AFRO*Strep* (SA): A surveillance system for group A streptococcal infection in South Africa

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AFRO*Strep* (SA): A surveillance system for group A streptococcal infection in South Africa

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Dedication

To the memory of my beautiful grandmother, Lorraine *Lolly* Maureen Barth, who was affected by cardiovascular disease and always took great pride in my research and academic achievements.

To the memory of Professor Bongani M Mayosi, a gifted scientist and inspirational leader who tragically passed away in July 2018. He will be greatly missed for his brilliant mind, infectious enthusiasm, modelling academic excellence, and humility. It was an honour and privilege to be one of his students.
His legacy will live on.

"*There is no problem that hard work can't solve*" – *Bongani Mayosi*

Publications arising from this thesis

- Barth D, Mayosi BM, Jabar A, Engel ME. Prevalence of Group A streptococcal disease in North and Sub-Saharan Africa: A systematic review protocol. BMJ Open, 2015;5:e0086. Available at: https://bmjopen.bmj.com/content/5/8/e008646

- Barth DD, Engel ME, Whitelaw A, Alemseged A, Sadoh WE, Ali SKM, Sow SO, Dale J, Mayosi BM. Rationale and design of the African group A streptococcal infection registry: the AFROStrep Study. BMJ Open, 2016.
  Available at: https://bmjopen.bmj.com/content/6/2/e010248

- Barth DD, Mayosi BM, Badri M, Whitelaw A, Engel ME. 2017. Invasive and non-invasive group A β-haemolytic streptococcal infections in patients attending public sector facilities in South Africa: 2003–2015. Southern African Journal of Infectious Diseases. 2018;33:12-17. Available at: http://dx.doi.org/10.1080/23120053.2017.1376546

Abstract

BACKGROUND

Group A β-haemolytic Streptococcus (GAS), a gram-positive bacterium also known as *Streptococcus pyogenes*, is responsible for a wide range of invasive and non-invasive group A streptococcal diseases. These diseases range from mild infections such as impetigo and pharyngitis to serious diseases, such as streptococcal toxic shock syndrome and necrotising fasciitis. Moreover, GAS may trigger autoimmune diseases following repeated episodes of infection, such as acute rheumatic fever, rheumatic heart disease and acute post-streptococcal glomerulonephritis; these are important causes of mortality and morbidity in developing countries. Prevalence and incidence data on GAS infections from developing countries, which include South Africa, are largely lacking when compared with industrialised nations. In South Africa, GAS infections are not notifiable; thus no surveillance programme exists to capture such information.

AIMS OF THE THESIS

This thesis sought to (1) establish the South African arm of the AFRO*Strep* biorepository and clinical database for patients with invasive and non-invasive GAS infection, (2) identify and summarise all published studies of laboratory-confirmed GAS infection in Africa, (3) describe, from national laboratory data, the incidence of invasive and non-invasive GAS isolates in South Africa and, (4) conduct a prospective, surveillance study in order to determine the molecular typing of GAS disease (including the molecular epidemiology) in Cape Town, South Africa over a 12-month period.

METHODS

A systematic review was conducted on population-based studies reporting on the prevalence of laboratory-confirmed GAS infection among patients living in Africa (Study 1). A retrospective

study of the incidence of GAS infection was conducted on data obtained from the National Health Laboratory Service between 2003 – 2015 (Study 2). The AFRO*Strep* registry and biorepository (based in Cape Town) was established and through passive surveillance, laboratory confirmed invasive and non-invasive GAS cases were collected over a 12-month period. The molecular analysis of invasive and non-invasive infection was determined using *emm* type sequencing to provide insight into vaccine development (Study 3).

RESULTS AND DISCUSSION

The pooled prevalence of GAS pharyngitis in Africa was determined to be 21% (95% CI, 13% to 30%). Our pooled prevalence was lower than previous systematic review findings of Shaikh who reported figures of 37% (95% CI, 32% to 43%) in children of all ages, and 24% (95% CI, 21% to 26%) among those younger than 5 years; nonetheless, GAS is still a significant infection amongst the people of Africa.

The incidence rates of laboratory-confirmed non-invasive GAS infection in the South African public sector appear to have declined over the last 13 years. This observation was in contrast with studies conducted elsewhere since the 1980s, which reported increases in the incidence of GAS infection. Given the possibility that the lower incidence of invasive and non-invasive GAS infection found in our study is due to infrequent submission of specimens for microbiological culture by health practitioners (i.e., ascertainment bias), our findings may be an underestimate of the true burden of disease in South Africa.

In our prospective surveillance study, Group A streptococcus were commonly isolated from pus swabs, blood, deep tissue and aspirates. Common clinical presentations included wound infections (20%), bacteraemia (15%), abscesses (9%) and septic arthritis (8%). Two-hundred and thirty-three isolates were available for *emm* typing, from which 46 different *emm* types were identified. The most

prevalent *emm* types were M76 (16% of isolates), M81 (10%), M80 (6%), M43 (6%), and M183 (6%) and were almost evenly distributed between non-invasive and invasive GAS isolates. Non-invasive and Invasive GAS infections were comparable in terms of prevalence and there was an association with M80 and patients presenting with non-invasive abscesses. When compared against the putative 30-valent vaccine under development, four of our most prevalent *emm* types are not included; vaccine coverage (i.e. vaccine type and non-vaccine type-killing) for non-invasive GAS and invasive GAS infection in our setting was 60% and 59% respectively, notably lower than coverage in developed countries.

CONCLUSION

This work provides evidence for a significantly high prevalence of GAS in Africa. While GAS surveillance in South Africa indicates a declining incidence of group A streptococcal disease in parts of the country over the last thirteen years, the findings may be an underestimate of the true burden of disease, demonstrating the need for accurate and comprehensive surveillance of GAS in South Africa. Finally, this research showed a low potential vaccine coverage in our setting and thus, emphasises the need for a reworking of the potential vaccine formulation to improve coverage in areas where the burden of disease is high.

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List of Abbreviations and Acronyms

AFRO*Strep*	The African group A streptococcal infection registry
GAS	Group A β-haemolytic streptococcus
*i*GAS	Invasive group A streptococcus
non-*i*GAS	Non-invasive group A streptococcus
ARF	Acute rheumatic fever
RHD	Rheumatic heart disease
NF	Necrotising fasciitis
STSS	Streptococcal toxic shock syndrome
APSGN	Acute post-streptococcal glomerulonephritis
Emm Type	M protein gene
MeSH	Medical subject heading
VT	Vaccine type
NVT-K	Nonvaccine type – killing
NVT-NK	Nonvaccine type – no killing
CI	Confidence interval
IQR	Interquartile range
ES	Effect size
N	Number
NS	Not stated
CSF	Cerebral spinal fluid
HIV	Human immunodeficiency virus
YR	Years
PY	Person-years
IR	Incidence rate
RD	Rate difference

HREC	Human Research Ethics Committee
PRISMA-P	Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols
PROSPERO	International prospective register of systematic reviews
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
REMEDY	Global Rheumatic Heart Disease Registry
ASAP	Awareness Advocacy Surveillance and Prevention Programme for Rheumatic Heart Disease
NHLS	National Health Laboratory Service
GSH	Groote Schuur Hospital
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
WHO	World Health Organisation
CDC	Centres for Disease Control and Prevention

1. CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

Introduction

Group A β-*haemolytic Streptococcus* (GAS), also known as Streptococcus *pyogenes,* is a gram-positive bacterium. The complications of GAS infection have been described in more detail in Section 1.2. Briefly, GAS infection may cause skin, mucosal, systemic and autoimmune diseases (Cunningham, 2000). Repeated GAS skin and pharyngeal infections may lead to serious autoimmune diseases, such as acute post-streptococcal glomerulonephritis, acute rheumatic fever (ARF) and rheumatic heart disease (RHD) (Cunningham 2012; Carapetis *et al.,* 2005).

Prevalence and incidence data on GAS from developing countries, which include South Africa, are largely deficient when compared with industrialised nations (Carapetis *et al.,* 2005). In South Africa, GAS infections are not notifiable (to the Department of Health) and hence, no surveillance programme exists in South Africa to capture GAS infection information.

This PhD project forms part of the Mayosi-led, Stop Rheumatic Heart Disease ASAP Programme (Robertson *et al.,* 2006), which has as one of its aims, the documenting of the epidemiology of GAS-related diseases by collecting comprehensive clinical and microbiological data on GAS infections in South Africa.

This thesis is nested within the AFRO*Strep* study (Barth *et al.,* 2016), which focuses on the establishment of a registry and biorepository for group A streptococcal infection in Africa. The pilot phase of AFRO*Strep* will focus on South Africa. This thesis serves to establish the Cape Town arm describing the epidemiology from passive surveillance of laboratory data on GAS isolated from patients with invasive (*i*GAS) and non-invasive (non-*i*GAS) group A streptococcal diseases.

Here, I summarise the current knowledge related to disease estimates for GAS with a particular focus on South Africa. I also review the literature with regard to the natural history of GAS infection and the need for public health surveillance systems. Finally, I present the Cape Town model for collecting non-invasive and invasive clinical and epidemiological information on GAS.

1.1 *Streptococcus Pyogenes*

Group A β-haemolytic *Streptococcus* (GAS), also known as *Streptococcus pyogenes*, is a gram-positive bacterium. Gram-positive refers to the chemical and physical properties of the cell wall, characterised by a thick cell wall protein called peptidoglycan. Gram-positive bacteria retain the crystal violet dye and the cells stain purple under the microscope (Baron, 1996).

1.1.1 History of group A *streptococcus* identification

The first description of streptococcal infection was described by the Austrian surgeon, Theodor Billbroth, in 1874, in patients presenting with erysipelas and wound infections (Billroth, 1874, 1877). He described the organism "kettenkokken" to be either isolated in pairs or in chains of four to twenty or more links The formal discovery of streptococci in history was recorded in 1879, when the organism was isolated from the uterus and blood of women with puerperal fever by Louis Pasteur (Alouf & Horaud 1997). He showed that streptococcus was responsible for the highest mortality rates of women and new-borns at the time. In 1884, Friedrich Julius Rosenbach, who studied bacteria isolated from suppurative lesions, further refined the name streptococcus to *streptococcus pyogenes* (*pyo*, pus and *genes*, forming) (Evans 1936).

1.1.2 Methods for identification and classification

Streptococcus pyogenes, also referred to as Streptococcus haemolyticus, is so named due to the pattern of haemolysis (seen as a clear zone around colonies) observed on 5% sheep blood agar plates, incubated under anaerobic conditions (Figure 1.1).

Figure 1.1 Blood agar plate with haemolysis surrounding streptococcus colonies. Photo: M Engel 2012

Dr Rebecca Lancefield, in 1933, subdivided the streptococci into groups based on differences in the surface antigen of the bacteria (Lancefield, 1933); Group A was classified by strains from human diseases; Group B by strains from bovine and dairy sources; Group C by various other animal sources etc. This was considered to be one of the most important advances in streptococci research since *streptococcus pyogenes* was identified as the organism responsible for streptococcal infections in humans and confirming the causative role in the development of Acute Rheumatic Fever (ARF) (Stollerman, 1975). These strains were then further classified based on the presence of a surface protein called the M protein (Figure 1.2).

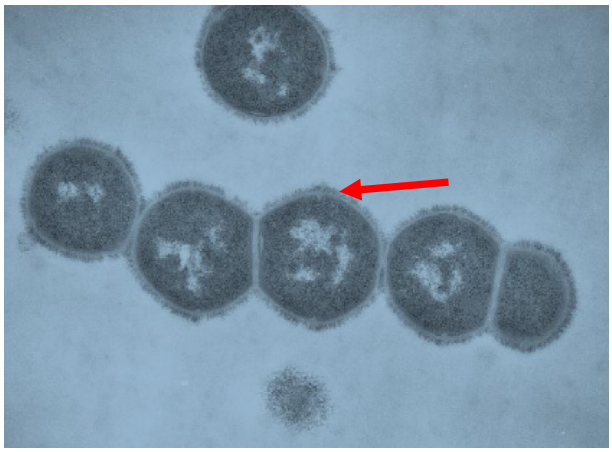


Figure 1.2 Electron micrograph of a chain of streptococci showing the surface M protein (Fischetti, 2016).

Lancefield was able to show more than 50 different GAS M types during her career (Lancefield, 1933). Since then, M protein serological typing was replaced by sequence typing of the M protein (*emm*) gene and more than 200 *emm* types have subsequently been identified (Facklam *et al.*, 1999).

1.1.3 M protein

The M protein consists of two polypeptide chains extending the cell wall of the bacteria, and composed of 4 blocks of amino acid residues (Fischetti, 1989). The N-terminus of the M protein (Figure 1.3) is hypervariable, thus allowing for the differentiation of GAS strains using specific M typing antisera (Cunningham, 2000). An *emm* type number is assigned to a sequence (e.g. "e*mm* 2"). Where there are differences in the 5' region of a particular type, this will determine the subtype e.g. *emm2.1*. *Emm* sequence typing is the preferred method to study the molecular epidemiology of GAS and has been widely applied in many regions of the world (Beall *et al.*, 1996).

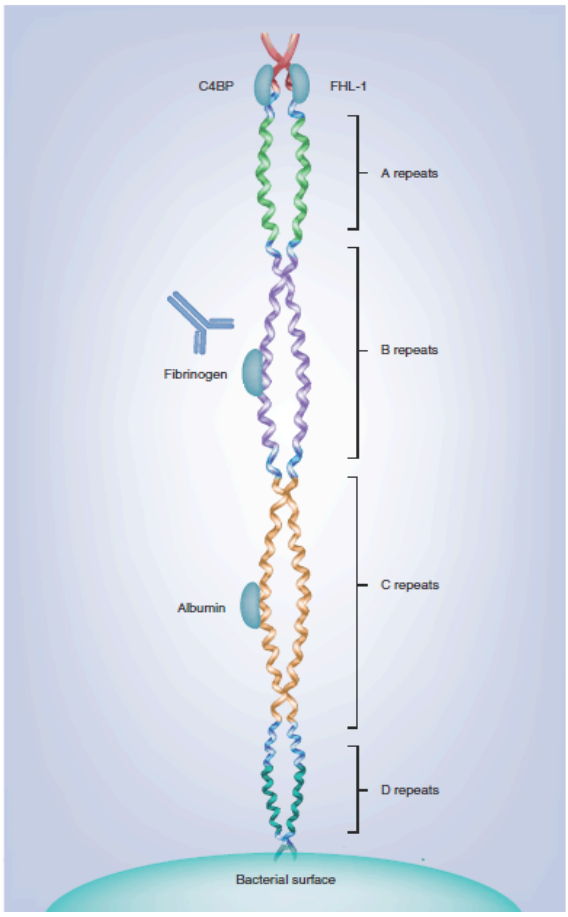


Figure 1.3 Schematic illustration of M protein. Individual M proteins form coiled coils, which extend from the bacterial surface. Two coils wind around each other, forming a dimer that is stabilized by intermolecular interactions between amino acids in both strands. The hypervariable N-terminal region, lacking a helical structure, is followed by the A, B, C and D repeat regions (Smeesters *et al.*, 2009).

1.2 Group A streptococcal infections and diseases

GAS causes skin (pyoderma) and mucosal diseases (pharyngitis) (Cunningham, 2000). Pyoderma is characterised by infection of the epidermis, i.e. blisters and inflammation of the skin, while GAS pharyngitis causes symptoms that include tonsillar swelling and exudate, swollen cervical lymph nodes, etc. (Cunningham, 2000). GAS can also be invasive, causing Streptococcal Toxic Shock Syndrome (STSS), which can occur with an infection at any site in the body. The disease is characterized by the occurrence of shock and failure of organs, such as the kidneys, lungs, liver, and brain, early in the course of GAS infection. Repeated GAS pharyngeal and skin infections following the initial infection may lead to serious GAS-related illnesses, which include acute post-

streptococcal glomerulonephritis (APSGN), ARF and RHD (Carapetis, Steer, *et al.*, 2005). RHD is associated with significant mortality and morbidity in children and young adults living in developing countries.

GAS infections can be divided broadly into three categories, namely non-invasive, invasive and autoimmune complications. Table 1.1 below lists the type of GAS diseases in each category.

Table 1.1. Non-invasive, invasive and autoimmune complications of group A streptococcus

Non-invasive infections	Invasive infections	Autoimmune complications
Pharyngitis	Bacteraemia	Acute Rheumatic Fever/ Rheumatic Heart Disease
Scarlet fever*	Cellulitis	
Impetigo	Necrotising fasciitis	Acute post-streptococcal glomerulonephritis
Erysipelas	Streptococcal toxic shock syndrome*	
	Puerperal sepsis	

**Toxin mediated diseases*

1.2.1 Non-invasive infections

Non-invasive skin infections are infections that occur in the outer layers of the skin, e.g. the epidermis.

1.2.1.1 Pharyngitis and scarlet fever

The most common cause of bacterial sore throat is caused by GAS; it mainly affects children between the ages of 5 and 15 years of age (Danchin *et al.*, 2007; Olubodun 1994). Outbreaks of GAS pharyngitis can affect people across all ages, especially in places like schools, households and public spaces where there are crowds and close contact with people (Wessels, 2016).

The clinical manifestation of streptococcal pharyngitis is characterised by a sudden onset of sore throat, fever and malaise (Wessels, 2016). Patients experience pain when swallowing, caused by the swollen and tender cervical lymph nodes. While most cases of sore throat are of viral etiology, symptoms of GAS pharyngitis may include fever, erythema and oedema of the posterior pharynx and tonsils which may be covered in yellowish or white exudate (Mandell, 2005) (Figure 1.4). If no treatment is administered, sore throat will usually self resolve within 3 – 6 days, however, throat cultures may remain positive for GAS for several weeks post the resolution of symptoms (Catanzaro *et al.*, 1954).

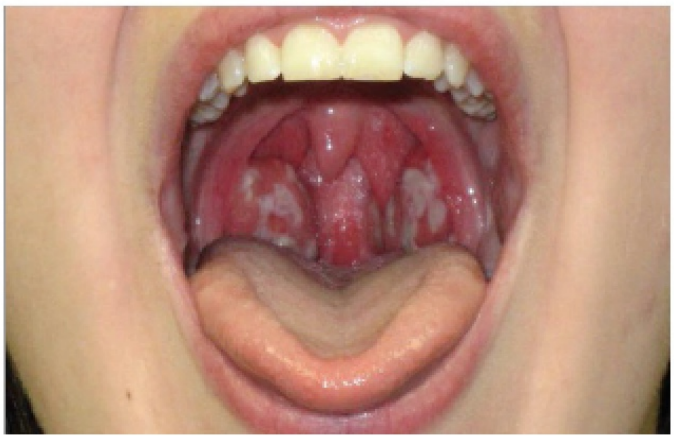


Figure 1.4. Patient with exudative GAS pharyngitis exudate (Mandell, 2005)

Although GAS is considered to be an extracellular pathogen, studies have shown the internalisation of GAS by human epithelial cells (LaPenta *et al.,* 1994; Schrager *et al.,* 1996). Complications of GAS pharyngitis include autoimmune diseases, such as APSGN.

Emm types commonly associated with pharyngitis include *emm* types 1, 3, 5, 6, 12, 14, 17, 19, and 24 (Cunningham 2000; Johnson *et al.*, 1992; Wessels 2016).

Scarlet fever is a clinical syndrome represented by a rash together with a group A streptococcal infection, commonly pharyngitis (Bisno *et al.,* 2002). The rash appears almost immediately

following infection and is characterised by minute papules, giving the skin a sandpaper-like feel. The rash last for 6 to 9 days. The tongue may also appear to look like a strawberry, characterised by its bright red colour and enlarged papillae on the surface of the tongue (Cunningham, 2000). Scarlet fever is thought to be caused by pyrogenic exotoxins; GAS strains containing the gene for one or more of 3 pyrogenic exotoxins A, B and C, cause scarlet fever (Bohach *et al.,* 1990). These exotoxins, also known as streptococcal superantigens, are responsible for the strawberry tongue and rash that is clinically presented by scarlet fever. *Emm* types associated with scarlet fever are similar to those described in GAS pharyngitis (Cunningham, 2000; Shulman and Tanz, 2010).

Treatment of pharyngitis/scarlet fever

Penicillin VK (orally) or benzathine penicillin (intramuscularly) remain the first-line antibiotic of choice for the treatment of GAS pharyngitis in South Africa (Brink *et al*., 2004), given that GAS isolates remain susceptible to penicillin antibiotics globally. Amoxicillin is recommended as an alternative, given its similar effectivenes to penicillin (Bisno *et al.,* 2002). The intramuscular administration of penicillin has been shown to be more effective than oral penicillin, by reducing the occurrence of ARF by as much as 91% (Manyemba 2003). For patients who are allergic to penicillin, alternatives such as clarithromycin, clindamycin, cephalexin, cefadroxil and azithromycin have been recommended (Shulman *et al*., 2012).

1.2.1.2. Pyoderma and other skin infections

It is important to understand the anatomy of the skin in order to differentiate skin disease caused by GAS. Figure 1.5 illustrates the different layers of the skin and deep tissues as these relate to the occurrence of different GAS conditions.

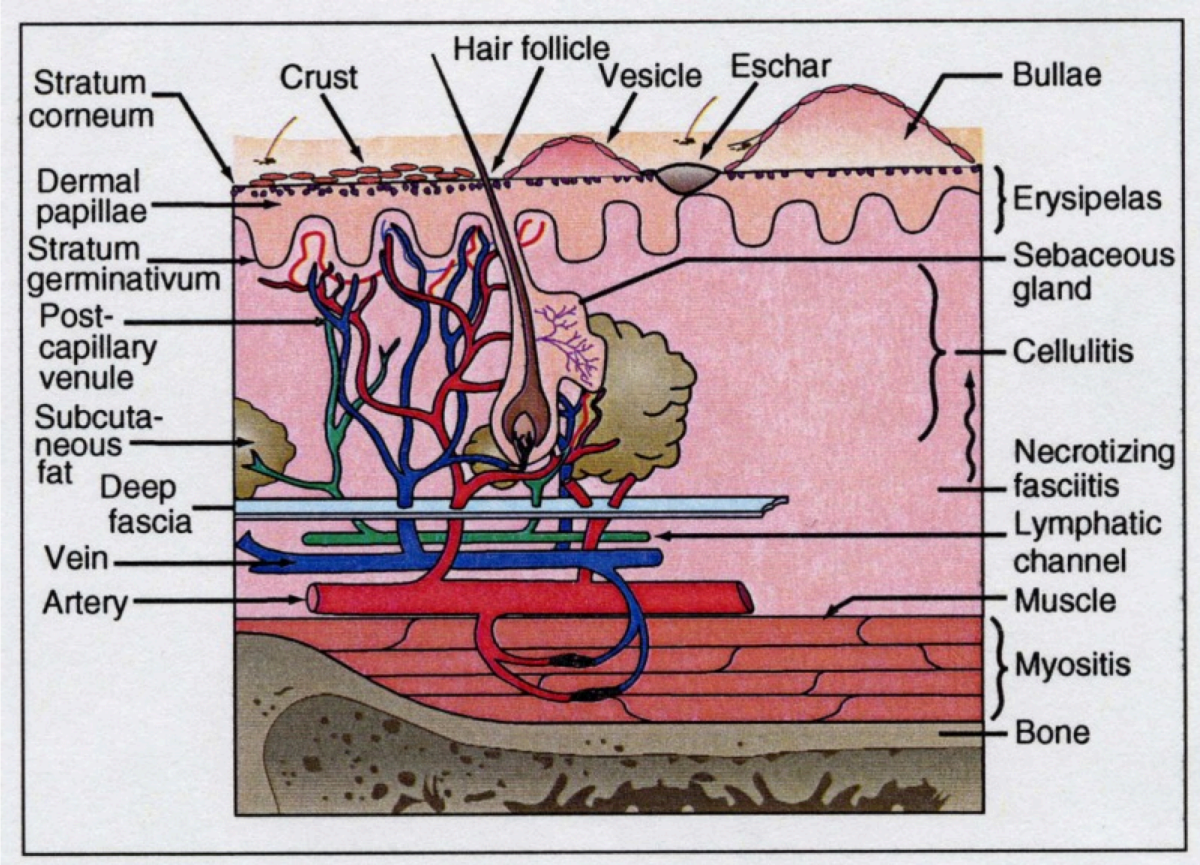


Figure 1.5. Skin structures and different GAS infections. (Source: Harrison's Principles of Internal Medicine, 19th edition) (Stevens, 2015)

1.2.1.3 Impetigo

Impetigo is limited to the outer layer of the skin and does not invade the epidermis (Bisno and Stevens, 1996). Impetigo is highly contagious and clinically presents as pustules on the surface of the skin, often the extremities or the face. These pustules may rupture and thick honey-coloured crusts may develop (Cole and Gazewood, 2007). Systemic symptoms are not often present, although lymphadenitis may occur. Impetigo, if left untreated, self-resolves after a few weeks without scarring. Strains that cause pyoderma are different to those that cause pharyngitis and ARF (Shulman and Bisno, 2014). GAS may penetrate the skin though abrasions and other types of trauma. The global burden of impetigo infection is estimated to be approximately 111 million cases per annum (Carapetis *et al.,* 2005).

1.2.1.4 Erysipelas

Erysipelas results from GAS penetrating the epidermis through abrasions and other skin traumas. Erysipelas affects the superficial epidermal layer of the skin, and the borders of infection are well distinguished from adjacent unaffected normal tissue. Furthermore, erysipelas is distinguished from other skin infections by its bright red appearance and raised lesions above the surrounding skin (Stevens and Bryant, 2016).

Treatment of skin lesions

The management of superficial GAS skin infections involves gentle cleansing of the wounds and honey-coloured scabs with a wash cloth and antibiotic soap in conjunction with antibiotic treatments, administered either orally and/or in the form of ointment (Walker *et al.,* 2014).

1.2.2 Invasive infections

Invasive GAS infection is defined by the entry of GAS into the blood stream, deep tissues and other sites in the body that are usually considered to be sterile (Invasive Group A streptococcus Sub-Committee 2006; Sharkawy *et al.,* 2002). Patients presenting with severe invasive GAS diseases, e.g. STSS, are associated with high rates of mortality.

The global incidence for invasive GAS infections is estimated to be around 600,000 cases per year and responsible for 160,000 deaths per year (Carapetis *et al.,* 2005).

1.2.2.1 Cellulitis

Cellulits occurs in the subcutaneous deep tissues of the skin (Bisno and Stevens, 1996). It results from infected burns, wounds or mild trauma. Clinical presentations include erythema (redness of the skin), tenderness, swelling and localised pain. Systemic manifestations include fever and

malaise. In addition, there may be associated bacteraemia and lymphangitis. The appearance, to a moderate extent, differs from erysipelas; lesions are not raised, the boundary between involved and uninvolved tissues is less defined, and lesions appear more pink than red in colour.

1.2.2.2 Necrotising fasciitis

Necrotising fasciitis (NF), also known as a "flesh-eating" disease, occurs when the deeper subcutaneous tissues and fascia are infected with GAS (Figure 1.5) (Stevens & Bryant 2016). NF is characteristed by the rapid spread of necrosis (also refered to as gangrene) of the skin and underlying organs. NF is initiated at the site of trauma or during a surgical incision. The lesion initially appears as mild erythema; however, 1 – 3 days later, the inflammation is more prounounced and the skin becomes purple in appearance. A large blister containing hemorrhagic or yellow fluid appears. Bacteraemia is often present and, following infection, an abscess may occur. By the fourth to fifth day, gangrene is evident in the affected skin and the spread is almost uncontainable.

The mortality rate associated with patients presenting with NF is high, even when appropriate treatment is administered (Stevens and Bryant, 2016). The delay in the correct diagnosis may result from the absence of clinical clues; it may only be recognised when the patient presents with STSS. The successful management of patients with NF is dependant on early recognition. Furthermore, severe pain and fever are among the earliest clinical manifestations and are important clues that point towards NF (Stevens and Bryant, 2016).

The *emm* types associated with NF are 1, 3 and 28 (Olsen and Musser, 2010).

1.2.2.3 Streptococcal toxic shock syndrome

STSS is toxin mediated and may present with other invasive GAS diseases in response to the production of superantigens, characterised by a sudden onset of hypotension, fever, shock and multiple organ failure (Lappin and Ferguson, 2009). These GAS antigens engage T-cell receptors, activating large numbers of T-cells and resulting in a massive cytokine reponse by T-cells; antigen-presenting cells cause tissue damage, intravascular thrombosis and organ failure (Lappin and Ferguson, 2009; O'Loughlin *et al.,* 2007).

The common sites of entry for GAS include the pharynx, mucosa, vagina and skin (Walker *et al.,* 2014). Surgical procedures have also provided entry points for GAS infection. An additional risk factor for STSS includes *emm* types 1 and 3 that have been associated with an increased incidence of STSS (Kiska *et al.,* 1997).

1.2.2.4 Bacteraemia

Bacteraemia refers to the presence of GAS in the blood stream, characterised by a sudden onset of fever, chills, nausea and vomiting, resulting from the pro-inflammatory cytokine response. The direct pathway into the blood stream may occur as a result of childbirth (puerperal sepsis), injury or as a consequence of superficial infection or colonisation of the skin or throat (Johansson *et al.,* 2010; Walker *et al.,* 2014).

1.2.2.5 Septic Arthritis

Septic arthritis refers to the presence of bacteria in the joint through direct inoculation or via the bloodstream. Patients with septic arthritis often present with hot, swollen or tender joints with a reduced range of movement (Gupta *et al.,* 2001).

1.2.2.6 Puerperal sepsis

Puerperal sepsis is an infection of the genital tract which occurs during labour or within 42 days of the postpartum period (Van Dillen *et al.,* 2010). Puerperal sepsis often presents with fever, pelvic pain, vaginal discharge and a delayed reduction in the size of the uteris. Puerperal sepsis is responsible for at least 75,000 maternal deaths per year occurring in predominantly low-income countries (Maharaj, 2007). Risk factors for puerperal sepsis include home birth in non-sterile environments, poor nutrition, prolonged labour with multiple vaginal examinations and caesarean section births (Kramer *et al.,* 2009; Maharaj 2007).

1.2.3 Mechanisms of invasion

Phagocytosis, the process by which a cell engulfs a solid particle (known as a phagosome), is one of the primary human defence mechanisms. GAS has many virulence factors; the four main mechanisms are adherence, antiphagocytosis, colonisation, and the production of pyrogenic exotoxins (Bisno *et al.,* 2003).

Prior to the colonisation and internalisation of GAS, adhesion to the host epithelial cells has been observed (Bisno *et al.,* 2003). Adhesins present in GAS include: pili, M protein, fibronectin binding properties, and lipoteichoic acid (Courtney *et al.,* 2002). Streptococcal pili have been shown to play a role in adhesins in GAS infections (Manetti *et al.,* 2007). The M protein adheres to Hep-2 cells in tissue cultures and skin keratinocytes (Wang and Stinson, 1994). Fibronectic binding protein, e.g. F1, adheres to cutaneous Langerhans cells (Hanski and Caparon, 1992). Lipoteichoic acid adheres to fibronectin in human buccal epithelial cells. Furthermore, anti-lipoteichoic acid serum has been shown to block colonisation and infection of the upper respiratory tract in mice (Courtney *et al.,* 2002).

GAS has the ability to avoid the natural defense mechanisms of the human body by means of an antiphagocytosis mechanism. Interference with opsonisation via the alternate complement pathway is achieved through the antiphagocytic properties of the M protein (Bisno, 1979). Control factors are further complemented by the binding of the M protein and other host proteins, preventing the activation of the alternate pathway and thus protecting the bacteria from host phagocytosis by polymorphonuclear leukocytes (Bisno *et al.,* 2003). In addition, the M proteins also binds to the Fc region of IgG to invade host phagocytosis. In addition, a capsule comprising hyaluronic acid also achieves an antiphagocytic effect (Wessels *et al.,* 1991). Encapsulated GAS are able to resist phagocytosis, while those lacking the capsule are susceptible to phagocytosis (Wessels *et al.,* 1991).

Colonisation of GAS is achieved by factors that evade immunoglobulins and the complementing cascade (Courtney *et al.,* 2002). The production of cytolytic toxins may increase, depending on whether or not there is a good nutrient supply, thus resulting in dominance over normal flora. C5a peptidase, produced by GAS, inactivates C5a, a component of the complement pathway, thereby minimising the influx of neutrophils and increasing survival (Courtney *et al.,* 2002; Walker *et al.,* 2014).

Exotoxins, namely streptolysin O and streptolysin S, play an important role in the pathogenesis of GAS diseases (Sumby *et al.,* 2005). Streptolysin O is ineffective in the presence of oxygen, and is thus lethal to erythrocytes and toxic to polymorphonuclear leukocytes, platelets, tissue-cultures and lysosomes. Similarly, Streptolysin S (which is not affected by oxygen) has a destructive effect on polymorphonuclear leukocytes, platelets, tissue-culture cells and lysosomes (Bisno *et al.,* 2003).

Treatment of invasive disease

Management of severe invasive GAS disease includes hemodynamic stabilisation and specific antimicrobial therapy (Wong and Stevens, 2013). If NF is suspected, prompt surgical intervention is required and fasciotomy or debridement may be necessary (Young *et al.*, 2006). Furthermore, penicillin G should be administered intravenously (3 to 4 million units every 4 hours), together with intravenous clindamycin (600 to 900mg), every 6 to 8 hours, for 10 to 14 days (Allen and Moore, 2010).

1.2.4 Sequelae of GAS

1.2.4.1 Acute rheumatic fever/rheumatic heart disease

Repeated GAS infections, such as pharyngitis, can give rise to auto-immune diseases, such as ARF and RHD. ARF/RHD is the most serious autoimmune sequela of GAS infection. The pathogenesis of RHD is thought to be initiated by molecular mimicry of the M protein to the human cardiac myocin, amongst others, resulting in permanent heart valve damage (Zabriskie, 1967) (see Figure 1.6). Briefly, following a GAS adhesion and invasion of the pharyngeal epithelial cells, GAS antigens activate both B and T cells. Molecular mimicry between GAS carbohydrate or serotype-specific M protein and the host heart, joint or brain tissues can lead to an inappropriate

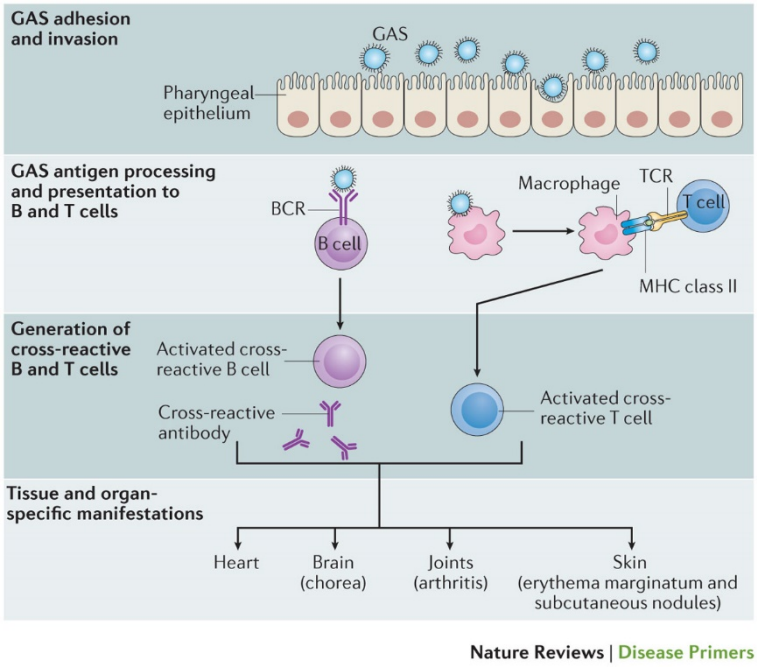


Figure 1.6. Generation of a cross-reactive immune response in ARF (Carapetis *et al.*, 2016)

autoimmune response, which causes the major manifestations of ARF. Damaged cardiac tissue, as a result of repeated episodes of ARF, leads to the development of RHD.

Prevention

The early detection, diagnosis and treatment of GAS pharyngitis with penicillin prevents the first attack of ARF (referred to as primary prevention) (Marijon *et al.*, 2012). If the first episode of ARF is not prevented, further episodes of a GAS pharyngitis may increase the chances of developing RHD. Secondary prevention refers to the prevention of episodes of ARF (Manyemba and Mayosi, 2003). Penicillin is administered intramuscularly as prophylaxis to prevent further episodes of ARF and worsening of RHD. Table 1.2 details the guidelines of the World Health Organisation (WHO) for primary and secondary prevention strategies in the treatment of ARF/RHD. Table 1.3 lists the duration of secondary prevention relating to the severity of carditis.

Table 1.2. WHO Guidelines for primary and secondary prevention of RF/RHD (World Health Organisation, 2004)

Antibiotic	*Administration*	*Dose*
Primary Prevention		
Benzathine benzylpenicillin	Single intramuscular injection	1200000 units 600000 units <27kg
Phenoxymethyl penicillin (Penicillin VK)	Orally 2-4 times/day for 10 full days	Children: 250mg bid or tid. Adolescents or adults: 250mg tid or qid, or 500mg bid
Amoxicillin	Orally 2-3 times/day for 10 full days	25-50mg/kg/day in three doses. Total adult dose is 750-1500mg/day
First-generation cephalosporins	Orally 2-3 times/day for 10 full days	Varies with agent
Erythromycin ethylsuccinate	Orally 4 times/day for 10 full days	Varies with formulation. Available as the stearate, ethylsuccinate, estolate or base
Secondary Prevention		
Penicillin	Intramuscular injection. Every 4 weeks (28 days)	Children: < 20kg: 600 000 U Children >20kg or adults: 1,200 000U
Penicillin VK	Orally bid	250 mg bid
Erythromycin	Orally 2-4 divided doses per day	40mg/kg per day; 400mg adolescents and adults bd.

mg, milligrams kg, killograms; bid, twice daily; tid, three times daily; qid, four times daily

Table 1.3. Period of dosage for secondary prevention (World Health Organisation, 2004)

Category	Duration of prophylaxis
All persons with ARF with no or mild carditis	Minimum of 10 years after most recent episode or age 21
All persons with ARF and moderate carditis	Minimum of 10 years after most recent episode or age 30
All persons with ARF and severe carditis	Minimum of 10 years after most recent episode or age 30 and then specialist review for need to continue. Post-surgical cases - life long

1.2.4.2 Acute post-streptococcal glomerulonephritis

APSGN is an immune disorder of the kidneys that is characterised by oedema, urinary abnormalities, hypertension and decreased levels of complement components in the serum (Shulman and Bisno, 2014). Risk factors for APSGN include poverty, poor hygiene and overcrowding (Shulman & Bisno 2014; Steer *et al.,* 2007). The incidence of APSGN is estimated to be approximately 470,000 cases per year and the cause of approximately 5,000 deaths per year, globally (Carapetis *et al.,* 2005). Finally, the *emm* types causing APSGN are different to the *emm* types causing ARF/RHD and they are associated with nephritogenic strains of GAS, e.g. 1,4, 12, 49, 55, 57, and 60 (Shulman & Bisno 2014; Shulman & Tanz 2010).

Treatment of APSGN

Antibiotic treatment of APSGN does not directly affect the clinical pathway; however, antibiotic therapy may eliminate GAS strains causing infection and reduce the risk of transmission. Penicillin is the antibiotic of choice, thus treatment is directed at the complications of the disease. Most children with APSGN will completely resolve symptoms of edema and kidney function following treatment. (Walker *et al.,* 2014).

1.3 The epidemiology of GAS disease

Streptococcus pyogenes is responsible for a wide range of diseases that are prevalent worldwide (Bailie *et al.,* 2005; Steer *et al.,* 2007). In 2005, Carapetis *et al.,* published a report, commissioned by the WHO, summarising the burden of GAS diseases in the world (Carapetis *et al.,* 2005). In this report, the prevalence of severe GAS disease is estimated to be 18.1 million cases with an annual incidence of 1.78 million cases, and responsible for approximately 517,000 deaths each year, globally (Carapetis, Steer, *et al*., 2005).

Together, ARF and RHD (comprising the greatest burden of GAS-related disease) affect around 15.6 million people, with 282,000 new cases (Carapetis *et al.,* 2005), and lead to the death of at least 233,000 people worldwide per annum (Carapetis *et al.,* 2005). Africa carries the highest burden of

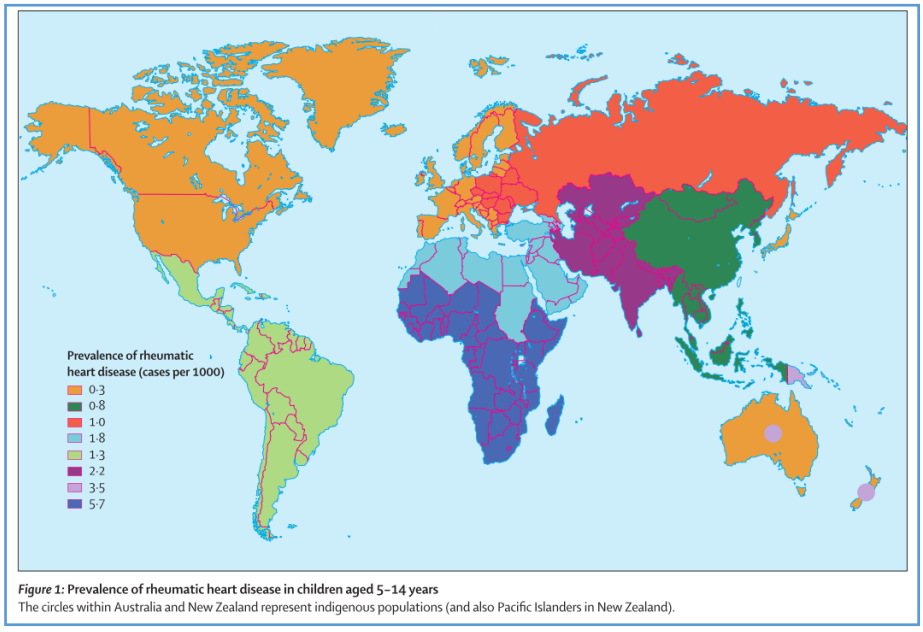


Figure 1.7. The global prevalence of rheumatic heart disease (Carapetis *et al*., 2005).

the disease compared with other regions (Figure 1.7). As a result of the failure to execute comprehensive prevention programmes, ARF and RHD are endemic in the developing countries of the world (Remenyi *et al.,* 2013). Approximately 40 – 60% of ARF episodes result in RHD (Allen 1982; Carapetis *et al.,* 2000; Ralph & Carapetis 2013) . The burden of invasive GAS disease

was reported to be 663,000 new cases and 163,000 deaths per annum. Furthermore, the prevalence of GAS pyoderma was estimated to be 111 million cases and over 616 million incident cases of GAS pharyngitis per year (Carapetis, Steer, *et al.*, 2005).

In the developed world, the incidence of many diseases has steadily declined; however, in many developing countries, the burden of *streptococcus pyogenes* continues to linger, causing millions of deaths every year (Carapetis, Steer, *et al.*, 2005), the majority of which are attributable to RHD. The epidemiology of GAS-related disease remains poorly understood, compared with other infectious diseases. Many countries have subsequently legislated *i*GAS infections as a notifiable condition, thus establishing *i*GAS surveillance.

Increases in the incidence of GAS diseases, particularly *i*GAS diseases, have been observed globally since the 1980s (Hoge *et al.,* 1993; Lynskey *et al.,* Lawrenson and Sriskandan, 2011). Outbreaks of infection and GAS sequelae have been reported during this time (Efstratiou, 2000) with similar increases also observed elsewhere (Darenberg *et al.,* 2013; Lamagni *et al.,* 2009; Report 2014). These increases in the incidence of *i*GAS infections have been associated with specific strains, leading to the possibility that these strains may represent more virulent properties, thus giving rise to the outbreak. Of interest, in most developing countries, the *emm1* strain was found to be dominant among *i*GAS infections (O'Loughlin *et al.,* 2007). The fluctuations in the incidence of *i*GAS infection vary by geographic region and time, and may be a reflection of the population's susceptibility to particular strains or a variation in the predominant strains (O'Brien *et al.,* 2002), which may also lead to fluctuations in the severity of infections and outcomes.

According to the WHO, GAS ranked as the ninth leading cause of human mortality on the global scale. The majority of deaths were attributed to invasive GAS infections and RHD (World Health Organisation, 2005) (Figure 1.8).

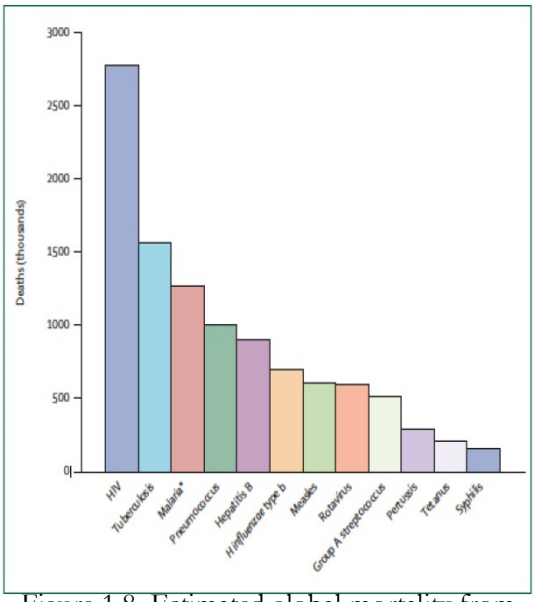


Figure 1.8. Estimated global mortality from various pathogens (Carapetis *et al.,* 2005)

In the developed world, a decline in the prevalence and incidence of ARF/RHD has been observed over the last 150 years as a result of improved living conditions and the extensive use of penicillin for the treatment of GAS pharyngitis (Carapetis, Mcdonald, *et al.*, 2005). Conversely however, developing countries, which account for 80% of the world's population, continue to experience high cases of ARF/RHD affecting some 2.4 million children aged five to fourteen years old, residing in developing countries (Carapetis *et al.,* 2005). The prevalence of GAS infection is higher in children due to multiple exposures, e.g. at schools, and host immunity. Higher rates of infection have also been observed among the elderly.

1.4 *Emm* type prevalence

In a recent review, Steer *et al.,* summarise the distribution of *emm* types in the world (Steer *et al.,* 2009). As previously mentioned (in Section 1.1.3), because of the diversity of the M protein, certain *emm* types have been associated with *i*GAS infections. In the developed world, *emm*1, *emm*3, *emm*12 and *emm*28 have been associated with *i*GAS disease (Creti *et al.,* 2007; O'Loughlin *et al.,* 2007; Steer *et al.,* 2009). Furthermore, *emm*1 has also been strongly associated with pharyngitis.

Many developed countries have undertaken *emm* typing surveillance over the last decade. The results from the Steer review have highlighted differences in the molecular epidemiology of GAS between developing and developed countries (Steer *et al.,* 2009). The *emm* types found to be circulating in developing regions were more diverse and did not have dominant *emm* types compared with those seen in developed regions. Figure 1.9 illustrates differences in *emm* type distribution between (A) developed countries, (B) Africa and (C) the Pacific region. The need for further studies looking at the molecular epidemiology of GAS is thus warranted in developing countries to understand the data better.

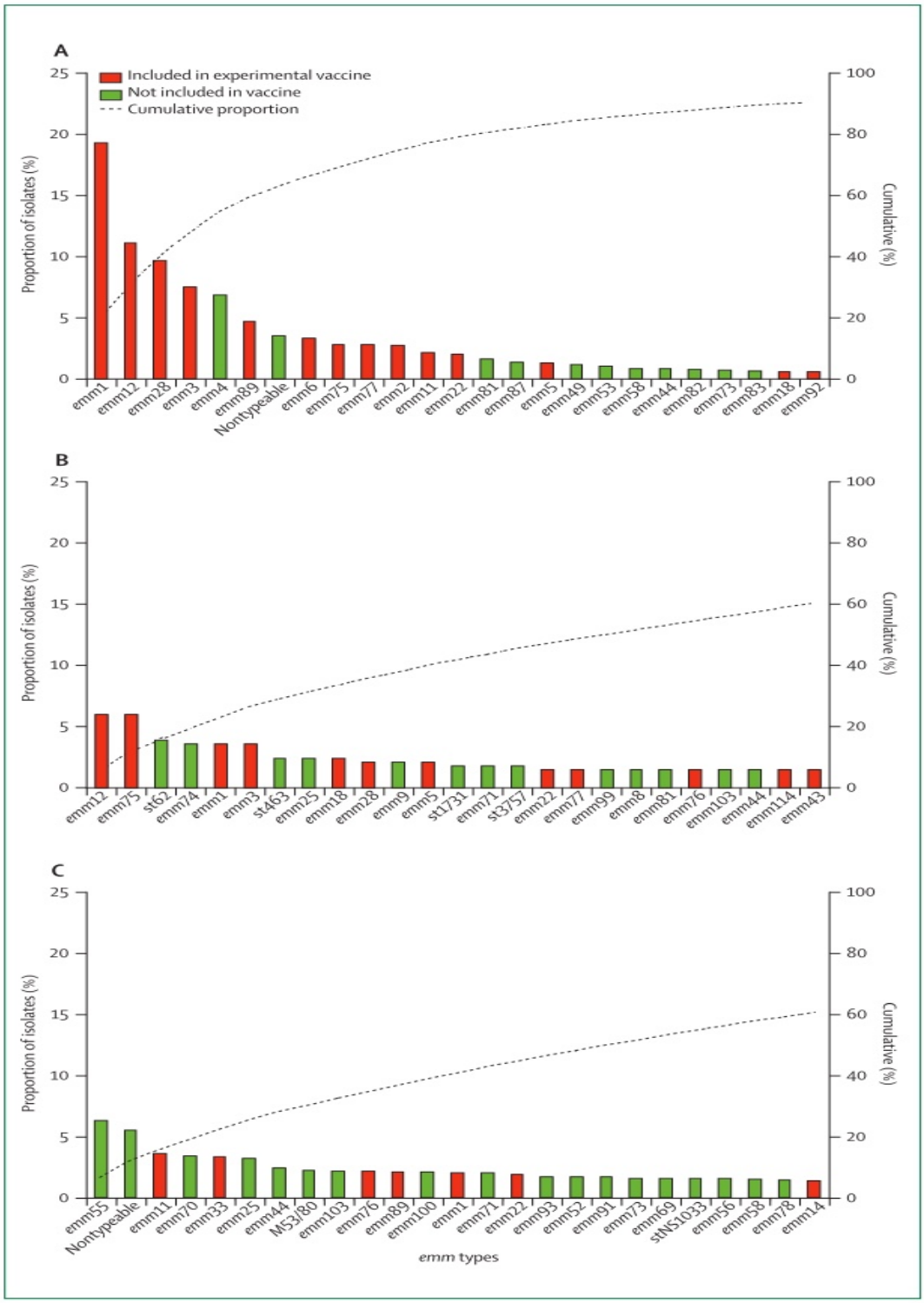


Figure 1.9. Proportions of the 25 most common *emm* types in (A) high income countries, (B) Africa, and (C) the Pacific regions (Steer *et al.,* 2009).

1.5 Risk factors associated with group A streptococcus

The highest prevalence rates for GAS have been observed among people in the lower socio-economic status category. A few studies reported higher prevalence estimates in environments considered to be of lower socioeconomic status when compared with studies in areas with a higher socioeconomic status (Carapetis *et al.*, 2005; Engel 2012).

Seasonal peaks have also been observed in a few studies. In a prospective (Active Bacterial Core) surveillance study of *i*GAS, over an eight-year period, consistent peaks in winter and early spring were observed (Nelson *et al.,* 2016). Similar peaks were also seen in European countries (Darenberg *et al.,* 2013; Lamagni *et al.,* 2008). GAS pharyngitis is seen to peak in autumn and winter (Cunningham 2000; Stollerman 1997). These seasonal variations are thought to be the result of a combination of concurrent viral infections, overcrowding, and exposure to children with pharyngitis (Factor *et al.*, 2003).

Studies on GAS and its sequelae, ARF and RHD, have observed higher rates of disease in females compared with males (Carapetis *et al.,* 2005). The reasons for this observation are not clearly understood.

Host factors associated with an increased risk of *i*GAS infections include HIV infection (Davies *et al.,* 1996), history of injectable drug use (Passaro *et al.,* 2002), diabetes, cardiac disease and cancer (Factor *et al.,* 2003). Environmental factors with an increased risk of *i*GAS infection are related to the presence of a child with a sore throat as well as household size (Factor *et al.,* 2003).

1.6 Primary prevention

1.6.1 Vaccines as a prevention strategy

Efforts to prevent and control GAS disease have been focussed on vaccine development, an important component of which requires an understanding of the burden and distribution of the *emm* types causing the disease. The current data are mostly informed by developed countries.

GAS-related diseases are endemic in developing countries of the world due to the failure to apply comprehensive prevention programmes (Remenyi *et al.*, 2013). Thus, the introduction of safe, effective and affordable vaccines to prevent GAS infections may be the most cost-effective method of primary prevention. A sequence based method, *emm* typing, of the N-terminal region of the M protein, widely used in many regions of the world, is the preferred method to study the molecular epidemiology of GAS strains (Beall *et al.,* 1996). Potential vaccine coverage in different geographic regions, especially those with high rates of ARF/RHD, requires a detailed understanding of the molecular epidemiology of GAS infections and the prevalent *emm* types circulating in the community (Steer *et al.,* 2009). Currently in the pre-clinical stage is a 30-valent vaccine (reformulated from a 26-valent vaccine) based on the variable N-terminal regions of the surface M protein of GAS (Dale *et al.*, 2011). Furthermore, another vaccine (J8 vaccine), based on antigens from the conserved C-repeat portion of the M protein, is undergoing phase 1 clinical trials (Dale *et al.,* 2013).

1.6.2 M protein vaccine

The target for vaccine development is based on the hypervariable N-terminus region of the M protein, which allows for the differentiating of GAS strains using specific M typing antisera (Cunningham, 2000). This region has been able to evoke antibodies with the greatest bactericidal properties, while showing no harmful cross-reaction with human tissue (Dale 2008; Dale *et al.,*

2013). The latest formulation comprises a 30-valent M protein vaccine, reformulated from a 26-valent M protein vaccine. This vaccine is informed by the epidemiology of GAS pharyngitis and invasive infections from the developed world. The 30 *emm* types and M proteins amino acid sequences can be seen in Figure 1.10.

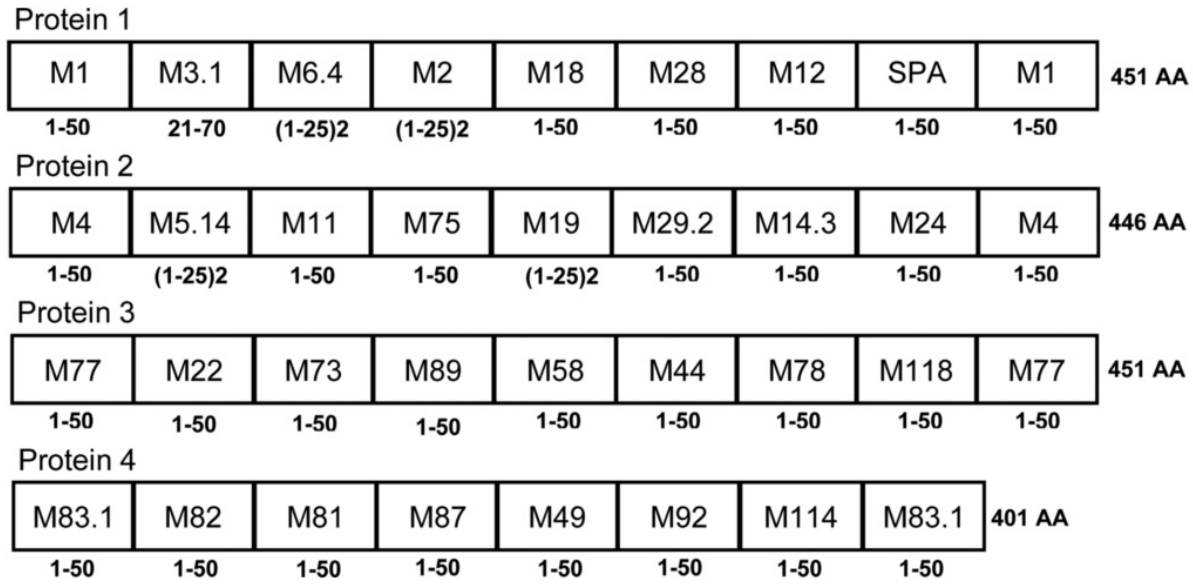


Figure 1.10. Diagram of the four proteins and *emm* types comprising the 30-valent M Protein-based vaccine.

Of interest, the current 30-valent formulation evoked bactericidal antibodies against a number of non-vaccine serotypes of GAS (Figure 1.11). A bactericidal killing of >50% was observed with 88% of isolates. Among non-vaccine types, 81% of isolates were killed (Dale *et al.,* 2013). This cross-optimization of non-vaccine types of GAS evoked by the current 30-valent M Protein formulation is not clearly understood, but more research is underway to understand the mechanisms involved better.

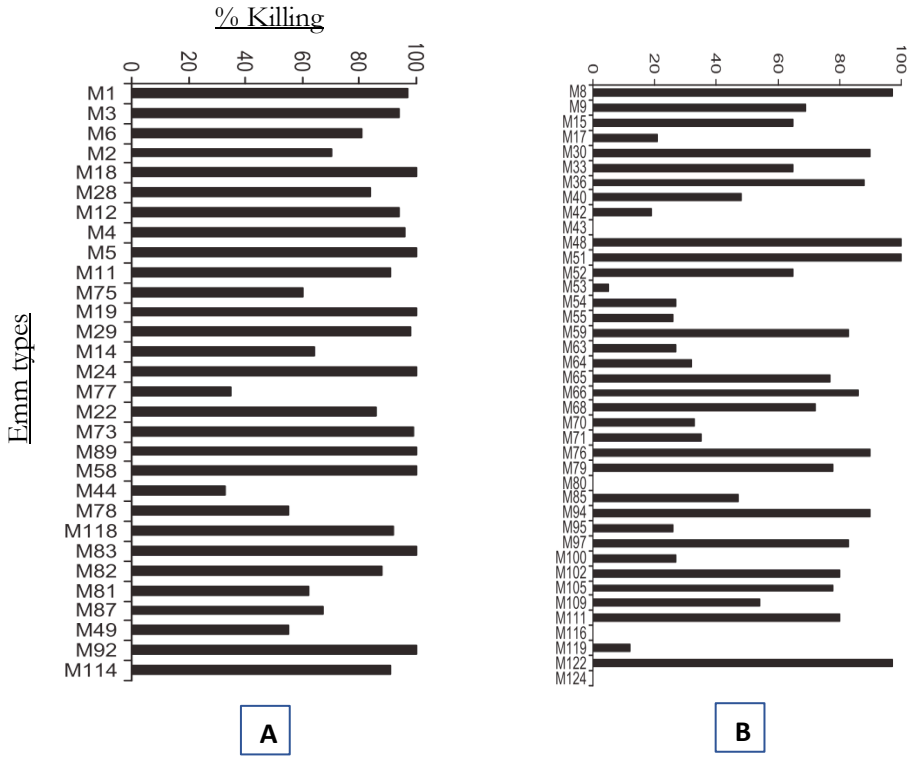


Figure 1.11. Indirect bactericidal activity of the 30-valent GAS vaccine.
A. Vaccine serotypes B. Non-vaccine serotypes (Dale *et al.*, 2011)

1.6.3 *Emm* clusters

The *emm* cluster system is a new typing system that classifies >200 *emm*-types into 48 *emm* clusters containing closely-related M proteins that share structural and binding properties (Sanderson-Smith *et al.,* 2014). Whilst this cluster system has not been applied widely to existing epidemiological data, data from which *emm*-clusters have been obtained indicate that *emm*-clusters associated with *i*GAS infection did not significantly change over time compared with *emm*-types. This system predicts the continent of the M protein vaccine antigen and is believed to serve as a framework to investigate the cross-protection phenomenon (currently not completely understood) and to provide a complementary hypothesis for the many variants from low-middle income countries (Sanderson-Smith *et al.*, 2014a). Furthermore, this concept could lead to the formulation

of an M Protein vaccine that will provide broad coverage in both developing and developed nations.

1.7 Public health surveillance

1.7.1 Importance of public health surveillance systems

Public health surveillance provides an essential platform to guide and inform prevention and control strategies to improve health outcomes of populations (Soucie, 2012). Public health surveillance is described as "the ongoing, systematic collection, analysis, and interpretation of health data, essential to the planning, implementation and evaluation of public health practice, closely integrated with the dissemination of these data to those who need to know and linked to prevention and control" (Thacker and Berkelman, 1988). The intention of surveillance is to empower decision-makers with effective, timely and useful evidence to lead a public health action plan. The design and implementation of surveillance systems are determined by their objectives and actions required from a particular intervention programme, with some requiring rapid interventions (infectious diseases), and others requiring timeous monitoring in order to plan interventions (chronic diseases) (Nsubuga *et al.*, 2006).

1.7.2 Laboratory surveillance

Laboratory-based surveillance systems involve training, resources, and facilities. A reference laboratory is important for quality control and support. Laboratory-based surveillance systems usually involve the systematic referral of isolates from sentinel clinics or health centres. A systematic sampling approach provides reliable data and is a better representation of the burden of disease compared with an arbitrary attempt at reporting trends of infections. Furthermore, the regular communication between a public health laboratory and an epidemiologist is essential for the successful execution of disease control programmes.

The use of serotyping is being widely adopted in surveillance laboratories and provides data on the subtypes of certain infectious diseases. A good example of this is the monitoring of *Salmonella*: laboratories in 61 countries around the world make use of serotyping for public health surveillance (Herikstad *et al.,* 2002). Molecular analysis empowers laboratory-based surveillance systems, allowing for the detection of prevalent or new strains that may be causing disease in populations.

1.7.3 The need for adequate registries

A patient disease registry is a powerful surveillance tool in epidemiology (McDonald *et al.*, 2005). Surveillance of infectious diseases forms the foundation of prevention and control strategies by identifying any changes in disease patterns, which may warrant further investigation, and by planning the implementation of control measures.

Disease registries are guided by research questions that are developed to serve multiple purposes. They provide platforms to study the natural history of disease, their clinical features, cost-effective treatment strategies and measures of improved quality of care (Glicklich and Dreyer, 2007).

Registries for streptococcal surveillance have been established in many developed countries, including Canada, England and the USA, where invasive group A streptococcus (*i*GAS) are a notifiable disease (CDC 1997; GOV.UK 2014; Davies *et al.,* 1996; Tyrrell *et al.,* 2005;). In 2004, the Eurosurveillance program began to capture comprehensive information on all cases of *i*GAS infection in Europe (Martin *et al.,* 2011; Meehan *et al.,* 2013). This surveillance programme was successful in tracking trends in *i*GAS infection, monitoring clusters and outbreaks, and conducting molecular epidemiological *emm* sequence typing on all isolates. In the United Kingdom, routine surveillance data indicate a significant increase of *i*GAS isolates from December 2008 (n=143) compared to the same period in 2007 (n=86) (Lamagni *et al.,* 2009). In Alberta, Canada, surveillance of *i*GAS infection collects information that informs vaccine development and

contributes to the implementation and evaluation of new intervention strategies for controlling GAS disease (Tyrrell *et al.,* 2005).

The size and severity of the burden of GAS-related diseases highlight the importance of epidemiologic surveillance to detect changes in disease distribution in various populations.

The incidence and prevalence of GAS infections in developing countries are largely unknown due to the lack of studies and surveillance from these regions (Carapetis *et al.,* 2005). Currently, *i*GAS infection is not notifiable in South Africa, thus there exists no registry for the documenting of GAS-related disease in the country (nor on the rest of the African continent), despite the importance of GAS infections in this region. Given that systematically collected data are essential for an effective disease-control programme (Nsubuga *et al.,* 2006), measuring the incidence and temporal trends is an essential first step toward reducing the burden of GAS disease in developing countries (Robertson *et al.,* 2006).

Objectives of this thesis:

1. To establish the AFRO*Strep* biorepository and clinical database composed of South African patients with a GAS-related diagnosis (both invasive and non-invasive).

2. To identify and summarize all studies reporting on the molecular epidemiology of Group-A streptococcal (GAS) isolates in Africa.

3. To describe from national laboratory data, the incidence of invasive and non-invasive Groups A streptococcal isolates in South Africa, 2003 – 2015.

4. To conduct a prospective, surveillance study in order to determine the burden of invasive disease associated with GAS in South Africa over a 12-month period.

5. To describe the molecular (*emm)* typing of invasive GAS isolates in South Africa, particularly with respect to how they compare and contrast with the molecular types of non-invasive GAS isolates.

2. CHAPTER TWO: THE ESTABLISHMENT OF THE AFRO*Strep* STUDY

This section has been published in part in the following peer-reviewed journal:
Barth DD, Engel ME, Whitelaw A, Alemseged A, Sadoh WE, Ali SKM, Sow SO, Dale J, Mayosi BM. Rationale and design of the African group A streptococcal infection registry: the AFROStrep Study. BMJ Open, 2016.

2.1 Introduction

Group A β-haemolytic streptococcus (GAS), a gram-positive bacterium also known as *Streptococcus pyogenes*, causes skin, mucosal, systemic and autoimmune diseases (Cunningham, 2000). Repeated pharyngeal and skin infections with GAS may lead to serious autoimmune diseases such as acute post-streptococcal glomerulonephritis, acute rheumatic fever (ARF) and rheumatic heart disease (RHD) (Cunningham 2012; Carapetis, Steer, *et al.*, 2005). Invasive GAS disease (*i*GAS) is associated with significant morbidity and mortality in children and young adults world-wide (Lees and Carrol, 2014). Increases in the number of cases of both invasive and non-invasive GAS diseases have been observed globally since the 1980s (Hoge *et al.*, 1993; Lynskey *et al.*, 2011). The reasons for these observations are not well understood and have subsequently, caused many countries to commence active surveillance systems for *i*GAS to closely document the epidemiology of the disease.

A patient disease registry is a powerful surveillance tool in epidemiology (McDonald *et al.*, 2005). Guided by research questions, registries are developed to serve multiple purposes and provide a platform to study the natural history of disease, clinical features, cost effectiveness of treatment strategies and care, to assess safety and harm, and to provide measures of improved quality of care (Glicklich and Dreyer, 2007). Registries for streptococcal surveillance have been established in some developed countries, for example Canada, England and USA, where *i*GAS is a notifiable disease (Tyrrell *et al.*, 2005; CDC 1997; GOV.UK 2014; H. Dele Davies *et al.*, 1996).
In 2004, the Eurosurveillance program began to capture comprehensive information on all cases of *i*GAS infection in Europe (Martin *et al.*, 2011; M. Meehan *et al.*, 2013). This surveillance

programme was successful in tracking trends in *i*GAS infection, monitoring clusters and outbreaks, and conducting molecular epidemiological *emm* sequence typing on all isolates. In the United Kingdom, routine surveillance data indicate a significant increase of *i*GAS isolates from December 2008 (n=143) compared to the same period in 2007 (n=86) (Lamagni *et al.*, 2009). In Alberta, Canada, surveillance of *i*GAS infection collects information that informs vaccine development and contributes to the implementation and evaluation of new intervention strategies for controlling GAS disease (Tyrrell *et al.*, 2005).

Currently, there exists no registry for the documenting of GAS-related disease in Africa, despite the importance of GAS infections in this region. Given that systematically collected data are essential for an effective disease-control programme (Nsubuga *et al.*, 2006), we have established the AFRO*Strep* Registry as an essential first step towards understanding the prevalence of laboratory-confirmed GAS disease in African countries.

2.2 Rationalé

In a World Health Organisation (WHO) report, GAS was put in the top ten leading causes of mortality world-wide, with the majority of deaths attributed to rheumatic heart disease, a chronic sequel of GAS pharyngitis (World Health Organisation, 2005). Prevalence and incidence data on laboratory-confirmed GAS infection from African countries are lacking when compared with industrialised nations (Carapetis, Mcdonald, *et al.*, 2005), although a number of studies in Africa have previously published data on GAS in a number of countries including Ethiopia, Mali, Nigeria, Sudan and Tunisia (Hraoui, Boutiba-Ben Boubaker, a. Doloy, *et al.*, 2011; Malik & Ali 2014; Mayosi 2006; Milagritos D. Tapia *et al.*, 2015; Tesfaw *et al.*, 2015b).

The AFRO*Strep* study is a collaborative study that aims to establish the first registry and biorepository of laboratory-confirmed GAS isolates in Africa, with one of its main objectives being

to collect comprehensive epidemiological, clinical, microbiological, and molecular data for GAS infections on the continent. AFRO*Strep* will serve as a platform for further investigations including molecular characterisation of isolates in order to contribute to the growing body of knowledge informing vaccine development.

The registry comprises two components;

1. Active surveillance of GAS pharyngitis cases from whom GAS has been isolated at clinics and community health centres.
2. Passive surveillance of laboratory data on GAS isolated from patients with invasive (*i*GAS) and non-invasive (non-*i*GAS) streptococcal disease.

Objectives of the AFRO*Strep* Registry

1. To collect demographic and clinical information from patients with *i*GAS and non-*i*GAS laboratory-confirmed infection.
2. To determine the molecular epidemiology of non-invasive and invasive GAS infection.
3. To assess strategies for treatment, control and prevention of GAS infection.
4. To conduct studies that contribute to the development of appropriate interventions such as vaccines.

2.3 Design of the study

AFRO*Strep* is a prospective, regional, multicentre, clinic- and laboratory-based registry initiative involving centres in Africa, many of which are part of the Stop Rheumatic Heart Disease A.S.A.P. Programme (Robertson *et al.*, 2006) and related studies such as the Global Rheumatic Heart Disease Registry (the REMEDY study) (Karthikeyan *et al.*, 2012). AFRO*Strep* seeks to document the prevalence, incidence, clinical and molecular characteristics of laboratory-confirmed GAS

infection in Africa. This thesis served to establish the Cape Town component, specifically focussing on the passive surveillance of laboratory data on GAS isolated from patients with *i*GAS and non-*i*GAS disease.

2.3.1 Study eligibility

All patients presenting with a sore throat (including tonsillitis) at participating clinics and community health centres regardless of age, and who have not had antibiotics in the previous 30 days, are eligible to participate in the active surveillance arm of AFRO*Strep*. For the passive surveillance component, all patients, irrespective of age, confirmed as having GAS disease, are eligible for inclusion.

Inclusion criteria. Anyone presenting with a sore throat with microbiological laboratory confirmation of GAS, providing informed consent (Appendix 9.11) is eligible for enrolment. *Exclusion criteria.* Patients will be excluded if no informed consent was obtained.

Clinical data and accompanying laboratory data will be entered into the AFRO*Strep* database designed on the OpenClinica platform version 3.0 (https://www.openclinica.com). In addition, isolates will be subjected to cryo-preservation for long-term storage in the AFRO*Strep* biorepository. This will enable further molecular investigations as part of the wider vaccine initiative spanning a number of sites worldwide.

2.3.2 Data collection

The study nurse in each site will seek participation in the study among eligible patients attending health care facilities for treatment of sore throat. Consenting patients will be examined clinically on a number of symptoms after which, a throat swab will be taken for microbiological culture. A

case report form will be used for recording data. Patient care will remain within the domain of the attending clinician or nurse. Antibiotics prescribed will also be documented.

Sample size. Active surveillance: To allow for comparison of prevalence across respective sites participating in AFRO*Strep*, a minimum sample of 246 participants with pharyngitis needs to be enrolled at each site. This calculation was based on existing prevalence estimates for GAS pharyngitis of around 21% (Engel *et al.*, 2014); 95% confidence interval (CI) and a precision level of 5% (Altman, 1980).

Passive surveillance. Eligible patients will be identified from positive GAS cultures isolated at participating laboratories. Clinical data will be obtained from hospital folders.

2.3.3 Governance

The institutions inputting data to the registry own their data. The registry committee will meet on a regular basis to address requests for data sharing which will be subjected to satisfactory evidence regarding the intended use of the data, maintenance of confidentiality and benefit to the entire community of patients, including the individual.

2.3.4 Data analysis plan

The information to be collected will include (but not be limited to) date of birth, gender, date and duration of illness, presenting clinical features and, microbiological findings. To protect the privacy of patients, a file will be created that will have no specific identifiers. Analysis will be conducted using Stata 11.2 (StatCorp, College Station, TX). Descriptive statistics will be used to describe the clinical syndromes associated with invasive and non-*i*GAS disease. The number of positive samples obtained each month will be analysed to determine prevalence of GAS among pharyngitis cases treated at the health facilities. Geographical Information Systems (GIS) will be used to plot

the residences of participants. Molecular characterization will be reported using *emm*-typing as previously described (Beall *et al.*, 1996).

2.3.5 Ethics and dissemination

Ethics approval for the AFRO*Strep* registry has been obtained from the Human Research Ethics Committee of the University of Cape Town (HREC/REF: R006/2015) (Appendix 9.1). Each recruiting site will seek ethics approval from their local ethics committee. Using standardized case report forms tailored to local requirements, eligible patients will be informed about the AFRO*Strep* registry, and their consent to participate will be recorded prior to enrolment. Children eight years of age and older will also be requested to provide assent (Appendix 9.10). Reports and publications emanating from the AFRO*Strep* Registry will not include any information that identifies either the patient themselves, their parents or guardians. Participants will be identified throughout the study duration by the study number allocated to them at the time of enrolment. All data will be stored on a password-protected computer and handled in the strictest confidence. The establishment of the biorepository will follow prescribed guidelines and documentation will be drawn up to afford maximum protection for the participants, who will be requested to provide specific consent for the long-term storage of their isolates. Findings and updates will be disseminated to collaborators, researchers, health planners and colleagues through peer-reviewed journal articles, conference publications and proceedings.

2.3.6 Status of the study and sites participation

The active and passive surveillance arms of AFRO*Strep* commenced in November 2015; initially, pilot sites in South Africa were anticipated to participate in the AFRO*Strep* Registry, after which, enrolment will expand across centres from the rest of Africa. All participating sites will enrol patients for a minimum of two years. A similar study had previously been conducted in Cape Town

(Engel *et al.*, 2014). A standard operating procedure for AFRO*Strep* with more information is available (Appendix 9.8).

2.3.7 Strengths and limitations

Health facilities collaborating in this study have huge catchment areas; thus, we anticipate large numbers of enrolment which will provide an acceptable population from which to draw conclusions on the general and molecular epidemiology of GAS in patients. However, the AFRO*Strep* study is a clinic- and laboratory-based registry and will not address the true burden of disease in the community. Furthermore, concerning the passive surveillance component, given the financial constraints facing many centres in Africa, it is conceivable that specimens submitted for laboratory evaluation will represent the more severe cases. Finally, in the light of the hypothesis that GAS impetigo plays a role in the pathogenesis of post-streptococcal diseases (Parks *et al.*, 2012), we acknowledge that the registry may be rendered incomplete by not including skin cultures, for reasons of the risk of mixed infections. But, given that many cases of *i*GAS begin on the skin, the molecular epidemiology of the most pathogenic GAS should be captured nevertheless. In addition, the physical examination will include notation of coexistent pyoderma. Nevertheless, despite the limitations, AFRO*Strep* will provide the first insights into the epidemiology of laboratory-confirmed GAS disease in African countries.

2.4 Discussion

To the best of our knowledge, the AFRO*Strep* study is the first prospective study of clinical, epidemiological and microbiological characteristics of group A streptococcal disease in Africa. Our registry, which includes the documenting of non-invasive GAS such as pharyngitis, represents an improvement on current registries limited to iGAS information. We will collect detailed data on clinical features at the time of presentation which will be stored together with corresponding

detailed laboratory information including molecular characterisation of GAS strains. The AFRO*Strep* study will also document current practices in treating group A streptococcal infection, with particular reference to prescription of penicillin antibiotics. Finally, AFRO*Strep*, because of its multicentre nature, presents the scope for examining regional similarities and differences in clinical and molecular features and outcomes of GAS infection.

3. CHAPTER THREE: METHODS

In this section, I will be describing the methods used in the inter-linked studies encompassed within this thesis. Firstly, I will provide a brief background followed by the detailed approaches used in each study.

3.1 Systematic Review: Prevalence of Group A streptococcal disease in Africa:

This protocol for this systematic review has been published in part in the following peer-reviewed journal:

Barth D, Mayosi BM, Jabar A, Engel ME. Prevalence of Group A streptococcal disease in North and Sub-Saharan Africa: A systematic review protocol. BMJ Open, 2015;5:e0086

3.1.1 Background

The true burden of group A streptococcal (GAS) disease in Africa is not known. GAS is a significant cause of mortality and morbidity on the global scale and in developing countries. According to Carapetis *et al*, the prevalence of severe GAS disease is at least 18.1 million cases with an incidence of at least 1.78 million cases per year. Systematic reviews represent a scientific approach to the aggregation of individual studies, that fits pre-specified eligibility criteria, so as to present a summary of the best available evidence to answer a specific research question. Systematic review are different from traditional reviews in that it aims to reduce bias in the selection of articles by making use of predefined, explicit methods (Higgins and Green, 2011). We conducted a systematic review and meta-analysis to investigate the burden of GAS disease among children and adults in Africa. This systematic review was based on and reported according to the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines (Moher *et al.*, 2009).

3.1.2 Specific Objectives

The primary objective of this review was to provide a quantitative summary, from published studies, of the prevalence of laboratory-confirmed GAS among people living in African countries. Secondary outcomes included evaluating the quality of included studies and analysing demographic and other characteristics which include the distribution of GAS *emm* types in Africa. This review will complement the findings of an existing review published in 2009 (Andrew C Steer *et al.*, 2009) and will serve to inform the development of putative vaccines.

3.1.3 Methods

The methods for this review follows those published previously (Mahmoud Werfalli *et al.*, 2014). This systematic review's protocol has been published in the PROSPERO International Prospective Register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO), registration number CRD42014012900, in a peer reviewed journal (Barth *et al.*, 2015), and is prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) 2015 Statement (Moher *et al.*, 2015).

3.1.3.1 Search methods and identification of studies

A broad search strategy was designed to maximise sensitivity (Table 3.1). The main search comprised individual searches using detailed medical subject heading (MeSH) terms for group A Streptococcal infection combined with terms relevant to Africa. In an attempt to maximise the likelihood of finding articles conducted in Africa, we applied an African search filter developed by Siegfried and colleagues (Pienaar *et al.*, 2011; Eisinga *et al.*, 2006).

Table 3.1. Search Strategy

((((((GAS) OR Group A Streptococc*) OR streptococc*) OR pharyngitis) OR streptococc* pyogenes) OR streptococc* pyogenes pharyngitis) OR streptococcus pyogenes[MeSH Terms] AND ((((“Africa”[MeSH] OR Africa*[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR “Burkina Faso”[tw] OR Burundi[tw] OR Cameroon[tw] OR “Canary Islands”[tw] OR “Cape Verde”[tw] OR “Central African Republic”[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR “Democratic Republic of Congo”[tw] OR Djibouti[tw] OR Egypt[tw] OR “Equatorial Guinea”[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR “Guinea Bissau”[tw] OR “Ivory Coast”[tw] OR “Cote d’Ivoire”[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 “Sao Tome”[tw] OR Senegal[tw] OR Seychelles[tw] OR “Sierra Leone”[tw] OR Somalia[tw] OR “South Africa”[tw] OR “St Helena”[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR “Western Sahara”[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw] OR “Central Africa”[tw] OR “Central African”[tw] OR “West Africa”[tw] OR “West African”[tw] OR “Western Africa”[tw] OR “Western African”[tw] OR “East Africa”[tw] OR “East African”[tw] OR “Eastern Africa”[tw] OR “Eastern African”[tw] OR “North Africa”[tw] OR “North African”[tw] OR “Northern Africa”[tw] OR “Northern African”[tw] OR “South African”[tw] OR “Southern Africa”[tw] OR “Southern African”[tw] OR “sub Saharan Africa”[tw] OR “sub Saharan African”[tw] OR “sub-Saharan Africa”[tw] OR “sub-Saharan African”[tw]) NOT (“guinea pig”[tw] OR “guinea pigs”[tw] OR ‘aspergillums Niger”[tw]))))

The search strategy was conducted independently by two reviewers (DB; ME) among multiple databases which include Medline (accessed via PubMed), Web of Science (accessed via IST Web of Knowledge), and Scopus databases from the earliest inception to the latest published data. In addition, we searched through proceedings from the XIX Lancefield International Symposium on *Streptococci* and *Streptococcal* disease (http://www.lancefield2014.com) and complemented our results with searches in Google Scholar, conference proceedings, scanning reference list of included articles, and theses databases. The search strategy was modified to suit the vocabulary of individual database(s). Our search was not restricted by any time period cut-off nor by language.

3.1.3.2 Inclusion criteria

We included studies describing the prevalence of GAS across all age groups, resident in countries belonging to the African continent, in the geographic regions of sub-Saharan and North Africa diagnosed with a laboratory-confirmed GAS isolate from all ethnicities, socioeconomic and educational backgrounds. All study designs were considered for inclusion. For the purpose of this review, the diagnosis of GAS was determined by culture and/or rapid antigen detection testing. The molecular characterisation of GAS was considered if the *emm* typing method was used to ascertain the strains. This technique is thought to be concurrent with the gold standard M-typing technique (Tewodros and Kronvall, 2005). We considered both published articles and unpublished studies. Articles published in other languages with full English abstracts were eligible for inclusion.

3.1.3.3 Exclusion criteria

We excluded narrative reviews, opinion pieces, letters and any other publications lacking primary data and/or explicit method descriptions. For publications of the same data, the most complete and recent versions were considered for inclusion.

3.1.3.4 Data extraction and management

Search results from all databases mentioned above and reference search results from published and unpublished data were managed with Mendeley referencing software (Copyright © 2009-2013 Mendeley Ltd). Data were extracted using predefined criteria using a data extraction form (Appendix 9.2).

3.1.3.5 Selecting studies for inclusion

Full text study articles were screened and scrutinised by title and abstract against predefined inclusion and exclusion criteria. Two reviewers (DB; ME) were assigned to evaluate and appraise the results of the searches based on the title and abstract. If the reviewer was uncertain, the study was marked as pending. Once all the studies were reviewed independently, the reviewers together compared their scripts; discrepancies were discussed and where necessary, a third reviewer (BM) was called upon to resolve any disagreements.

3.1.3.6 Quality appraisal of included studies

Internal, external validity and generalizability of the included study results were evaluated for risk of bias. An assessment of the risk of bias informed the evaluation of heterogeneity in the pooled analysis. A quality assessment tool for evaluating prevalence studies as suggested by Hoy and colleagues (and adapted by Werfalli and colleagues) were employed. The revised version allows for a composite score to assist with relative comparison between the studies, thereby reducing reviewers' subjectivity (Hoy *et al.*, 2012; Werfalli *et al.*, 2014). In a nutshell, Werfalli and colleagues added a quantitative scoring system to the Risk of Bias table, allocating four points for external validly 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 3.2).

Table 3.2. Quality assessment criteria for prevalent studies with scoring (Hoy *et al.*, 2012; Werfalli *et al.*, 2014)

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

3.1.3.7 Data synthesis

To calculate the unadjusted prevalence estimates of symptomatic group A streptococcal infection in our study population, we individually recalculated the reported prevalence estimates to confirm numerators and denominators. We confirmed the prevalence estimates reported by the authors were correct; however, some studies had to be adjusted to obtain numerators and denominators relevant to our study population. Secondly, where sample populations included both symptomatic GAS and asymptomatic GAS (van Zyl *et al.*, 1981; Bélard *et al.*, 2015; O'Meara *et al.*, 2015; Mzoughi *et al.*, 2004; Tewodros *et al.*, 1992), we only extracted information from symptomatic GAS patients.

Using Stata® (version 13.1) the Freeman-Tukey double arcsine transformation *metaprop* routine was used to calculate the combined prevalence estimate, with the standard error across the unadjusted estimates from the included studies. The Freeman-Turkey stabilises the variance of study-specific prevalence, minimizing the influence from studies with extremely small prevalence or extremely large prevalence estimates (Nyaga *et al.*, 2014).

We stratified the aggregated prevalence by region in order to confirm earlier reports of regional differences in GAS (Andrew C Steer *et al.*, 2009), thus, we endeavoured to assess similarities and difference within the Africa continent. Our hypothesis was that GAS infection would differ regionally because of geographical and climatic differences. We also evaluated prevalence estimates according to study design so as to assess methodological influences on overall estimates. Our hypothesis was that GAS prevalence's would not be statistically different across study designs. We intended to perform sub-analyses of GAS colonization risk factors including gender, crowding, and seasonality.

3.1.3.8 Sensitivity analysis

Where heterogeneity was statistically significant, sub-group and sensitivity analysis was conducted to establish if the meta-analysis results are influenced by the effect of study designs as well as the geographical settings (low income versus middle income countries). Sensitivity analysis was performed to determine potential sources and explanations for the heterogeneity. These analyses included plotting studies of a high quality and comparing results to see how it differs from the overall result. Studies which were considerably heterogeneous and where pooling data was not possible, the findings were narratively explained together with tables and figures where applicable. Any discrepancies or disagreements were discussed by the reviewers and if necessary an independent reviewer was asked to provide clarification.

3.1.3.9 Ethics and dissemination

Systematic reviews draw on publicly available data and therefore do not require formal ethical review. The findings of this systematic review will be disseminated through peer-reviewed journal publications and conference proceedings.

To our knowledge, there are no systematic reviews that have specifically looked at the burden of laboratory-confirmed GAS infection in Africa. We expect this review to compliment that of Steer and colleagues in 2009 which reported on the global *emm* type distribution of GAS. Finally, we believe that the results of this systematic review will have implications for policy, practice and vaccine development, informed by data solely from Africa where the burden of GAS disease is among the greatest.

3.1.3.10 Funding

No funding was received for this review.

3.2 Retrospective Study: A review of invasive and non-invasive GAS infection in South Africa

3.2.1 Background

The epidemiology of GAS infection has been discussed in detail in section 1.3. Briefly, increases in the annual number of cases for GAS disease, especially invasive GAS (*i*GAS) diseases have been observed globally since the 1980s. The incidence and prevalence of both *i*GAS and non-*i*GAS infections in developing countries, which includes South Africa, are largely unknown (Carapetis, Mcdonald, *et al.*, 2005). Systematically collected data are essential for a functioning disease-control programme (Nsubuga *et al.*, 2006) and thus, the measurement of incidence and temporal trends are an essential first step toward reducing the burden of GAS disease in developing countries (Robertson *et al.*, 2006). We conducted a retrospective study of cases of *i*GAS and non-*i*GAS infection identified at the National Health Laboratory Services to determine the incidence of GAS infection in South Africa.

3.2.2 Specific Objectives

The aim of this study was to describe the epidemiology of *i*GAS and non-*i*GAS infection and associated clinical characteristics in the public sector of South Africa, over a recent 13-year period (2003 – 2015).

3.2.3 Methods

3.2.3.1 Study design and population

This study was conducted under the auspices of the AFRO*Strep* Study, an initiative aimed at documenting the epidemiology of GAS in Africa (Barth *et al*., 2016). We performed a retrospective analysis of *i*GAS and non-*i*GAS isolates cultured at National Health Laboratory Service (NHLS) centres from January 2003 to December 2015. The NHLS is the largest diagnostic pathology service in South Africa, supporting both national and provincial health departments in healthcare delivery in the nine provinces of South Africa (http://www.nhls.ac.za). Following for the application and approval for data use from the NHLS information systems, we were able to retrieve all recorded cases of GAS infection from January 2003 to December 2015, in a Microsoft excel spreadsheet. Data fields not relevant to the objectives and analysis of this review were removed.

3.2.3.2 Case Definition

*i*GAS was defined as GAS isolated in culture from a sterile site such as blood, cerebrospinal fluid (CSF) and pleural fluid (Sharkawy *et al*., 2002; Invasive Group A streptococcus Sub-Committee 2006). GAS isolated from a non-sterile site such as the skin and throat was considered to be non-invasive (Su *et al*., 2009). In addition to microbiological data (including site of isolation), we also abstracted demographic information and clinical data, where available.

3.2.3.3 Statistical analysis

The overall and age-specific incidence rates of *i*GAS and non-*i*GAS infection were calculated using annual census population data for South Africa, reported per 10^5 person-years (py). A statistical

exploratory analysis was also conducted to identify changes in the trend of GAS infection (increasing, decreasing or remain unchanged) over the 13-year time interval. The change in GAS infection was not expected to be linear from year to year (with no correlation between measurements collected at different times), and therefore we employed the Mann–Kendall Test for Monotonic Trend (MK test)(Kendall, 1975), rather than parametric linear regression analysis which requires that the residuals from the fitted regression line be normally distributed; an assumption not required by the MK test. Linear trend (median drop per year in GAS infection rates) was assessed using the robust linear regression Theil–Sen estimator (Birkes and Dodge, 1993); this technique was shown to be significantly more accurate than simple linear regression for skewed and heteroskedastic data and compares well against no robust least squares even for normally distributed data in terms of statistical power. The strength of the trend was assessed using the MK test statistic. The test for significance was set at the 5% level. All statistical analyses were performed using STATA® version 13 (StataCorp, Texas, USA).

3.2.3.4 Ethical approval

Ethics approval has been obtained from the Human Research Ethics Committee at the University of Cape Town (HREC/REF: R006/2015) (Appendix 9.1).

3.3 Prospective laboratory-based surveillance Study

3.3.1 Background

GAS is responsible for a wide range of non-*i*GAS and *i*GAS diseases (Bailie *et al.*, 2005; Steer *et al.*, 2007). Primary prevention efforts for GAS infection include the development of a 30-valent serotype vaccine, reformulated from a 26-valent vaccine (Dale *et al.*, 2011); the M serotypes of GAS included in this reformulation were based on data from the developed world and cross coverage of certain *emm* types were observed. *i*GAS *emm* type data from sub-Saharan Africa is essential for assessing potential vaccine coverage. Three studies (Engel *et al.*, 2014; Milagritos D Tapia *et al.*, 2015; Tewodros & Kronvall 2005) reported on the molecular epidemiology of non-*i*GAS and a single study (Seale *et al.*, 2016b) reported on the molecular epidemiology of *i*GAS, in sub-Saharan Africa. By means of a one-year prospective laboratory study, under the auspices of AFRO*Strep*, we determined the clinical characteristics and molecular epidemiology of non-*i*GAS and *i*GAS infection among South African patients attending Groot Schuur Hospital, a tertiary institution in Cape Town, so as to inform the development of M protein-based vaccines.

3.3.2 Specific Objectives

This surveillance study aims to describe the clinical and molecular *emm* type distribution of non-*i*GAS and *i*GAS infections from the National Health Laboratory Services in Cape Town between February 2016 and March 2017.

3.3.3 Methods

3.3.3.1 Clinical Surveillance and Case Definitions

In order to determine the extent of GAS infections and the serotypes that cause disease in South Africa, we conducted a prospective laboratory surveillance study among samples from February 2016 – March 2017 submitted to the National Health Laboratory Service (NHLS) for processing. The NHLS is the largest diagnostic pathology service in South Africa (http://www.nhls.ac.za). *i*GAS was defined as GAS isolated in culture from a sterile site including blood, cerebrospinal fluid (CSF) and pleural fluid (Invasive Group A streptococcus Sub-Committee 2006; Sharkawy *et al.*, 2002). GAS isolated from a non-sterile site such as the skin and throat was considered to be non-invasive (Su *et al.*, 2009). We documented demographic data, clinical presentation, laboratory data and *emm* types that cause non-*i*GAS and *i*GAS infections. At the time of a laboratory-confirmed GAS diagnosis, a standardized case report form (Appendix 9.3) was completed and clinical information were determined by patient folder review and medical notes by a microbiologist practitioner. Isolates were collected and stored at -80 degrees °C until transfer to the AFRO*Strep* laboratory.

3.3.3.2 Study eligibility

All patients, irrespective of age, confirmed as having a positive GAS culture isolated from the NHLS laboratory at Groote Schuur Hospital, were eligible for inclusion. Inclusion into AFRO*Strep* is subject to anyone with a microbiological laboratory confirmation of GAS and the availability of clinical data.

3.3.3.3 Data collection

Clinical data and accompanying laboratory data were entered into the AFRO*Strep* database designed on the OpenClinica platform version 3.0 (https://www.openclinica.com) (Appendix 9.7). In addition, isolates were subjected to cryo-preservation for long-term storage in the AFRO*Strep* biorepository to enable further molecular investigations as part of the wider vaccine initiative spanning a number of sites worldwide.

3.3.3.4 Study procedure

GAS isolated from sterile or non-sterile sites in the human body were processed and stored on microbeads by the Microbiology Laboratory at the NHLS. GAS isolates were collected once a month from the NHLS and taken to the AFRO*Strep* laboratory housed in the department of Medicine at the University of Cape Town. GAS isolates were stored in the AFRO*Strep* laboratory at -80°C in cryopreservative microbeads until DNA extraction. Each isolate was inoculated onto 5% sheep blood agar plates according to the standard protocol, inverted and incubated anaerobically at 37 °C for 24-48 hours (Appendix 9.4). All cultures of beta-haemolytic colonies were further identified by Gram stain, catalase, and serogrouping, as appropriate.

The *emm* typing procedure was performed according to established protocols (Beall *et al.*, 1996). Briefly, DNA was extracted from single colonies of GAS and subjected to PCR-amplification using primers targeting the N terminal of the *emm* gene. The PCR products are separated and visualized using agarose gel electrophoresis and purified (Appendix 9.6). DNA was extracted using the Wizard Genomic DNA Purification Kit (Appendix 9.5) and the DNA quality and quantity were determined using the NanoDrop technique. The amplified DNA product was sent to the University of Stellenbosch for sequencing according to guidelines by the Centres for Disease Control and Prevention (CDC) (https://www.cdc.gov/streplab/protocol-emm-type.html). The

BLAST programme (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp) was used to compare the DNA sequences with published sequences in the CDC databases. More detailed methods are described below in section 3.4. In addition, we also recorded the cluster sequence types.

3.3.3.5 Data analysis plan

The information collected included; date of birth, gender, presenting clinical features, and microbiological findings. To protect the privacy of patients a separate database was created that contained no specific identifiers. Analysis was conducted using Stata 13 (StatCorp, College Station, TX). We evaluated the association between *emm* type and clinical symptoms by means of a Chi-square or Fishers exact test. A p value of <0.05 was considered to be statistical significant.

3.3.3.6 Ethics and dissemination

Ethics approval for the AFRO*Strep* registry was obtained from the Human Research Ethics Committee of the University of Cape Town (HREC/REF: R006/2015) (Appendix 9.1). Reports and publications emanating from the AFRO*Strep* Registry will not include any information that identifies either the patient themselves, their parents or guardians. Participants were identified throughout the study duration by the study number allocated to them at the time of enrolment. All data are stored on a password-protected computer and handled in the strictest confidence. Findings and updates will be disseminated to collaborators, researchers, health planners and colleagues through peer-reviewed journal articles, conference publications and proceedings.

3.4 emm Typing

3.4.1 Culture and identification of Beta Haemolytic Streptococci

Group A streptococcal isolates were stored at -80°C in the AFRO*Strep* laboratory upon receipt at the microbiology laboratory at the NHLS. GAS isolates were stored in storage media containing microbeads. GAS samples were inoculated on 5% sheep blood agar and incubated for 48 hours at 37°C, in the presence of 5% CO_2. Isolation of *streptococcus pyogenes* using culture remains the gold standard for identification (Murray *et al.*, 1976). Contaminated specimens were sub-cultured on a fresh blood agar plate. Isolates confirmed as Lancefield group A were investigated further.

3.4.2 Genomic DNA extraction

The Wizard Genomic DNA Purification Kit,500 was used to extract DNA from the bacteria. Following incubation, single colonies were removed from the agar plate and suspended in 1ml nucleus free water, stored in Eppendorf tubes. The protocol for DNA extraction as per the instruction of the manufacturer was followed. For a detailed step-by-step guide, see appendix 9.6.

3.4.3 Polymerase chain reaction

Following the extracted DNA from the previous step in 3.4.2, the *emm* gene was amplified in accordance with the CDC protocol (Beall *et al.*, 1996). This method amplifies a portion of the gene using sequence specific primers. These primers, in pairs, bind to either side of the double-stranded DNA. The primers used (Table 3.3), were synthesized at the Department of Molecular and Cell Biology at the University of Cape Town. *Taq* Polymerase, the enzyme responsible for the PCR amplification, is isolated from the bacterium *thermas aquaticus* (Saiki *et al.*, 1988) *Taq* Polymerase is able to survive high temperatures during PCR cycles. To ensure the successful amplification of the *emm* gene, the CDC recommends a combination of reagents at specific concentrations (CDC,

2011). PCR reagents comprised 1X buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 70 picomol/Ql of the forward and reverse primers and 1.5U of SuperTherm Taq (JMR, Holdings, London UK), and then brought to a final volume of 50 Ql with DNAse-free water.

Table 3.3. Primers used for *emm* typing

Target gene	Primer	Primer Sequence
Emm gene	Primer 1 (forward)	TAT T(C/G) GCT TAG AAA ATT AA
	Primer 2 (reverse)	GCA AGT TCT TCA GCT TGT TT
	*Emm*seq2	TAT TCG CTT AGA AAA TTA AAA ACA GG

During PCR, cycling conditions for amplification of the *emm* gene involves three principle amplification steps; denaturation, annealing and elongation. The cycling conditions for *emm* typing, recommended by the CDC (CDC, 2011) begins with initial denaturation at 94°C for 60 seconds, followed by 30 cycles of denaturation at 94°C for 15 seconds, annealing at 46.5°C for 30 seconds and extension at 72°C for 75 seconds. An additional 20 cycles followed with denaturation at 94°C, annealing at 46.5°C for 30 seconds, extension at 72°C for 75 seconds with a 10 second increment in between each of the subsequent 19 cycles. The final extension was carried out at 72°C for 10 minutes.

3.4.4 Agarose gel electrophoresis

The technique used to separate DNA fragments, based on their sizes (Stellwagen, 2009) when subjected to an electrical current is referred to as electrophoresis. DNA is negatively charged because of the phosphate groups on its sugar-phosphate backbone thus migrating to the positive end of the agarose gel when an electric current is passed through. The percentage of agarose within

the gel determines the pore size; hence different percentages of gels are prepared based on the size of DNA fragments to be separated. The larger the DNA fragments, the slower they migrate through the agarose gel compared with smaller fragments (Stellwagen, 2009).

Agarose gel electrophoresis was used to separate the DNA fragments following PCR. As the expected sizes of *emm* amplicons range from 750 bp to 1400 bp, a 2% agarose gel was used to separate the PCR products. Agarose (SeaKem. LE Agarose, Lanza, USA) was dissolved in 1X TAE buffer and ethidium bromide (10 ng/Ql), which intercalates between base pairs of DNA and fluoresces under ultraviolet light. Eight microliters (µl) of the PCR product were loaded into each well, with 2 Ql of loading dye. The fragments were separated by passing an electric current through the agarose for 3 hours of 60V to allow the negatively charged DNA fragments to migrate towards the positive electrode. The rate of migration is inversely proportional to the size of the fragments. A molecular marker (HyperLadder IV, Bioline, UK) was included to enable estimation of *emm* amplicon sizes.

3.4.5 Sequencing and assigning emm types

DNA purification and sequencing were conducted at the University of Stellenbosch. Sequencing of purified DNA was done using the ABI Prism. BigDyeTM Terminator Cycle Sequencing kit (Applied Biosystems, USA) at Stellenbosch University, South Africa. The DNA concentration was adjusted to 10 ng/Ql for amplicon less than 1000-bp and 20 ng/Ql for amplicons more than 1000-bp as recommended by the sequencing facility at Stellenbosch University, South Africa. Primer emmseq2 (Table 1.1), recommended by the CDC, was prepared to 1.1 ng/Ql and used for sequencing reaction (CDC, 2011). Sequences generated were analysed using BioEdit v7.0.9 (Ibis Biosciences, USA). The sequences were submitted electronically to the *streptococcus pyogenes emm* sequence database centre at the CDC which assigned all the *emm* types and subtypes (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp).

4. CHAPTER FOUR:

STUDY 1: PREVALENCE OF GROUP A STREPTOCOCCAL DISEASE IN AFRICA: A SYSTEMATIC REVIEW

4.1 Introduction

The prevalence of severe group A streptococcal (GAS) disease is estimated to be 18.1 million cases with an incidence of 1.78 million cases, and is responsible for approximately 517,000 deaths each year, globally. As discussed in more detail in the Literature review, chapter 1, section 1.3, the burden of GAS-disease remains poorly defined in Africa.

The introduction of safe, effective and affordable vaccines to prevent GAS infections may be the most cost-effective method of primary prevention of severe GAS-related diseases. Potential vaccine coverage in different geographic regions, especially those with high rates of disease, requires a detailed understanding of the molecular epidemiology of GAS infections (Andrew C Steer *et al.*, 2009). A sequence based method, *emm* typing, of the N-terminal region of the M protein, widely used in many regions of the world, is the preferred method to study and define the molecular epidemiology of GAS strains (Beall *et al.*, 1996). Steer and colleagues, in an earlier systematic review, documented the global distribution of *emm* types of GAS to define prevalent strains and to assess the coverage and implications for the experimental multivalent vaccine (Andrew C Steer *et al.*, 2009). One of the main limitations were reported to be the small number of studies in regions such as Africa causing a potential bias in the results, and may not be generalizable to all countries in this region.

We conducted a systematic review and meta-analysis to investigate the prevalence of GAS disease among children and adults in Africa. In addition, we also documented the frequency and distribution of *emm* types among isolates, thereby informing the development of putative vaccines.

4.2 Methods

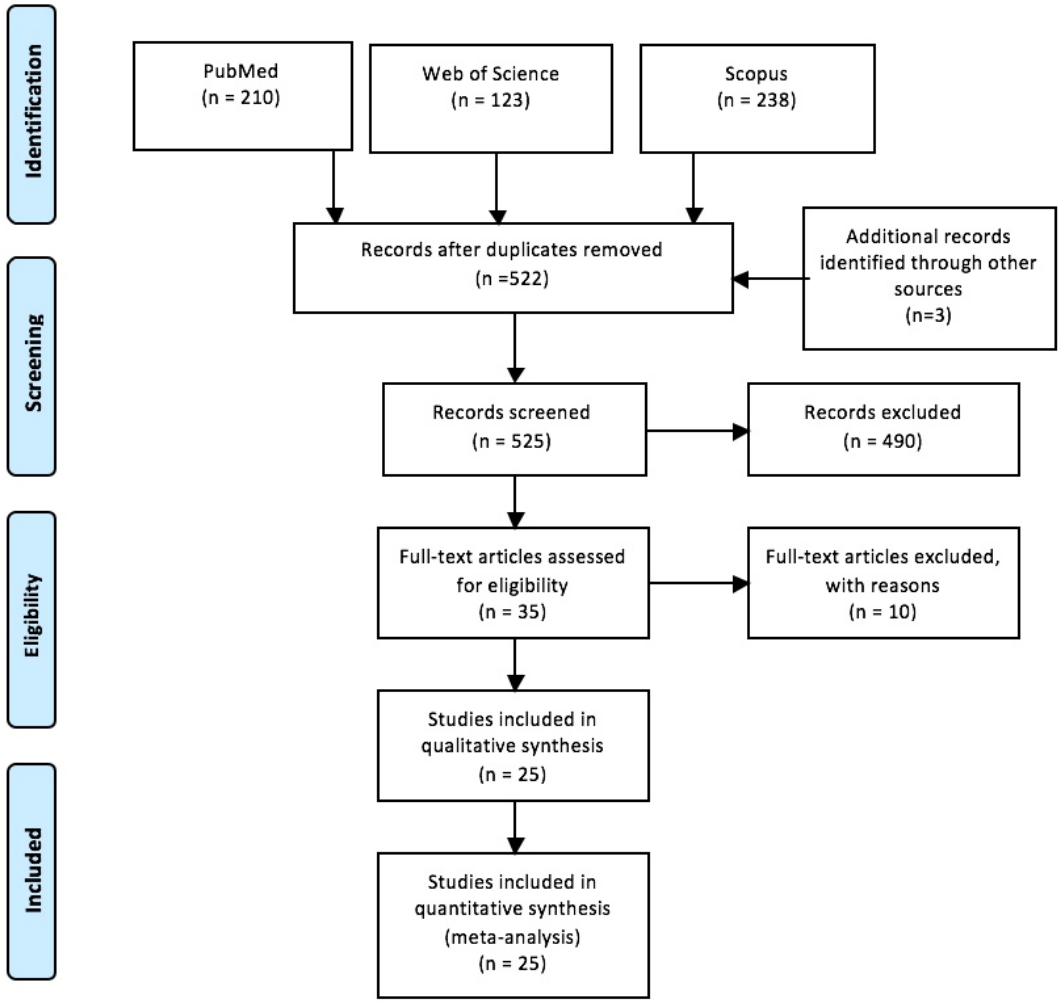
The protocol for this study has been published (Barth *et al.*, 2015) and is registered on PROSPERO (International Prospective Register of systematic reviews, registration number CRD42014012900, http://www.crd.york.ac.uk/PROSPERO). The detailed methods for this systematic review can be found in Chapter 3, section 3.1.

4.3 Results

This systematic review is reported according to the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines (Moher *et al.*, 2009). The PRISMA checklist can be seen in Table 4.4.

We retrieved 525 articles from searches in electronic databases (Figure 4.1). After screening the titles, we excluded 490 articles. We reviewed the abstracts of the remaining articles and further excluded articles not relevant to our study; thirty-five articles were identified to be eligible and included in the review. Twenty-five articles met inclusion criteria (Ringertz *et al.*, 1993; Ibekwe & Okafor 1983; Fourati *et al.*, 2009; Abd El-Ghany *et al.*, 2015; Zegeye *et al.*, 2016; Pius *et al.*, 2016; Seale *et al.*, 2016; Engel *et al.*, 2017; Tesfaw *et al.*, 2015a; A. Benouda *et al.*, 2009; Boukadida *et al.*, 2003; Bassili *et al.*, 2002; Steinhoff *et al.*, 1997; Bélard *et al.*, 2015; Gonsu *et al.*, 2015; Rimoin *et al.*, 2011; SEDKI *et al.*, 2010; Mezghani Maalej *et al.*, 2010; Olivier & de Graad 1978; van Zyl *et al.*, 1981; Taplin & Lansdell 1973; Mzoughi *et al.*, 2004; O'Meara *et al.*, 2015; Tewodros *et al.*, 1992; Milagritos D Tapia *et al.*, 2015)(Table 4.1). Ten articles (El Kholy *et al.*, 1980; Iroezindu *et al.*, 2014; Beekmann *et al.*, 2005; Hraoui, Boutiba-Ben Boubaker, A. Doloy, *et al.*, 2011; A Benouda *et al.*, 2009; Rimoin *et al.*, 2008; Liebowitz *et al.*, 2003; Rimoin *et al.*, 2010; Engel *et al.*, 2014; Andrew C Steer *et al.*, 2009) did not meet inclusion criteria and reasons for exclusion are listed in Table 4.2.

Figure 4.1: PRISMA flow diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. ~~PLoS~~ Med 6(7): e1000097. doi:10.1371/~~journal.pmed~~1000097

For more information, visit www.prisma-statement.org.

4.3.1 Characteristics of included studies

The included studies comprised twenty-five peer-reviewed journal articles published between 1973 and 2016 and sample sizes ranging between 47 and 64,671 (Ringertz *et al.*, 1993; Ibekwe & Okafor 1983; Fourati *et al.*, 2009; Abd El-Ghany *et al.*, 2015; Zegeye *et al.*, 2016; Pius *et al.*, 2016; Engel *et al.*, 2017; Tesfaw *et al.*, 2015a; A. Benouda *et al.*, 2009; Boukadida *et al.*, 2003; Bassili *et al.*, 2002; Steinhoff *et al.*, 1997; Bélard *et al.*, 2015; Gonsu *et al.*, 2015; Rimoin *et al.*, 2011; SEDKI *et al.*, 2010; Mezghani Maalej *et al.*, 2010; Olivier & de Graad 1978; van Zyl *et al.*, 1981; Taplin & Lansdell 1973; Mzoughi *et al.*, 2004; O'Meara *et al.*, 2015; Tewodros *et al.*, 1992; Milagritos D Tapia *et al.*, 2015; Seale *et al.*, 2016) (Table 4.1). Of these, 22 were cross-sectional study designs (n=73,311), while 3 were longitudinal studies (n= 2,138). The age distribution aged from 1 day to 92 years. Microbiological culture was used to identify cases of GAS. Studies were conducted in clinics/outpatient departments, and in schools.

All the studies were conducted across the African continent: Southern Africa (3 studies, n= 1326 (Olivier & de Graad 1978; Engel *et al.*, 2017; van Zyl *et al.*, 1981)), North Africa (10 studies, 5018 (Abd El-Ghany *et al.*, 2015; Boukadida *et al.*, 2003; A. Benouda *et al.*, 2009; Bassili *et al.*, 2002; Fourati *et al.*, 2009; Mezghani Maalej *et al.*, 2010; Mzoughi *et al.*, 2004; Rimoin *et al.*, 2011; SEDKI *et al.*, 2010; Steinhoff *et al.*, 1997)), East Africa (7 studies, n=67,015 (Ringertz *et al.*, 1993; O'Meara *et al.*, 2015; Taplin & Lansdell 1973; Tesfaw *et al.*, 2015a; Tewodros *et al.*, 1992; Zegeye *et al.*, 2016; Seale *et al.*, 2016)), West Africa (3 studies, n=1931 (Ibekwe & Okafor 1983; Milagritos D Tapia *et al.*, 2015; Pius *et al.*, 2016)), Central Africa (2 studies, n=159 (Bélard *et al.*, 2015; Gonsu *et al.*, 2015). Studies were done in urban, rural and semi-urban areas.

Among the studies included in this review, only 3 reported the molecular characterisation of GAS isolates (Engel *et al.*, 2014; Milagritos D Tapia *et al.*, 2015; Seale *et al.*, 2016). All studies employed *emm* typing methods to determine the molecular epidemiology of GAS isolates.

A list of excluded studies and reasons for exclusion can be found in Table 4.2.

4.3.2 Investigating Heterogeneity

Given the notably large heterogeneity, I^2= 96.3% (95% CI, 95% to 97%), we conducted a sensitivity analysis to assess the influence of various study characteristics such as quality of the study and sample size.

Table 4.1. Characteristics of included studies

Study ID	Region	Country	Design	Setting (Local, social context)	Geographical Setting	Population description	Inclusion criteria	Selection Methods	Site of recruitment	Age(s)
Abd El-Ghany 2015	Northern Africa	Egypt	longitudinal	Pediatric outpatient clinic in a Children's Hospital, Cairo, Egypt	Urban	Children attending outpatient clinic	Fever, sore throat and unequivocal erythema of the pharynx were classified as cases	Convenience sampling	Pediatric Outpatient Clinic	4 - 16 years
Bassili 2002	Northern Africa	Egypt	cross-sectional	Private health clinics (Higher SES) /Public Health Service (Lower SES) in Alexandria	Urban	Children attending private and public health services in Alexandria, Egypt	Sore throat and or difficulty swallowing	Systematic randon sampling	Public and Private health clinics	1 - 15 years
Bassili 2002	Northern Africa	Egypt	cross-sectional	Private health clinics (Higher SES) /Public Health Service (Lower SES) in Alexandria	Periurban	Children attending private and public health services in Alexandria, Egypt	Sore throat and or difficulty swallowing	Systematic randon sampling	Public and Private health clinics	1 - 15 years
Bassili 2002	Northern Africa	Egypt	cross-sectional	Private health clinics (Higher SES) /Public Health Service (Lower SES) in Alexandria	Rural	Children aged attending private and public health services in Alexandria, Egypt	Sore throat and or difficulty swallowing	Systematic randon sampling	Public and Private health clinics	1 - 15 years
Bélard 2015	Central Africa	Gabon	cross-sectional	Rural and urban areas within the province of Moyen-Ogooué.	Rural	children and adults (pre-school, school and adults)	Sore throat; (1) provision of written informed consent and (2) residence within the province of Moyen-Ogooué.	convenience sampling	Recruitment, interviews, and sampling took place at the participants'	2 months to 92 years
Benouda 2009	Northern Africa	Morocco	cross-sectional	Four primary health care in Rabat and Sale cities	NS	Children and adults presenting to primary health clinics	Children and adults seen by a doctor who proposed treatment for a sore throat infection	convenience sampling	primary health clinics	0 - 71 years
Boukadida 2003	Northern Africa	Tunisia	cross-sectional	Three primary health care centres in Sousse, Tunisia	NS	Anyone presenting to one of three clinics with a sore throat	All children older than 3 years with acute pharyngitis were eligible	convenience sampling	Primary health clinics	3 - 72 years
Ebekwe 1983	Western Africa	Nigeria	cross-sectional		NS					NS
Engel 2016	Southern Africa	Cape Town	cross-sectional	3 community clinics in Langa and Bonteheuwel, Cape Town and South Africa.	Urban	children 3–15 years of age who presented with sore throat	Sore throat	convenience sampling	Public Health Centres	3 - 15 years
Fourati 2009	Southern Africa	Tunisia	cross-sectional	Emergency department and walk-in clinics - multicentre	NS		Children with pharyngitis	convenience sampling	Public Health Centres	3 - 17 years
Gonsu 2015	Eastern Africa	Cameroon	cross-sectional	Paediatric Ear, Nose and Throat units of the University Teaching Hospital and the Central Hospital, Yaounde	NS	Cases presenting at a Paediatric Hospital	3 to 72 years consulting for pharyngitis or sore throat at the Paediatric	convenience sampling	University Teaching Hospital	16 - 72 years

Study ID	Region	Country	Design	Setting (Local, social context)	Geographical Setting	Population description	Inclusion criteria	Selection Methods	Site of recruitment	Age(s)
Maalej 2010	Northern Africa	Tunisia	cross-sectional	Primary health care clinic	NS	Children attending a primary health care clinic in Tunisia.	Sore throat	convenience sampling	Public health care clinic	2 - 9 years
Mzoughi 2004	Northern Africa	Tunisia	cross-sectional	Two paediatric outpatient clinics in Sousse (Lower SES)	NS	Children attending two paediatric outpatient clinics in Tunisia	Sore throat	convenience sampling	Two paediatric outpatient clinics in Sousse (Lower SES)	2 - 8 years
O'Meara 2015	Eastern Africa	Kenya	longitudinal	rural village, 52% households fall below the poverty line (Lower SES)	Rural	Children attending the outpatient department of Webuye District Hospital (WDH)	Children presenting with fever	convenience sampling	Outpatient health centre	1 - 12 years
Olivier 1978	Southern Africa	Bloemfontein	cross-sectional	Outpatient clinic in Bloemfontein	periurban	Children with sore throat	Sore throat	convenience sampling	Outpatient clinic	2 - 19 years
Pius 2016	Northern Africa	Nigeria	cross-sectional	Special care baby unit, hospital	NS	Neonates admitted to the SCBU who were at risk for sepsis or had presumptive diagnosis of NNS	(1) preterm/ term neonates with clinical features suggestive of NNS, (2) presence of maternal perinatal risk factors for septicaemia, (3) history of unclean delivery and poor cord care and (4) consent.	convenience sampling	Special care baby unit, hospital	0 - 28 days
Rimoin 2011	Northern Africa	Egypt	longitudinal	Pediatric outpatient clinic in Egypt (Lower SES)	Urban	children presenting to a pediatric outpatient clinic in Egypt	Sore throat	convenience sampling	Pediatric outpatient clinic	2 - 12 years
Ringertz 1993	Eastern Africa	Ethiopia	cross-sectional	Rural and Urban public health facilities (3 health care centres)	Both		Sore throat	convenience sampling	Health care centres	< 5 years
Seale 2016	Northern Africa	Kenya	cross-sectional	Rural Kilifi County Hospital in coastal Kenya (Lower SES)	Rural	cases of invasive GAS admitted to Kilifi County Hospital.	Cases of invasive GAS infection	convenience sampling	Kilifi County Hospital in Kenya	0 - 12 years
Sedki 2010	Southern Africa	Egypt	cross-sectional	Rural region of Eastern Cairo - Outpatient health centre and school dispensary room	Rural	Children with clinical diagnoses of non-treated acute pharyngitis	1-Child presenting with acute pharyngo-tonsillitis. 2-Clinical based on 3 diagnostic criteria.	convenience sampling	mixed (outpatient clinic of health centre or the school dispensary room)	3 - 15 years
Steinhoff 1997	Northern Africa	Egypt	cross-sectional	Urban Outpatient clinic of the Abu Reesh Children's Hospital, University of Cairo	Urban	Children who presented with sore throats to the outpatient clinic of the Abu Reesh Children's Hospital, University of Cairo	history of sore throat; unequivocal erythema of the pharynx; and parental consent for the study	convenience sampling	Outpatient health clinic	2- 13 years

Study ID	Region	Country	Design	Setting (Local, social context)	Geographical Setting	Population description	Inclusion criteria	Selection Methods	Site of recruitment	Age(s)
Tapia 2015	Western Africa	Mali	cross-sectional	Four public schools (Lower SES)	Periurban	Children attending one of four public schools in Mali.	Student complaining of sore throat	convenience sampling	Four public schools	5 - 16 years
Taplin 1973	Southern Africa	Uganda	cross-sectional	not stated but assumed Rural setting	NS	Infants and school children (no further information given).	sore throat and skin infections	not stated	not stated	2 - 15 years
Tesfaw 2015	Eastern Africa	Ethiopia	cross-sectional	Two Public health centres in Jimma	Periurban	children years with pharyngitis presenting at two Health Centers in Jimma town	Sore throat	convenience sampling	Public Health Centres	5 - 15 years
Tewodros 1992	Eastern Africa	Ethiopia	cross-sectional	three public elementary schools located near a paediatric teaching hospital, located in the centre of Addis Ababa.	periurban	Children attending three elementary schools	Sore throat	convenience sampling	Paediatric teaching hospital	3 - 8 years
Van Zyl 1981	Southern Africa	Pretoria	cross-sectional	Outpatient hospital and two private practices	Urban	Children and adults with sore throat	Sore throat	convenience sampling	Outpatient hospital and two private practices	5 - 25 years
Zegeye 2016	Eastern Africa	Ethiopia	cross-sectional	Pediatric Cardiac Clinic of TASH in Addis Ababa, Ethiopia	Both	Children on secondary prophylaxis for RHD participated in this study	children who were on secondary prophylaxis for RHD were enrolled in the study.	convenience sampling	recruited consecutively as they came to the cardiac clinic for their follow up appointment	5 - 15 years

Table 4.2. List of excluded studies

Author	Year	Title	Reason for exclusion
Hraoui	2011	Epidemiological markers of Streptococcus pyogenes strains in Tunisia	No Denominator/Not population-based
Steer	2009	Global emm type distribution of group A streptococci: systematic review and implications for vaccine development.	No denominator
Liebowitz	2003	National surveillance programme on susceptibility patterns of respiratory pathogens in South Africa: moxifloxacin compared with eight other antimicrobial agents	No denominator
El Kholy	1980	A controlled study of penicillin therapy of group A streptococcal acquisitions in Egyptian families.	Objective of study not applicable
Iroezindo	2014	Sputum bacteriology and antibiotic sensitivity patterns of community-acquired pneumonia in hospitalized adult patients in Nigeria: a 5-year multicentre retrospective study.	Denominator not representative of the population
Beekmann	2005	Antimicrobial resistance in Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and group A -haemolytic streptococci in 2002–2003	No denominator
Rimoin	2008	Variation in Clinical Presentation of Childhood Group A Streptococcal Pharyngitis in Four Countries	Duplicate study data. Chose later version
Engel	2014	Group A Streptococcal emm Type Prevalence among Symptomatic Children in Cape Town and Potential Vaccine Coverage	Duplicate study data. Chose later version
Rimoin	2010	The utility of rapid antigen detection testing for the diagnosis of streptococcal pharyngitis in low-resource settings.	Duplicate study data. Chose later version
Benouda	2009	Antimicrobial Resistance of Respiratory Pathogens in North African Countries	Duplicate study data. Chose later version

4.3.3 Assessment of risk bias in included studies

Risk of bias was assessed using the Hoy criteria as modified by Werfalli and colleagues (Hoy *et al.*, 2012; Mahmoud Werfalli *et al.*, 2014). Twelve studies had a low risk of bias (Bassili *et al.*, 2002; Bélard *et al.*, 2015; Boukadida *et al.*, 2003; Mezghani Maalej *et al.*, 2010; Milagritos D Tapia *et al.*, 2015; Tewodros *et al.*, 1992; Steinhoff *et al.*, 1997; SEDKI *et al.*, 2010; Gonsu *et al.*, 2015; van Zyl *et al.*, 1981; Olivier & de Graad 1978; Engel *et al.*, 2017), Twelve had a moderate risk of bias (A. Benouda *et al.*, 2009; Abd El-Ghany *et al.*, 2015; Mzoughi *et al.*, 2004; O'Meara *et al.*, 2015; Rimoin *et al.*, 2011; Ringertz *et al.*, 1993; Tesfaw *et al.*, 2015; Pius *et al.*, 2016; Zegeye *et al.*, 2016; Milagritos D Tapia *et al.*, 2015; Fourati *et al.*, 2009; Seale *et al.*, 2016) and one study was deemed to have a high risk of bias (Ibekwe and Okafor, 1983)(Figure 4.2, Table 4.3).

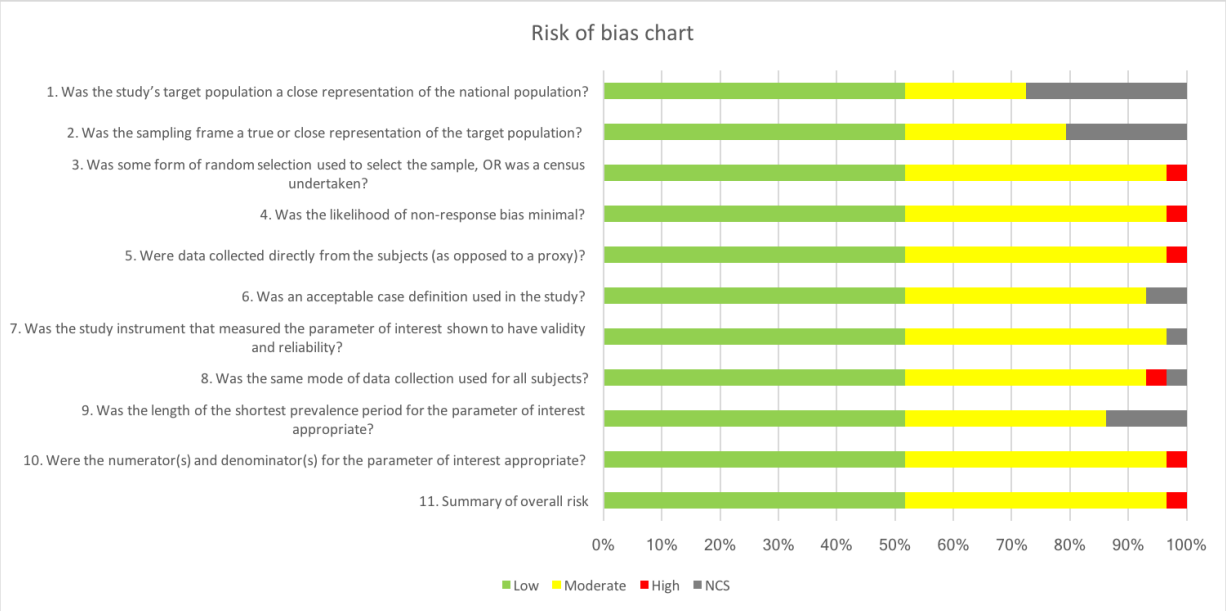


Figure 4.2. Risk of Bias Chart.

Table 4.3. Summary of risk of bias evaluation

		Internal Risk of Bias				External Risk of Bias							
Study ID	**Year**	**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**	**Quality score**	**Risk of bias**
Abd El-Ghany	2015	NCS	NCS	0	1	1	1	1	1	1	1	7	Moderate
Bassili	2002	1	1	1	1	1	1	1	1	1	1	10	Low
Bélard	2015	1	1	0	1	1	1	1	1	1	1	9	Low
Benouda	2009	NCS	NCS	0	1	1	1	1	NCS	1	1	6	Moderate
Boukadida	2003	1	1	0	1	1	1	1	1	1	1	9	Low
Maalej	2010	1	1	0	1	1	1	1	1	1	1	9	Low
Tapia	2015	1	1	0	1	1	1	1	1	1	1	9	Low
Mzoughi	2004	NCS	1	0	1	1	1	1	1	1	0	7	Moderate
O'Meara	2015	0	1	0	1	1	1	1	1	1	1	8	Moderate
Rimoin	2011	0	0	0	1	1	1	1	1	1	1	7	Moderate
Ringertz	1993	1	1	0	1	1	NCS	1	1	NCS	1	7	Moderate
Steinhoff	1997	1	1	0	1	1	1	1	1	1	1	9	Low
Tesfaw	2015	NCS	NCS	0	1	1	1	1	1	1	1	7	Moderate
Tewodros	1992	1	1	0	1	1	1	1	1	1	1	9	Low
Zegeye	2016	0	1	0	1	1	1	1	1	1	1	8	Moderate
Ebekwe	1983	NCS	NCS	0	1	1	NCS	NCS	1	NCS	1	4	High
Pius	2016	0	1	0	1	1	1	1	1	1	1	8	Moderate
Seale	2016	0	1	0	1	1	1	1	1	1	1	8	Moderate
Gonsu	2015	1	1	0	1	1	1	1	1	1	1	9	Low
Fourati	2009	NCS	NCS	0	1	1	1	1	1	NCS	1	6	Moderate
Sedki	2010	1	1	0	1	1	1	1	1	1	1	9	Low
Taplin	1973	NCS	NCS	0	1	1	1	1	1	NCS	1	6	Moderate
Engel	2016	1	1	0	1	1	1	1	1	1	1	9	Low
Olivier	1978	1	1	0	1	1	1	1	1	1	1	9	Low
Van Zyl	1981	1	1	0	1	1	1	1	1	1	1	9	Low

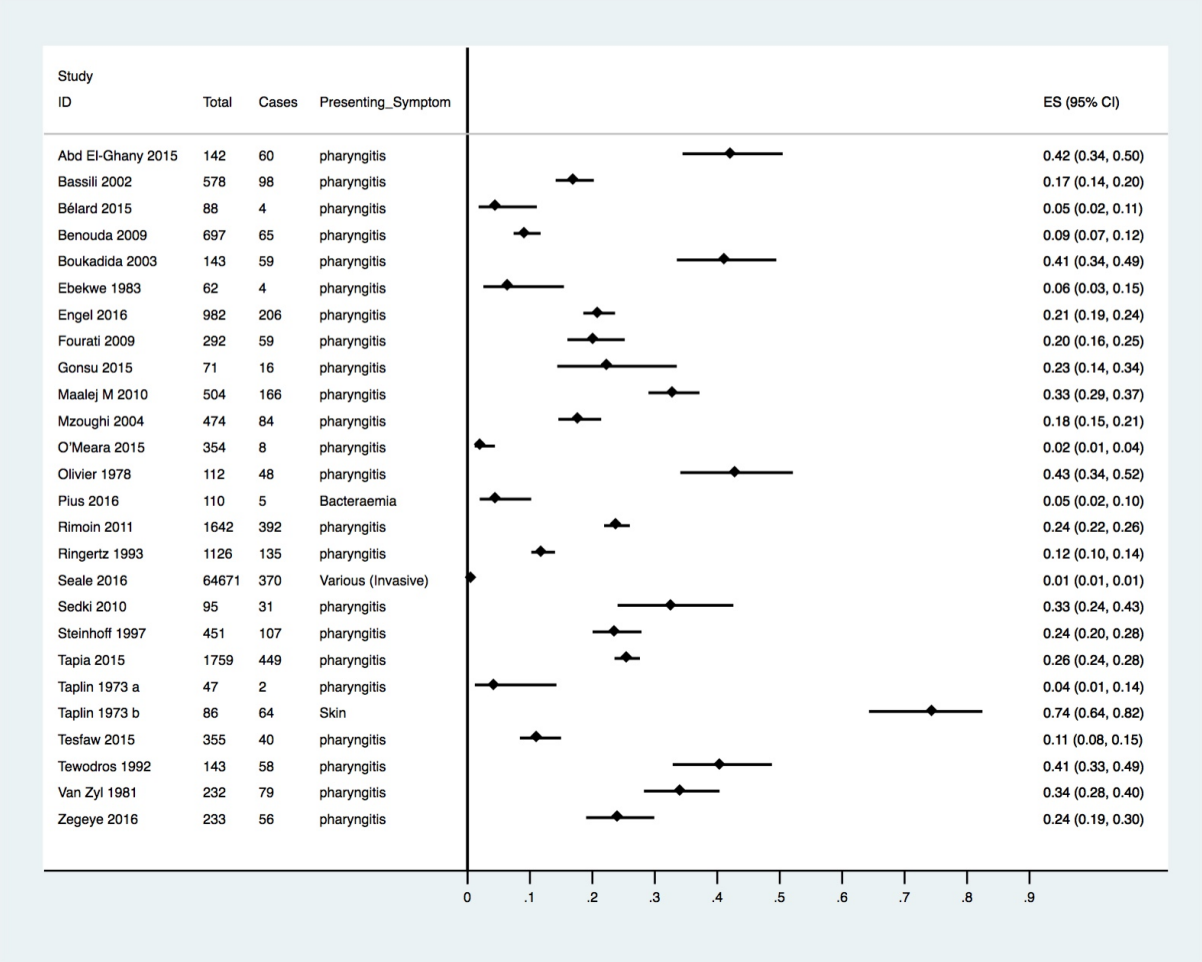
NCS. Not clearly stated

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

4.3.4 Prevalence of included studies

4.3.4.1 Overall

The prevalence of GAS in 25 studies, (n=75,449) among patients aged between 2 months and 92 years, presenting with pharyngitis, skin infection, fever, rheumatic fever and rheumatic heart disease living in Africa are shown below (Figure 4.3). One study comprised two groups of participants, presenting with skin infection and pharyngitis (Taplin and Lansdell, 1973).



ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.3. Studies included in the systematic review

4.3.4.2 Invasive Disease

Two studies (n=64,781) reported respective prevalence estimates of 5% and 0,6% (Pius *et al.*, 2016; Seale *et al.*, 2016) in GAS isolated from normally-sterile sites including blood, CSF and soft tissue.

4.3.4.3 Non-invasive Disease

Skin infections

A single study reported on skin infections consisting of 86 participants with a prevalence of 74% (95% CI, 64% to 82%).

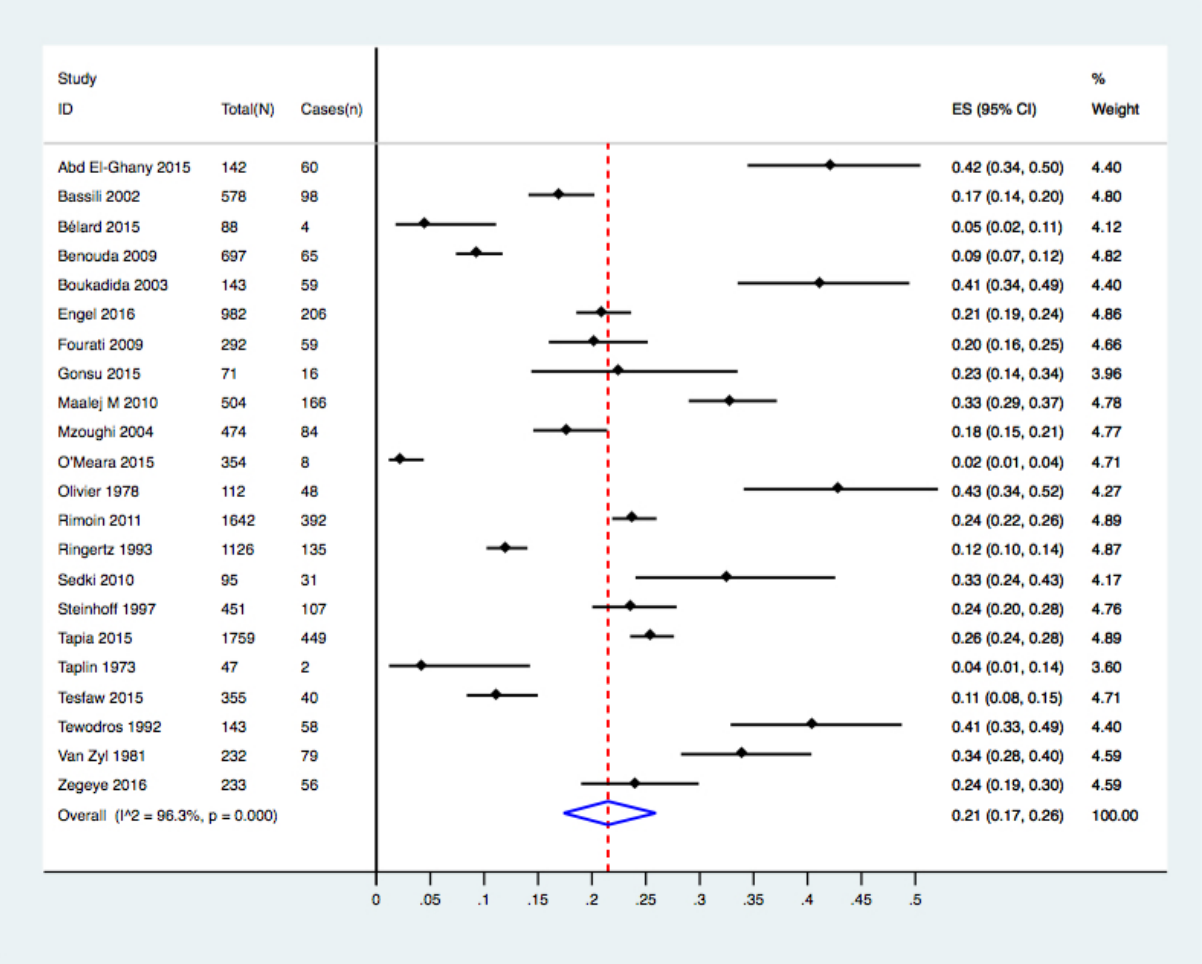
Pharyngitis

Twenty-two studies reported on the prevalence of GAS pharyngeal isolation and were considered for meta-analysis. All data synthesis in this review will be based on GAS pharyngitis.

4.3.5 Data synthesis: GAS Pharyngitis

4.3.5.1 Overall

The overall pooled prevalence estimate for GAS pharyngitis was 21% (95% CI, 17% to 26%; 22 studies, n=10,520) for streptococcal pharyngitis among people within the age range of 2 months - 92 years living in Africa, I^2= 96.25% (Figure 4.4).



ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.4. Pooled estimate of GAS Pharyngitis prevalence in Africa

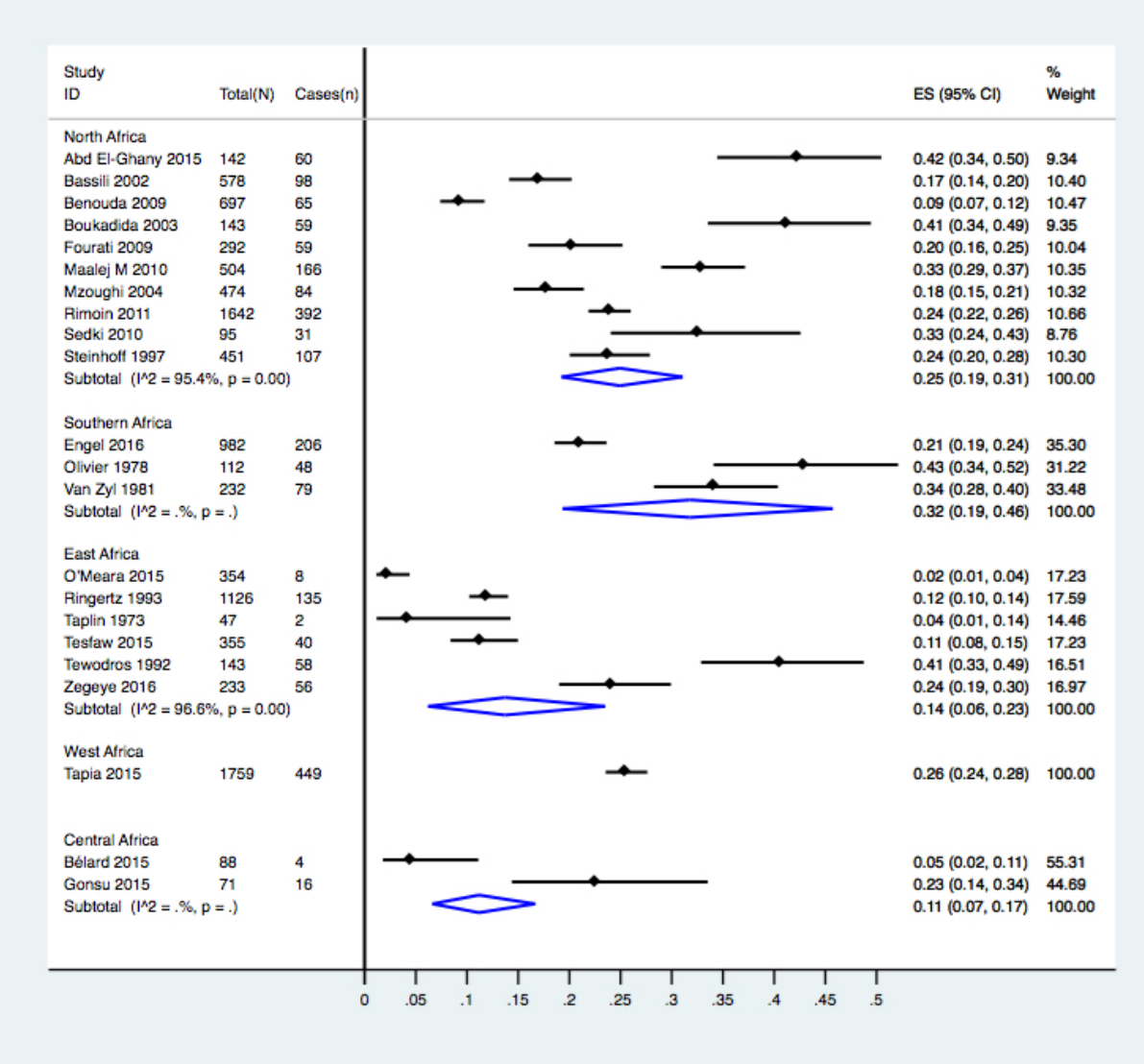
4.3.5.2 Regional Analysis

The map below (Figure 4.5) shows the countries that contributed data to this systematic review.

Figure 4.5: Countries contributing to this review

4.3.5.3 Regional analysis: Pharyngitis

The pooled prevalence estimates in North Africa were similar to a single study in West Africa, 25% (95% CI, 19% to 31%) and 26% (95% CI, 24% to 26%) respectively. Southern regions had a pooled prevalence estimate of 32% (95% CI, 19% to 46%). Central and East African regions had the lowest prevalence's of 11% (95% CI, 7% to 17%) and 14% (95% CI, 6% to 23%; age range of 2 months - 92 years; I^2= 95.40% (Figure 4.6).

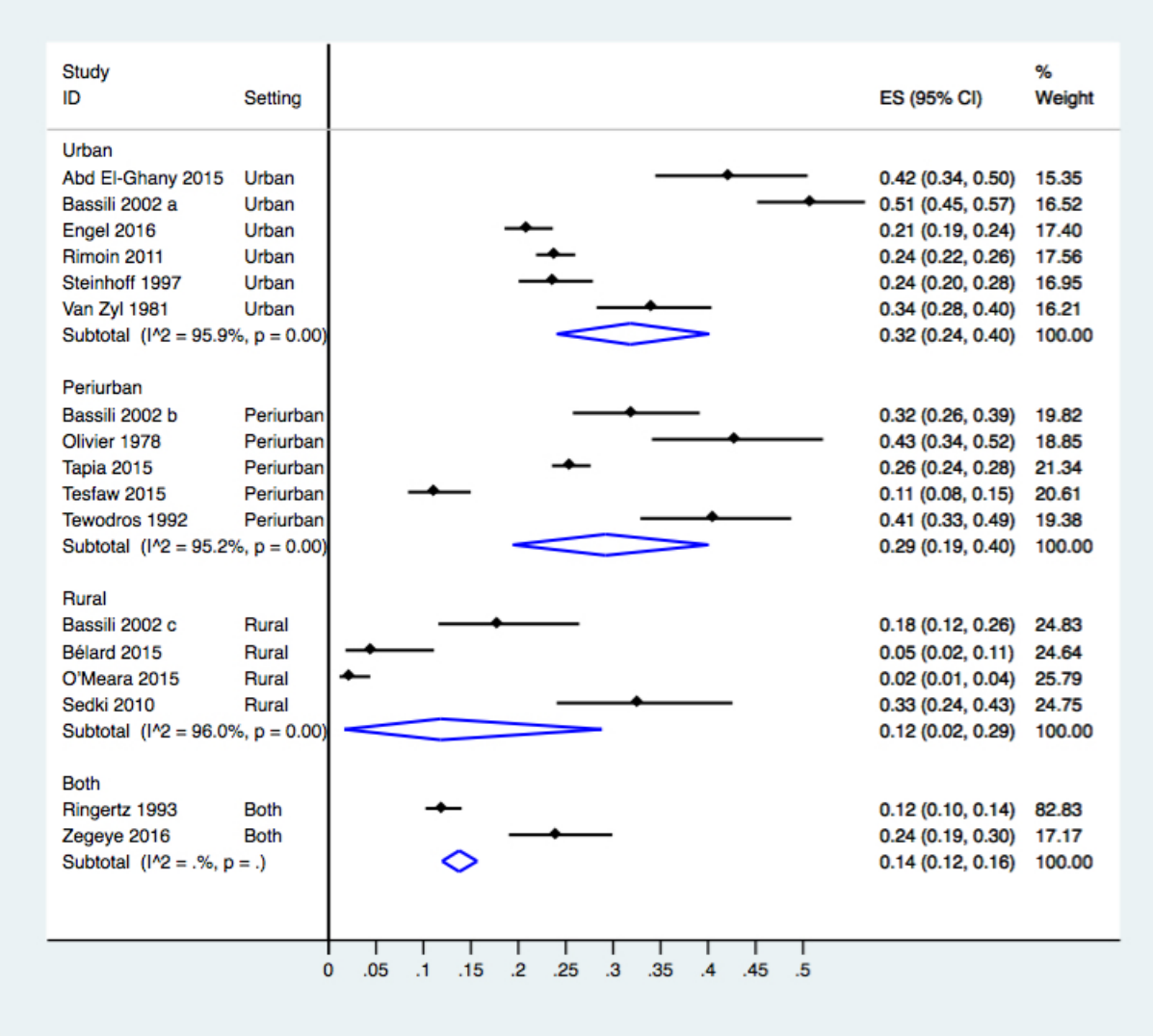


ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.6. Regional analysis: pharyngitis

4.3.5.4 Urban vs rural: Pharyngitis

The pooled prevalence estimates by rural, peri-urban and urban geographical settings were 12% (95% CI, 2% to 29%), 29% (95% CI, 19% to 40%) and 32% (95% CI, 24% to 40%) respectively. Studies reporting prevalence estimates in both urban and rural geographical regions had a pooled prevalence estimate of 14% (95% CI, 12% to 16%); this difference was statistically significant, (P<0.0001). (Figure 4.7)

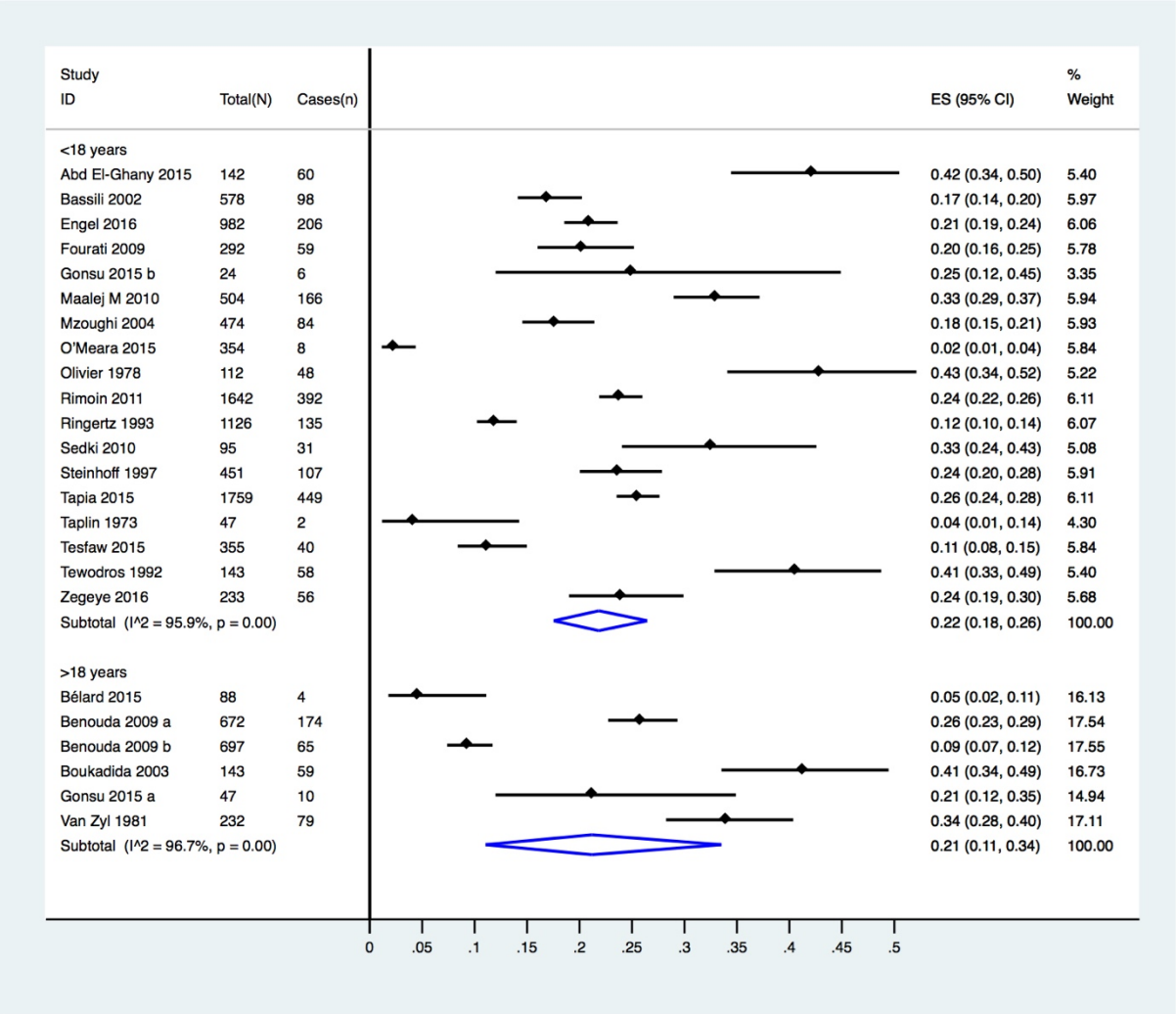


ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.7. Setting: pharyngitis analysis

4.3.5.5 Age range: Pharyngitis

Patients <18 years old had a pooled prevalence estimate of 22% (95% CI, 18% to 26%) and patients >18 years old had a pooled prevalence estimate of 21% (95% CI, 11% to 34%); this difference was not statistically significant, (P=0.90). (Figure 4.8)



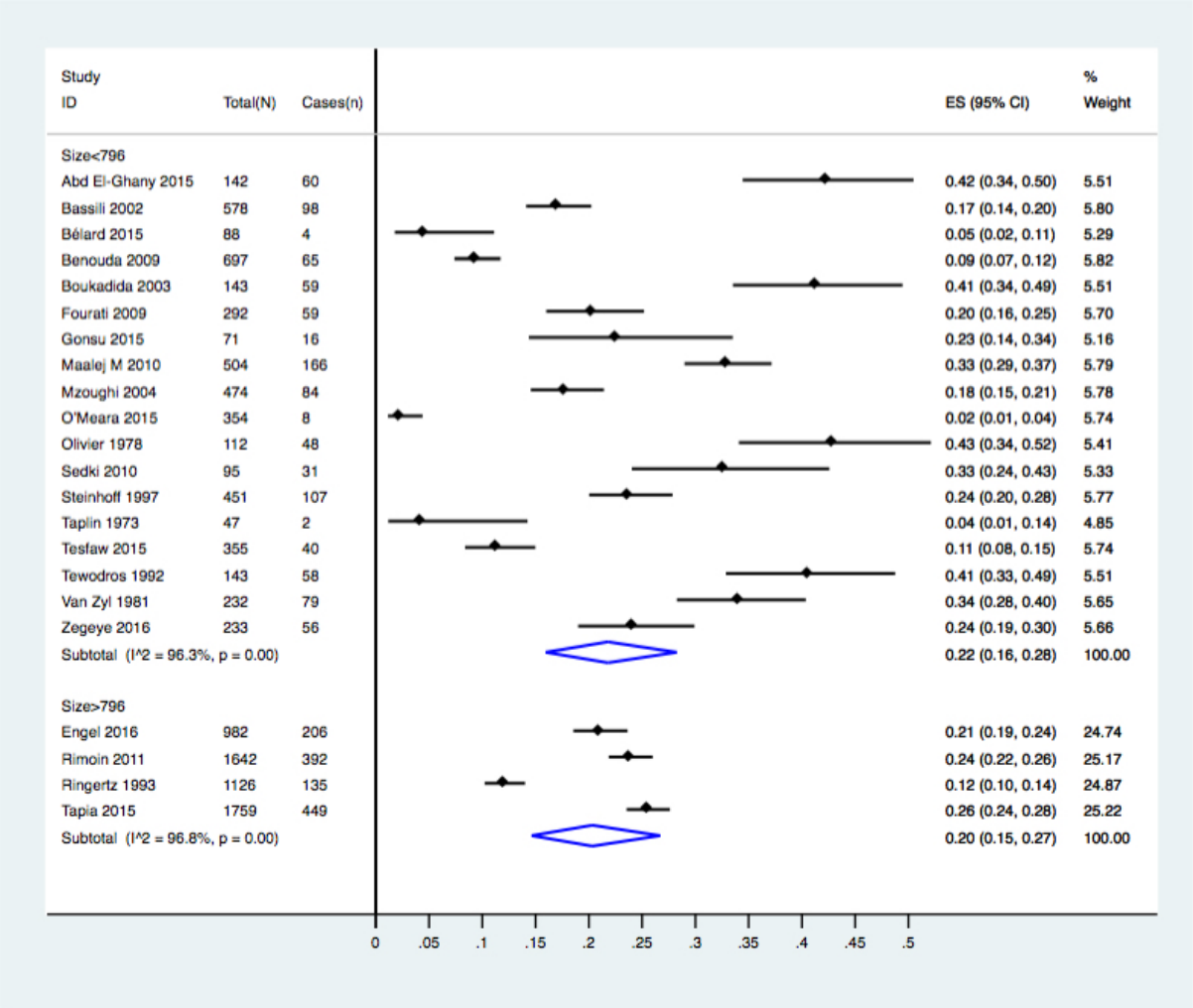
ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.8. Age analysis: pharyngitis

4.3.5 Sensitivity analysis

4.3.6.1 Sample size: Pharyngitis

Studies considered to be adequately powered had a prevalence estimate of 20% (95% CI, 15% to 27%). In comparison, studies considered to be inadequately powered had a prevalence of 22% (95% CI, 16% to 28%). P=0.71. (Figure 4.9)

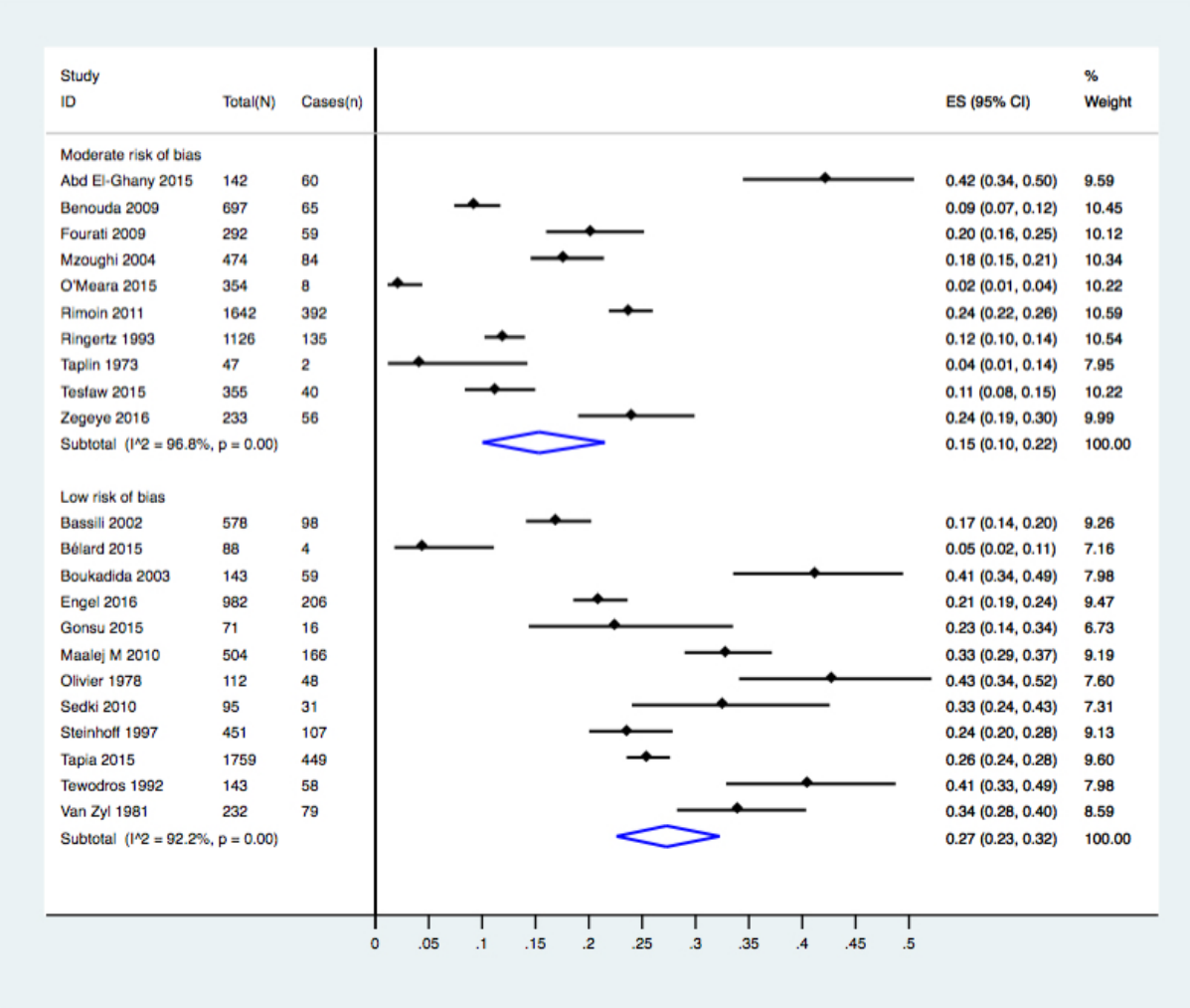


ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.9. Sample size analysis: pharyngitis

4.3.6.2 Quality: Pharyngitis

When considering quality assessment among pharyngitis cases, studies considered having a low risk of bias studies (n=12) had a pooled prevalence of 27% (95% CI, 23% to 32%) and those who had a moderate risk of bias (n=10) had a pooled prevalence of 15% (95% CI, 10% to 22%); this difference was statistically significant (P<0.0001) (Figure 4.10).



ES, effect size; CI, confidence interval; N, denominator; n, number

Figure 4.10. Quality of the studies: pharyngitis

4.3.7 Molecular characterisation of symptomatic group A *streptococcus* isolates

Two included studies reported on the molecular characterisation of GAS pharyngitis (Engel *et al.*, 2014; Tapia *et al.*, 2015). The first study observed 26 different *emm* types; the most prevalent was emm48 which accounted for 15% of the total isolates. The most prevalent *emm* types were *emm*48, *emm*89, *emm*4, *emm*12, *emm*75, *emm*1, *emm*94, *emm*22, *emm*9 and accounted for 73% of isolates typed. These GAS isolates showed a 65% coverage with an additional 23% cross-reactivity compared with current 30-valent vaccine formulation. The second study observed 70 different *emm* types; most prevalent being *emm*65 which accounted for 5.6% of the total isolates typed. The most prevalent types were *emm*65 *emm*18 *emm*55 *emm*42 *emm*81 *emm*58 *emm*25 *emm*109 *emm*11 *emm*169 *emm*89 *emm*77 *emm*75 *emm*64, representing 49% of the total isolates. These GAS isolates showed a 31% vaccine coverage and 44.1% *emm* cross-reactivity. One study reported on the molecular characterisation of invasive GAS disease (Seale *et al.*, 2016), observing 88 different *emm* types in the study population. No *emm* type represented >5% of the total isolates and showed a 28% coverage and 29% *emm* cross-reactivity.

4.4 Discussion

This systematic review is the first comprehensive synthesis of the prevalence of group A streptococcus infection, particularly GAS pharyngitis, among people living in Africa.

There are three important findings: (1) There is a high prevalence of GAS pharyngitis among people living in Africa, (2) There are regional differences with Central Africa having a significantly lower prevalence of GAS compared with the North, Southern and West African regions, (3) There are geographical differences; urban settings have a significantly higher prevalence rate compared with rural settings.

The pooled prevalence of GAS pharyngitis in Africa was determined to be 21% (95% CI, 13% to 30%). A single study reported on the prevalence of GAS skin infections with a prevalence of 74% (95% CI, 64% to 82%). Two studies, reporting on the molecular characterisation of GAS pharyngitis, showed a vaccine coverage and cross-reactivity of 75% and 88% respectively, with reference to a 30-valent vaccine currently under development. A single study reporting on the molecular characterisation of invasive GAS (*i*GAS) indicated a low coverage (28%), and cross-reactivity (29%), together providing a potential coverage of 57%. In addition, given the paucity of *i*GAS studies highlighted in this review, we conducted a prospective surveillance study to collect information on *i*GAS infection (Chapter 5).

Shaikh and colleagues, in an earlier systematic review, reported pooled prevalence estimates of 37% (95% CI, 32% to 43%) and 24% (95% CI, 21% to 26%) in children among all ages and those younger than 5 years respectively, presenting with pharyngitis and residing in low, middle and high income countries (Shaikh *et al.*, 2010). A single study in this review from Egypt, reported a higher prevalence estimate of 27% (95% CI, 25% to 30%). Our pooled estimate of 21% (95% CI, 17%

to 26%) for GAS pharyngitis, indicates that the prevalence of GAS infection remains high among African people.

In a review by Steer *et al*, the *emm* types included in the reformulated vaccine covered less than 65% of all isolates in four regions; Middle East, Asia, Pacific region and with particularly poor coverage in Africa (Andrew C Steer *et al*., 2009).

Central Africa had the lowest pooled prevalence of 11% (95% CI, 7% to 17%) and contrasted significantly with pooled prevalence rates in the North (25%), Southern (32%), and West African (26%) regions. Of interest, compared with pooled prevalence estimates of GAS carriage, West African regions had a significantly lower prevalence compared with other regions (Moloi, 2015).

We considered the impact of geographical setting on the overall prevalence estimates of GAS in Africa. Urban and peri-urban settings had a similar prevalence of 32% and 29% respectively. As expected, a pooled prevalence estimate of 12% (95% CI, 2% to 29%) in rural settings, were lower than urban settings, and may be explained by the socioeconomic factors which influence infection such as overcrowding and the lack of suitable housing etc. (Factor *et al*., 2003).

We also considered the effect of age on prevalence estimates and found no significant difference between older and younger people living in Africa. This finding was unexpected given that GAS pharyngitis is thought to be most commonly observed in younger children between the ages 5 - 15 years old (Carapetis, Steer, *et al*., 2005; Shulman *et al*., 2012; Steer *et al*., 2009).

Our study results were robust when considering the impact of sample size on pooled prevalence estimates using n=796 as a cut-off as recommended by Daniel W (Daniel, 1999). Studies considered to be adequately powered had a slightly lower pooled prevalence estimate of 20% (95%

CI, 15% to 27%), compared with those considered not adequately powered, 22% (95% CI, 16% to 28%).

We also considered the impact of quality of included studies for GAS Pharyngitis. Studies with a moderate risk of bias had significantly lower prevalence estimate of 15% (95% CI, 10% to 22%) compared with those of low risk of bias, 27% (95% CI, 23% to 32%). This finding suggests our overall pooled results may underestimate the prevalence of GAS pharyngitis in Africa.

This systematic review provides further evidence for the high burden of disease caused by GAS infection in Africa, thus providing support for a vaccine. The direct and indirect costs of GAS disease in the US alone in 2005 were in excess of 2 billion dollars per year (A. L. Bisno *et al.*, 2002). A GAS vaccine would undoubtedly be a cost effective primary level intervention to prevent GAS infections. Currently, a 30-valent vaccine (reformulated from a 26-valent vaccine) based on the variable N-terminal regions of the surface M protein of GAS is being developed (Dale *et al.*, 2011).

One of the main strengths of this review is attributed to multiple databases searched, using an African search filter and a robust approach to the meta-analysis of the data. We systematically and purposefully assessed all the data available with no language exclusions, using the most recently published standard quality assessment tools for prevalence studies. A limitation to the results of our systematic review is the significant heterogeneity in the prevalence estimates produced in the meta-analysis, however, this is expected when pooling prevalence studies. We made use of the Freeman-Tukey double arc-sine transformation 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, as well as across major subgroups (Nyaga *et al.*, 2014).

Attempts to fully explain the heterogeneity was unsuccessful in terms of subgroup analyses. Given the variance of standardised epidemiological and laboratory efforts used in various studies,

comparisons between populations, and conclusions about the differences in prevalence rates should be made with caution. We also intended to perform sub-analysis of GAS colonization risk factors including crowding, gender and seasonality; however, due to data limitations, these could not be performed.

Our results, which we believe are an underestimate of the true prevalence of GAS pharyngitis, confirm the high prevalence of GAS infections in Africa, warranting the need for new and improved prevention strategies which include well-functioning disease registries such as AFRO*Strep* (Barth *et al.*, 2016) to collect robust clinical, microbiological and molecular data. Improved rapid diagnostic tools and the need for training health care professionals with updated clinical prediction rules is vital to reduce the prevalence of pharyngitis due to missed diagnoses. Regional data were scarce on differences and associated risk factors, and thus studies are needed to provide these missing figures. Our findings support the call for the development of a vaccine as a primary preventative measure in attempt to reduce the burden of GAS diseases in Africa and around the globe (Dale *et al.*, 2011; Steer *et al.*, 2009).

Table 4.4. PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	80
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.	81
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	62
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	63
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.	63
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.	65
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.	64
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	64
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).	66
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.	66
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	64

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.	67
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	68
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	68 -69

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).	67
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	69
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.	83
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.	86 – 88
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).	91
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.	92 – 98
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	92 – 98
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]).	99 – 100
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).	102
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).	104

Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	105
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.	69

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

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5. CHAPTER FIVE:

STUDY 2: INVASIVE AND NON-INVASIVE GROUP A B-HAEMOLYTIC STREPTOCOCCAL INFECTIONS IN PATIENTS ATTENDING PUBLIC SECTOR FACILITIES IN SOUTH AFRICA: 2003 - 2015

5.1 Introduction

Increases in the annual number of cases for invasive GAS (*i*GAS) and non-invasive GAS (non-*i*GAS) diseases have been observed globally since the 1980s. In the United Kingdom, a report from routine surveillance data indicate a significant increase of invasive GAS isolates from December 2008 (n=143) compared to the same period in 2007 (n=86) (Lamagni *et al.*, 2009). The reasons for these observations are not clearly understood and have subsequently caused many countries to commence active surveillance systems for invasive GAS to document the epidemiology of the disease. However, data from the Centers for Disease Control and Prevention surveillance programme reported stable incidence rates for invasive GAS disease from 1996 – 1999, and from 2002 – 2009 (O'Loughlin *et al.*, 2007; Stockmann *et al.*, 2012).

The incidence and prevalence of both invasive and non-invasive GAS infections in developing countries are largely unknown (Carapetis *et al.*, 2005). The aim of this study was to describe the epidemiology of *i*GAS and non-*i*GAS infection and associated clinical characteristics in the public sector of South Africa, over a recent 13-year period (2003 – 2015).

5.2 Methods

The detailed methods for this study are described in Chapter 3, section 3.2. Briefly, we performed a retrospective analysis of *i*GAS and non-*i*GAS cultured at the National Health Laboratory Service (NHLS) from January 2003 to December 2015.

5.3 Results

Clinical characteristics

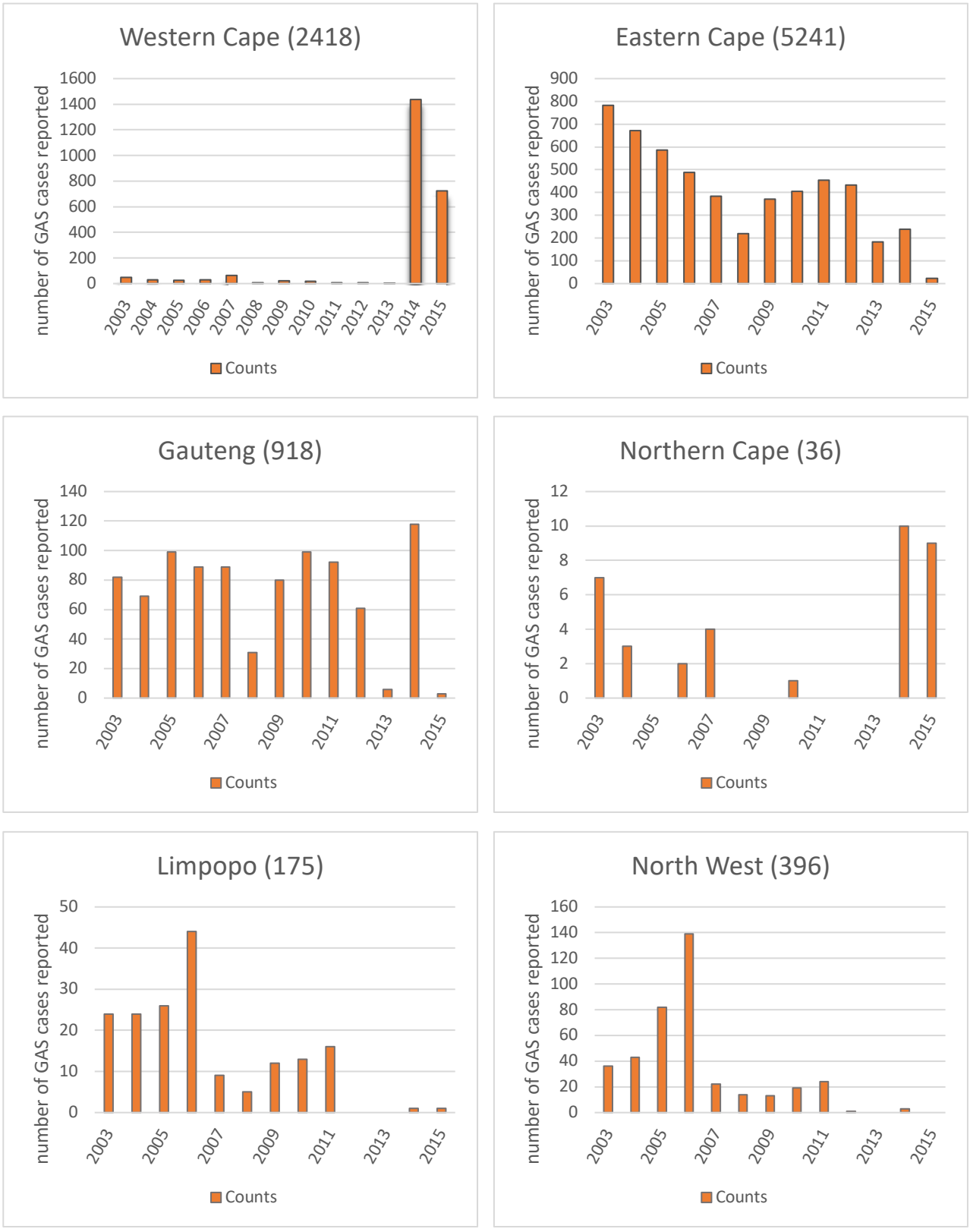
The clinical characteristics of patients with *i*GAS infection and non-*i*GAS are presented in Table 5.1. For the period between 2003 and 2015, 10,091 GAS isolates were recovered from South African patients. Sources of isolates included blood, CSF, abscesses, joint aspirates, pleural fluid, burn swabs, ear, eye and pus swabs (Table 5.2). Blood and aspirates were common sources of isolation among *i*GAS infections and pus swabs were the most common source of isolation for non-*i*GAS infections.

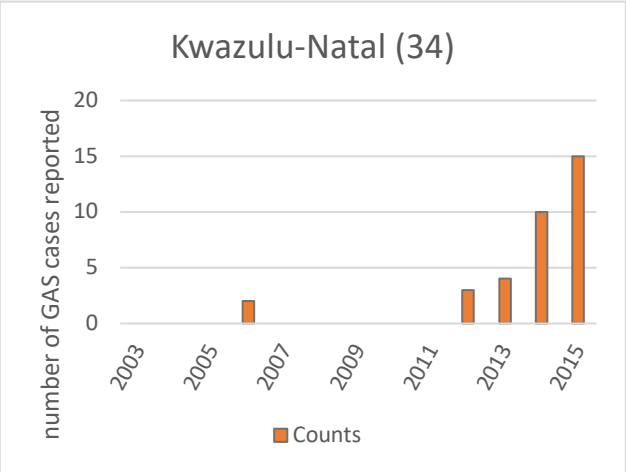
By Province

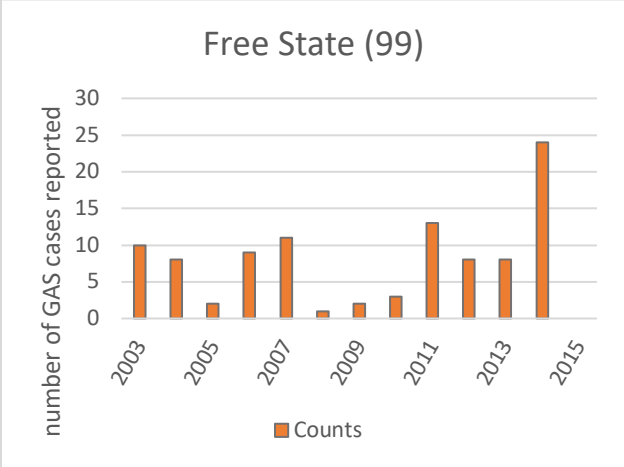
When considering the GAS data at a provincial level, a number of anomalous data collection trends were highlighted. The Northern Cape Province represented 0.4% of the total GAS infections in South Africa over the study period; data were missing for years 2005, 2008-2009, 2011-2013), (Figure 5.1.) In the Western Cape, low reporting rates were seen for the first 11 years of the study period, followed by major peaks in reporting rates in 2014 and 2015, (Figure 5.1.) In Gauteng, the overall number of GAS reported over the study period was low considering it has the largest population compared to other provinces in South Africa. The very low number of cases in 2013 and 2015 is also notable, (Figure 5.1). The Free State province showed low reporting of GAS in 2005, 2008 – 2011, (Figure 5.1).

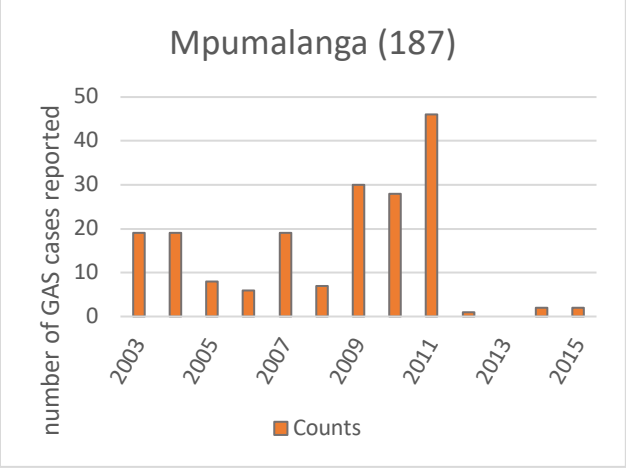
The provinces of Limpopo and the North West show similar patterns of reporting with many cases reported in the beginning of the study period followed by a decline after 2006 and almost no data reported after 2011, (Figure 5.1). The Mpumalanga province also showed inconsistent reporting with a peak in 2011 followed by almost no data thereafter. Kwazulu-Natal recorded the least number cases of GAS in South Africa (n=34) despite it being home to the second largest population, after Gauteng. It must be noted that the Kwazulu-Natal province was the last to be integrated into the NHLS electronic system, with reporting only commencing in the later years. The Eastern Cape province accounted for 52% of the total GAS cases throughout the country (Figure 5.1).

Figure 5.1. GAS isolation by Province




Kwazulu-Natal (34)
number of GAS cases reported
20
15
10
5
0
2003
2005
2007
2009
2011
2013
2015
Counts


Free State (99)
number of GAS cases reported
30
25
20
15
10
5
0
2003
2005
2007
2009
2011
2013
2015
Counts


Mpumalanga (187)
number of GAS cases reported
50
40
30
20
10
0
2003
2005
2007
2009
2011
2013
2015
Counts

GAS Disease incidence in South Africa

The provincial level data revealed a low ascertainment and inconsistent reporting practices of GAS infections throughout the study period and we were unable to determine any variation in IRs of GAS over the study period across the provinces. The Eastern Cape was the only province with complete data for each of the years within the study period, thus making it possible to evaluate incidence rates and describe trends of GAS infection over the study period.

GAS Disease incidence in Eastern Cape only

The mean annual IR for GAS infection was 6.00 cases per 10^5 py. The annual IR decreased significantly from 2003 to 2015 from $12.04/10^5$ py to $0.45/10^5$ py (rate difference (RD), $11.59/10^5$; 95% (CI, $10.73/10^5$; $12.45/10^5$) (Figure 5.2). Twenty-eight percent of isolates (n=1,474) were from patients younger than 18 years of age, 58% from patients (n=3,014) aged 18 – 64 years and, 14% (n=722) from those older than 64 years. The mean age was 30 years; Range, 2 days – 102 years). The mean age-specific IRs were as follows: <18 years, $3.62/10^5$ py; 18-64 years, $7.29/10^5$ py; ≥65 years, $14.06/10^5$ py. The increase in IRs with age was apparent in both the *i*GAS, and non-*i*GAS sub-sets of patients (Table 5.1).

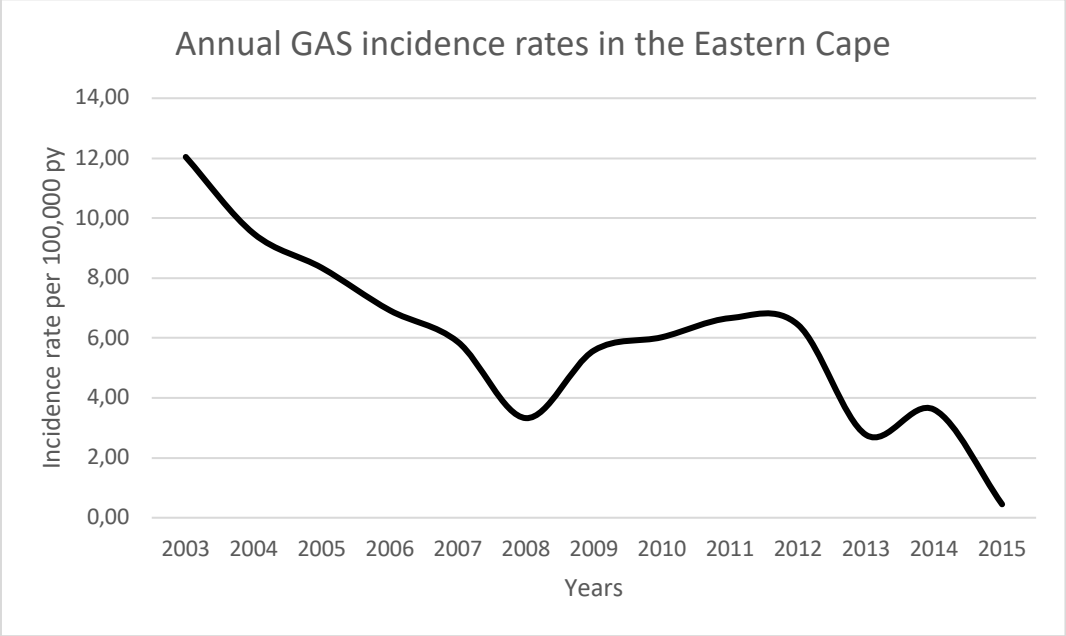


Figure 5.2. Annual GAS incidence rates among 5, 256 patients with invasive and non-invasive group A streptococcal infection, Eastern Cape, South Africa, 2003-2015

Table 5.1. Year of isolation, age and sex of the 10,091 cases with invasive and non-invasive group A streptococcal infection, South Africa, 2003-2015

Year of isolation	N (%)
2003	1079 (10.7)
2004	915 (9.1)
2005	931 (9.2)
2006	959 (9.5)
2007	640 (6.3)
2008	301 (3.0)
2009	571 (5.7)
2010	628 (6.2)
2011	683 (6.8)
2012	521 (5.2)
2013	213 (2.1)
2014	1863 (18.5)
2015	786 (7.8)
Patient age, years	
< 18	2920 (29)
19 - 64	5902 (59)
≥ 65	1199 (12)
Sex#	
Male	5080 (51.4)
Female	4809 (48.6)

N, number; GAS, Group A Streptococcus # where data were reported

Table 5.2. Source of isolates taken from the 10,091 patients with invasive and non-invasive group A streptococcal infection, South Africa, 2003-2015

Source of Isolate	N (%)
Invasive GAS *	**1,646 (16)**
Blood culture	900 (8.9)
Other aspirates	194 (1.9)
Pleural fluid	121 (1.2)
Cerebrospinal fluid	63 (0.6)
Joint aspirate	41 (0.4)
Intra-abdominal fluid	36 (0.4)
Biopsy	9 (0.1)
Other sites (≤5 cases)	282 (2.8)
Non-invasive GAS*	**8,445 (84)**
Pus swab	6421 (63.6)
Urine	562 (5.6)
Burn swab	474 (4.7)
Ear swab	366 (3.6)
Wound swab	147 (1.5)
Sputum	142 (1.4)
Throat swab	82 (0.8)
Vaginal swab	72 (0.7)
Eye	56 (0.6)
Other sites (≤5 cases)	123 (1.2)

* data shown for sites where number of isolates > 5 per site.

N, number; GAS, Group A Streptococcus

Eastern Cape:

Invasive GAS

Four hundred and twenty-eight cases of *i*GAS isolates (8% of total GAS cases in the Eastern Cape) were documented in the NHLS database. A multi-modal curve was observed over the study period (Figure 5.3), with a mean annual IR of 0.48 cases per 10^5 py (mean age, 27 years; Range, 2 days – 84 years).

Over the study period, there was a decrease in the annual IR, RD, 0.23/10^5 py; 95% CI, 0.02 - 0.44 /10^5 py. Breakdown by age-specific categories was 36% among those <18 years of age, 51%, 18 – 64 years, and 13%, ≥ 65 years. The mean age-specific IRs for *i*GAS were as follows: < 18 years, 0.38/10^5 py; 18-64 years, 0.52/10^5 py; >65 years, 1.03/ 10^5 py. The IRs remained stable between 2003 and 2012, with slight increases and decreases. The IR for *i*GAS peaked in 2014 followed by a rapid decline in 2015.

Non-invasive GAS

Four thousand eight hundred and twenty-eight cases making up 92% of the dataset met with the case definition for non-*i*GAS. The mean age was 31 years (Range, 2 days – 102 years); GAS isolation for age-specific categories were 28%, <18 years of age, 58%, 18 – 64 years and 14%, ≥ 65 years (Table 5.3).

Mean annual incidence rates for non-*i*GAS were 5.48 cases per 10^5 py. Over the complete study period, there was a decrease in the rate of infections, RD, 11.36/10^5 py; 95% CI, 10.53 - 12.19/10^5 py. The mean age-specific IRs for non-*i*GAS were as follows (Table 5.3): <18 years, 3.24/10^5 py; 18-64 years, 6.77/10^5 py; >65 years, 13.03/10^5 py. A multi-modal shaped curve for incidence was observed; GAS IRs declined steadily from the beginning of the study period to 2008. This was followed by a slight increase before steadily declining to the end of the study period. There was a

marked decline in the numbers of GAS organisms isolated in 2008 and 2013 (Figure 5.3). The Mann-Kendall test and the Theil-Sen estimator showed a significant decreasing trend in the incidence of non-*i*GAS infection over the study period (P=0.002) (Table 5.4).

Table 5.3. Mean age-specific incidence rates of GAS, Eastern Cape, 2003-2015

	Age Category (yr)			Total
	<18	18 – 64	≥ 65	Mean IR
All GAS *n* (%)	1474 (28)	3014 (58)	722 (14)	5210 (100)
IR (per 10^5 py)	3.62	7.29	14.06	6.00
Invasive GAS *n* (%)	153 (36)	214 (51)	53 (13)	420 (100)
IR (per 10^5 py)	0.38	0.52	1.03	0.48
Non-invasive GAS *n* (%)	1321 (28)	2800 (58)	669 (14)	4790 (100)
IR (per 10^5 py)	3.24	6.77	13.03	5.48

yr, years; n, number; IR, Incidence rate; py, person-years

Age data were not available for n=31 (0.99%)

Figure 5.3. Annual Incidence rates of invasive and non-invasive group A streptococcal (GAS) infection. Eastern Cape, South Africa, 2003-2015

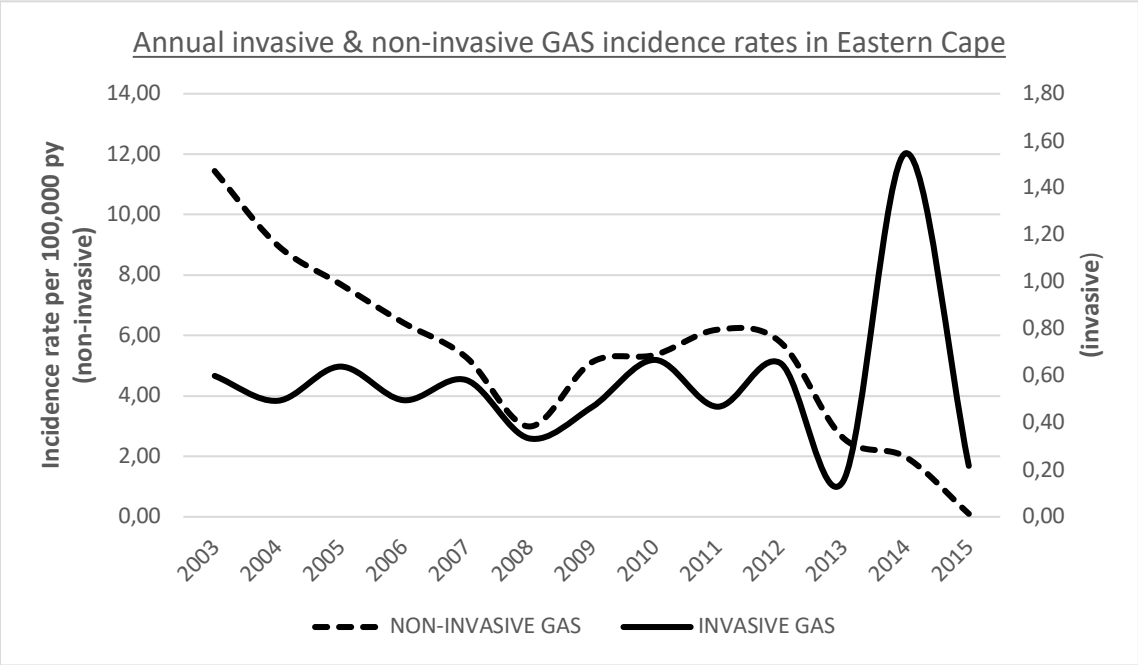


Table 5.4: Mann-Kendall trend test and Sen slope estimation for GAS infection in the public sector over a 13-year period (Eastern Cape).

GAS infection	Mann-Kendall	Sen estimation (median change (95% CI))
Invasive GAS	P = 0.2	-0.01 (-0.03 to 0.01)
Non-invasive GAS	P = 0.002	-0.65 (-0.82 to -0.50)

CI, confidence interval

5.4 Discussion

We sought to determine the incidence of GAS infection in all the provinces of South Africa. However, incomplete data were pervasive, with the Eastern Cape province being the only one with information for all the years under review, and accounted for 52% of the isolates collected in this study. Therefore, the incidence study was confined to the Eastern Cape.

The incidence rates (IR) of laboratory-confirmed *i*GAS and non-*i*GAS infection reported in the Eastern Cape Province of the South African public sector appears to have declined over the last 13 years. Specimens were retrieved from a variety of sources, the bulk of which were pus swabs, blood and urine samples. Of interest, the age-specific IRs increased with increasing age category, with those older than 65 years having the highest IR of 14.06 per 10^5 person-years. Non-*i*GAS infections showed a statistically significant decrease over the study period.

The laboratory data, retrieved from the National Health Laboratory Service database of South Africa, represent the public health sector serving 84% of the total South African population (Mayosi & Benatar, 2014).

In the Eastern Cape, the public health sector serves a slightly higher proportion (89%) of the population in the province than other provinces (ECDOH, 2015). To our knowledge, only two studies have reported incidence data on *i*GAS in Africa (Seale *et al.*, 2016; Berkley *et al.*, 2005). In a study conducted among children in rural Kenya, the IRs per 10^5 py for all (definite and probable) *i*GAS were as follows: children <5 years, 35, (95% CI, 30 - 40); and children <1 year was 101, (95% CI, 83 - 121) (Seale *et al.*, 2016). The second study reported *i*GAS and non-*i*GAS IRs per 10^5 py: children <5 years, 29 and children <1 year was 96 (Berkley *et al.*, 2005). These figures are among the highest reported compared with studies done in other resource limited settings and contrasts noticeably with our low mean IR of 3.62 /10^5 py amongst children < 18 years of age. The high

IR reported in Kenya was thought to be due to the presence of a number of risk factors including severe acute malnutrition, malaria and HIV infection which confirm reports from an earlier study conducted in Kenya, where *i*GAS was associated with malnutrition and HIV infection (Berkley *et al.*, 2005). Unfortunately, for our study, data regarding these putative risk factors were unavailable. Elsewhere, in Fiji, high IRs per 10^5 py were also reported; among those <5 years, IRs were 26 (95% CI, 19 - 34), and > 65 years, IRs were 85 (95% CI, 61 - 116) (Steer *et al.*, 2008; Andrew C. Steer *et al.*, 2009), while in New Caledonia, the all-ages (definite and probable) *i*GAS IR was $43/10^5$ py (95% CI, 35 - 52) (Baroux *et al.*, 2014). It is possible that the low incidence of GAS infection in our study is due to infrequent submission of specimens for microbiological culture by health practitioners in the Eastern Cape Province, particularly in more remote areas of the province.

The slight decrease in *i*GAS incidence in our data is in contrast with those reported elsewhere in the world since the 1980s (Efstratiou, 2000). In a surveillance study in Utah, USA, the overall incidence of *i*GAS disease increased significantly from 2002 to 2010 (Stockmann *et al.*, 2012). Ireland also experienced an increase in IRs with the highest annual IR observed in 2013 since *i*GAS became notifiable in 2004 (Meehan *et al.*, 2013). Similar increases have been observed elsewhere in Europe (Darenberg *et al.*, 2013; Report 2014). Slightly more males were affected compared with females; and the increase in incidence of *i*GAS and non-*i*GAS disease with age is in keeping with trends seen in studies published in New Caledonia and Fiji (Baroux *et al.*, 2014; Steer *et al.*, 2009).

The fluctuations in the IRs, observed over the study period for both *i*GAS and non-*i*GAS infection, followed a marked decrease observed in 2008 and 2013 in both *i*GAS and non-*i*GAS infection and are not clearly understood. We acknowledge that the variations in the incidence rates may be due to one of two, or both of the following reasons: (1) it may represent a reflection of the true variance in incidence, or (2) it may be a reflection in the number of samples submitted for

processing by NHLS laboratories which may be attributed to the rate of the reporting of cases at any given point in time and/or laboratory budget restrictions. The implications for these would thus be, in the case of the former, an indication that non-*i*GAS-related diseases are indeed on the decline in the South African setting. However, should the latter be the case with lower numbers of sample submitted to the laboratory, these data would nevertheless, represent the minimum incidence rates for GAS-related diseases. Also, the higher number of GAS cases reported from the Eastern Cape provinces may be due to close proximity of laboratories to academic hospitals and specimen-taking practices which may vary between the different institutions of the country, rather than a higher prevalence of GAS-related diseases in these provinces. It should also be noted that specimen collection practices may differ among provinces. Also, provinces such as Kwazulu-Natal only joined the digital system in the latter years. Thus, these findings emphasize the need for further research to allow for a more accurate estimation of the extent of GAS-related conditions.

The collection of epidemiological data in many developing countries is poor. A 2005 WHO report on *i*GAS disease showed that GAS is an important cause of mortality and morbidity on the global scale and in Africa (Carapetis *et al.*, 2005); thus, valid estimates of GAS infection in South Africa and Africa are needed to inform the revision of current prevention and control strategies and the development of new primary prevention strategies including the development of a universal GAS vaccine to reduce the burden of *i*GAS disease and their consequences (Dale *et al.*, 2013).

The main strength of this study is the large sample size of GAS isolates (>10,000) that were collected from the NHLS database over a 13-year period from all provinces of South Africa and representing 84% of the population. It is to be noted that IRs were calculated based on data from the Eastern Cape Province only (>5,000), where NHLS serves 89% of the population. This study, however, has several limitations including the retrospective design and source of data, and the lack of detailed clinical information in the NHLS database. Moreover, this study may underestimate

the incidence of non-*i*GAS infection, specifically for pharyngitis, given that microbiological throat swabs are not done routinely as standard clinical practice in South Africa. Similarly, for *i*GAS, our data are likely to underestimate the true incidence given widespread empiric use of antibiotics without culture in clinical practice.

Public health surveillance systems provide valid and reliable scientific information essential to inform appropriate decision making for the best possible action and intervention (Nsubuga *et al.*, 2006). In response to the need for an enhanced surveillance system, to collect high quality data, the AFRO*Strep* registry has been established to collect prospective and comprehensive information on *i*GAS and non-*i*GAS infection in Africa as an initial attempt to address the dearth of information on *i*GAS disease on the continent (Barth *et al.*, 2016).

In conclusion, the decrease in IRs among non-*i*GAS infection and persistent IRs for *i*GAS infection in the Eastern Cape, emphasizes the need to remain vigilant in diagnosing *i*GAS correctly and for systematically collecting data so as to ensure an efficient and functioning disease-control programme (Nsubuga *et al.*, 2006). Moreover, our data are almost certainly an under-estimate of the true burden of GAS disease which needs to be documented appropriately in a prospective surveillance study proposed by the AFRO*Strep* registry.

6. CHAPTER SIX:

STUDY 3: THE MOLECULAR TYPING OF NON-INVASIVE AND INVASIVE GROUP A STREPTOCOCCAL INFECTION AT GROOTE SCHUUR HOSPITAL IN CAPE TOWN

6.1 Introduction

The World Health Organization (WHO) ranked group A β-haemolytic *streptococcus* (GAS) (also known as *Streptococcus pyogenes*) as the ninth leading cause of human mortality, with the majority of deaths attributable to invasive group A streptococcal (*i*GAS) diseases (World Health Organisation, 2005). The majority of cases occur in developing countries (Carapetis *et al.*, 2005). It is believed that >600,000 cases of *i*GAS infection occur annually, with >160,000 deaths. Despite these alarming numbers, data on GAS infection are scant in developing countries (Carapetis *et al.*, 2005).

GAS is responsible for a wide range of invasive and non-invasive group A streptococcal (non-*i*GAS and *i*GAS) diseases (Bailie *et al.*, 2005; Steer *et al.*, 2007). These diseases range from mild infections such as impetigo and pharyngitis to serious diseases such as streptococcal toxic shock syndrome, and necrotising fasciitis. Moreover, GAS may trigger autoimmune diseases following repeated episodes of infection, such as acute rheumatic fever (RF)/rheumatic heart disease (RHD) and acute post-streptococcal glomerulonephritis (APSGN) (Carapetis *et al.*, 2005).

There is a dearth of *emm* type data in sub-Saharan Africa; three studies (Engel *et al.*, 2014; Tapia *et al.*, 2015; Tewodros & Kronvall 2005) have reported on the molecular typing of non-*i*GAS and a single study (Seale *et al.*, 2016) reported on the molecular epidemiology of *i*GAS.

The African GAS infection registry (the AFRO*Strep* Study) was established to collect epidemiological data on GAS in Africa, where surveillance information is largely lacking. Launched in 2016 with a pilot project in South Africa, it aimed to provide an understanding of GAS disease in Africa (Barth *et al.*, 2016).

Primary prevention of GAS has been focused on the development of a vaccine; the most advanced being a 30-valent vaccine formulation (Dale *et al.*, 2011). The M serotypes of GAS included in the current vaccine formulation were based on data from the developed world with cross coverage of certain *emm* types being observed. Information about the *emm* types causing *i*GAS disease is crucially important to assess potential vaccine coverage, especially in regions such as sub-Saharan Africa, where the burden of *i*GAS disease are among the highest (Carapetis *et al.*, 2005). By means of a one-year prospective laboratory study, under the auspices of AFRO*Strep*, we determined the clinical characteristics and molecular types of non-*i*GAS and *i*GAS infection among patients attending Groote Schuur Hospital, a tertiary institution in Cape Town, so as to inform the development of M protein-based vaccines. Specifically, we aimed to describe the molecular characteristics of *i*GAS isolates, particularly with respect to how they compare and contrast with the molecular characteristics of non-*i*GAS isolates.

6.2 Methods

6.2.1 Study design and participants

In order to determine the extent of GAS infections and the serotypes that cause disease in Cape Town, we conducted a prospective laboratory surveillance study among samples from February 2016 – March 2017 submitted to the National Health Laboratory Service (NHLS) from inpatients and outpatients attending Groote Schuur Hospital (GSH) in Cape Town. GSH is a tertiary level hospital serving a catchment population of approximately one and a half million people (Myer *et al.*, 2013) and forms part of a network of clinics and hospitals that are affiliated to the University of Cape Town. GSH provides care to more than five-hundred and sixty thousand referrals and inpatient admissions every year of adults (>12 years) and neonates (Groote Schuur Hospital, no date).

We documented demographic data, clinical presentation, laboratory data and *emm* types that cause non-invasive group A streptococcal (non-*i*GAS) and invasive group A streptococcal (*i*GAS) infections. The study was approved by the Human Research Ethics Committee at the University of Cape Town (HREC/REF: R006/2015).

6.2.2 Clinical Surveillance and Case Definitions

At the time of a laboratory-confirmed GAS diagnosis, a standardized case report form was completed by the study microbiologist. Clinical information was obtained by accessing the patient's medical record. Isolates were collected and stored at -80 degrees Celsius until transfer to the AFRO*Strep* laboratory.

*i*GAS was defined as GAS isolated from a sterile site such as blood, cerebrospinal fluid and pleural fluid (Invasive Group A streptococcus Sub-Committee 2006; Sharkawy *et al.*, 2002), or isolated from a wound culture with a clinical diagnosis of necrotising fasciitis or streptococcal toxic shock syndrome (Nelson *et al.*, 2016). GAS culture from deep tissue (e.g. abscess) or a biopsy sample following surgery were also considered to be invasive infection (Steer *et al.*, 2009). GAS isolated from a non-sterile site such as the skin and throat was considered to be non-invasive (Su *et al.*, 2009).

6.2.3 Molecular assays

Emm typing has been discussed in chapter 3, section 3.4. Briefly, GAS isolates were stored in the AFRO*Strep* laboratory at -80°C in cryopreservative microbeads until DNA extraction. These isolates were sub-cultured on 5% sheep's blood agar media by isolation, streaking and the plate was incubated for 24 to 48 hours at a temperature of 37°C. DNA was extracted using the Wizard Genomic DNA Purification Kit and the DNA quality and quantity were determined using the NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) technique. The

amplified DNA product was sent to Stellenbosch University for sequencing according to guidelines by the Centres for Disease Control and Prevention (CDC) (https://www.cdc.gov/streplab/protocol-emm-type.html). The BLAST programme (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp) was used to compare the DNA sequences with published sequences in the CDC databases.

6.2.4 Statistics

We evaluated the association between *emm* type and clinical symptoms using Chi-Square or Fishers Exact tests. A p value of <0.05 was considered to be statistical significant. All statistical analysis was performed using Stata® (version 13.1; StataCorp, College Station, TX).

The sample size was calculated using a prevalence of 21% for GAS pharyngitis as reported in a previous study conducted in Cape Town (Engel *et al.*, 2017). The minimum reliable sample size was n=255 to be able to detect possible differences between non-*i*GAS and *i*GAS infection groups. The confidence level was set at 95% and the margin of error was set at 5%. More information on the methods for this study can be found in Chapter 3, section 3.3.

6.3 Results

From February 2016 to March 2017, 488 laboratory-confirmed GAS cases were identified at the NHLS based at GSH in Cape Town. The median age was 31 years (IQR, 21 – 45 years). GAS was more commonly isolated from males (63%). *i*GAS accounted for 46% of GAS cases. Patients with *i*GAS infection were older, with a median age of 36 years (IQR, 22 – 53 years) compared with patients who had non-*i*GAS infection with a median age of 29 years (IQR, 20 – 40 years). The proportion of *i*GAS infections were more common among newborns and patients ≥65 years old compared with non-*i*GAS infections. Characteristics of patients with non-*i*GAS and *i*GAS infection are listed in Table 6.1.

Clinical information was available for 460 (94%) isolates (Table 6.2). Among non-*i*GAS, the most common clinical manifestations were wound infections (34%), abscesses (11%) and hand sepsis (11%). For *i*GAS infections, the most common clinical presentations were bacteraemia (33%), septic arthritis (18%), and abscesses (7%). *Emm* 80 was significantly associated with patients presenting with non-*i*GAS abscesses (P=0.007).

Information on the site of sampling was available for 475 isolates (97%); data were recorded as detailed on the laboratory requisition form. In addition to those listed in Table 6.3, bone, nasal swabs and tissue samples were contained under "other". Thirteen isolates had no site of isolation information; however, classification into non-*i*GAS and *i*GAS infections were based on clinical data and additional information recorded in the notes section of the CRF.

Table 6.1. Gender and age distribution of cases with non-invasive and invasive group A streptococcal infection in Cape Town

	non-*i*GAS (n=262)	*i*GAS (n=226)	Total (n=488)
female	100 (38%)	76 (34%)	176 (36%)
male	162 (62%)	143 (63%)	305 (63%)
NS		7 (3%)	7 (1%)
≤12 months	4 (2%)	10 (4%)	14 (3%)
>1 – 5 years	20 (8%)	12 (5%)	32 (7%)
>5 - 12 years	25 (10%)	11 (5%)	36 (7%)
>12 - 18 years	12 (5%)	6 (3%)	18 (4%)
>18 - 64 years	192 (72%)	140 (62%)	332 (68%)
≥65 years	9 (3%)	26 (12%)	45 (9%)
unknown age		21 (9%)	21 (2%)

Non-iGAS, non-invasive group A streptococcus; iGAS, invasive group A streptococcus; n, number; NS, not stated

Table 6.2. Clinical manifestations of non-invasive and invasive GAS infection by age category

	Number of patients by age category						
Clinical Manifestation	≤12 Months	1 - 5 Years	6 - 12 Years	13 – 18 Years	19 – 64 Years	≥65 Years	Total N (%)
Non-invasive GAS infection (n=262)							
Wound infection	1	6	11	5	63	3	89 (34%)
Abscess	1	4	2	2	19	1	29 (11%)
Hand sepsis*	0	2	4	2	20	0	28 (11%)
Hand infection	0	0	1	1	15	0	18 (6%)
Lower limb infection	0	1	0	0	15	2	18 (7%)
Other	2	6	6	0	42	2	58 (26%)
NS							22 (8%)
Invasive GAS infection (n=226)							
Bacteraemia	7	5	2	1	41	18	74 (33%)
Septic Arthritis	0	3	3	1	29	5	41 (18%)
Abscess	2	0	1	0	13	0	16 (7%)
Necrotizing Fasciitis	0	0	0	1	10	1	12 (5%)
Wound infection	0	0	0	0	7	1	8 (4%)
Cellulitis	0	1	0	0	4	0	5 (2%)
Osteomyelitis	0	0	1	0	4	0	5 (2%)
Erysipelas	0	0	1	0	3	0	4 (2%)
Other†	1	2	3	2	25	1	34 (15%)
NS							6 (3%)
Missing age data							21 (9%)

GAS, group A streptococcus; NS, not stated; N, number of cases with clinical manifestations.

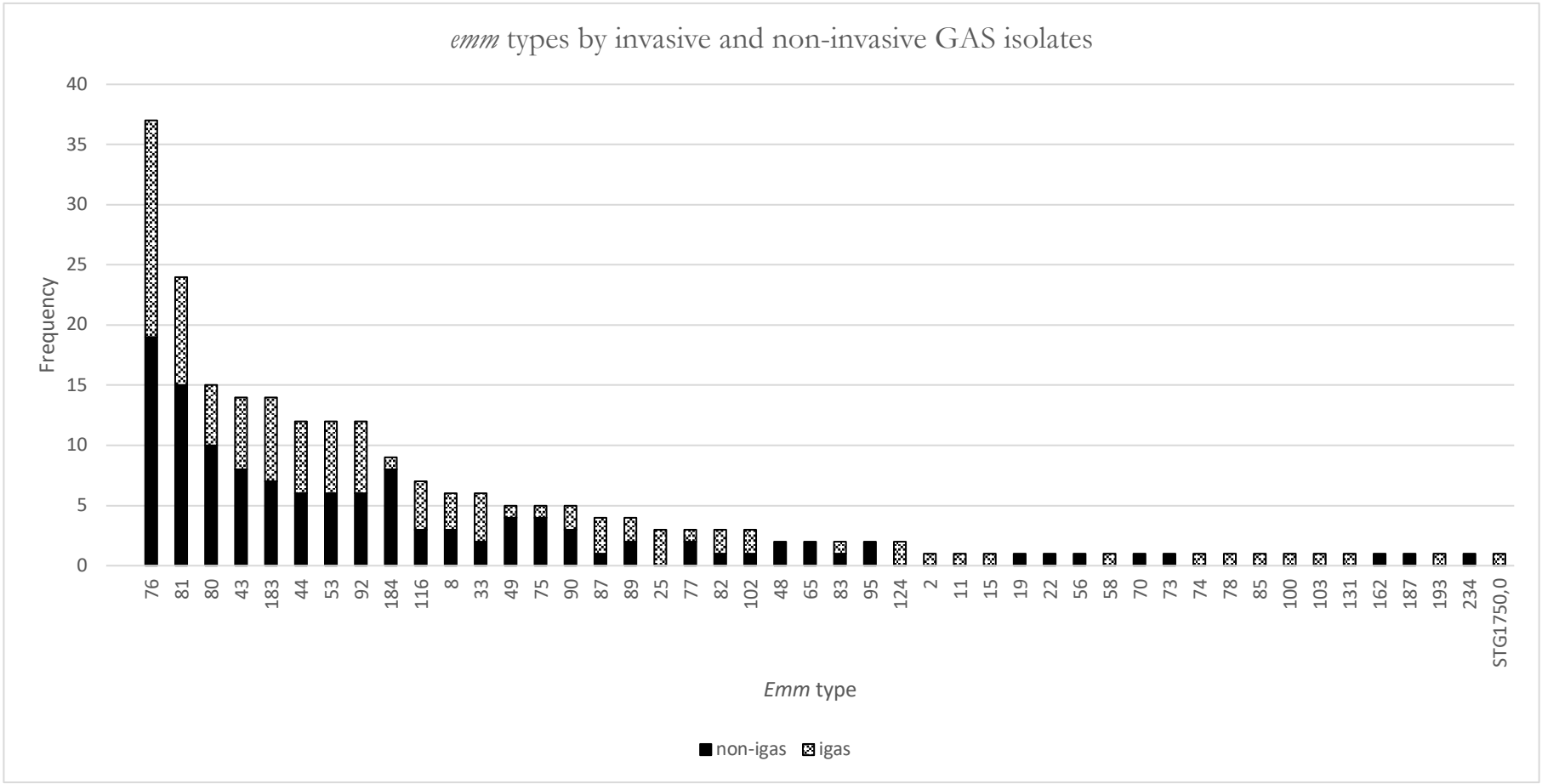
† Symptoms occurring in <5 patients included osteitis, osteomyelitis, empyema and meningitis amongst others.

** Hand sepsis is considered non-invasive because infection was inoculated through the skin.*

Table 6.3. Sample types submitted for processing

Sample	N (%)
Pus swab	258 (53%)
Blood	90 (18%)
Deep tissue	47 (10%)
Abscess	36 (7%)
Aspirate	31(6%)
CSF	5 (1%)
Other	8 (2%)
NS	13 (3%)
Total	488 (100%)

CSF, cerebrospinal fluid; NS, not stated; N, number



non-iGAS, non-invasive group A streptococcus; iGAS, invasive group A streptococcus,

Figure 6.1. Distribution of *emm*-types identified by non-invasive and invasive GAS isolates

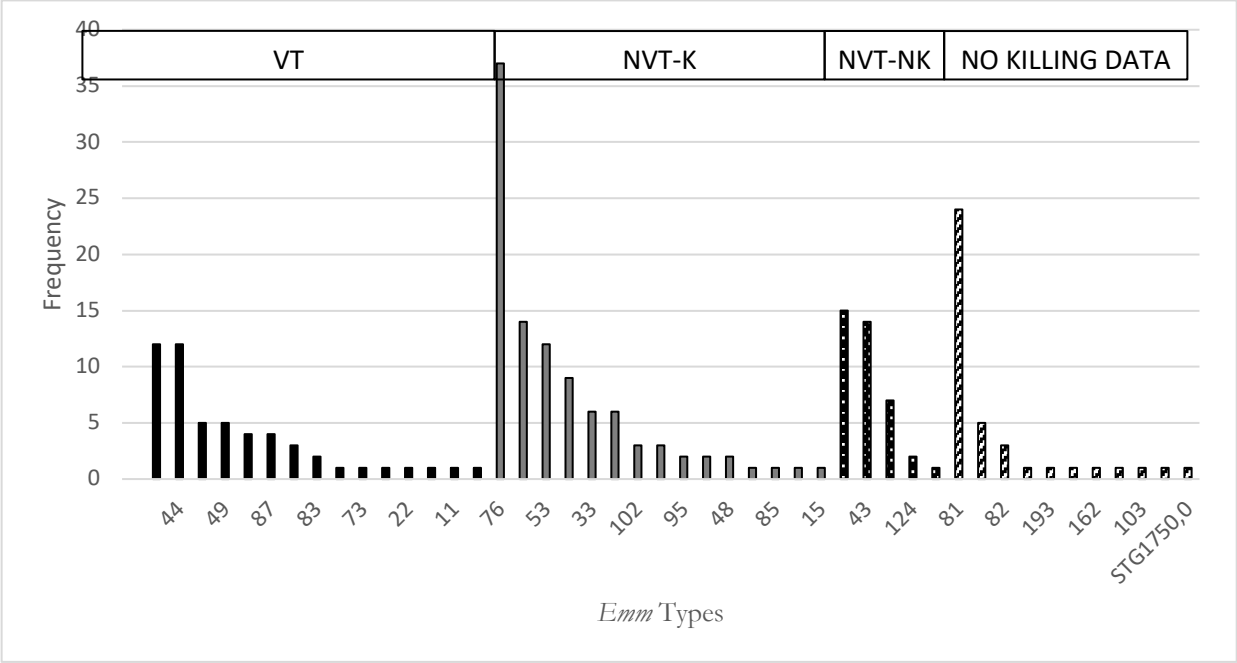
Distribution of M types

Molecular evaluation was conducted on 238/488 isolates; reasons included contaminated agar plates (following sub-culture of GAS isolates), failed PCR reactions, and remaining isolates are pending sequencing. Five isolates failed to report *emm* types with results reading as "no hits found". Forty-six *emm* types were identified in 233 non-*i*GAS and *i*GAS isolates (Figure 6.1). The 10 most prevalent *emm* types accounted for >67% of the isolates; these were, in descending order, M76 (16%), M81 (10%), M80 (6%), M43.7 (6%), M183.2 (6%), M44 (5%), M53 (5%), M92 (5%), M184 (4%), and M116(3.0%). Twenty different *emm* types accounted for 86% of GAS isolates. STG1750.0 was a newly characterized *emm* type identified in one isolate. Twenty *emm* types were represented only once.

Vaccine coverage

We assessed the proportion of *emm* types that were included in the 30-valent GAS vaccine currently being developed (Dale *et al*., 2011). Fifteen *emm* types amongst our cohort are included in the vaccine are represented by 54 GAS isolates (23%) (Figure 6.2). Fifteen non-vaccine *emm* types represented 100 isolates (43%) have shown cross protection, demonstrating >50% bactericidal killing in the presence of rabbit antisera generated after vaccination with the 30-valent vaccine (Dale *et al*., 2011). Our most commonly isolated *emm* type (M76) is not included in the 30-valent vaccine but is among the *emm* types that evoked bactericidal antibodies.

Of 233 GAS isolates, 54 were vaccine types (VT) and 100 were non-vaccine types indicating cross coverage (NVT-K). Forty (17%) isolates had no information regarding potential vaccine coverage (No killing data). This vaccine could cover 65% of *emm* types and 66% of GAS cases in our setting.

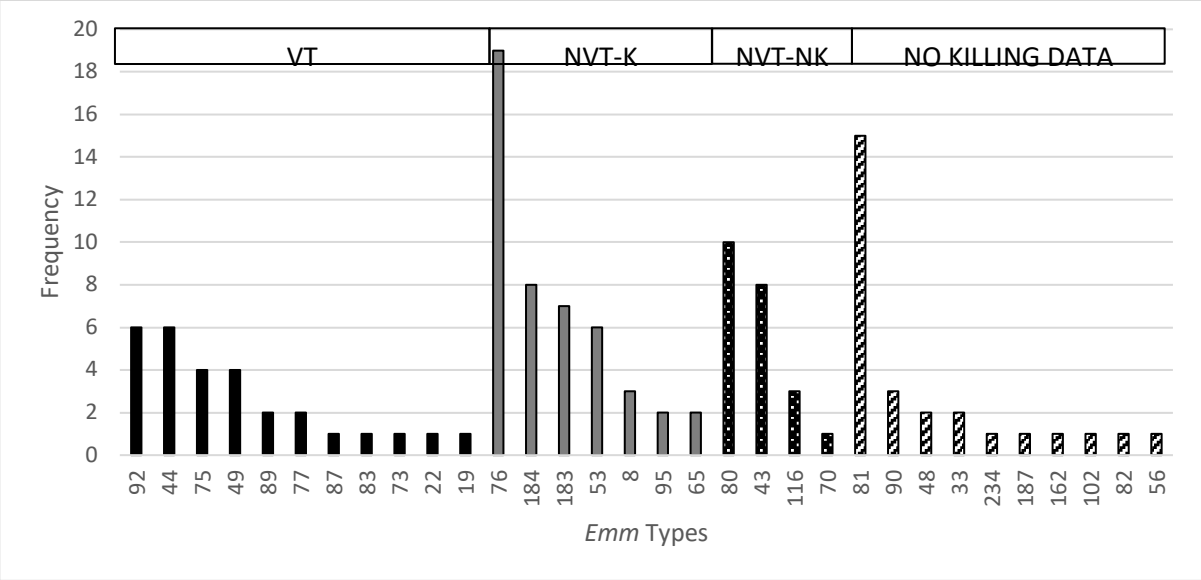


VT, vaccine type, NVT-K, nonvaccine type-killed, NVT-NK, nonvaccine type-not killed

Figure 6.2. Frequency of non-invasive and invasive *emm* types observed: VT (vaccine types), NVT-K (non vaccine type coverage), NVT-NT (non-vaccine types not covered) and no killing data.

Non-iGAS

A total of 32 *emm* types were identified in 126 non-*i*GAS isolates (Figure 6.3). Of these, the most prevalent *emm* types, with a frequency of >3% in the population were M76 (15%), M81 (12%), M80 (8%), M43.7 (6%), M184 (6%), M183.2 (6%), M44 (5%), M53 (5%), M92 (5%), and M49 (3%). The 10 most prevalent *emm* types accounted for 71% of the isolates; 20 different *emm* types accounted for 90% of non-*i*GAS isolates. No new *emm* types were observed. Twelve *emm* types were presented only once.



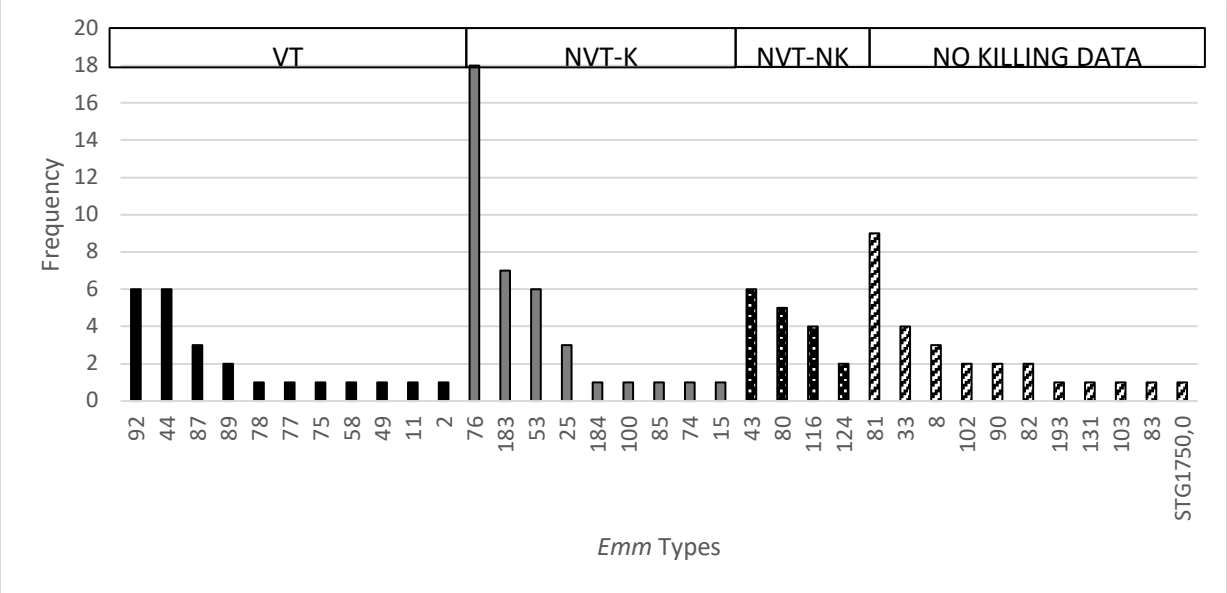
VT, vaccine type, NVT-K, nonvaccine type-killed, NVT-NK, nonvaccine type-not killed

Figure 6.3. Frequency of non-invasive *emm* types observed: VT (vaccine types), NVT-K (non vaccine type coverage), NVT-NT (non-vaccine types not covered) and no killing data.

The proportion of non-*i*GAS *emm* types included in the 30-valent vaccine was 29 (23%), representing 11 different *emm* types. An additional 47 non-*i*GAS isolates (37%) representing 7 *emm* types were included among cross-protection isolates. The most commonly isolated *emm* type for non-*i*GAS infection, (M76) was not included in the 30-valent vaccine. The potential coverage for non-*i*GAS infection in our setting is 60%. Twenty-eight (22%) isolates had no information regarding potential vaccine coverage (No killing data).

iGAS

Thirty-five *emm* types were identified in 107 *i*GAS isolates (Figure 6.4). Among these isolates, the most prevalent *emm* types, with a frequency of >3% in the population were M76 (17%), M81 (8%), M183.2 (7%), M43.7 (6%), M44 (6%), M53 (6%), M92 (6%), M80 (5%), M116.1 (4%), M33 (4%), and M8 (3%). The 10 most prevalent *emm* types accounted for 66% of the isolates; 20 different *emm* types accounted for 84% of the GAS cases isolated. STG1750.0 was identified in 1 isolate as a newly characterized *emm* type. Seventeen *emm* types were only represented once.



VT, vaccine type, NVT-K, nonvaccine type-killed, NVT-NK, nonvaccine type-not killed

Figure 6.4. Frequency of non-invasive *emm* types observed: VT (vaccine types), NVT-K (non vaccine type coverage), NVT-NT (non-vaccine types not covered) and no killing data.

The proportion of *i*GAS *emm* types included in the 30-valent vaccine was 24 (22%), representing 11 different *emm* types. An additional 39 *i*GAS isolates (36%) representing 9 more *emm* types were included among the cross-protection isolates. The most commonly isolated *emm* type for *i*GAS infection (M76) was not included in the 30-valent vaccine. The potential coverage for *i*GAS infection in our setting is 59%. Twenty-seven (25%) had no information regarding potential vaccine coverage (No killing data).

Clusters

Of the 233 GAS isolates, we were able to assign an *emm* cluster designation to 231 isolates (Table 6.4) according to the cluster classification method (Baroux *et al*., 2014). Ten *emm* type clusters were observed among the GAS isolates (Table 6.4). Five *emm*-clusters namely D4, E2, E3, E6 and E4 comprised 90% of the *emm* types.

Table 6.4: *Emm* Cluster types

Cluster	Freq.	Percent	Cum %
D4	59	25.54	
E2	54	23.37	48.91
E3	41	17.74	66.65
E6	34	14.71	81.36
E4	20	8.65	90.01
NS	9	3.89	93.09
stG6.6	9	3.89	97.79
Clade Y	2	0.86	98.65
D2	1	0.43	90.08
E1	1	0.43	99.51
Formerly st3211.0	1	0.43	100.00
Total	231	100.00	

Seasonal variation

There was an association between the type of GAS infection across the seasons, however, this did not reach statistical significance (Chi-square for trend, P=0.06). Non-*i*GAS infections showed a peak in the winter months. *i*GAS infections troughed in the winter months and peaked in summers months (Figure 6.5). Furthermore, a higher proportion of *i*GAS infections were observed during the winter months compared with non-*i*GAS infection and this difference was statistically significant (Z test, P=<0.001).

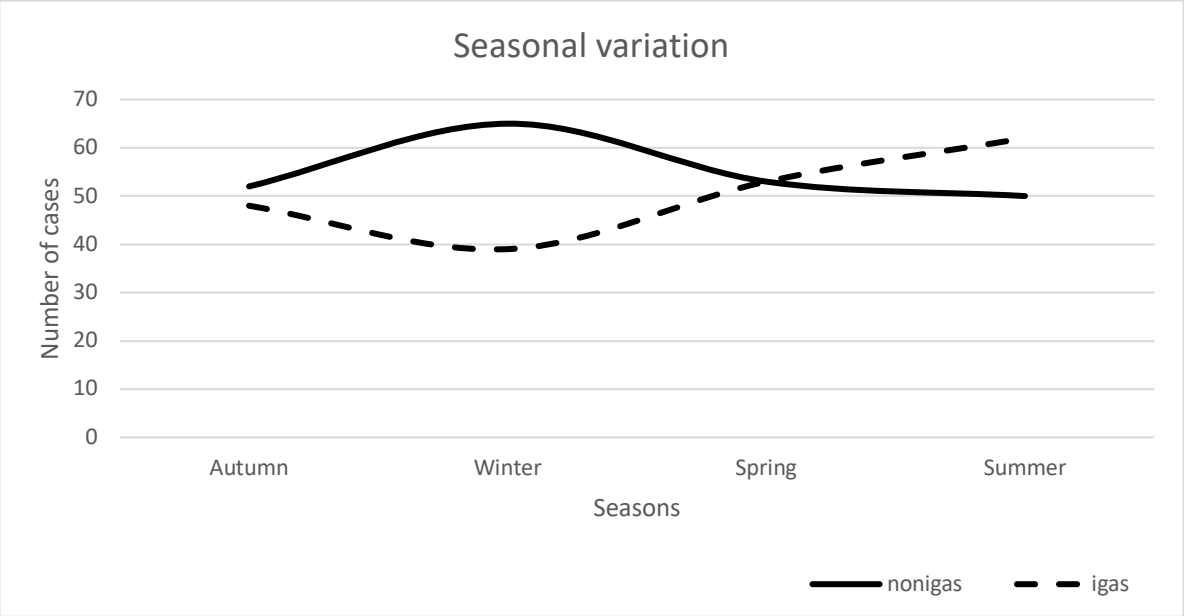


Figure 6.5. Number of cases by season

6.4 Discussion

This is the first prospective study to describe the molecular types of both non-invasive and invasive GAS infections in South Africa. The most prevalent *emm* types were almost evenly distributed between non-*i*GAS and *i*GAS isolates; few *emm* types accounted for the majority of non-*i*GAS (90%) and *i*GAS (84%) cases. The proportion of *i*GAS cases was remarkably high, accounting for almost half (46%) of GAS infections in our surveillance study.

Compared with the 30-valent vaccine, only one-third of the 46 *emm* types in our study (15/46), were included translating to a vaccine coverage (vaccine type and nonvaccine type-killing) for non-*i*GAS and *i*GAS infection of 60% and 59%, respectively. Notably, our most prevalent strains, in both non-*i*GAS and *i*GAS groups, were not included in the 30-valent vaccine. Interestingly, a new *emm* type, STG1750.0 not yet assigned an *emm* type in the CDC database, was obtained from one patient presenting with bacteraemia.

We showed a lower diversity of *emm* types, a result similar to others found in high-income countries (Steer *et al*., 2009). In our study, 20 *emm* types represented 86% of GAS isolates, which is lower than studies conducted in Africa which reported 25 *emm* types representing 70% of 70 *emm* types (Tapia *et al*., 2015), 26 *emm* types representing 63% of 91 *emm* types (Steer *et al*., 2009) for GAS pharyngitis, and 48 *emm* types representing 62% of 78 *emm* types for GAS skin and pharyngeal infections (Tewodros and Kronvall, 2005). Another study of *i*GAS isolates, conducted in Kenya reported 88 different *emm* types (Seale *et al*., 2016); 74% of our strains were common in their study. Of interest, our study findings were similar to a surveillance study conducted in Tunisia (Hraoui *et al*., 2011). Their twenty most prevalent *emm* types represented 82% of the total *emm* types and the proportion of *i*GAS cases was 46%, compared with our surveillance study of 49%. Furthermore, the most

common *emm* types (M1, M12, M28, M3, and M4) isolated in high-income countries were not represented in our study.

Epidemiological studies have shown significant associations between *emm* type and GAS disease manifestations. *Emm* types 1, 3, 5, 6, 12, 14, 17, 44, and 61 have been associated with superficial GAS disease (Cunningham 2000; Johnson *et al.*, 1992; Shea *et al.*, 2011) and *emm* types 1, 3 and 28 associated with *i*GAS diseases (Olsen and Musser, 2010). In addition, *emm* types have also been associated with clinical manifestations which include APSGN (*emm* types 1, 4, 12, 49, 55, 57, and 60)(Shulman and Tanz, 2010), and ARF (*emm* types 1, 3, 5, 6, 11, 12, 14, 17, 18, 19, 24, 27, 29, 30, 32, and 41)(Shulman and Bisno, 2014)(Shulman and Tanz, 2010). Our study represents *emm* types that have been previously shown to be associated with pharyngitis, impetigo, ARF, and PSGN.

Seasonal variation in the frequency of GAS cases have been observed in studies conducted in the United States of America. GAS Infection have been shown to peak in the winter and early spring months, and trough in the summer and autumn months (Nelson *et al.*, 2016). Similar seasonal variations have also been observed in Europe (Darenberg *et al.*, 2013a; Lamagni *et al.*, 2008). Non-*i*GAS infections in our study are in keeping with this observation, however, for *i*GAS infection, a higher number of cases were observed in the summer months.

A new *emm* cluster typing system classifies >200 *emm*-types into 48 *emm* clusters containing closely related M proteins that share structural and binding properties (Sanderson-Smith *et al.*, 2014). This system predicts the M protein vaccine antigen content and serves as a framework to investigate the cross-protection phenomenon and provide complementary hypothesis for the many variants from low-middle income countries (Sanderson-Smith *et al.*, 2014). Five *emm* clusters were responsible for 90% of the disease burden. It is thus conceivable, that the *emm* cluster typing system could be an

important typing tool to identify vaccine antigen candidates that may prove to be effective at preventing a larger proportion of GAS infections, especially in South Africa (Sanderson-Smith *et al.*, 2014).

Our study had a number of limitations:

1. We were unable to assess the variation in the distribution of *emm* types over time, as reported in other studies (Meehan *et al.*, 2013; Nelson *et al.*, 2016), since our data collection was over a one-year period.
2. This was a hospital-based study and therefore could not calculate population-based incidence rates over the study period.
3. GSH is mainly an adult hospital hence the low number of cases in young patients, therefore, caution must be applied when generalising these findings to the lower age category.
4. *Emm* data were not available for all GAS isolated over the study period. We compared the isolates that were typed with those not typed and found no significant difference with regard to gender (Chi-Square, P=0.92) and non-*i*GAS and *i*GAS groups (Chi-Square, P=0.87). We also considered age group analysis and found no difference among patients between the ages of 13 – 18 (Z test for proportions, P= 0.84), 19 – 64 years (Z test, P=0.79) and those older than 64 years (Z test, P=0.90). There was a difference in the younger population, among the newborns (Z test, P= 0.02) and 6 - 12 years, (Z test, P=0.02). This difference could be due to the small sample size in these age categories.

Our results have implications for current vaccine development initiatives. The current 30-valent vaccine formulation is informed by high-income countries accounting for 90% of strains causing disease in those regions. By comparison, vaccine coverage in our study was considerably lower than the coverage in high-income countries. Even though our five most prevalent *emm* types (M76, M81,

M80, M183 and M43), accounting for 45% of our cases, were not included in the current 30-valent vaccine formulation, there is evidence of cross-protection based on bactericidal antibodies that recognize shared epitopes in the N-terminal region of the *emm* types (Dale *et al.*, 2011; Dale *et al.*, 2013). Few *emm* types are responsible for the majority of GAS cases in our setting, thus, an effective vaccine will not require diverse *emm* serotypes. Furthermore, an important finding in our study is that bactericidal activity against 33% of the non-vaccine *emm* types in our study could translate to additional protective coverage of 43%.

The same *emm* types caused both *i*GAS and non-*i*GAS infections in our study, thus suggesting that host immune factors have a role to play in determining the severity and outcome of GAS infections in different individuals (Davies *et al.*, 1996). Patients with serious GAS infections who present with severe clinical manifestations are inclined to produce elevated levels of proinflammatory cytokines in response to GAS products (Norrby-Teglund *et al.*, 2000).

Although we were unable to calculate incidence rates, the proportion of *i*GAS infection at GSH was high, however, this is to be expected given that GSH is a tertiary level hospital where patients with severe disease are referred for care. In contrast, at a community health centre, we would expect to see less *i*GAS infections and more non-*i*GAS infections e.g. GAS pharyngitis.

*i*GAS infection is responsible for a substantial burden of disease and its clinical manifestations are associated with important causes of premature mortality and morbidity. Following the first comprehensive review, published more than a decade ago, there remains a challenge in quantifying the burden of GAS disease around the world. Although more data are slowly becoming available, more work needs to be done especially in resource limited areas such as sub-Saharan Africa. Understanding the epidemiology and true burden of GAS diseases will help target efforts and settings

in which the vaccine and other trials could be conducted. While vaccine development, targeting areas other than *emm*-protein are underway, it must be noted that the *emm*-protein vaccine is currently at the most-advanced stage of development, thus warranting documenting the variation thereof. Furthermore, the reporting of *i*GAS through passive surveillance provides a platform to evaluate trends, identify new strains causing disease and in so doing, inform the development of vaccine efforts.

7. CHAPTER SEVEN: CONCLUSIONS AND FUTURE DIRECTION

SUMMARY AND CONCLUSIONS

IMPLICATIONS FOR POLICY, PRACTICE AND FUTURE RESEARCH

This thesis details the establishment of the AFRO*Strep* registry as a platform for GAS-related research with three studies of GAS infection in South Africa providing insights into the burden of GAS-related disease. The synopsis below presents the unique contributions made by these studies. The implications for policy and practice, as well as recommendations for future research are also represented.

7.1 Originality / Novel Insight

The primary purpose of this thesis was to determine the epidemiology for GAS infection in Africa. To provide context, I endeavoured to identify and summarise all studies reporting on the prevalence and molecular epidemiology of GAS isolates in Africa. In South Africa specifically, I sought to (1) describe from national laboratory data, the incidence of invasive and non-invasive GAS isolates, 2003 – 2015, (2) to establish the AFRO*Strep* biorepository and clinical database composed of South African patients with invasive and non-invasive GAS infection, (3) to conduct a prospective, surveillance study in order to determine the burden of invasive disease associated with GAS in South Africa over a 12-month period and, finally, (4) describe the molecular *emm* types of invasive GAS isolates in South Africa, particularly with respect to how they compare and contrast with *emm* types of non-invasive isolates.

There are three principal findings from this series of studies:

Study one: This systematic review provided the first comprehensive estimates on the prevalence of GAS infection in Africa and provides strong justification for improved prevention strategies and efforts to support the development of vaccine initiatives, given the high prevalence of GAS infection in African people. In addition, this work highlights the dearth of molecular type data of GAS-related diseases in Africa and emphasises the need for further research to address this need. In addition, this work emphasises the need for healthcare workers to be vigilant, ensuring accurate diagnosis and treatment of GAS infections.

Study two: This study suggests that laboratory-confirmed non-invasive GAS infection in the South African public sector appears to have declined over the last 13 years. There is the possibility that the lower incidence of invasive and non-invasive GAS infection found in our study is due to infrequent submission of specimens for microbiological culture by health practitioners; thus, our findings may be an underestimate of the true burden of disease in South Africa. Interestingly, this study showed an increase in incidence with age in our cohort, consistent with findings reported previously. This work further highlights the need for accurate and comprehensive surveillance of GAS in South Africa.

Study three: This is the first prospective study to describe the molecular types of both non-invasive and invasive GAS infections in South Africa. The most prevalent *emm* types were almost evenly distributed between non-*i*GAS and *i*GAS isolates; few *emm* types accounted for the majority of non-*i*GAS (90%) and *i*GAS (84%) cases. The proportion of *i*GAS cases was remarkably high, accounting for almost half (46%) of the total cohort. Our most prevalent *emm* types causing the majority of invasive and non-invasive GAS disease were not included in the 30-valent vaccine currently under

development. Finally, this research showed a low potential vaccine coverage in our setting (60% and 59% for non-*i*GAS and *i*GAS respectively) and thus emphasises the need for revised primary prevention efforts and a vaccine formulation with improved coverage in areas where the burden of disease is high.

This thesis highlights the need for comprehensive public health surveillance systems to record complete and accurate information on the epidemiology of GAS in South Africa and Africa, as an essential step to reduce the burden of GAS-related diseases in Africa. Thus, surveillance, we believe will allow us to be confident in our disease estimates and disease characteristics so as to better inform prevention strategies including the development of an effective vaccine.

7.2 Implications for policy and practice

The findings of this thesis provide important baseline information for clinicians, health care workers and policy makers regarding the burden of GAS in South Africa. The pooled prevalence estimate of 32% (95% CI, 19% to 46%) in Southern Africa, provides support to the need to remain vigilant in accurately diagnosing GAS pharyngitis infections.

While some developed countries have invasive GAS infections listed as a notifiable disease so as to monitor outbreaks and new strains, more African data is needed to allow for a similar directive, especially given resource and personnel constraints.

7.3 Implications for future research

This work highlights the need for more studies on the molecular epidemiology of invasive GAS infections so as to have a better understanding of the epidemiology of GAS in areas where the

burden of GAS disease is high e.g. South Africa and sub-Saharan Africa. Longitudinal, population-based surveillance studies are essential to determine trends in the incidence of GAS, changes in the distribution of prevalent and new *emm* types circulating in our setting, and to be confident in our disease estimates so as to better inform public health prevention strategies.

This thesis provides important information with regard to a 30-valent GAS vaccine currently under development. Our findings suggest that an effective vaccine formulation with good coverage does not require a diverse group of serotypes since few *emm* types caused the majority of GAS disease in our setting.

This work further alludes to the need for an alternative approach to the vaccine development, perhaps such as the *emm* cluster tool to supplement the *emm* type distribution data, given the diversity and change in *emm* types over time, especially in sub-Saharan Africa.

8. CHAPTER EIGHT: REFERENCES

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9. CHAPTER NINE: APPENDICES

Appendix 9.1: University of Cape Town IRB Ethics approval

Appendix 9.2: Systematic Review data collection

Appendix 9.3: AFRO*Strep* Case Record Form

Appendix 9.4: Lab SOP: Culture procedure

Appendix 9.5: Lab SOP: DNA Extraction

Appendix 9.6: Lab SOP: Polymerase Chain Reaction

Appendix 9.7: OpenClinica database: Standard Operating Procedure

Appendix 9.8: AFRO*Strep* Standard operating procedure

Appendix 9.9: AFRO*Strep* assent form

Appendix 9.10: AFRO*Strep* consent form and information sheet

Appendix 9.1: University of Cape Town IRB Ethics approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 February 2015

REF NO: R006/2015

Dr M Engel
Department of Medicine
Old Main Building

Dear Dr Engel

PROJECT TITLE: THE AFRO*Strep* REGISTRY

Thank you for your response letter dated 09th February 2015, addressing the issues raised by Human Research Ethics Committee (HREC).

The HREC has **approved** the registration of your registry.

The registration of this registry is valid until 30 March 2018.

Please provide the HREC with an update if the registry continues beyond this period.

Please Note: All research, including that undertaken for a master's or doctoral degree, using registered databases, registries and repositories, requires submission as a new study. It requires an application form (FHS013) and a protocol which has undergone departmental review. The study will receive its own HREC REF number which will be linked to the main database or repository.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

pp TJBurgess)

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • **Facsimile** [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

19 February 2015

HREC/REF: 092/2015

Prof B Mayosi
Department of Medicine
J46-43
OMB

Dear Prof Mayosi

Project Title: THE AFROSTREP STUDY: A SURVEILLANCE SYSTEM FOR GROUP A STREPTOCOCCAL INFECTION IN SOUTH AFRICA (PHD candidate- D Barth)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 28 February 2016.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:-Dylan Barth is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Hrec/ref:092/2015

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/01/2018
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	10/1/2017

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	09 January 2017		
HREC REF Number	092/2015	Current Ethics Approval was granted until	30/01/2017
Protocol title	The AFROStrep Study: A surveillance system for group A streptococcal infection in South Africa (PhD Candidate – D Barth)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ☒ No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	A/Prof Mark Engel		
Department / Office Internal Mail Address	Medicine. Room J46.43, Old Main Buidling. GSH		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☑ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☑ No

HUMAN RESEARCH ETHICS COMMITTEE
09 JAN 2017
HEALTH SCIENCES FACULTY

Appendix 9.2: Systematic review data extraction form

Review Title:	**The Prevalence of Group A Beta Haemolytic Streptococci in Africa: A Systematic Review**

Study ID (e.g. Barth 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 ☐ No☐ Unclear☐
Participants	**Including children of ages 5-15 years**	Yes ☐ No☐ Unclear☐
	Have throat swab/RADT	Yes ☐ No☐ Unclear☐
Setting	**Study took place in an African country**	Yes ☐ No☐ Unclear☐
Types of Outcome measure	**Primary outcome: Prevalence of Beta haemolytic Group A streptococcus**	Yes ☐ No☐ Unclear☐
	Secondary outcomes: Distribution of Beta haemolytic Group A streptococcus, M-Protein gene types.	Yes ☐ No☐ Unclear☐
Results	**Prevalence**	Yes ☐ No☐ Unclear☐
INCLUDE ☐	**EXCLUDE**☐	**PENDING**☐
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** ☐ **No** ☐ **Unclear** ☐
Ethical approval needed/ obtained for study	**Yes** ☐ **No** ☐ **Unclear** ☐
Notes:	

Participants

	Descriptions as stated in report/paper
Country	
Population description	
Setting (location, social context)	
Inclusion criteria	
Exclusion criteria	
Method of recruitment of participants (e.g. mail, phone)	
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 symptomatic GAS

Crude Prevalence
BHS

Total # recruited	Withdrawals and exclusions	Total sample size analysed	No of symptomatic GAS cases analysed	Numerator Denominator	Crude Prevalence (%)
Note:					

GAS

Total # recruited	Withdrawals and exclusions	Total sample size analysed	No of symptomatic GAS cases analysed	Numerator Denominator	Crude Prevalence (%)
Note:					

Stratified Prevalence

Factors adjusted for in this study.	Total sample size analysed per factor	No of symptomatic GAS cases analysed per factor	Numerator Denominator	Adjusted Prevalence (%)
Age				
Gender				
Household Number				
Setting (e.g. rural vs urban)				
Antibiotics				
Notes:				

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

No of symptomatic GAS cases analysed	No of symptomatic GAS cases typed	Numerator Denominator	Crude Prevalence (%)	Frequency of emm types	Distribution of emm types
Note:					

Discussion

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

Other Information

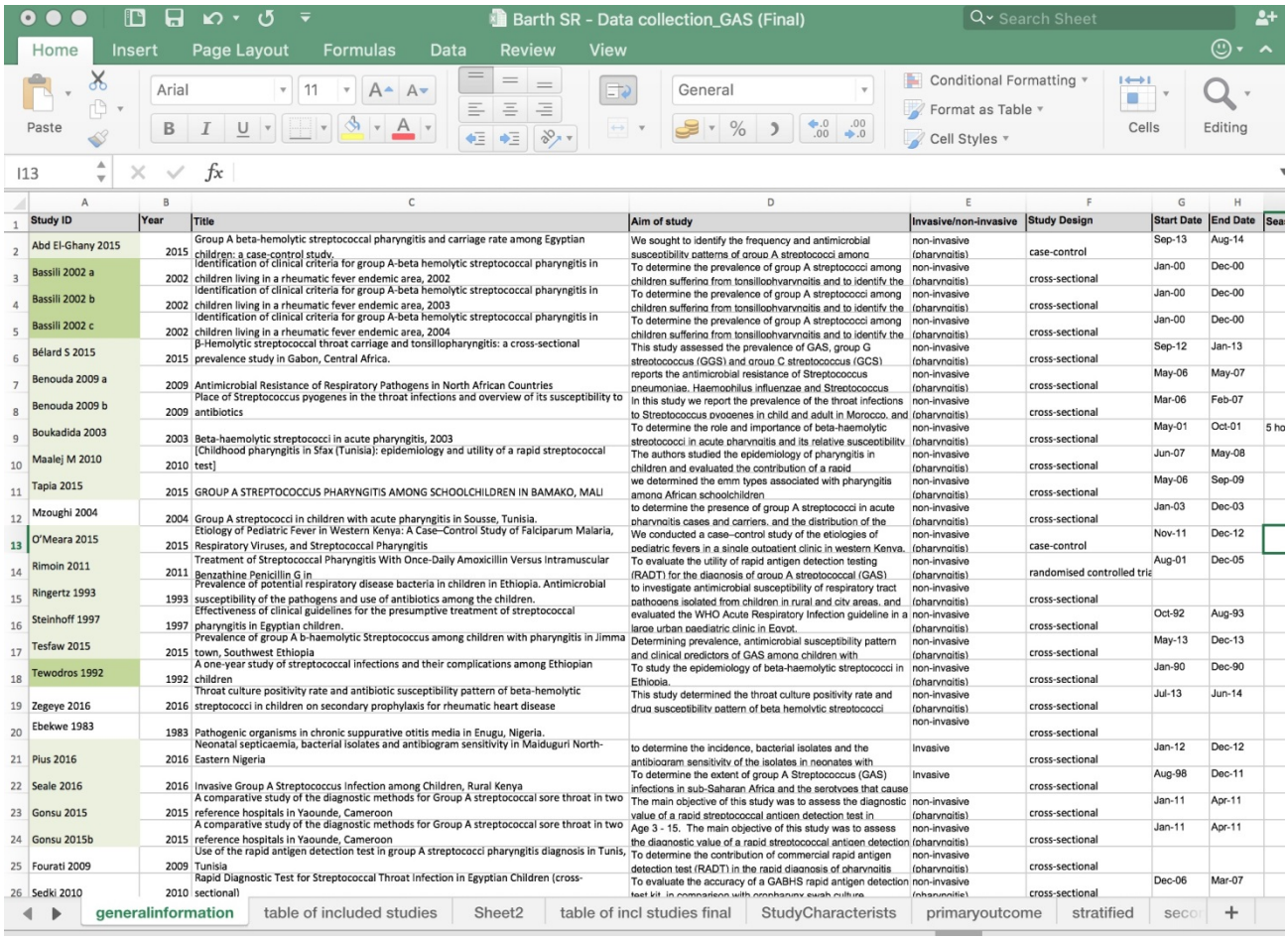
Study funding sources (Including role of funders)	
Possible conflicts of interest (For study authors)	
Missing data	
Correspondence required for further study information (from whom, what and when)	
Notes:	

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** ☐	**No** ☐	**Unclear** ☐
Explain:			
2. Was the sampling frame a true or close representation of the target population?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
3. Was some form of random selection used to select the sample, OR was a census undertaken?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
4. Was the likelihood of nonresponse bias minimal?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
5. Were data collected directly from the subjects (as opposed to a proxy)?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
6. Was an acceptable case definition used in the study?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
7. Was the study instrument that measured the parameter of interest shown to have validity and reliability?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
8. Was the same mode of data collection used for all subjects?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
9. Was the length of the shortest prevalence period for the parameter of interest appropriate?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
11. Authors' reported limitations of study's methods/results	**Yes** ☐	**No** ☐	**Unclear** ☐
Explain:			
Notes:			

Quality of the study
After considering items 1-11, rate the overall quality of the study as “Good,” “Fair,” or “Poor” using the guidance below.

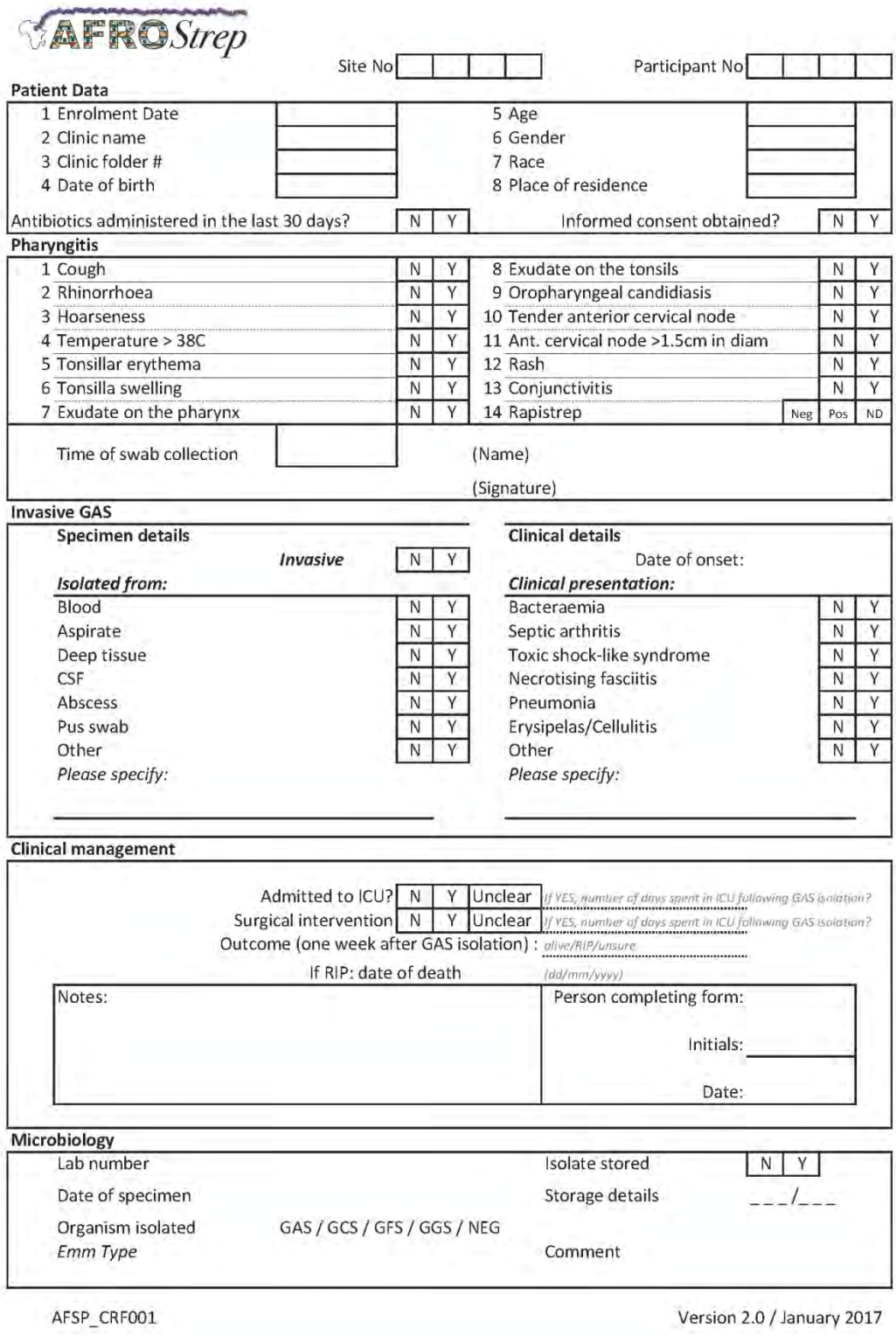
Quality	**Tick one**
Good: Few of the items are rated “No” and none of the limitations are thought to decrease the validity of the conclusion. If items 3,4,7,or 8 are rated “no”, then the study id likely to have major flaws.	
Fair: Enough items are rated “No”, with at least some important limitation. Thus introduce some uncertainty about the validity of the conclusions.	
Poor: Several items are rated “No”, introducing serious uncertainty about the validity of the conclusions.	
Notes:	



	A	B	C	D	E	F	G	H
1	Study ID	Year	Title	Aim of study	Invasive/non-invasive	Study Design	Start Date	End Date
2	Abd El-Ghany 2015	2015	Group A beta-hemolytic streptococcal pharyngitis and carriage rate among Egyptian children: a case-control study.	We sought to identify the frequency and antimicrobial susceptibility patterns of group A streptococci among	non-invasive (pharyngitis)	case-control	Sep-13	Aug-14
3	Bassili 2002 a	2002	Identification of clinical criteria for group A-beta hemolytic streptococcal pharyngitis in children living in a rheumatic fever endemic area, 2002	To determine the prevalence of group A streptococci among children suffering from tonsillopharyngitis and to identify the	non-invasive (pharyngitis)	cross-sectional	Jan-00	Dec-00
4	Bassili 2002 b	2002	Identification of clinical criteria for group A-beta hemolytic streptococcal pharyngitis in children living in a rheumatic fever endemic area, 2003	To determine the prevalence of group A streptococci among children suffering from tonsillopharyngitis and to identify the	non-invasive (pharyngitis)	cross-sectional	Jan-00	Dec-00
5	Bassili 2002 c	2002	Identification of clinical criteria for group A-beta hemolytic streptococcal pharyngitis in children living in a rheumatic fever endemic area, 2004	To determine the prevalence of group A streptococci among children suffering from tonsillopharyngitis and to identify the	non-invasive (pharyngitis)	cross-sectional	Jan-00	Dec-00
6	Bélard S 2015	2015	β-Hemolytic streptococcal throat carriage and tonsillopharyngitis: a cross-sectional prevalence study in Gabon, Central Africa.	This study assessed the prevalence of GAS, group G streptococcus (GGS) and group C streptococcus (GCS)	non-invasive (pharyngitis)	cross-sectional	Sep-12	Jan-13
7	Benouda 2009 a	2009	Antimicrobial Resistance of Respiratory Pathogens in North African Countries	reports the antimicrobial resistance of Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus	non-invasive (pharyngitis)	cross-sectional	May-06	May-07
8	Benouda 2009 b	2009	Place of Streptococcus pyogenes in the throat infections and overview of its susceptibility to antibiotics	in this study we report the prevalence of the throat infections to Streptococcus pyogenes in child and adult in Morocco. and	non-invasive (pharyngitis)	cross-sectional	Mar-06	Feb-07
9	Boukadida 2003	2003	Beta-haemolytic streptococci in acute pharyngitis, 2003	To determine the role and importance of beta-haemolytic streptococci in acute pharyngitis and its relative susceptibility	non-invasive (pharyngitis)	cross-sectional	May-01	Oct-01
10	Maalej M 2010	2010	[Childhood pharyngitis in Sfax (Tunisia): epidemiology and utility of a rapid streptococcal test]	The authors studied the epidemiology of pharyngitis in children and evaluated the contribution of a rapid	non-invasive (pharyngitis)	cross-sectional	Jun-07	May-08
11	Tapia 2015	2015	GROUP A STREPTOCOCCUS PHARYNGITIS AMONG SCHOOLCHILDREN IN BAMAKO, MALI	we determined the emm types associated with pharyngitis among African schoolchildren	non-invasive (pharyngitis)	cross-sectional	May-06	Sep-09
12	Mzoughi 2004	2004	Group A streptococci in children with acute pharyngitis in Sousse, Tunisia.	to determine the presence of group A streptococci in acute pharyngitis cases and carriers, and the distribution of the	non-invasive (pharyngitis)	cross-sectional	Jan-03	Dec-03
13	O'Meara 2015	2015	Etiology of Pediatric Fever in Western Kenya: A Case–Control Study of Falciparum Malaria, Respiratory Viruses, and Streptococcal Pharyngitis	We conducted a case–control study of the etiologies of pediatric fevers in a single outpatient clinic in western Kenya.	non-invasive (pharyngitis)	case-control	Nov-11	Dec-12
14	Rimoin 2011	2011	Treatment of Streptococcal Pharyngitis With Once-Daily Amoxicillin Versus Intramuscular Benzathine Penicillin G in	To evaluate the utility of rapid antigen detection testing (RADT) for the diagnosis of group A streptococcal (GAS)	non-invasive (pharyngitis)	randomised controlled tria	Aug-01	Dec-05
15	Ringertz 1993	1993	Prevalence of potential respiratory disease bacteria in children in Ethiopia. Antimicrobial susceptibility of the pathogens and use of antibiotics among the children.	to investigate antimicrobial susceptibility of respiratory tract pathogens isolated from children in rural and city areas, and	non-invasive (pharyngitis)	cross-sectional		
16	Steinhoff 1997	1997	Effectiveness of clinical guidelines for the presumptive treatment of streptococcal pharyngitis in Egyptian children.	evaluated the WHO Acute Respiratory Infection guideline in a large urban paediatric clinic in Egypt.	non-invasive (pharyngitis)	cross-sectional	Oct-92	Aug-93
17	Tesfaw 2015	2015	Prevalence of group A b-haemolytic Streptococcus among children with pharyngitis in Jimma town, Southwest Ethiopia	Determining prevalence, antimicrobial susceptibility pattern and clinical predictors of GAS among children with	non-invasive (pharyngitis)	cross-sectional	May-13	Dec-13
18	Tewodros 1992	1992	A one-year study of streptococcal infections and their complications among Ethiopian children	To study the epidemiology of beta-haemolytic streptococci in Ethiopia.	non-invasive (pharyngitis)	cross-sectional	Jan-90	Dec-90
19	Zegeye 2016	2016	Throat culture positivity rate and antibiotic susceptibility pattern of beta-hemolytic streptococci in children on secondary prophylaxis for rheumatic heart disease	This study determined the throat culture positivity rate and drug susceptibility pattern of beta hemolytic streptococci	non-invasive (pharyngitis)	cross-sectional	Jul-13	Jun-14
20	Ebekwe 1983	1983	Pathogenic organisms in chronic suppurative otitis media in Enugu, Nigeria.		non-invasive	cross-sectional		
21	Pius 2016	2016	Neonatal septicaemia, bacterial isolates and antibiogram sensitivity in Maiduguri North-Eastern Nigeria	to determine the incidence, bacterial isolates and the antibiogram sensitivity of the isolates in neonates with	Invasive	cross-sectional	Jan-12	Dec-12
22	Seale 2016	2016	Invasive Group A Streptococcus Infection among Children, Rural Kenya	To determine the extent of group A Streptococcus (GAS) infections in sub-Saharan Africa and the serotypes that cause	Invasive	cross-sectional	Aug-98	Dec-11
23	Gonsu 2015	2015	A comparative study of the diagnostic methods for Group A streptococcal sore throat in two reference hospitals in Yaounde, Cameroon	The main objective of this study was to assess the diagnostic value of a rapid streptococcal antigen detection test in	non-invasive (pharyngitis)	cross-sectional	Jan-11	Apr-11
24	Gonsu 2015b	2015	A comparative study of the diagnostic methods for Group A streptococcal sore throat in two reference hospitals in Yaounde, Cameroon	Age 3 - 15. The main objective of this study was to assess the diagnostic value of a rapid streptococcal antigen detection	non-invasive (pharyngitis)	cross-sectional	Jan-11	Apr-11
25	Fourati 2009	2009	Use of the rapid antigen detection test in group A streptococci pharyngitis diagnosis in Tunis, Tunisia	To determine the contribution of commercial rapid antigen detection test (RADT) in the rapid diagnosis of pharyngitis	non-invasive (pharyngitis)	cross-sectional		
26	Sedki 2010	2010	Rapid Diagnostic Test for Streptococcal Throat Infection in Egyptian Children (cross-sectional)	To evaluate the accuracy of a GABHS rapid antigen detection test kit, in comparison with oropharynx swab culture	non-invasive (pharyngitis)	cross-sectional	Dec-06	Mar-07

generalinformation | table of included studies | Sheet2 | table of incl studies final | StudyCharacterists | primaryoutcome | stratified | seco

Appendix 9.3: AFROStrep Case Record Form



AFROStrep

Site No ☐☐☐☐ Participant No ☐☐☐☐

Patient Data

1 Enrolment Date		5 Age	
2 Clinic name		6 Gender	
3 Clinic folder #		7 Race	
4 Date of birth		8 Place of residence	

Antibiotics administered in the last 30 days? N Y — Informed consent obtained? N Y

Pharyngitis

1 Cough	N	Y	8 Exudate on the tonsils	N	Y
2 Rhinorrhoea	N	Y	9 Oropharyngeal candidiasis	N	Y
3 Hoarseness	N	Y	10 Tender anterior cervical node	N	Y
4 Temperature > 38C	N	Y	11 Ant. cervical node >1.5cm in diam	N	Y
5 Tonsillar erythema	N	Y	12 Rash	N	Y
6 Tonsilla swelling	N	Y	13 Conjunctivitis	N	Y
7 Exudate on the pharynx	N	Y	14 Rapistrep	Neg	Pos ND

Time of swab collection

(Name)

(Signature)

Invasive GAS

Specimen details

Invasive N Y

Isolated from:

Blood	N	Y
Aspirate	N	Y
Deep tissue	N	Y
CSF	N	Y
Abscess	N	Y
Pus swab	N	Y
Other	N	Y

Please specify:

Clinical details

Date of onset:

Clinical presentation:

Bacteraemia	N	Y
Septic arthritis	N	Y
Toxic shock-like syndrome	N	Y
Necrotising fasciitis	N	Y
Pneumonia	N	Y
Erysipelas/Cellulitis	N	Y
Other	N	Y

Please specify:

Clinical management

Admitted to ICU? N Y Unclear *If YES, number of days spent in ICU following GAS isolation?*

Surgical intervention N Y Unclear *If YES, number of days spent in ICU following GAS isolation?*

Outcome (one week after GAS isolation) : *alive/RIP/unsure*

If RIP: date of death *(dd/mm/yyyy)*

Notes:

Person completing form:

Initials:

Date:

Microbiology

Lab number — Isolate stored N Y

Date of specimen — Storage details ___/___

Organism isolated GAS / GCS / GFS / GGS / NEG

Emm Type — Comment

AFSP_CRF001 Version 2.0 / January 2017

Appendix 9.4: Lab SOP: Culture procedure

SOP TITLE:	Culture Procedure	**SOP#**	S###-01
WRITTEN BY:	Dylan Barth Kelin Engel Babu Mohammed	**VERSION**	1.0
		SUPERSEDES	
		LINKED DOCS	
APPROVED BY:		**EFFECTIVE DATE**	01 Mar 2017

1. Purpose: To describe the procedures for sub-culture of GAS isolates from AFRO*Strep* sites to the Reference Laboratory

2. Applies to: All sites participating in the AFRO*Strep* research project

3. Responsibilities: Principal Investigator or designee

---

4. Procedure:

- Sub-culture the GAS isolates on 5% blood agar.
- Incubate overnight at 37°C in 5% CO_2.
- Check plate growth. If not satisfactory, incubate again.

Reference

Wasas AD, Huebner RE, De Blanche M, Klugman KP. Long-term survival of Streptococcus pneumoniae at room temperature on Dorset egg medium. J Clin Microbiol 1998;36:1139-40.

For questions regarding collection, please email Dr Mark Engel mark.engel@uct.ac.za or contact the AFRO*Strep* Coordinating Office by phone (+27214045735) or fax (+27214486815)

Appendix 9.5: Lab SOP: DNA Extraction

SOP TITLE:	DNA Extraction	**SOP#**	S###-01
WRITTEN BY:	Dylan Barth Kelin Engel Babu Mohammed	**VERSION**	1.0
		SUPERSEDES	
		LINKED DOCS	
APPROVED BY:		**EFFECTIVE DATE**	01 Mar 2017

1. Purpose: To describe the procedures for DNA extraction of GAS isolates from AFRO*Strep* sites to the Reference Laboratory

2. Applies to: All sites participating in the AFRO*Strep* research project

3. Responsibilities: Principal Investigator or designee

---

4. Materials

- 2% blood agar plates

- sterile loops

- CO2 incubator

Sample Sub-culture

1. Collect samples from -80C
2. Samples are placed on lab bench until they defrost
3. Record the sample id and date on the agar plates
4. Ensure sample has defrosted & use a sterile loop to sub culture the GAS (from cryovials)
5. Leave agar plates on bench for 10-15 mins (upright)
6. Incubate at 37 C in presence of 5% CO2

DNA Extraction (Wizard Genomic DNA Purification Kit,500)

1. Heat the waterbath to 37 C
2. Heat the waterbath to 80 C

Materials to Be Supplied by the User

• 1.5ml microcentrifuge tubes
• water bath, 80ÅãC
• water bath, 37ÅãC
• isopropanol, room temperature
• 70% ethanol, room temperature
• water bath, 65ÅãC (optional; for rapid DNA rehydration)
• 50mM EDTA (pH 8.0) (for gram positive bacteria)

• 10mg/ml lysozyme (Sigma Cat.# L7651) (for gram positive bacteria)
• 10mg/ml lysostaphin (Sigma Cat.# L7386) (for gram positive bacteria)

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.
2. Centrifuge at 13,000–16,000 Å~ g for 2 minutes to pellet the cells. Remove the supernatant. For Gram Positive Bacteria, proceed to Step 3. For Gram Negative Bacteria go directly to Step 6.
3. Resuspend the cells thoroughly in 480µl of 50mM EDTA.
4. Add the appropriate lytic enzyme(s) to the resuspended cell pellet in a total volume of 120µl, and gently pipet to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place. Note: For certain Staphylococcus species, a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin is required for efficient lysis. However, many Gram Positive Bacterial Strains (e.g., Bacillus subtilis, Micrococcus luteus, Nocardia otitidiscaviarum, Rhodococcus rhodochrous, and Brevibacterium albidium) lyse efficiently using lysozyme alone.
5. Incubate the sample at 37ÅãC for 30–60 minutes. Centrifuge for 2 minutes at 13,000–16,000 Å~ g and remove the supernatant.
6. Add 600µl of Nuclei Lysis Solution. Gently pipet until the cells are resuspended.
7. Incubate at 80ÅãC for 5 minutes to lyse the cells; then cool to room temperature.
8. Add 3µl of RNase Solution to the cell lysate. Invert the tube 2–5 times to mix.
9. Incubate at 37ÅãC for 15–60 minutes. Cool the sample to room temperature.
10. Add 200µl of Protein Precipitation Solution to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.
11. Incubate the sample on ice for 5 minutes.
12. Centrifuge at 13,000–16,000 Å~ g for 3 minutes.
13. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol. Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.
14. Gently mix by inversion until the thread-like strands of DNA form a visible mass.
15. Centrifuge at 13,000–16,000 Å~ g for 2 minutes.
16. Carefully pour off the supernatant and drain the tube on clean absorbent paper. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.
17. Centrifuge at 13,000–16,000 Å~ g for 2 minutes. Carefully aspirate the ethanol.
18. Drain the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.
19. Add 100µl (concentration changed to 60µl) of DNA Rehydration Solution to the tube and rehydrate the DNA by incubating at 65ÅãC for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4ÅãC.
20. Store the DNA at 2–8ÅãC.

Reference

Wasas AD, Huebner RE, De Blanche M, Klugman KP. Long-term survival of Streptococcus pneumoniae at room temperature on Dorset egg medium. J Clin Microbiol 1998;36:1139-40.

https://www.promega.com/-/media/files/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol.pdf Wizard Genomic DNA Purification Kit,500 protocol

For questions regarding collection, please email Dr Mark Engel mark.engel@uct.ac.za or contact the AFRO*Strep* Coordinating Office by phone (+27214045735) or fax (+27214486815)

Appendix 9.6: Lab SOP: Polymerase Chain Reaction

As per the cdc website:

https://www.cdc.gov/streplab/protocol-emm-type.html#modalIdString_CDCTable_2

1. Spin down lysate at full speed for 1 min.
2. For each sample, aliquot 24 µL master mix in PCR tubes/plate.
3. Add no more than 1.0 µL lysate supernatant – vortex gently – centrifuge for few seconds to collect reaction mix at the bottom of the PCR tube/plate.
4. Place the PCR reaction tubes/plate on thermocycler under the following conditions.

Appendix 9.7: OpenClinica database: Standard Operating Procedure

Completion of eCRFs in OpenClinica SOP

Introduction

This Standard Operating Procedure (SOP) describes how to navigate OpenClinica and enter data through the electronic database interface.

Scope

Abbreviations

CRF Case record form
eCRF Electronic case record form

Note: Study events

- Study events are essentially study visits. Each study event in OpenClinica is associated with the CRFs available for that study event. All study events are created upfront by the data manager and associated with the relevant CRFs.
- Study events happen at a specific date and time, whether they were planned or not. A study event and its CRFs for a specific patient become available for data capture once a study event has been assigned a date and time for the specific patient. OpenClinica calls this 'Scheduling' an event.

Procedures

1. Logging in to OpenClinica

- Use bookmark to access OpenClinica via Firefox browser at http://srvwinocs002.wf.uct.ac.za:8080/OpenClinica/pages/login/login
- Log in with unique login details as provided by the data manager.
- The landing page is an overview of the study (See Figure 1).
- Upon login to OpenClinica, always check the left---side bar for 'Alerts & Messages'.
- The left---side bar also contains the key to icons used in OpenClinica for ease of reference (See Figure 2).
- All the available activities and views may be found under the 'Tasks' tab (See Figure 1), so when in doubt, start there.

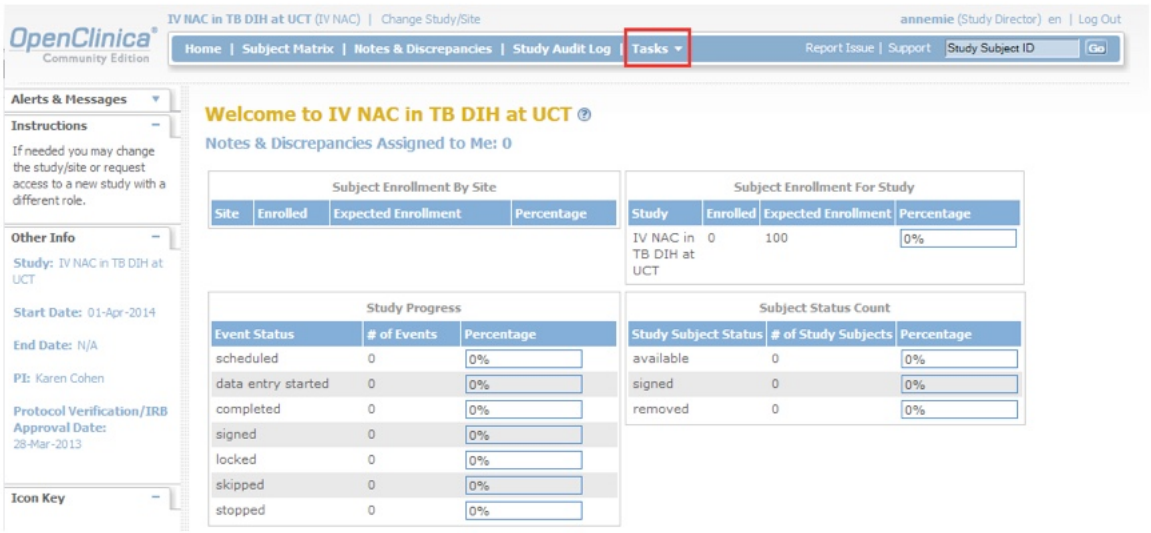


FIGURE 1 OPENCLINICA LANDING PAGE IS OVERVIEW OF STUDY

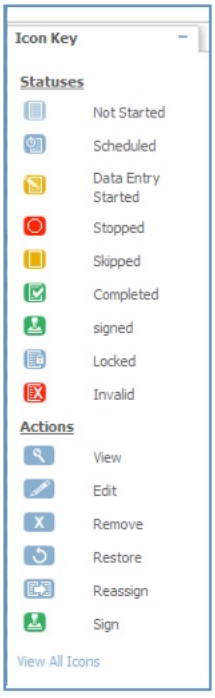


FIGURE 2 KEY TO ICONS FREQUENTLY USED IN OPENCLINICA

2. Adding a newly enrolled participant to the study database

2.1 Under 'Tasks', select 'Add Subject'.

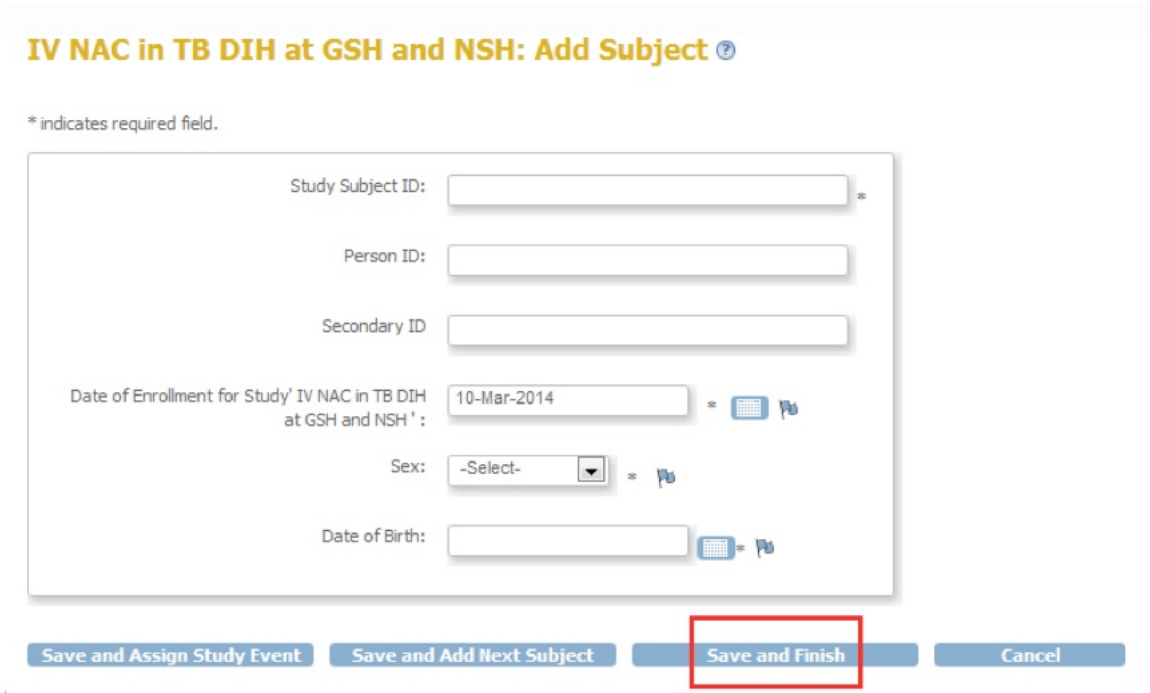


FIGURE 3 ADDING A NEWLY SCREENED PATIENT TO THE STUDY DATABASE

2.2 Enter the screening number as the 'Study Subject ID'.

2.3 Leave 'Person ID' and 'Secondary ID' blank.

2.4 Enter the 'Date of Enrolment'.

2.5 Enter patient's sex.

2.6 Enter patient's date of birth.

2.7 Select 'Save and Finish' (See Figure 3, this will automatically take you to step 3 of the next section).

2.8

3. Scheduling a study event for a patient

3.1 Under 'Tasks' select 'Subject Matrix'.

FIGURE 4 THE SUBJECT MATRIX

3.2 Under the heading 'Actions' click on the 'View' icon to view a participant's record (See Figure 4Figure 5).

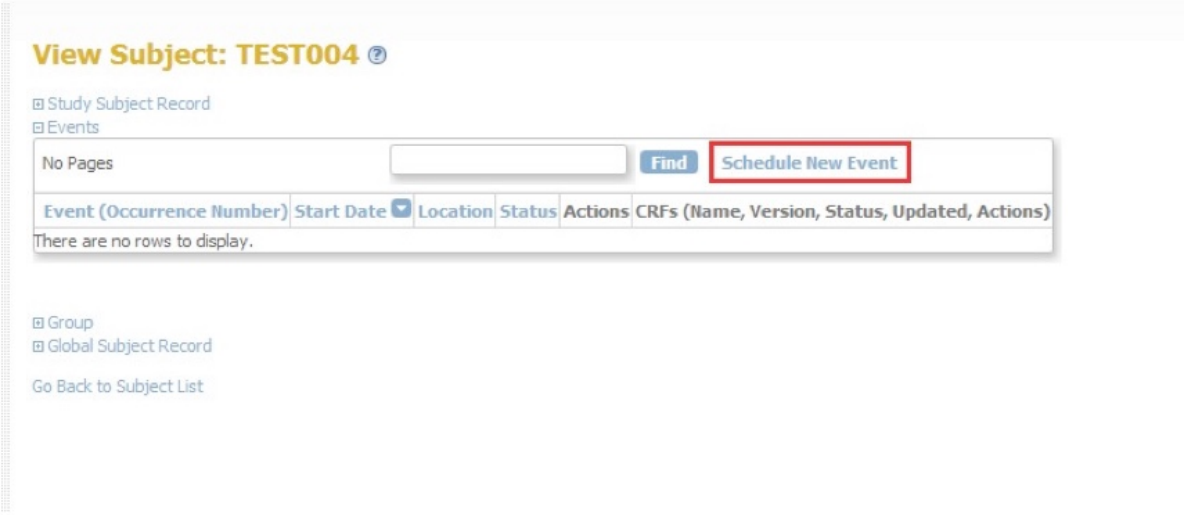


FIGURE 5 THE SUBJECT RECORD

3.3 Select 'Schedule New Event' (See Figure 5).

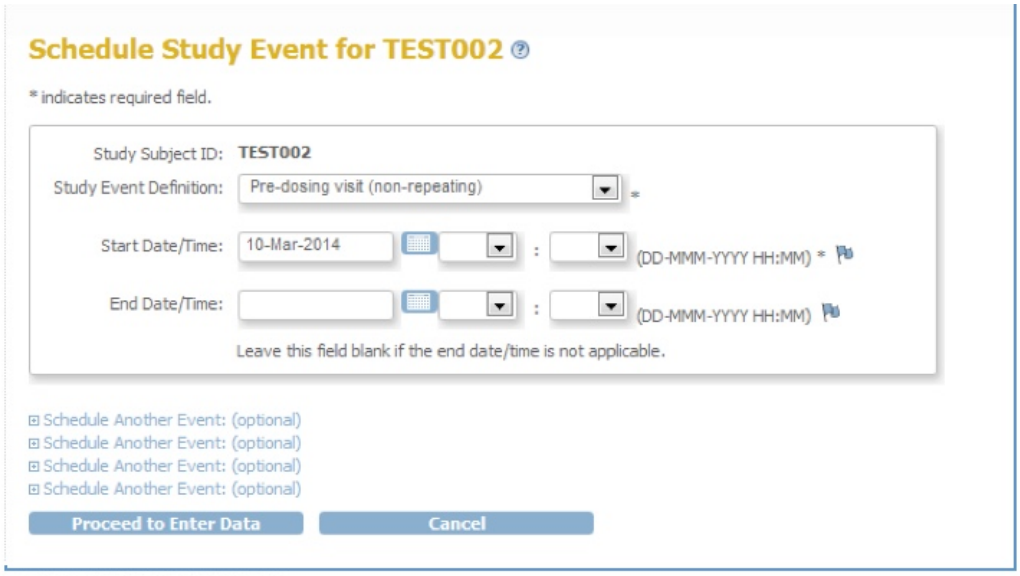


FIGURE 6 SCHEDULING A STUDY EVENT

3.4 Select the appropriate study visit under 'Study Event Definition'.

3.5 Enter the date and time that the study visit occurred.

3.6 Leave the end date and time blank.

3.7 Select 'Proceed to Enter Data' (See Figure 6, this will automatically take you to step 3 of the next section).

4. Entering data for a patient

4.1 Under 'Tasks' select 'Subject Matrix'.

4.2 Under the heading 'Actions' click on the 'View' icon to view a participant's record with applicable CRFs for each scheduled study event in grid form (See Figure 4).

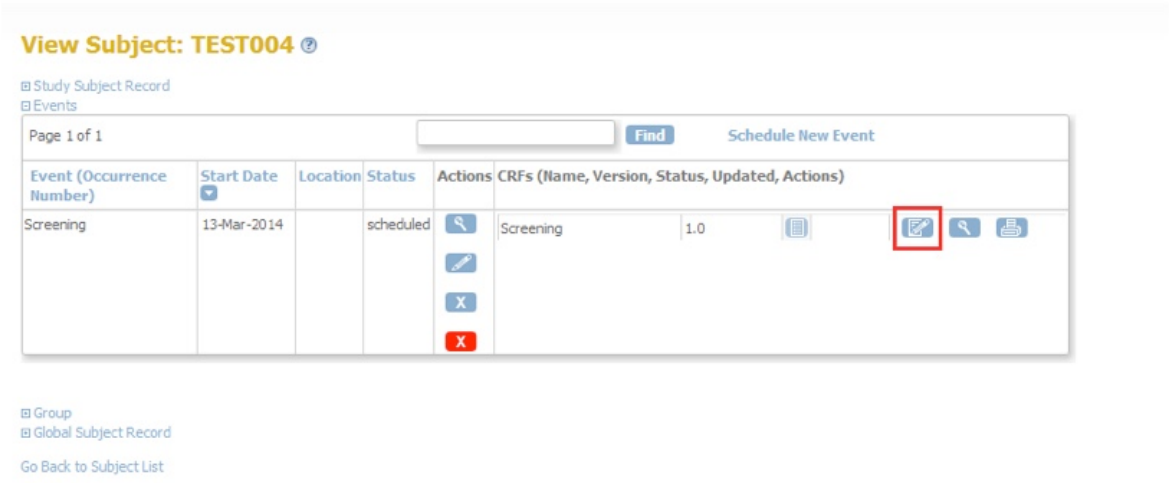


FIGURE 7 THE SUBJECT RECORD WITH AN EVENT SCHEDULED

4.3 Under the heading 'CRFs' click on the 'Enter Data' icon to open an eCRF for data entry (See Figure 7).

4.4 Required fields are marked with a * and the eCRF cannot be saved if these are not completed correctly. Once completed, click 'Save' (See Figure 8).

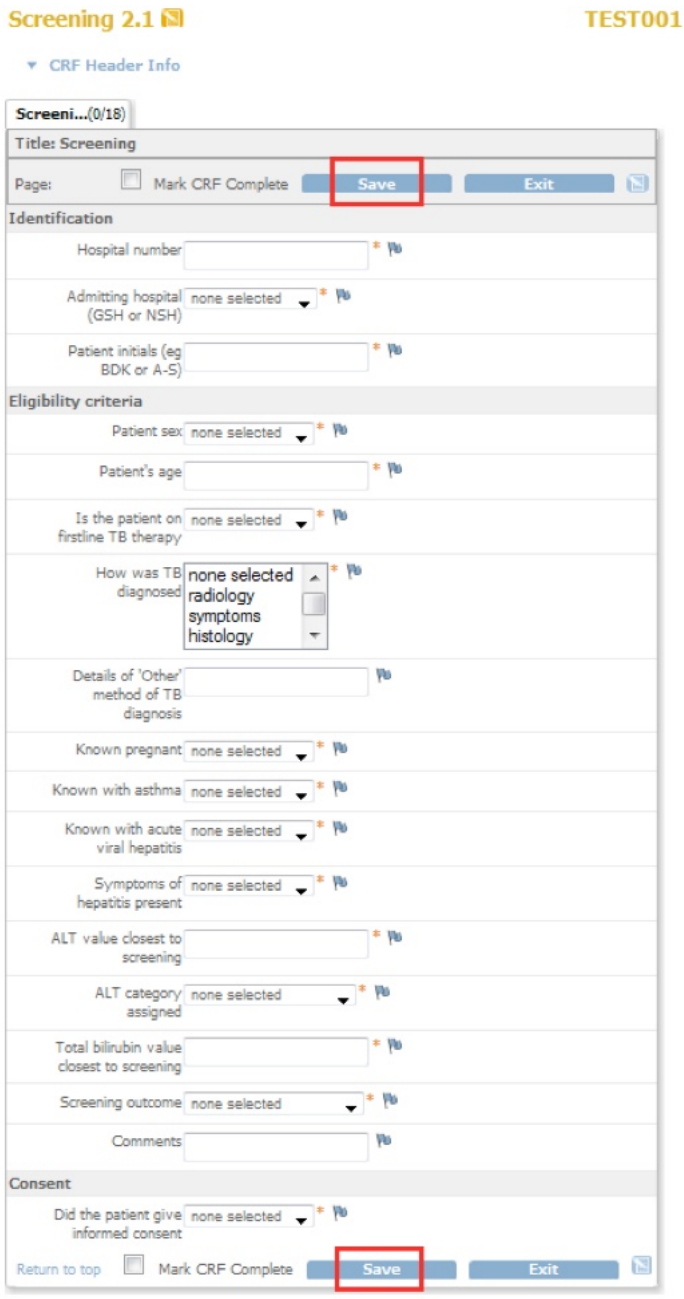


FIGURE 8 CRF OPEN FOR DATA ENTRY

5. Reviewing notes and discrepancies

5.1 Under 'Tasks' select 'Notes & Discrepancies' to display a list of Notes and Discrepancies assigned to the current user in a grid form.

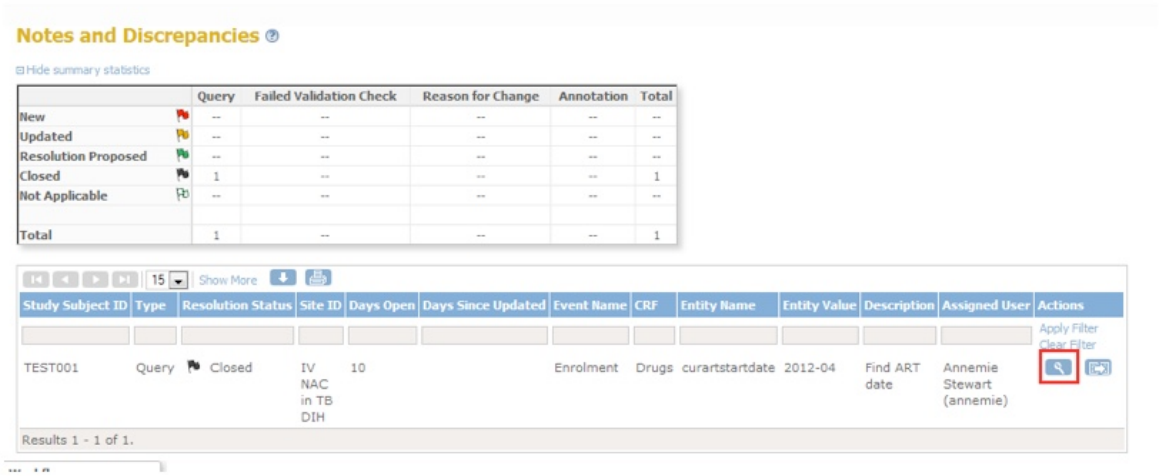


FIGURE 9 LIST OF NOTES AND DISCREPANCIES

5.2 Under the heading 'Actions' click the 'View' icon in order to view the details of the note (SeeFigure 9).

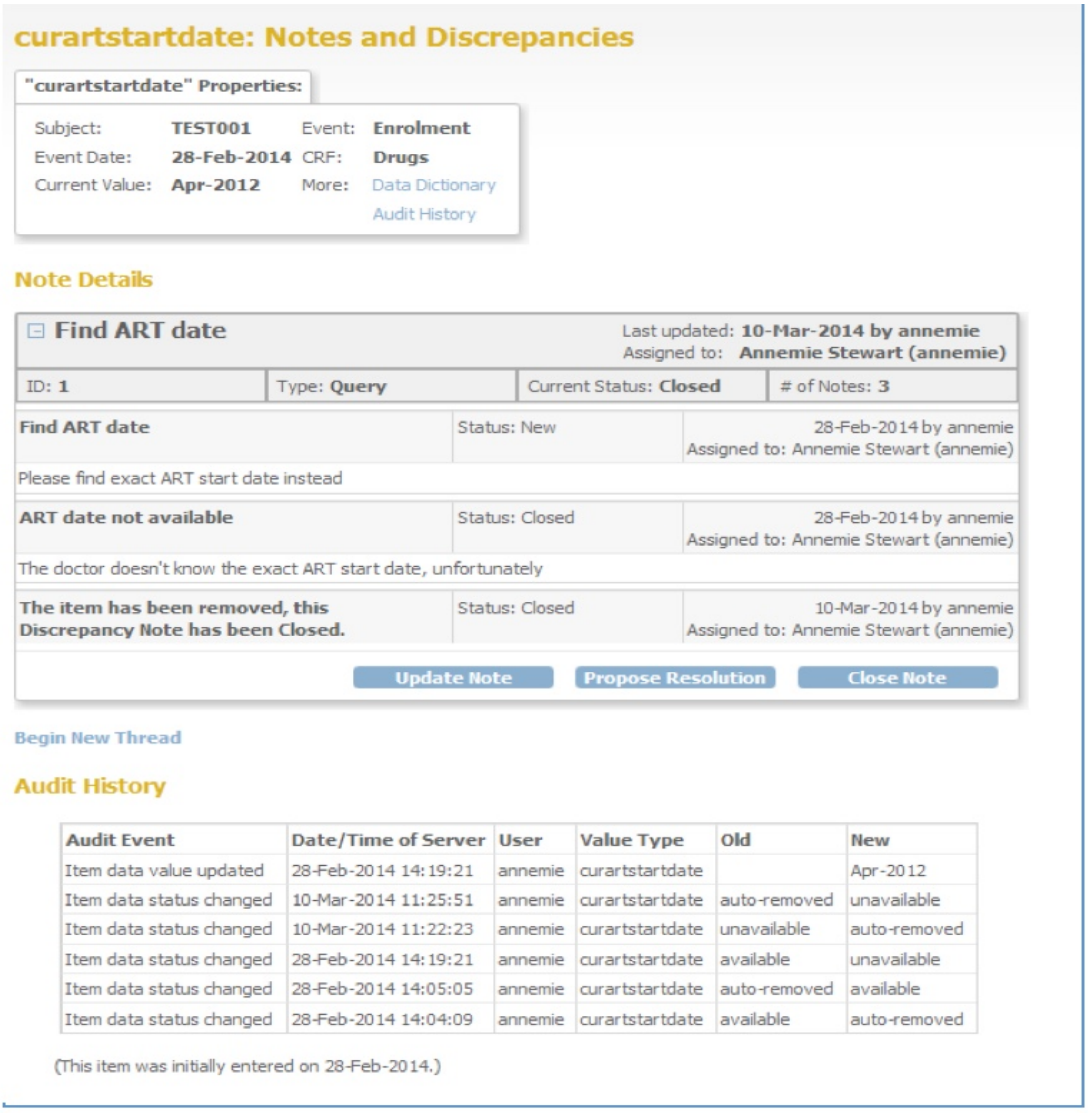
5.3 Review source data as necessary and reply to the 'notes' by

a) Changing/correcting the data and clicking 'Update Note', or

b) Typing an explanation of the data and clicking 'Update Note', or

c) Typing a proposed resolution that will be sent back to the data manager and clicking 'Propose Resolution'.

5. All notes, changes and corrections made to the data are recorded in the study audit log which is available to the study monitor for verification.



curartstartdate: Notes and Discrepancies

"curartstartdate" Properties:

Subject:	TEST001	Event:	Enrolment
Event Date:	28-Feb-2014	CRF:	Drugs
Current Value:	Apr-2012	More:	Data Dictionary
			Audit History

Note Details

Find ART date — Last updated: 10-Mar-2014 by annemie; Assigned to: Annemie Stewart (annemie)

ID: 1 | Type: Query | Current Status: Closed | # of Notes: 3

Find ART date	Status: New	28-Feb-2014 by annemie Assigned to: Annemie Stewart (annemie)
Please find exact ART start date instead		
ART date not available	Status: Closed	28-Feb-2014 by annemie Assigned to: Annemie Stewart (annemie)
The doctor doesn't know the exact ART start date, unfortunately		
The item has been removed, this Discrepancy Note has been Closed.	Status: Closed	10-Mar-2014 by annemie Assigned to: Annemie Stewart (annemie)

Update Note | Propose Resolution | Close Note

Begin New Thread

Audit History

Audit Event	Date/Time of Server	User	Value Type	Old	New
Item data value updated	28-Feb-2014 14:19:21	annemie	curartstartdate		Apr-2012
Item data status changed	10-Mar-2014 11:25:51	annemie	curartstartdate	auto-removed	unavailable
Item data status changed	10-Mar-2014 11:22:23	annemie	curartstartdate	unavailable	auto-removed
Item data status changed	28-Feb-2014 14:19:21	annemie	curartstartdate	available	unavailable
Item data status changed	28-Feb-2014 14:05:05	annemie	curartstartdate	auto-removed	available
Item data status changed	28-Feb-2014 14:04:09	annemie	curartstartdate	available	auto-removed

(This item was initially entered on 28-Feb-2014.)

FIGURE 10 DETAILS OF A NOTE

Appendix 9.8: AFROStrep Standard operating procedure

A REGISTRY
FOR GROUP A STREPTOCOCCAL INFECTION
IN AFRICA

MANUAL OF PROCEDURES
VERSION 1.0

Prepared by

Dylan D Barth
Mark E Engel

For the AFRO*Strep* operations committee

Background

The AFRO*Strep* Study: A registry for group A streptococcal (GAS) infection in Africa

Group A β-haemolytic Streptococcus (GAS), a gram-positive bacterium also known as Streptococcus pyogenes, results in skin conditions, such as pyoderma and mucosal diseases such as pharyngitis. Repeated GAS infections may lead to severe GAS-related illnesses which include acute post-streptococcal glomerulonephritis, acute rheumatic fever (ARF) and rheumatic heart disease (RHD). RHD is associated with significant morbidity and mortality in children and young adults living in developing countries. Increases in the number of cases for invasive and non-invasive GAS diseases have been observed globally since the 1980s. The reasons for these observations are not clearly understood and have subsequently caused many countries to commence active surveillance systems for invasive GAS to closely document the epidemiology of the disease. Prevalence and incidence data on GAS infections from Africa are largely lacking especially given that GAS infections are not notifiable.

The AFRO*Strep* project is a proposed multi-centre study with the objective to collect comprehensive data on GAS disease in Africa. The study comprises two approaches: (1) an active community-based surveillance of (non-invasive) GAS pharyngitis at sentinel sites and, (2) passive data collection of invasive GAS disease diagnosed at laboratories across South Africa. Currently, the existing database and repository contains data and isolates of >230 participants obtained from a community-based pilot Demonstration Site in Cape Town. Isolates are to be subjected to DNA isolation to allow for characterization by molecular methods and cryo-preservation for long-term storage. It is anticipated that AFROStrep will allow for the evaluation of a number of entities including rates of severe GAS infection, sequence typing of the emm gene, etc.

The objectives of the AFRO*Strep* Registry:

1. To collect demographic and clinical information from patients with non-invasive and invasive laboratory-confirmed GAS infection.
2. To establish a bio repository of isolates so as to allow for studies to determine the molecular epidemiology on non-invasive and invasive GAS infection.
3. To conduct studies that contribute to the growing body of knowledge informing vaccine development
4. To assess strategies for treatment, control and prevention of GAS infection

1. GLOSSARY

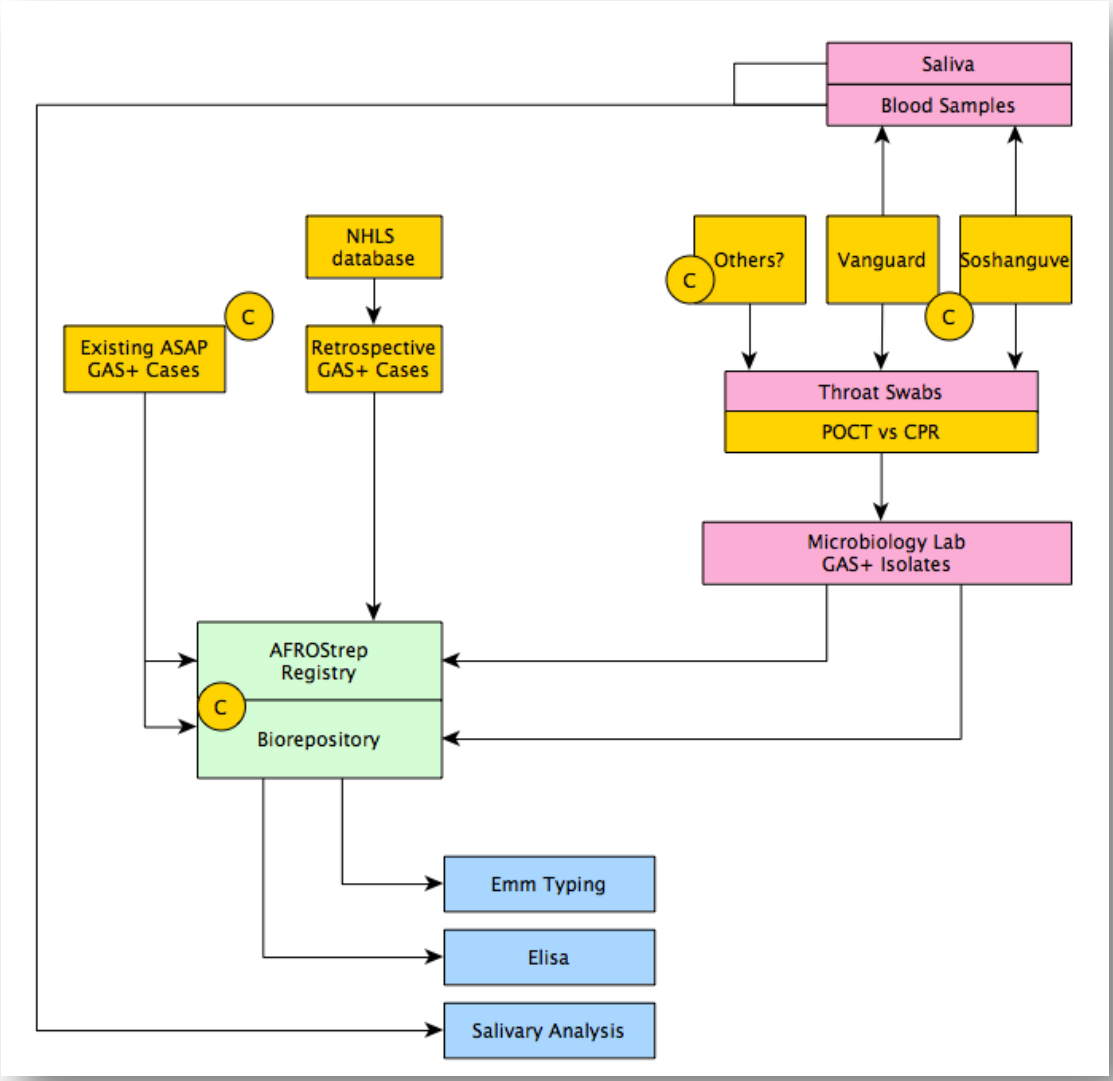
GAS	**group A streptococcus**
CRF	Case Report Form
GCP	Good Clinical Practice
MOP	Manual of Operations
SOP	Standard Operating Procedures
PCO	Project Coordinating Office
PI	Principal Investigator
ERF	Event Record Form

2. STUDY OVERVIEW

TITLE	THE AFRO*STREP* STUDY: A SURVEILLANCE SYSTEM FOR GROUP A STREPTOCOCCAL INFECTION IN AFRICA
STUDY SIZE	OPEN-ENDED
STUDY DESIGN	ITERNATIONAL MULTI-CENTRE CROSS-SECTIONAL DESIGN
OBJECTIVES	• TO COLLECT DEMOGRAPHIC AND CLINICAL INFORMATION FROM PATIENTS WITH NON-INVASIVE AND INVASIVE LABORATORY-CONFIRMED GAS INFECTION IN AFRICA • TO ESTABLISH THE AFRO*STREP* BIOREPOSITORY AND CLINICAL DATABASE COMPOSED OF AFRICAN PATIENTS WITH A GAS-RELATED DIAGNOSIS. • TO CONDUCT A PROSPECTIVE, SURVEILLANCE STUDY IN ORDER TO DETERMINE THE BURDEN AND REGIONAL DISTRIBUTION OF INVASIVE DISEASE ASSOCIATED WITH GAS AT COLLABORATING SITES IN AFRICA OVER A 12-MONTH PERIOD • TO DESCRIBE THE MOLECULAR EPIDEMIOLOGY OF INVASIVE GAS ISOLATES IN AFRICA, PARTICLUARLY WITH RESPECT TO HOW THEY COMPARE AND CONTRAST WITH THE MOLECULAR EPIDEMIOLOGY OF PHARYNGEAL ISOLATES
INCLUSION CRITERIA	INCLUSION INTO AFROSTREP IS SUBJECT TO: • ANYONE PRESENTING WITH A SORE THROAT • MICROBIOLOGICAL LABORATORY CONFIRMATION OF GAS • INFORMED CONSENT • AVAILABILITY OF CLINICAL DATA

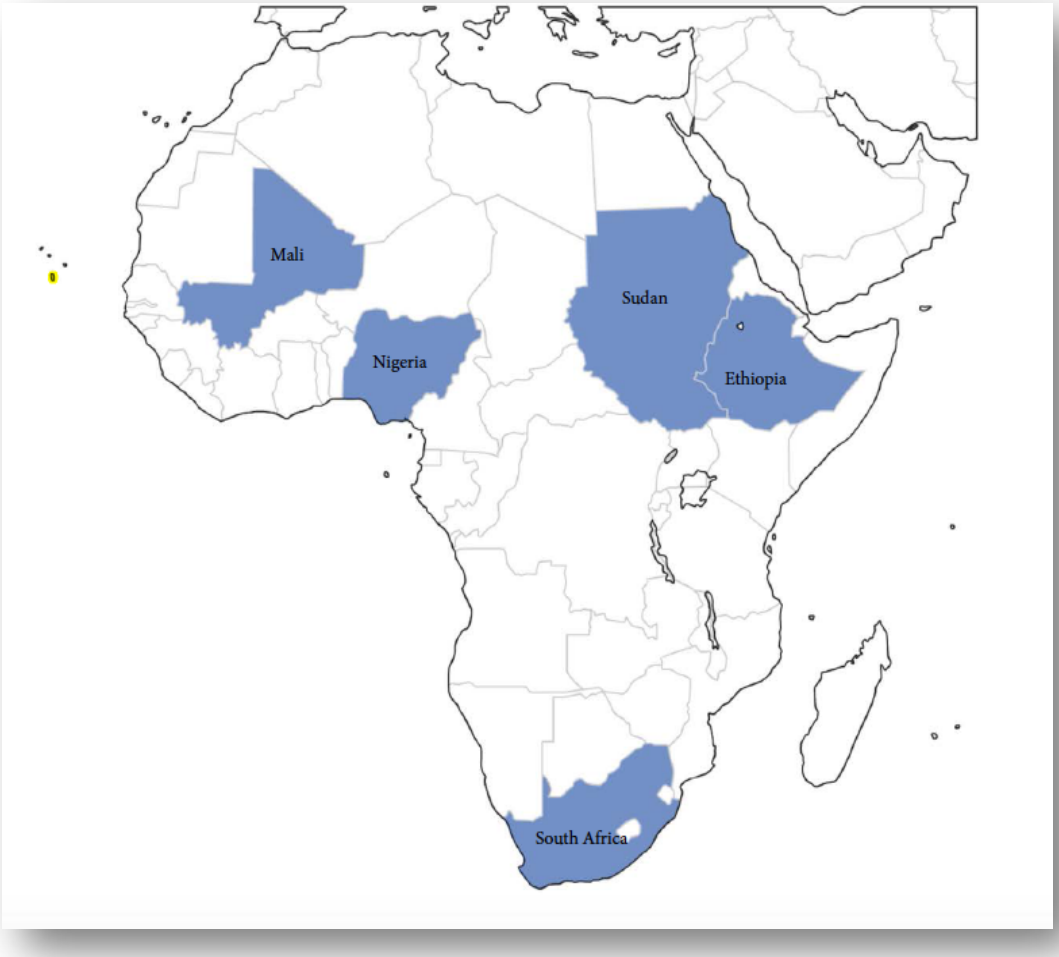
EXCLUSION CRITERIA	PATIENTS WILL BE EXCLUDED IF NO INFORMED CONSENT WAS OBTAINED
PROJECT COORDINATING OFFICE: AFRICA (PCO)	DR MARK ENGEL (PI) MR DYLAN BARTH (CO-PI) DEPARTMENT OF MEDICINE, J 46.47 OLD MAIN BUILDING, GROOTE SCHUUR HOSPITAL UNIVERSITY OF CAPE TOWN, OBSERVATORY 7925 TELEPHONE: +27-21-4047676; FAX: +27-21-4472765 EMAIL ADDRESSES: MARK.ENGEL@UCT.AC.ZA DYLAN.BARTH@UCT.AC.ZA
COORDINATING OFFICE:	

2.1 AFROSTREP PROCESS FLOW CHART



Comprehensive information of cases obtained from both components will be captured into the AFRO*Strep* registry database and specimens will be stored in a bio repository.

2.2 COLLABORATING AFRICAN SITES (CURRENT)



Coordinating centres: Ethiopia, Jimma University; Mali, Centre pour le Développement des Vaccins; Nigeria, University of Benin; South Africa, University of Cape Town; Sudan, University of Khartoum.

The map above displays the current collaborating sites in the AFROStrep Registry. Geographical Information Systems (GIS) flowing out of recent work (Barth *et al*, 2015) will be used for spatial analyses including clustering of cases, prevalence maps, etc.

3. PATIENT ELIGIBILITY

*** Please maintain a SCREENING LOG of all patients screening for enrolment in the AFRO*Strep* registry .**

3.1 INCLUSION AND EXCLUSION CRITERIA

I. Inclusion Criteria [ALL criteria must be present for eligibility to be confirmed]

- Informed consent

  AND

  One of the following
- Anyone presenting with a sore throat
- Microbiological laboratory confirmation of GAS

II. Exclusion Criteria [none of the criteria must apply for the patient to be eligible]

- Inability to provide informed consent/assent

3.2 INFORMED CONSENT

Written Informed consent must be obtained prior to enrolling patients in the study.

If the patient or person providing the informed consent cannot sign the Informed Consent Form, a thumbprint will suffice.

For future reference, please record in the patient's file:

- The date and time of consent.
- The name of the person obtaining consent.
- The informed consent form.

**Refer to appendices for consent forms*

A template *Informed Consent Form* is included in appendices. This should be adapted and translated for use in each site. A copy of the consent form must be provided to the participant.

ASSENT:

Children who are 8 years and older will be required to sign an assent form in addition to the consent form for inclusion into the study.

**Refer to appendices for assent forms*

3.3 PATIENT CONFIDENTIALITY

All patients will be identified by a unique number assigned at enrollment. The number consists currently of a combination of the center number plus the order in which the patient was enrolled.

For example: A patient at center 100 (Clinic 01 - Cape Town), who was the 4th patient enrolled, will be identified by Patient Identification Number 100-04.

All Case Report Forms and all other study documentation, including source documents and correspondence regarding the patient, must document the center and subject number. Please ensure this number is placed on ALL documents. Patient confidentiality must be upheld in all study documentation. The registration form with contact details must be kept separately from the study documentation to maintain confidentiality as regards data.

3.4 CENTRE NUMBERS AS AT 1 JULY 2015

100	Cape Town: Clinic 1
101	Cape Town: Clinic 2
102	Cape Town: Hospital 1
110	Pretoria: Clinic 1
111	Polokwane: Clinic 1
120	Kwazulu-Natal: Clinic 1
200	Ethiopia
300	Mali

4. CASE REPORT FORM

To complete the case report form:

- Ensure that the participant has signed the consent form.
- Ensure that the participant meets all of the inclusion criteria.
- Ensure that all exclusion criteria are assessed and none apply.
- Confirm that participant meets the eligibility criteria
- Please complete Centre and Participant number on each page.
- Please answer each question by marking an X in one box on each line OR writing numbers in the spaces provided OR writing neatly and legibly on the lines provided.

General Instructions:

- When completing forms please **use black pen only.** Never use pencil or red pen.
- **For questions where a number is required for a response and there is more than one box provided, please fill the boxes from right to left and zero fill all empty boxes. For example, a response of 16 years of age would be recorded as below.**

0	**1**	**6**

- **If it is necessary to make a correction, please draw a single line through the incorrect value and write the correct value nearby. Please initial and date each correction.** Never use Liquid Paper/Tippex/correction fluid.

ME 24/03/2015

0	**1**	**6**

To complete the contact information sheet, please record patient information including:

- Name, initials, home address and phone number(s) of the patient. Include the GPS coordinates if available and hospital number.
- Contact information of at least two contacts including family members, friends or neighbours.
- The contact information of the local clinic.

5. DATA MANAGEMENT

PROCEDURE:

1. The data manager will receive complete, quality assured, and corrected CRFs

2. All CRFs will be captured within 2 weeks of the study visit utilizing the OpenClinica database program. The standard operating procedure for data entry using OpenClinica is attached as an appendix.

3. All data entry will be double-checked for accuracy using the following methods:
   (a) Visual proofing of the CRF with the computer screen post scanning/data entry.
   (b) Cross-referencing with source documents as appropriate.

4. All CRFs captured into the OpenClinica database program will be initialed and dated in a designated location on the CRF.

5. Data entry will take place on a designated internet based password protected computer.

6. All completed and verified CRFs will be filed in its appropriate CRF folder.

** Refer to the OpenClinica SOP attached as an appendix*

6. STUDY ORGANISATION AND RESPONSIBILITIES

6.1 ORGANISATION AND GOVERNANCE

Coordinators:

Dylan Barth, Mark Engel

Responsibilities include:

- Day-to-day running of the registry under the supervision of the Steering Committee
- Keeping and disseminating the minutes of the Steering Committee.
- Preparation of the trial protocol, a master copy of the study materials and aids, development of the case record forms, study database, data internal consistency checks and data analysis.
- Preparation of the interim reports for the independent data safety and monitoring board
- Applications for research grants to fund the study.

Operations Committee: Mark Engel (Chair), Dylan Barth, Andrew Whitelaw, Bongani Mayosi

- It is responsible for monitoring the Registry's function on a short–term basis and for implementing decisions of the Steering Committee.
- The Operations Committee will meet every three months and its decisions and minutes will be circulated to all participants by means of a newsletter.

The Steering Committee:

- The Steering Committee consists of the members of the Operations Committee and Site Principal Investigators. Mark Engel (Chair).

- It is proposed that at least one member of the Steering Committee should be from each of the countries actively recruiting.

- The Steering Committee is responsible for the overall policy of the Registry and for supervising its successful conclusion. Publication policy and sub-study policy are decided by the Steering Committee. Decisions of the Steering Committee will be communicated to all active participants by dissemination of its minutes through the regular newsletter.

- The Steering Committee will attempt to meet annually (dependent on finance). It is recognized that many meetings will be "opportunistic" and will be held at the time of national meetings which most members will attend. If this is not possible in any one year an international tele-conference will be held in its place.

6.2 PUBLICATION POLICY

- Publication policy and authorship will be co-ordinated through the **Publications Committee** that reports directly to the Steering Committee of which it is a sub-committee. Guidelines for the Publications Committee are as follows:

- **Mark Engel** will chair the Publications Committee and will be responsible for selecting, through a process of consultation with members of the Steering Committee.

- The overall purpose of the Publications Committee is to:

    - Ensure the timely writing and publication of high quality manuscripts from Registry data;

    - Solicit, review and prioritise analysis plans and manuscripts from the Registry database;

    - Prioritise authorship of manuscripts / abstracts / posters arising from the Registry database in a fair and just manner;

    - Review manuscripts / abstracts / posters arising from the Registry database.

6.3 AUTHORSHIP GUIDELINES

- *Primary Publications*: it is anticipated that at least two primary publications should result if the registry is successful. The first (methods) will describe the rationale, design and methodology of the registry. The second (results) will describe the outcome of the registry. Authorship of the primary publications will be in the name of the AFRO*Strep* registry – pilot study. All actively recruiting centres will be listed in an appendix under the name of the principal investigator and his / her organisational affiliation.

- *Secondary Publications:* These may arise from sub-studies or from suggestions from participants who wish to analyse the Registry database in areas of personal interest. In such instances the **Publications Committee** will be responsible for ensuring that first and last authors are the people that have developed the project idea and done the writing and also for ensuring that all listed authors meet conventionally accepted authorship credit guidelines. Co-ordinating centre workers may be included as authors at the discretion of the **Publications Committee**.

6.4 SUB-STUDIES

- Actively recruiting centres and investigators are encouraged to propose sub-studies and sub-analyses of the data in the Registry database.

- A Sub-study Committee (**Chair: Mark Engel**), which reports to the Steering Committee, will review all such proposals to ensure that they are feasible and do not compromise the integrity of the database and primary publications.

- Proposed sub-studies:

  - Rapi-Strep Trial

  - Laboratory investigations using molecular techniques

6.5 MEMORANDUM OF AGREEMENT

- Signed after ethics approval has been obtained

- Outline site specific deliverables

- Confirm the arrangement and details for the transfer of funds to the site

6.6 PROJECT COORDINATING OFFICE:

Department of Medicine, Groote Schuur Hospital, University of Cape Town, South Africa.

Responsibilities include:

- Ensuring good collaboration with the study sites.
- Assisting sites in obtaining ethical & regulatory approvals
- Preparation and distribution of study materials and study aids.
- Receiving CRFs and other relevant material from study sites.
- Data entry, data validation and quality control checks.
- Organisation of Investigator's meetings
- Communication help lines
- Monitoring of study sites
- Preparation and distribution of study updates
- Assisting sites with smooth running of the study
- Training site teams on study procedures

6.7 INVESTIGATING SITES

RESPONSIBILITIES INCLUDE:

- Forming the ASAP AFRO*Strep* team and raise awareness among all medical and nursing staff involved in the study.
- Obtaining ethics and other regulatory approvals.
- Ensuring that all patients admitted with a diagnosis of group A streptococcal infection are considered for screening, making sure that all eligible consenting patients are enrolled.
- Completing all necessary Case Report Forms and sent to the designated data capturer for data entry.
- Responding promptly to all inquiries from the Project Coordinating Office regarding patient CRFs, lab forms etc. or other important study matters.
- Participating in monitoring visits by responding to all queries raised.

6.8 MAINTENANCE OF RECORDS

The Principal Investigator must:

- Maintain a Screening Log of all patients with a sore throat considered for enrolment.
- Make sure that all patients folders and related all documents are kept in an office in a securely locked cabinet , and accessible to study staff.
- Obtain a correctly completed Patient Informed Consent Form for each patient enrolled into the study.
- Maintain a list of Patient ID and corresponding patient names to enable records to be found at a later date (this should be kept in a locked cabinet away from CRFs to maintain confidentiality).
- Retain all records, including the signed Informed Consent Forms and the Patient Identification List for **at least 2 years** after the official study closeout.

The PCO will inform the Investigator when these documents no longer need to be retained.

6.9 COMMUNICATION

The first line of contact for study related questions would be the Project Coordinating Office.

Contacts

PROJECT COORDINATING OFFICE

Department of Medicine

H47, Old Main Building

Groote Schuur Hospital

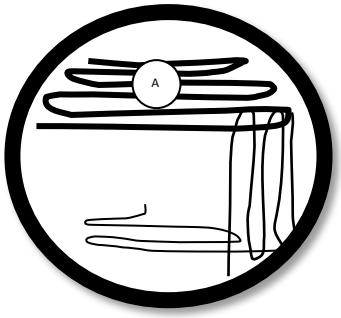
University of Cape Town

7. THROAT CULTURE - SOP

The throat culture is an important procedure to identify pathogens that cause pharyngitis or that colonize the throat. It must be performed with care and in an identical fashion from subject to subject. When performed incorrectly, the sensitivity of the culture falls; in other words, some subjects who have a pathogen in their pharynx will not be identified. The most commonly identified bacterial organism that causes pharyngitis is Streptococcus pyogenes, or Group A Strep (GrAS). This Standard Operating Procedure (SOP) describes the procedure for obtaining and plating a throat culture.

Culture Procedure:

1. Verify the identity of the subject and label a sterile culture swab and, when used at the point of contact, the back of an agar plate, with the information requested by the protocol (typically 2 patient identifiers, the date, and an identifier for the person plating the organism).
2. Put on gloves. These gloves need not be sterile.
3. Hold a bright flashlight or penlight in one hand or have the subject face a bright fixed source of light.
4. Remove the swab from its sterile container taking care to keep the cotton tip end sterile. Do not use swabs found in packages in which the sterile packaging has been violated.
5. Ask the subject to open the mouth widely and protrude the tongue and say, “aghhh” or pant. If the tonsils or tonsillar fossae and the posterior pharynx are easily seen, the culture may be done without a tongue depressor. If not, then use a tongue depressor, placed about ½ to ¾ of the way to the posterior edge of the tongue and firmly push the tongue inferiorly. Do not put the tongue depressor so far posteriorly as to gag the subject.
6. Rub the swab on one tonsil, the posterior pharynx, and the other tonsil. Do not touch the tongue or oral cavity, if possible.
7. If the protocol calls for it, place the swab back in its cap and crush the ampoule that holds the transport media in the tip. On the other hand, if the protocol allows for it, one may swab the specimen directly onto an appropriate agar plate or other solid media, most commonly 5% sheep blood agar.
8. Transport the labellled swab or the inoculated agar plate to the laboratory as directed in the individual protocol.
9. If GrAS is to be identified, an antibiotic disk containing 0.04 U of bacitracin (an “A” disk) should be placed on the agar in an area of expected heavy growth. It is placed firmly on the agar using forceps or other sterile means.
10. The agar plate is incubated and analyzed as per the protocol.



Appendix 9.9: AFROStrep Assent form

Assent Form for children 8 years and older

The AFRO*Strep* REGISTRY: A surveillance system for group A streptococcal (GAS) infection in South Africa

Principal Investigator: Prof Bongani M. Mayosi, D.Phil.
Groote Schuur Hospital and University of Cape Town
Tel: 27-21-406-6200
Email: bongani.mayosi@uct.ac.za

We are doctors and nurses from the University of Cape Town and Groote Schuur Hospital and we are doing a study of sore throat in children. Sore throat can be caused by many kinds of germs.

We would like to ask you and your parent / legal guardian a few questions about your sore throat. After that, the nurse will ask to look inside your mouth at your throat and feel your neck for swollen glands.

To find out if you have a germ in your throat, we will use a long soft stick (like an earbud) and lightly touch the back of your throat. This will not hurt at all and is very quick, but may be a little uncomfortable. This stick will then be sent away to test for a germ that causes sore throat. If you allow us to examine you, you will be helping doctors understand sore throat better.

Your information will help doctors and nurses to learn how to treat sore throat in children. You are allowed to say that you don't want to be in the study. Nobody will be angry with you if you say no. The clinic nurses will still treat your sore throat if you don't want to be in the study. Before you decide, you can ask us questions. If you want to be in the study, you must write your name on this sheet of paper. This means that you are happy to be involved in the study.

Participant:

_____

Printed name Signature Date

Study doctor or research nurse:

_____

Printed name Signature Date

Witness:

_____

Printed name Signature Date

Appendix 9.10: AFROStrep consent form and patient information sheet

The AFRO*Strep* REGISTRY:
A surveillance system for group A streptococcal infection in South Africa

Information Sheet: Sore Throat Study

Principal Investigators: Mark Engel, BSc (Hons), MPH (Epid/Bios), PhD
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Co-Investigators: Dylan Barth, BTech (Env. Epid), MPH
Groote Schuur Hospital and University of Cape Town

Bongani M. Mayosi, D.Phil.
Groote Schuur Hospital and University of Cape Town

Andrew Whitelaw, M.B., Ch.B.
Tygerberg Hospital and Stellenbosch University

INTRODUCTION

Your child has been asked to participate in the AFRO*Strep* registry because he/she has a sore throat. A research registry is a collection of information about patients who have a particular disease or condition, or who receive a particular treatment to be used for future research studies. The collection of data is essential for a functioning disease-control programme and allows researchers to conducts studies directed at increasing our knowledge on diseases. This information sheet provides you with information that you should know and understand before agreeing to be included in the AFRO*Strep* registry.

After reading this information sheet, please feel free to ask any questions or voice concerns that you may have. You may keep a copy of this consent form and patient information sheet for your records and referral.

An unlimited number of participants will be enrolled in the AFRO*Strep* registry. Your child's participation in this registry will not involve any visits in addition to routine care and he/she may withdraw from participating in the AFRO*Strep* Registry at any point.

WHY IS THIS REGISTRY BEING ESTABLISHED?

The purpose of the AFRO*Strep* registry is to collect comprehensive demographic, clinical, microbiological and epidemiological data from patients with group A streptococcal (GAS) infection. Information in this registry may be used to enable a wide range of future genomic and genetic studies, biologic and immunologic research, epidemiological, clinical, and health services research.

Future research studies emanating from the AFRO*Strep* registry will only use the information in this registry and will not require additional informed consent from participants in the registry. The information in this registry may also be used to identify patients who may be eligible to participate in certain future research studies conducted by the University of Cape Town that relate to their particular disease, condition, or treatment and for which information is needed that is not in the registry. If your child is identified (based on information about you in the registry) as being potentially eligible for a future research study that relates to a particular disease, condition, or treatment, you may be contacted to find out if he/she would be interested in participating in the research study. If you are interested at that point, the research study would be fully explained to you, and informed consent will be required before participating.

WHY ARE WE DOING THIS STUDY?

There are many causes of sore throat. Most often a sore throat is caused by a virus. A virus is a germ which can cause illnesses, for example colds and flu. Antibiotics do not work against viruses. Antibiotics should only be used when a doctor or clinical nurse believes that a patient's illness is caused by a bacterium, which is a different kind of germ which can be killed by antibiotics.

If antibiotics are used too often and incorrectly, bacteria become resistant to the antibiotics. This means that antibiotics no longer work against them and so in future, we may be unable to treat infections caused by these bacteria.

A particular type of bacterium called *Streptococcus pyogenes* can cause sore throat. As a complication of this type of sore throat, a small percentage of patients can later develop disease of the heart valves (Rheumatic heart disease) or kidney disease. Antibiotics can prevent these complications from developing.

There are many different genetic types of *Streptococcus pyogenes* and it is not known which types of this bacterium are most common in your area. In the study, we will take throat swabs. Bacteria isolated from these swabs will be sent to a laboratory in Cape Town to examine the genetic structure of the bacteria. The genes of the bacteria, and not those of yourself, will be investigated.

It is hoped that if we know which genetic types are most common, it will help researchers to produce an effective vaccine against S*treptococcus pyogenes*. A vaccine is a substance that is used to protect a healthy person from developing an infection.

IF YOUR CHILD PARTICIPATES, WHAT WILL BE EXPECTED OF HIM/HER?

If you agree to participate in the study, the study nurse or doctor will ask you questions about your child's illness. After a brief examination, a throat swab will then be performed. Using a long plastic stick with cotton wool at the end (much like an earbud) a sample of the mucous or pus from the back of the throat will be taken. This sample will be tested immediately using a rapid strep test, thereafter being sent to a laboratory where we will look for the presence of *Streptococcus pyogenes*.

Following the swab being taken, the clinic doctor or nurse will examine and treat your child as usual.

WHAT WILL HAPPEN TO THE THROAT SWAB AT THE END OF THE STUDY?

Throat swabs collected from your child during this study will be labelled with a unique code. It will not be possible to determine your child's identity from this information. Should your child's swab contain bacteria, the sample will be stored. We will conduct further molecular investigations on the bacteria

later on. You will not be informed of any results of these studies, nor benefit financially from any medicines or vaccines which are produced by these studies.

Any future research with your child's samples will be reviewed by the University of Cape Town's Institutional Review Board before the research can begin. Your permission is required for these later studies. If you do not agree to the storage and later investigations of the bacteria, you can still be involved in the rest of the study procedures.

WHAT ARE THE RISKS OF BEING INVOLVED IN THE STUDY?

Taking the throat swab can be uncomfortable, especially for younger children. Touching the back of the throat may make your child feel nauseous for a little while. We will try to make the procedure as quick and comfortable as possible.

WHAT ARE THE BENEFITS OF TAKING PART IN THIS STUDY?

The results of the laboratory diagnosis of your child's swab will be made available to the clinic staff to be added to his/her folder. Your child's participation in this research will enable researchers to better understand the prevalent *Streptococcal pyogenes* strains in South Africa and will assist researchers to develop a vaccine that is effective in our setting and which may one day help in the prevention of the complications of sore throat.

HAS THIS STUDY BEEN APPROVED?

This study is being performed by investigators from the Department of Medicine and the Department of Medical Microbiology at Tygerberg Hospital.

The study has been approved by the Human Research Ethics Committee of the University of Cape Town. If you have any problem with this study, please contact this committee at 021 406 6338.

YOUR RIGHTS AS A PARTICIPANT IN THIS STUDY

You have the right to refuse to participate in this trial. Your child will still be treated in the same way if you do not participate. You have the right to withdraw from the study at any time.

CONFIDENTIALITY

All information collected during the course of this study will be kept securely and confidentially. Reports about the study and results that may be published in scientific journals will not include any information which identifies the participant personally.

If the investigators have any further questions about your child's medical information which may be important for this study, we may need to find the clinic folder to gather such information. This information will be treated as confidential.

INFORMED CONSENT: The AFRO*Strep* REGISTRY: A surveillance system for group A streptococcal (GAS) infection in South Africa

- I hereby confirm that I have been informed about the purpose, benefits and risks of my child's participation in this study.
- I am aware that some information regarding my child's health will be used anonymously by the investigators and researchers.
- I have been informed that participation is voluntary.
- I have had an opportunity to ask questions and these have been answered
- I have read and understood the contents of this document.

I agree to the storage and later investigations of bacterial specimens obtained from my child's throat.

YES / NO

Participant:

_____

Printed name Signature Date

Study Doctor or Research Nurse:

_____

Printed Name Signature Date

Witness:

_____

Printed Name Signature Date