's[tw] OR africain[tw] OR africaine[tw] OR africaine's[tw] OR africanes[tw] OR african's[tw] OR africal[tw] OR africanum[tw] OR african[tw] OR african'[tw] OR african"[tw] OR african's[tw] OR african1[tw] OR african2[tw] OR africana[tw] OR africanae[tw] OR africanalles[tw] OR africanamerican[tw] OR africanan[tw] OR africanane[tw] OR africananes[tw] OR africanasian[tw] OR africanstrongylus[tw] OR africanalotropis[tw] OR africaner[tw] OR africaners[tw] OR africaner[tw] OR africanendemic[tw] OR africanene[tw] OR africanenes[tw] OR africanensis[tw] OR africanenvironment[tw] OR africaner[tw] OR africanes[tw] OR africanit[tw] OR africanised[tw] OR africanism[tw] OR africanist[tw] OR africanists[tw] OR africanity[tw] OR africanium[tw] OR africanizada[tw] OR africanization[tw] OR africanization'[tw] OR africanize[tw] OR africanized[tw] OR africanized'[tw] OR africanizing[tw] OR africanjournal[tw] OR africanum[tw] OR africano[tw] OR africanoides[tw] OR africano[tw] OR africanos[tw] OR africanoside[tw] OR africanpatients[tw] OR africanpiper[tw] OR africans[tw] OR africans'[tw] OR africanon[tw] OR africantrinervitermes[tw] OR africantriol[tw] OR africanum[tw] OR africanum'[tw] OR africanumsp[tw] OR africanum[tw] OR africanus[tw] OR africanus'[tw] OR africanusgen[tw] OR africanz[tw] OR africare[tw] OR africarice[tw] OR africanas[tw] OR africanasia[tw] OR africanive[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR Burundi[tw] OR Cameroon[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR Djibouti[tw] OR Egypt[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR Jamahiriya[tw] OR Jamahiriya[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libya[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayotte[tw] OR Morocco[tw] OR Mozambique[tw] OR Mozambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR Senegal[tw] OR Seychelles[tw] OR Somalia[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw]</p>
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Appendix 2: Data extraction form

Review Title:	The Prevalence of Group A Beta Hemolytic Streptococci Carriage in children in Africa: Systematic Review
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Study ID (e.g. Moloi 2015)	
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General information

Date form completed (dd/mm/yyyy)	
Name of person extracting data	
Reference citation (e.g. Medline)	
Study author contact details	
Publication type (e.g. full report, abstract)	
Bibliography hand search, References for possible eligible studies	
Notes:	

Study Eligibility

Study Characteristics	Eligibility criteria	Eligibility criteria met?
Types of study	Observational study reporting on Prevalence.	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Participants	Including children of ages 5-15 years	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Diagnosis	Have cultured throat swab/RADT	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Setting	Study took place in an African country	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Outcome measure	Primary outcome: Prevalence of asymptomatic Group A streptococcus	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
	Secondary outcomes: Typing of asymptomatic Group A streptococcus isolates	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Results	Prevalence	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
INCLUDE <input type="checkbox"/> EXCLUDE <input type="checkbox"/> PENDING <input type="checkbox"/>		
Reason for exclusion/ pending?		
Notes:		

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

Characteristics of the Study

	Descriptions as stated in report/paper
Aim of study	
Study Design	
Start date	
End date	
Seasons of participation	
Diagnosis criteria (RADT, Throat swab)	
No of Diagnosis/tests on individual child	
Informed consent obtained	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Ethical approval needed/ obtained for study	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Notes:	

Participants

	Descriptions as stated in report/paper
Country	
Population description	
Setting (location, social context)	
Inclusion criteria	
Exclusion criteria	
Site of recruitment of participants (e.g. clinic, schools)	
Selection methods (e.g. randomized)	
Recruited sample size	
Age(s)	
Gender	
Ethnicity	
Notes:	

Outcome

Primary outcome: Prevalence of Group A *Streptococcus*

Total # recruited	Withdrawals and exclusions	Total sample size analysed	No of asymptomatic GAS cases analysed	<u>Numerator</u> Denominator	Crude Prevalence
Note:					

Risk factors for Group A *Streptococcus* colonization

Factors	Total sample size analysed per factor	No of asymptomatic GAS cases analysed per factor	<u>Numerator</u> Denominator	Prevalence
Gender				
Household Number (crowding)				
Seasons				
Notes:				

Secondary Outcome: Molecular characterisation of asymptomatic Group A *Streptococcus*, Isolates, (emm) types.

No of asymptomatic GAS cases analysed	No of asymptomatic GAS cases typed	<u>Numerator</u> Denominator	Crude Prevalence (%)	Emm types and their Frequency
Note:				

Discussion

	Descriptions as stated in report/paper
Key conclusions of study authors	
Study limits as reported by authors	
Recommendations	
Notes:	

Other Information

	Descriptions as stated in report/paper
Study funding sources (Including role of funders)	
Possible conflicts of interest (For study authors)	
Missing data	
Correspondence required for further study information (from whom, what and when)	
Notes:	

Appendix 3: Risk of Bias for Prevalence Studies

1. Was the study's target population a close representation of the national population in relation to relevant variables?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
2. Was the sampling frame a true or close representation of the target population?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
3. Was some form of random selection used to select the sample, or was a census undertaken?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
4. Was the likelihood of nonresponse bias minimal?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
5. Were data collected directly from the subjects (as opposed to a proxy)?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
6. Was an acceptable case definition used in the study?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
7. Was the study instrument that measured the parameter of interest shown to have validity and reliability?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
8. Was the same mode of data collection used for all subjects?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
9. Was the length of the shortest prevalence period for the parameter of interest appropriate?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	Yes <input type="checkbox"/> No <input type="checkbox"/> Unclear <input type="checkbox"/>
Support for judgment:	
Notes:	

Appendix 4: Notes on re- calculation

Author	Notes on calculation re-adjustments.
Cross Sectional	
Abdissa 2011.	NO re-calculations adjustments were made.
Bélar 2014.	Sample population with GAS tonsillopharyngitis, GGS or GCS and those with symptomatic β -hemolytic were excluded
Brait 2004.	The final GAS carriage denominator was 2140. This number excluded 143 samples, lost due, to withdrawal and various exclusions.
Engel 2012.	NO re-calculations adjustments were made.
Frederiksen 1988.	Study outcomes included both carriage of <i>s. pyogenes</i> and carriage of <i>s. pneumonia</i> . We excluded all calculations of <i>s. pneumonia</i> carriage. Made NO re-calculations adjustments made for GAS carriage.
Gaumer 1972.	
Khemiri 1982.	Both articles were in French and were translated by an independent translator who is not part of the review team. As the reviews we made NO re-calculations adjustments.
Lawal1990.	Sample population with skin lesions were excluded in all calculation
McLaren 1975.	Study outcomes included both GAS carriage and Rheumatic heart disease. We excluded all calculations of Rheumatic heart disease study. There were NO re-calculations adjustments made for GAS carriage.
Mzoughi 2004.	Sample population with acute pharyngitis were excluded in all calculations. While GAS carriage sample population was taken as the controls of this study and NO re-calculations adjustments were made for GAS carriage.
Ransome 1983.	This study reported the prevalence of GAS carriage seasonally, stratified according to ethnicity. To calculate the overall denominator and numerator for GAS carriage, we initially took an average of the denominators and numerators of ethnic groups for only the first season only. This gave us the overall numerator and denominator for that season.
O'Meara 2015.	Sample population who were afrile were excluded in all calculations. While GAS carriage population was as the case controls. For GAS carriage population the authors' recruited 184 children, however, they analysed 161 children. There is no transparent report of what happened to the other 23 children.
Van Staden 1982.	
Van Zyl 1981.	Both articles in Afrikaans and was translated by DB who is part of the review team. NO re-calculations adjustments.
Longitudinal	
El Kholya 1973.	The study reported the percentage of prevalence for GAS carriage each year. We used the only the first year's results for the numerator and denominator calculations.
Ogunbi 1974.	The study reported on skin and throat infection and carriage. We excluded sample population with skin and throat infection, and those who were GAS carriers in the nose, ankle, back and wrist. Only throat carriage was included and they were NO re-calculations adjustments made for GAS carriage.

Appendix 5: Unadjusted Prevalence

Study ID	Region	Country	Design	Outcome Measure (s)	Total # recruited	Withdrawn	Total (N)	Cases of GAS (n)	Prevalence (%)	SEM	Lower CI	Higher CI
Abdissa 2011.	Eastern Africa	Ethiopia	Cross sectional	Prevalence of GAS carriers	941	4	937	91	9.71	0.01	0.08	0.12
Bélard 2014.	Middle Africa	Gabon	Cross sectional	Prevalence of GAS carriers	1050	377	673	48	7.13	0.01	0.05	0.09
Braito 2004.	Eastern Africa	Tanzania	Cross sectional	Prevalence of GAS carriers	2286	143	2140	184	8.56	0.01	0.07	0.99
El Kholya 1973.	Northern Africa	Egypt	Longitudinal	Prevalence GAS carrier & typing of isolates	300	144	156	20	12.82	0.27	0.08	0.191
Engel 2012.	Southern Africa	South Africa	Cross sectional	Prevalence GAS carrier & typing of isolates	950	0	950	31	3.26	0.01	0.02	0.05
Frederiksen1988.	Southern Africa	Zambia	Cross sectional	Prevalence GAS carrier & typing of isolates	246	0	246	46	18.70	0.02	0.14	0.24
Gaumer 1972.	Northern Africa	Tunisia	Cross sectional	Prevalence of GAS carriers	174	0	174	65	37.36	0.00	0.02	0.45
Khemiri 1982.	Northern Africa	Tunisia	Cross sectional	Prevalence of GAS carriers	1008	0	1008	42	4.17	0.01	0.03	0.06
Lawal 1990.	Western Africa	Nigeria	Cross sectional	Prevalence of GAS carriers	4395	0	4395	83	1.89	0.00	0.02	0.02
McLaren 1975.	Southern Africa	South Africa	Cross sectional	Prevalence of GAS carriers	12050	0	12050	627	5.18	0.00	0.05	0.06
Mzoughi 2004.	Northern Africa	Tunisia	Cross sectional	Prevalence GAS carrier & typing of isolates	155	0	155	14	9.03	0.24	0.05	0.15
O'Meara 2015	Eastern Africa	Kenya	Cross sectional	Prevalence of GAS carriers	5300	0	5300	1	0.62	0.00	0.00	0.03
Ogunbi 1974.	Western Africa	Nigeria	Longitudinal	Prevalence of GAS carrier	184	23	161	87	1.64	0.00	0.01	0.02
Ransome 1983.	Southern Africa	South Africa	Cross sectional	Prevalence of GAS carriers	246	0	246	56	11.38	0.02	0.08	0.16
Tewodros 1992.	Eastern Africa	Ethiopia	Longitudinal	Prevalence GAS carrier & typing of isolates	506	0	506	86	16.00	0.02	0.12	0.19
Van Staden 1982.	Southern Africa	South Africa	Cross sectional	Prevalence of GAS carriers	247	0	247	4	1.62	0.01	0.00	0.04
Van Zyl 1981.	Southern Africa	South Africa	Cross sectional	Prevalence of GAS carriers	165	0	165	20	12.0	0.03	0.08	0.18

Supplementary Materials

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	61
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	62
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	67
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	67
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	67
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	76
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	74
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	105
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	67
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	67
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	80
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	80
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	74

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	79
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	78
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	84
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	83
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	90
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	85
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	94
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	90
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	93
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	104
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	106
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	106
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	80

Submission Guidelines for 
Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, RTF, or PDF. Microsoft Word documents should not be locked or protected.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information.
Font	Use any standard font and a standard font size.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file.
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses "Vancouver" style.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

- Beginning section** *The following elements are required, in order:*
- Title page: List title, authors, and affiliations as first page of manuscript
 - Abstract
 - Introduction
- Middle section** *The following elements can be renamed as needed and presented in any order:*
- Materials and Methods
 - Results
 - Discussion
 - Conclusions (optional)
- Ending section** *The following elements are required, in order:*
- Acknowledgments
 - References
 - Supporting Information Captions (if applicable)
- Other elements**
- Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately.
 - Tables are inserted immediately after the first paragraph in which they are cited.
 - Supporting information files are uploaded separately.

Systematic reviews and meta-analyses

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as Supporting Information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Include the PRISMA flow diagram as Fig 1

Include the PRISMA checklist as Supporting Information