An investigation of group A *Streptococcus.*

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LIST OF ABBREVIATIONS

AU	Absorbance units
BA	bacteriological agar
CDC	Centers for Diseases Control and Prevention
CFU	coliform forming units
GAS	Group A streptococcus
g/L	grams per litre
HIV	Human immunodeficiency virus
iGAS	invasive group A streptococcus
IBC	Institutional Biosafety Committee
µL	microlitre
mL	millilitre
mm	millimetres
nm	nanometres
OD_{600}	optical density at 600 nm
PC2	Physical Containment Level 2
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PYR	Pyrrolidonyl Arylamidase
qPCR	quantitative polymerase chain reaction assay
rpm	revolutions per minute
THB	Todd Hewitt broth
TNTC	too numerous to count
WHO	World Health Organization

ABSTRACT

Streptococcus pyogenes or group A streptococcus (GAS) is a gram-positive bacterium transmitted primarily through respiratory droplets, or skin contact with broken skin that has secretions from infected sores on the skin. Foodborne GAS transmission also exists. Additionally, the environment is also a potential reservoir and facilitates transmission through contaminated equipment, surfaces, dust and fomites. GAS is responsible for causing a range of infections that include rheumatic fever, necrotising fasciitis, post streptococcal glomerulonephritis, pharyngitis, strep throat and scarlet fever. These infections are classified as either mild or invasive. Anyone in a population can be infected by GAS. However, the immunocompromised, children 5-15 years old, the elderly and pregnant women are at most risk of these infections.

GAS is endemic among Indigenous communities in Australia with the prevalence rate ranging from 23.9 to 82.5 cases per population of 100,000 people. This has been attributed to factors including low socioeconomic status, characterised by overcrowding and poor housing conditions. This research was undertaken, in part, to inform health care workers in a remote community that had expressed concern about the lack of information available about the risk factors of GAS. Since GAS infections are not nationally notifiable in Australia, their control and prevention has faced challenges. Making GAS notifiable at the national level would help to inform public health and research initiatives aiming to reduce the impact of this condition.

An extensive literature review was undertaken to examine all published studies from the past ten years that identified group A streptococcus infections, their risk factors and the prevention and control strategies in place for the control of these bacteria. The findings showed that GAS infections exist with higher prevalence among the Indigenous populations and that the incidence of the infections has its peak during childhood. Schools, hospitals and residential care homes were also found to be high risk areas for the transmission of the infections. This literature review also highlighted the current prevention and control measures in place for GAS transmission. Hand and personal hygiene were reported to be key in prevention of these infections.

Hand hygiene can be achieved through hand washing with soap and water or use of hand sanitisers. The effectiveness of hand sanitisers in the destruction of pathogenic microorganisms varies depending on different factors. Samples of hand sanitisers available in the local supermarkets were purchased and tested in August 2020. A total of five samples were purchased. Four samples were hand sanitisers: the fifth sample was a dish washing liquid, used as a comparison. All samples were tested for their effectiveness in the destruction of GAS bacteria using a bacteriological culture method. Results of the study showed that three of the four hand sanitiser samples tested, and the

dishwashing liquid, demonstrated a log reduction of log 7 and were therefore effective in killing *S. pyogenes*. One hand sanitiser sample recorded a log reduction of log 1, which indicates its inability to effectively kill *S. pyogenes*.

Future research should involve a bigger sample size to determine the effectiveness of more brands of available hand sanitisers. Further testing of hand sanitisers that are ineffective in killing *S. pyogenes* should also be done. Testing of the effectiveness of alcohol-free hand sanitisers on the destruction of GAS should also be undertaken. Additionally, this kind of testing should be done on high touch surfaces to determine whether disinfection methods effectively destroy GAS. Swabs can be collected from the hands of the people at most risk to determine presence and numbers of GAS microorganisms on their hands before and after the use of different brands of hand sanitisers, to determine their effectiveness. To determine the effectiveness of hand hygiene in control of GAS infections, work involving comparison of morbidity due to GAS before and after implementation of hand hygiene using hand sanitisers, or a combination of both hand hygiene techniques, in populations which are more susceptible to GAS infections, should also be done.

These findings, coupled with the literature review, show the importance of understanding GAS disease risk factors and the feasible community prevention and control measures. The findings will ensure reduced disease morbidity and mortality and therefore improved quality of life in populations of at-risk individuals.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed: Nelly Janira Avire.

Date: 19th October 2020.

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Statement of co- authorship

The following people contributed to the development of the research article manuscript sent for publication which was work undertaken as part of this thesis. The co-authors are listed in the order that the co-authored manuscript appears in the thesis.

Dr Harriet Whiley

Dr Kirstin Ross

All above listed contributions equated to no more than 25% of the work necessitated for the preparation of the research manuscript.

This thesis includes a manuscript submitted for publication, therefore some repetition between chapters occurs. Some variations may be seen in the referencing style used and this is due to the referencing style used by the journal.

1.0 INTRODUCTION

This chapter presents a review of the relevant literature of the key concepts of this study. This includes literature defining GAS, its transmission, the populations at risk of getting GAS infections, the risk factors of transmission and the available prevention and control strategies for these infections. It also explores the burden of GAS infections worldwide. It further narrows down the literature review on GAS infections' burden, with a focus on Australia, and the prevention and control of the infections using hand hygiene, specifically, by the use of hand sanitisers. Lastly, this chapter outlines the research questions, the aims and the objectives of the study.

1.1 Group A *Streptococcus*

Streptococcus pyogenes or group A *Streptococcus* (GAS) is a gram-positive, non-motile, non-spore forming coccus that occurs in chains or in pairs of cells that are round to ovoid, measuring 0.5 to 1.0 µm in diameter (Patterson 1996; Reglinski & Sriskandan 2015, p. 667; Spellerberg & Brandt 2016, p. 3). GAS colonises epithelial surfaces, primarily of the throat and skin, but also colonises other surfaces such as the vagina and rectum, from where it can cause a wide variety of mild or severe infections (Walker et al. 2014, p. 266; Cunningham 2000, p. 471).

GAS is primarily spread by respiratory secretions, contact with skin sores caused by GAS or through contact with contaminated material or equipment (Sosa 2016, p. 126; Steer et al. 2016, p. 2955; Hancock-Allen et al. 2014, pp. 1136-37). However, foodborne infections also occur (Chen et al. 2017, p. 7; Liu et al. 2014, p. 545; Kemble 2013, p. 650-652). Moreover, foodborne transmission and transmission through environmental reservoirs like contaminated surfaces at community level or in enclosed social settings have not been extensively explored. Infection can also be transmitted by asymptomatic carriers (Deutscher et al. 2011b, pp. 992-93; Lamden 2011, p. 396). GAS has an incubation period of 1-3 days (Centers for Disease Control and Prevention 2020). The signs and symptoms for GAS infections include high fever, severe muscle aches, localised muscle tenderness and sometimes redness at the site of a wound (WHO 2005; Zhang et al. 2017, p. 1; Stevens 1992, pp. 3-7).

Diagnosis of GAS is mostly done by culturing the bacteria of clinical specimens (Spellerberg & Brandt 2016, p. 2). However, *S. pyogenes* diagnosis can also be done using rapid tests like the Lancefield antigen determination, PYR (Pyrrolidonyl Arylamidase) test, bacitracin test, antibiotic resistance testing and direct antigen detection. Moreover, serological tests and typing of *S. pyogenes* and rapid molecular techniques like qPCR can also be used in the diagnosis of *S. pyogenes* (Spellerberg & Brandt 2016, pp. 5-11; Fox, Marcon & Bonsu 2006, p. 2593; Reijtman et al. 2020, pp.1-5). Culturing of *S. pyogenes* in the laboratory is undertaken using specified media. Growth of streptococci preferably uses an agar media supplemented with blood, however, other media like Oxoid® blood-

agar base, Bacto heart infusion broth and Todd-Hewitt broth can also be used to grow *S. pyogenes* (Spellerberg & Brandt 2016, p. 2; Williams 1958, p. 153; Roobthaisong et al. 2017, p. 3/22).

Morphologically the appearance of cultured *S. pyogenes* colonies after 24 hours of incubation at 35-37°C is small dome-shaped with a smooth or moist surface and clear margins. They display a white-greyish colour and have a diameter of > 0.5 mm. When cultured in agar supplemented with blood, *S. pyogenes* colonies appear surrounded by a zone of β-haemolysis that is often two to four times as large as the colony diameter (Spellerberg & Brandt 2016, p. 3), however, *S. pyogenes* colonies grown on Todd Hewitt agar do not have the β-haemolysis zone. THB (CMO 189) contains 10.0 g/L of infusion from 450 g fat free minced meat, 20.0 g/L of tryptone, 2.0 g/L each of glucose, sodium bicarbonate and sodium chloride, 0.4 g/L disodium phosphate and a pH 7.8 ± 0.2 at 25^{o}C. These ingredients and conditions favour the growth of β-haemolytic bacteria which includes *S. pyogenes* (Oxoid Australia 2020a). Moreover, BA (LP0011) which is added to the THB to make culture plates has a low mineral content, which enables free diffusion of antimicrobial substances: it also has a very high working gel strength (Oxoid Australia 2020b). For successful growth of *S. pyogenes* colonies, incubation temperature of 36^{o}C or 37^{o}C is recommended.

When treated appropriately with antibiotics, GAS is communicable for 24–48 hours. Communicability can last for 10–21 days in untreated cases that are uncomplicated. Additionally, communicability can be prolonged in untreated complicated cases (Vincent, Celestin & Hussain 2004, p. 1466). Invasive group A streptococcal infections occur when a person's immunity is unable to fight the bacteria (Olp, Chamales & Schmiedecke 2020, p. e3; Zhang et al. 2017 p. 3; Olajuyigbe et al. 2018, p. 435). Additionally, sores or other breaks in the skin may allow the bacteria to get into the tissue (Stevens & Bryant 2016, p. 6; Adebanjo et al. 2018, p. 1785).

GAS causes severe invasive and sometimes irreversible infections like rheumatic fever or rheumatic heart disease, necrotising fasciitis, post streptococcal glomerulonephritis and mild superficial infections like pharyngitis, strep throat and scarlet fever (Efstratiou & Lamagni 2017, pp. 9-11; WHO 2005, p. 1; Rivera-Hernandez et al. 2020, pp. 1-2). There are certain strains of GAS that are more likely to cause severe disease than others (Bisno, Brito & Collins 2003, p. 192). The reason some strains cause more severe illness is not totally clear, however, it's believed that this is related to their ability to produce substances (toxins) that cause shock and organ damage or enzymes that cause tissue destruction (Stevens 1992, pp. 2-3; Department of Health New York 2020).

1.2 GAS worldwide

Group A *Streptococcus* bacteria are of interest because of their ability to cause a wide range of infections that are associated with high levels of morbidity and mortality worldwide. These infections have remained endemic in some communities and have also been reported to re-emerge in communities where they had earlier been eradicated.

The systematic review (below), has been submitted for consideration for publication. The review identified population groups at most risk of contracting GAS infections, the common areas where the infections are likely to spread faster, the risk factors associated with GAS transmission and available prevention and control strategies for GAS infections worldwide. This research identified hand hygiene as one of the key public and environmental health prevention measures for GAS transmission. This informed the study design regarding community GAS transmission prevention among the Indigenous populations in Australia, where GAS infections are most prevalent (Francis et al. 2019, p. 288; May, Bowen & Carapetis 2016, p. 201). The results from this study are presented below.

1.2.1 A review of group A streptococcus: Risk factors, prevention and control.

1.2.1.1 Abstract:

Background: Group A streptococcus (GAS), or *Streptococcus pyogenes,* is a pathogen of public health significance, infecting 18.1 million people worldwide and resulting in 500,000 deaths each year. It is commonly transmitted via respiratory droplets, touching skin sores caused by GAS or through contact with contaminated material or equipment. Foodborne transmission is also possible, although there is need for further research to quantify this route of infection.

Methods: This systematic literature review examined published articles on the risk factors and the prevention and control strategies for mitigating GAS infections based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement reporting guidelines for systematic literature reviews. Scopus and Web of Science databases were used to find required articles.

Results: It was demonstrated that GAS is highly prevalent in developing countries and among Indigenous populations in developed countries. Children aged 5-15 years and the elderly were at greatest risk of GAS infection, with transmission rates being higher in schools, kindergartens, hospitals and residential care homes. This was attributed to overcrowding and the higher level of social contact in these settings.

Conclusion: This review demonstrated that GAS infections are responsible for high disease burdens worldwide. Prevention and control measures should target improvement of living conditions and personal and hand hygiene. Adherence to infection prevention and control practices, which includes proper use of personal protective equipment, constant disinfection of shared equipment and proper waste disposal should be emphasised in high risk settings. Resource distribution by governments especially in developed countries should also be considered.

Keywords: group a step; group A streptococcus; Strep A; GAS; Streptococcus pyogenes; S. pyogenes; Group A streptococci AND control; prevention; public health; infection control; intervention*; management; risk factor*; risk*

1.2.1.2 Background

Streptococcus pyogenes, or group A streptococcus, (GAS), is a gram-positive bacterial pathogen commonly found in the throat and skin of humans (Cummins et al. 2012; Banigo et al. 2018; You et al. 2018). GAS has been found to persist for weeks or months in the carrier state in the anus, vagina, pharynx and skin of human hosts without causing disease (Rasi & Pourheydari 2009; Mahida et al. 2018). However, in postpartum and menopausal women, GAS is not considered a vaginal flora and its presence should be investigated immediately (Iwata & Iwase 2017; Olp, Chamales & Schmiedecke 2020). GAS can opportunistically exploit weakened immune systems to cause diseases that range from localised and mild to systemic and severe (World Health Organization 2005; Hupp, Kallstrom & Myers 2018; Oliver et al. 2019a; Olp, Chamales & Schmiedecke 2020; Thielemans et al. 2020). Mild GAS infections include pharyngitis, strep throat and scarlet fever (WHO 2005; Efstratiou & Lamagni 2017; Rivera-Hernandez et al. 2020; Worthing et al. 2020) whereas invasive GAS infections (iGAS) include rheumatic fever, necrotising fasciitis and post streptococcal glomerulonephritis (Aziz & Kotb 2008; Xie et al. 2010; Olp, Chamales & Schmiedecke 2020; Worthing et al. 2020). Approximately 18.1 million people currently suffer from a serious GAS disease with 1.78 million new cases and 500,000 deaths occurring each year (WHO 2005; Walker et al. 2014; Turner et al. 2017; Tyrrell et al. 2018; Barth et al. 2019).

During the 20th century there was a decrease in the incidence of GAS diseases in developed countries largely as a result of improved living conditions (WHO 2005; Ralph & Carapetis 2012; Kumar et al. 2014; Wu et al. 2016). However, genetic changes in circulating GAS strains and/or changes in host susceptibility to infection can lead to dramatic increases in the rates of specific diseases (Gendron et al. 2014; Boie et al. 2015; Ikebe et al. 2015; Tagini et al. 2017; You et al. 2018). No situations exemplify this more than the global upsurge of invasive GAS disease that originated in the 1980s, and the regional increases in scarlet fever in North-East Asia and the United Kingdom (Nelson et al. 2016; Zhang et al. 2017). In each case, increased disease rates have been associated with the emergence of new GAS strains with increased disease-causing capability. Epidemiological studies also show re-emergence of these diseases in developed countries. Studies from the United States indicated that iGAS infection rates from 2005 to 2012 remained steady with 3.8 cases per 100,000 persons and resulting in 1116 deaths per year (Nelson et al. 2016). In 2015, the United States reported >15000 cases of iGAS and 1600 deaths (Mosites et al. 2018). GAS cases have also been reported to have increased over time in Canada. The incidence rate for GAS infections in Canada in 2015 was 5.24 cases per 100,000 persons up from 2.4 cases per 100,000

persons in 2003 (Tyrrell et al. 2018). In the United Kingdom, the incidence rate of GAS is reported to be 2.9 cases per 100,000 persons per year (Watts et al. 2019). Outbreaks of GAS infections have been reported from community settings, institutions and within households (Sivagnanam et al. 2015).

Globally, GAS infections are not a public health notifiable disease except in a few countries like England, Wales, Japan, Canada, Norway, China and the United States although only specific GAS infections are reported (Yang et al. 2013; Ikebe et al. 2015; Oppegaard, Mylvaganam & Kittang 2015; Sivagnanam et al. 2015; Tyrrell et al. 2018; CDC 2019). In some countries it's only notifiable in some states. In Australia for example GAS is notifiable in the Northern Territory and Queensland only (Francis et al. 2019). In countries where GAS infections are notifiable, specific conditions are notifiable while others are not (Chen et al. 2017; Banigo et al. 2018; Mosites et al. 2018).

GAS infections are diseases of poverty (Carapetis et al. 2005; May, Bowen & Carapetis 2016; Barnett, Bowen & Carapetis 2019). They remain a significant problem in developing countries and the disadvantaged populations within developed countries where household crowding and social disadvantage exists (Francis et al. 2019). These communities report higher numbers of cases when compared with the number of cases in non-Indigenous populations. For example, the incidence rate of iGAS in Alaska in the United States in 2015 was 12.3 cases per 100,000 persons, more than twice that of the rest of the United States. In Canada, the incidence rate in Alberta where the Indigenous population live was 10.24 cases per 100,000 persons compared with 5.28 cases per 100,000 persons nationally (Tyrrell et al. 2018). In New Zealand, the incidence of GAS infections among Indigenous populations is 10-20 times higher than that of non-Indigenous populations (Chen et al. 2017).

The aim of this review was to examine population risk factors for GAS and the information regarding the success of different prevention and control strategies. This information will provide targeted advice to prevent the spread of GAS and reduce morbidity and mortality rates as a consequence.

1.2.1.3 Methods

The databases Scopus (*n* = 732) and Web of Science (*n* = 780) were searched for articles written in English over the last ten years with the keywords; group a step; group A streptococcus; Strep A; GAS; Streptococcus pyogenes; S. pyogenes; Group A streptococci AND control; prevention; public health; infection control; intervention*; management; risk factor*; risk*. Figure 1.1 presents the systematic approach to inclusion or exclusion. Articles were screened by reading titles and abstracts and initially excluded if they did not refer specifically to group A streptococcus or *Streptococcus pyogenes* or if they were review articles or not written in English. Further screening was done by reading the text in full to exclude articles that did not describe either at risk groups for the spread of these infections, risk factors for the spread of these infections or the prevention and control strategies

of these infections. Any studies that focused on laboratory isolation of different GAS species only and a treatment regimen for GAS infections were also excluded. Since the aim of this study was to provide overall knowledge on GAS infections, risk factors for their spread and prevention and control strategies, all studies that met the inclusion criteria were included, regardless of any perceived faults in the study design.

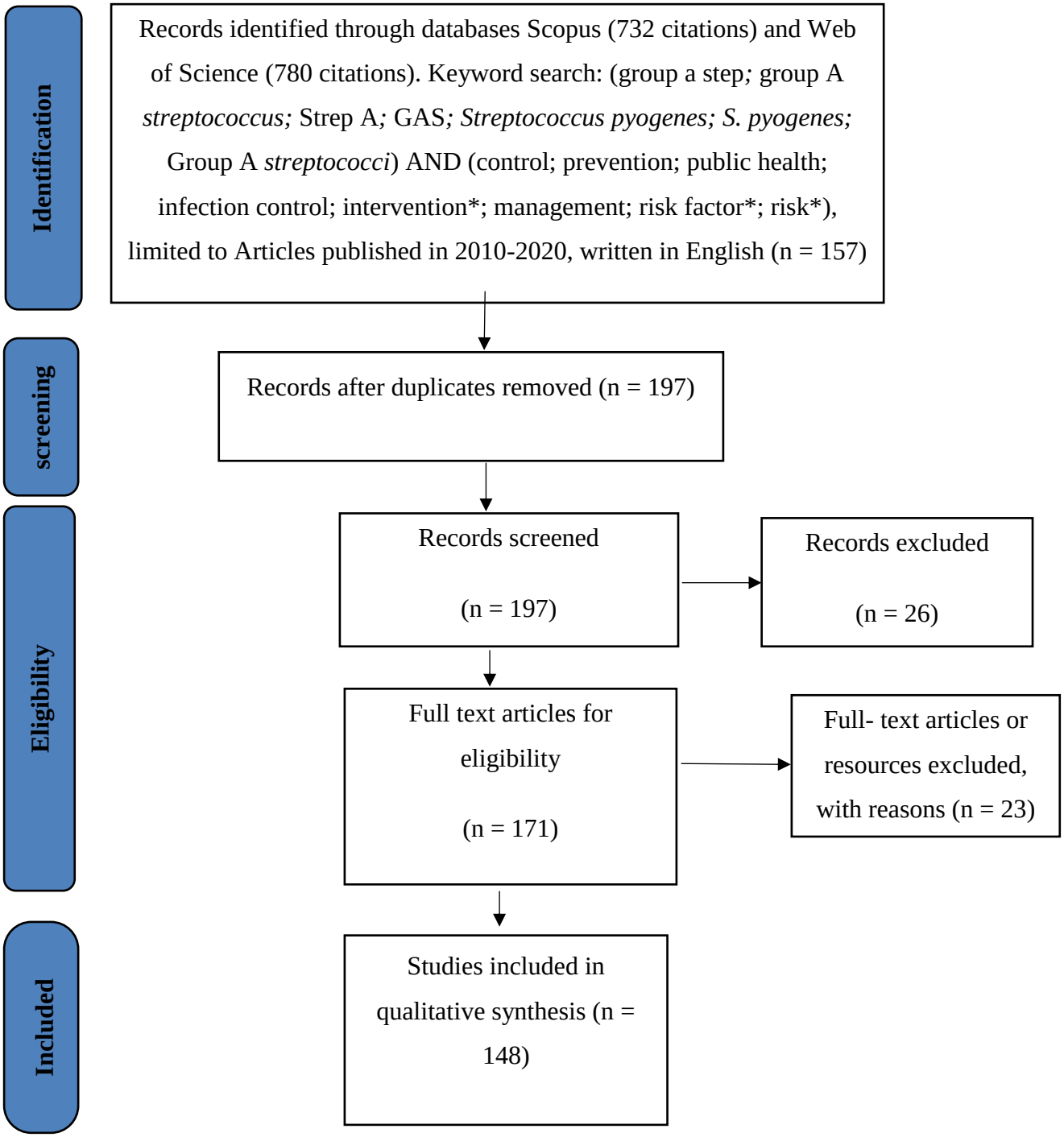


Figure 1. 1: Overview of search methods and articles' inclusion and exclusion criteria

1.2.1.4 Results

After the inclusion and exclusion criteria were applied a total of 148 articles were included in this review. The key findings of these articles relating to GAS risk factors, routes of transmission and the prevention and control strategies are presented in Table 1.1.

Areas of prevalence

Group A streptococcus (GAS) infections were reported to be common in developing countries as seen in 15% of the reviewed studies and among the Indigenous populations in developed countries as reported by 20% of all studies reviewed. Specifically, GAS infections are reported to be endemic in low socioeconomic communities (Shetty, Mill & Eggleton 2014; Oliver et al. 2017; Ralph et al. 2019; Thielemans et al. 2020).

Mode of transmission

The mode of transmission for GAS infections was reported to be respiratory droplets and direct contact with infected people and surfaces as seen in 98% of all studies reviewed. However, a few studies demonstrated that transmission could also be foodborne through contaminated food (Kemble et al. 2013; Liu et al. 2014; Chen et al. 2017).

At risk groups

The population groups at most risk of being infected with GAS infections included people with underlying medical conditions (15%) of reviewed studies, school going children of 5-15 years (11%) and the elderly (10%). Additionally, pregnant women, women with postpartum status and neonates (9%) were also identified as groups at risk for GAS infections. When gender was considered, boys and men were reported to be more at risk of getting infected by GAS infections compared with girls and women (Yang et al. 2013; Langley et al. 2016; Lee, Cowling & Lau 2017; Liu et al. 2018; Tyrrell et al. 2018).

Common environments of exposure

The common environments for GAS infections included schools, nurseries and kindergartens (8%), hospitals (6%), homeless shelter environments (6%), care homes (4%) and military training facilities (1%) of reviewed studies.

Risk factors

Seven percent of the reviewed studies identified overcrowding as a major risk factor for the spread of GAS infections. Housing conditions such as dampness, poor ventilation and house temperature were also responsible for the transmission of GAS infections (3%). Contaminated surroundings and

surfaces (6%) and low socioeconomic status (Bennett et al. 2019; Cannon et al. 2019) also favoured the transmission of GAS infections

Other factors highlighted in the review that propagated the spread of GAS infections, especially in hospitals and care homes, included poor infection control practices (15%), cross infection by health care workers and other patients or residents (9%), contaminated hospital equipment (3%) and contaminated devices like intra uterine devices, catheters and penile constriction rings among others (Baker et al. 2019; Krughoff et al. 2020). Additionally, poor personal and hand hygiene (8%), exposure to asymptomatic persons or asymptomatic status due to previous GAS infection (Abd El-Ghany et al. 2015; Kobayashi et al. 2016; Lev-Sagie et al. 2017; Mearkle et al. 2017; Yokchoo et al. 2019), a high number of social contacts (Torres et al. 2016; Chen et al. 2017; Baker et al. 2019), limited household resources including sharing of personal items (Zhang et al. 2017; Adebanjo et al. 2018; Baker et al. 2019; Dauby et al. 2019) were also highlighted to favour the transmission of GAS infections. This review also identified knowledge gaps on diagnosis and treatment guidelines among medical practitioners (Mathan, Erkart & Houlding 2017; Knoderer et al. 2019; Di Muzio et al. 2020), change of host immunity due to conditions like pregnancy or underlying medical conditions (Boie et al. 2015; Oliver et al. 2019a; Olp, Chamales & Schmiedecke 2020; Thielemans et al. 2020), environmental tobacco smoke exposure (Marshall et al. 2015; Bennett et al. 2019), exposure to biting insects and skin injuries or diseases (Sivagnanam et al. 2015; Adebanjo et al. 2018; Chadha et al. 2018; Baker et al. 2019; Francis et al. 2019) as risk factors for GAS transmission. Poor wound care (10%) long term exposure to severe air pollutants and mutating or diverse gene types of GAS (6%) and inability to pay for quality health care services (Tartof et al. 2010; Allen et al. 2011; Torres et al. 2016; Adebanjo et al. 2018; Francis et al. 2019) were also noted as factors that made people vulnerable and susceptible. Seasonal variations (Oppegaard, Mylvaganam & Kittang 2015; Tyrrell et al. 2018; You et al. 2018; Barth et al. 2019; Oliver et al. 2019a), poor treatment success due to poor health seeking behaviour and antibiotic resistance (Lin et al. 2011; Abd El-Ghany et al. 2015; Siemens et al. 2016; Mathan, Erkart & Houlding 2017; Shakoor et al. 2017; Hammond-Collins et al. 2019) also favour the spread while intravenous drug injection and use of alcohol were also mentioned as risk factors (Adebanjo et al. 2018; Mosites et al. 2018; Tyrrell et al. 2018; Oliver et al. 2019a). However, one study contradicted this finding (Bundle et al. 2017).

Prevention and control strategies

In addressing prevention and control strategies for GAS infections, many studies (26%) suggested that early diagnosis and treatment was an effective way of prevention and control. Fifteen percent of the reviewed studies recommended effective use of infection control procedures in hospitals and care homes as preventive measures. Additionally, 9% suggested screening of asymptomatic cases and post exposure prophylaxis of vulnerable groups as a control measure. Occasional screening of health workers for GAS infections (7%) was also mentioned as a measure to prevent the spread.

Improved surveillance and epidemiological investigation were also emphasised (13%) as preventive measures for GAS infections. Four percent of the reviewed articles advocated for improved access to health care services as a control measure for GAS infections. Furthermore, health education at hospital and community levels (9%) including capacity building of health care workers on proper diagnosis and management of GAS infections (6%) were also advocated as preventive measures. Improved quality of housing was mentioned in 3% of the articles as a control measure for the infections.

Other prevention and control measures for these infections included environmental sanitation (Cummins et al. 2012; Zhang et al. 2017; Dickinson et al. 2019; Wang et al. 2019), reduced or no overcrowding (Shetty et al. 2014; Cannon et al. 2019; Wang et al. 2019), improved personal hygiene (Lee, Cowling & Lau 2017; Adebanjo et al. 2018; Dauby et al. 2019), proper handwashing (Sosa 2016; Zhang et al. 2017; Dickinson et al. 2019; Francis et al. 2019; Hammond-Collins et al. 2019) and avoiding sharing of personal items (Zhang et al. 2017; Adebanjo et al. 2018).

According to Seale et al. (2016) and Zhang et al. (2017), reduced malnutrition rates and reduced Human Immunodeficiency Virus (HIV) infections were considered preventive measures for the spread of GAS infections. Reduced movement of children to new areas (Cannon et al. 2019; Francis et al. 2019), encouraging physical activity for children (Liu et al. 2015; Zhang et al. 2017), sustainable skin disease control programs (May, Bowen & Carapetis 2016; Dauby et al. 2019) and early and improved treatment of skin infections and burns (Anderson et al. 2016; Seale et al. 2016; Ahmed et al. 2018; Francis et al. 2019) also helped to prevent or reduce the transmission.

For pregnant mothers and neonates, use of sepsis measures at delivery and antiseptic neonatal cord care (Seale et al. 2016) were suggested to be important measures to put in place to prevent GAS infections.

Improved microbial detection methods for GAS was also highlighted by 10% of reviewed articles as an efficient way to identify these bacteria and hence provide better management of infected persons, which is a way of reducing disease transmission.

Other methods that were proposed to reduce GAS transmission but are currently not used included vaccination (8%) and screening for GAS during pregnancy (Hamilton et al. 2013; Shinar et al. 2016). Currently, there is no licenced vaccine for GAS infections (Seale et al. 2016; Chochua et al. 2017; Makthal et al. 2017), moreover, there is no standard protocol in place to follow when screening for GAS in pregnancy (Hamilton et al. 2013).

1.2.1.5 Discussion

Areas of prevalence

The burden of GAS diseases and the association of these diseases with poverty cannot be ignored. Despite being in existence for hundreds of years, GAS still causes a substantial burden of disease and death on a global scale, mainly in children and young adults in less developed countries (Steer et al. 2016; Bono-Neri 2017; Barth et al. 2019; Oliver et al. 2019a; Ralph et al. 2019; Di Muzio et al. 2020). In low and middle income countries and in disadvantaged populations in high income countries like the United States and Australia, GAS infections have remained endemic over time (Harris et al. 2011; Mosites et al. 2018; Francis et al. 2019; Nakauyaca et al. 2019; Palladino et al. 2019). GAS infections also remain relatively important diseases in more developed countries. According to this review, the high diversity of GAS genotypes has led to the persistence of these infections globally (Ralph & Carapetis 2012; Ikebe et al. 2015; Tagini et al. 2017; Tyrrell et al. 2018; You et al. 2018). Strain variations also exist for GAS between developed and developing countries. Low income settings report high GAS strain diversity compared with high income settings (de Almeida Torres et al. 2013; Karaky et al. 2014; Barth et al. 2019). The reason as to why this is so is not clear (Steer et al. 2009), although Tarof et al. (2010) argue that local factors, such as crowding, may enhance the frequency of GAS transmission and horizontal gene transfers that contribute to increased strain diversity in such settings. A study carried out in South Africa showed that there were similarities of GAS strains when compared with the ones in Tunisia and Kenya but were different when compared with those of developed countries (Barth et al. 2019). Similarities in strain diversity were also reported in settings with similar living conditions which include Indigenous populations in developed countries, Africa and the Pacific region (de Almeida Torres et al. 2013; Mosites et al. 2018). This review also found that socially disadvantaged communities are heavily burdened by these infections due to their low socioeconomic status characterised by poor housing conditions and inability to afford medical care, among other factors (Ralph & Carapetis 2012; Kumar et al. 2014; Shetty, Mills & Eggleton 2014; Gray et al. 2017; Mathan, Erkart & Houlding 2017; Oliver et al. 2017; Francis et al. 2019). According to a study from the United Kingdom, interviews conducted with the patients, teachers and parents of children affected with rheumatic fever revealed that low socioeconomic status was common among most of the respondents (Cannon et al. 2019). Furthermore, poor living conditions and inability to afford medical care, evidence of low socioeconomic status (Steer et al. 2016), were reported by these respondents. In Australia, records indicate that GAS infections and their sequalae among the Indigenous Australians continue to persist at equal or higher levels when compared with cases in developing countries (May, Bowen & Carapetis 2016). Tropical regions of Northern Queensland and the Northern Territory, the only states that report GAS infections as notifiable diseases, have the highest reported rates, with incidence in Indigenous populations ranging from 23·9 to 82·5 cases per 100,000 persons and non-Indigenous from 4·7 to 10·3 cases per 100,000 persons. This has been linked to the higher proportion of Indigenous people in northern Australia who experience high levels of socioeconomic disadvantage and household overcrowding (Boyd et al. 2016). In Alaska in the United States, a region where

Indigenous Americans live, the incidence rate of GAS infections in 2015 was 12.3 cases per 100,000 persons, a rate that was more than twice that of the rest of the United States (Mosites et al. 2018).

Transmission

GAS is responsible for causing a range of infections which include rheumatic fever, necrotising fasciitis, post streptococcal glomerulonephritis, pharyngitis, strep throat and scarlet fever (Cunningham 2000; WHO 2005; Efstratiou & Lamagni 2017; Barth et al. 2019; Rivera-Hernandez et al. 2020; Worthing et al. 2020). The common symptoms for these infections include high fever, severe muscle aches, localised muscle tenderness and sometimes redness at the site of a wound (WHO 2005; Bono-Neri 2017; Worthing et al. 2020). The incubation period for these infections is 1-3 days after exposure (Vincent, Celestin & Hussain 2004).

Humans are the only natural reservoir for GAS (Ralph & Carapetis 2012; Qing-Zeng et al. 2013), although animals like cows can also be reservoirs for some GAS species. When infected by humans, they become intermediate hosts and pass on the bacterium in milk which if consumed unpasteurised can infect other humans (McDaniel et al. 2014).

Findings of this review showed that transmission of GAS is majorly through respiratory droplets or skin contact with broken skin that has secretions from infected sores on the skin (Al-ajmi et al. 2012; Beaudoin et al. 2014; Walker et al. 2014; Sosa 2016; Steer et al. 2016; Efstratiou & Lamagni 2017; Zhang et al. 2017; Sharma et al. 2019; Olp, Chamales & Schmiedecke 2020). The environment is also a potential reservoir and facilitates transmission through contaminated equipment, surfaces, dust and fomites (Beaudoin et al. 2014; Mahida et al. 2014; Sosa 2016; Banigo et al. 2018; Mahida et al. 2018; Dickinson et al. 2019; Sharma et al. 2019), however, very few current studies have explored this area hence it is an area for further research. Foodborne GAS infections as a result of contaminated food sources also exist (Katzenell, Shemer & Bar-Dayan 2001; Kemble et al. 2013; Walker et al. 2014; Chen et al. 2017) as seen in this review. GAS has been proven to survive in ice cream (18 days), raw and pasteurised milk at 15-37 ºC (96 hours), room temperature butter (48 hours) and neutralised butter (12-17 days) (International Commission on Microbiological Specifications for Foods 1996). Additionally, GAS has been found to last several days in salads at room temperature (Katzenell, Shemer & Bar-Dayan 2001). An investigation of a GAS outbreak in China in 2014 among a film crew demonstrated that foodborne outbreaks due to GAS infections exist, although they are very rare. However, these outbreaks are always difficult to recognise at early stages and hence usually ignored by health care workers (Liu et al. 2014). From the literature reviewed, very few studies have been done with regards to foodborne GAS and therefore more research needs to be done on the same.

Common areas of infection

GAS infections are commonly spread in schools, nurseries and kindergartens, hospitals, care homes, military camps and homeless shelters (Cummins et al. 2012; Beaudoin et al. 2014; Engelthaler et al. 2016; Sosa 2016; Lee, Cowling & Lau 2017; Zhang et al. 2017; Hammond-Collins et al. 2019; Sharma et al. 2019; Watts et al. 2019; Worthing et al. 2020) as highlighted in this review. A review of epidemiological data for scarlet fever for the period 2005-2015 conducted by Zhang et al. (2017) in Hong Kong showed that the infections were higher during the months when schools were open. Additionally, more cases were reported among children who attended nurseries and kindergartens. This was attributed to low immunity in this population group and high populations in these settings (Lee, Cowling & Lau 2017; Zhang et al. 2017; You et al. 2018).

Cummins et al. (2012) review of 20 outbreaks in care homes showed high infection rates existed as a result of cross infection from infected home care residents to the healthy residents as well as infections from home care staff to residents. However, cross infection from care staff was slightly lower compared with cross infection among residents. This is supported by Dooling et al. (2013) and Deutscher et al. (2011b). Incidence of GAS in residents of long-term care facilities is also higher (3-8 fold) than among community residents of the same age (Saavedra-Campos et al. 2017; Nanduri et al. 2019).

This review identified hospitals as high-risk areas for infections (Beaudoin et al. 2014; Sosa 2016; Ahmed et al. 2018; Mahida et al. 2018; Sharma et al. 2019). This was attributed to poor surgical procedures, contaminated medical instruments or hospital environment and cross infection from other patients and healthcare workers (Beaudoin et al. 2014; Sosa 2016; Sharma et al. 2019).

According to Engelthaler et al. (2016), evidence from a study conducted on clients from homeless shelters and jails in the United States showed that conditions in these settings favoured transmission of GAS infections. A comparable study conducted in Canada also reported similar findings (Athey et al. 2016). Hammond-Collins et al. (2019) conducted a study on GAS infected cases between August 2016 to January 2018 in Belgium. The results showed incidence rates of 2333 cases per 100,000 persons in homeless groups and 25 cases per 100,000 persons in non-homeless groups showing a higher incidence (100 times higher) for homeless persons compared with non-homeless persons. An outbreak investigation in homeless shelters in Canada in 2019 supported the same findings (Dohoo et al. 2020).

At risk groups

GAS affects anyone in any population (Lanitis et al. 2012; Okuzono et al. 2018). However, GAS infections are most common in children 5-15 years old and the elderly (Giraldez-Garcia et al. 2011; Steer et al. 2016; Lee, Cowing & Lau 2017; Liu et al. 2018; Baker et al. 2019; Cannon et al. 2019; Oliver et al. 2019b; Di Muzio et al. 2020) as demonstrated in this review. Eleven percent of the total reviewed articles reported children of school going age as the most vulnerable group for these

infections. Findings of a population-based case control study undertaken in New Zealand between 2010 and 2014 showed that 79.1% of new cases were reported in children 5-17 years old and cases were rare in children 4 years and below (Baker et al. 2019). This was also reported in studies from the United Kingdom in 2013 where most cases were children 5-14 years old (Cannon et al. 2019). Additionally, a review of scarlet fever cases for the period 2005-2015 in Hong Kong revealed high incidence among children 5-15 years, although those at most risk were children 3-5 years of age. A finding that slightly contradicted the general findings of the study. However this age group includes children just entering kindergarten since the average Hong Kong kindergarten admits children from the age of two years and eight months and above (Lee, Cowling & Lau 2017). Even though any person of any age group can be an asymptomatic case of GAS (Sosa 2016; Drayß et al. 2019) most of these asymptomatic cases are children (Yokchoo et al. 2019). Asymptomatic cases are rare in young adults and the elderly (Pearson et al. 2017; Drayß et al. 2019). Asymptomatic persons act as reservoirs for these bacteria and therefore pose a great risk of transmission (Vijaya, Sathish & Tanakaram 2013; Yokchoo et al. 2019).

According to this review, the elderly population is also vulnerable to GAS infections (Lin et al. 2011; Dooling et al. 2013; Lin et al. 2013; Efstratiou & Lamagni 2017; Teatero et al. 2018). A study of the review of GAS outbreaks in Europe conducted by Cummins et al. (2012) established that 20 out of 31 outbreaks that occurred between 1992 and 2008 were related to residential care homes. Chalker et al. (2016) also showed that many cases of GAS infections were common in the elderly population especially those over 70 years. Mearkle et al. (2017) argue that there is increased risk for GAS infections among couples 75 years and older when exposed to asymptomatic cases.

People with underlying medical conditions are equally highly vulnerable to GAS infections as seen in the reviewed articles (Malota et al. 2015; Follmann, Huang & Gabriel 2016; Seale et al. 2016; Sosa 2016; Brennan & LeFevre 2019; Cannon et al. 2019; Oliver et al. 2019a; Dohoo et al. 2020; Thielemans et al. 2020). Most respondents to an interview carried out on cases, their parents, spouses and teachers of 59 cases of acute rheumatic fever, from the United Kingdom reported that they had an underlying medical condition (Cannon et al. 2019). This review also showed that previous skin conditions and recent wounds were some of the medical conditions that favoured the transmission of GAS infections (Hodgins et al. 2015; Athey et al. 2016; Adebanjo et al. 2018; Mosites et al. 2018; Tyrrell et al. 2018; Dohoo et al. 2020). Pre-existing medical conditions and co-infections like influenza, malnutrition, diabetes mellitus, HIV and malaria also expose people to GAS infections due to reduced immunity to fight infections (Lin et al. 2013; Hodgins et al. 2015; Follmann, Huang & Gabriel 2016; Seale et al. 2016; Gray et al. 2017; Linder et al. 2017). Immunocompromised persons are susceptible to GAS infections regardless of age (Laatiris et al. 2012; Malota et al. 2015; Linder et al. 2017). Sosa (2016) argues that pre-existing medical conditions in pregnancy can cause GAS infections to progress to toxic shock syndrome or necrotising fasciitis, severe types of GAS infections. This review also reported changes in host immunity especially during pregnancy as a

factor that exposed women to GAS infections (Anderson 2014, Gendron et al. 2014; Boie et al. 2015; Olp, Chamales & Schmiedecke 2020), however, GAS should not be underestimated because it can infect even healthy individuals (Lanitis et al. 2012).

From the reviewed articles it can be seen that pregnant women, women with postpartum status and neonates are also classified as vulnerable groups (Deutscher et al. 2011a; Anderson 2014; Sosa 2016; Steer et al. 2016; Mearkle et al. 2017; Kawaguchi et al. 2019; Riad et al. 2020). Results of a study carried out by Rottenstreich et al. (2019) reported that pregnant women were 20 times more at risk of GAS infections than non-pregnant women. This has been attributed to changes in host immunity due to pregnancy or postpartum status (Anderson 2014; Gendron et al. 2014; Boie et al. 2015; Olp, Chamales & Schmiedecke 2020). Studies carried out on pregnant women also reveal that GAS infections can cause abortions, still births and neonatal deaths (Deutscher et al. 2011a). Additionally, caesarean sections undertaken with contaminated medical instruments expose pregnant women to GAS infections (Sosa 2016). Lactation also reduces the availability of protective vaginal flora like lactobacillus hence increases the chances of the growth of other microorganisms like GAS (Verstraelen et al. 2011; Kawaguchi et al. 2019). Increased incidence has also been reported in infants (Steer et al. 2016; Gray et al. 2017) mainly through exposure to asymptomatic persons in the households or mother to neonate cross infection (Waddington, Snelling & Carapetis 2014; Mearkle et al. 2017).

In terms of gender, the review showed that higher incidence rates of GAS infections are reported in men compared with women (Lin et al. 2011; Yang et al. 2013; Langley et al. 2016; Efstratiou & Lamagni 2017; Liu et al. 2018; Tyrrell et al. 2018). More cases are also reported in boys compared with girls (Lee, Cowling & Lau 2017; Liu et al. 2018). According to a study carried out in China, scarlet fever cases data extracted for the period 2004-2016 showed that incidence among boys and men was 1·54 times greater than that among girls and women before the upsurge, and 1·51 times greater after the upsurge (Liu et al. 2018). Lee, Cowling and Lau (2017) attribute this high risk to more physical interactions and poorer personal hygiene among boys. In the United Kingdom however, there is an even distribution of cases across genders (Mearkle et al. 2017). Very little is known about why the incidence is higher in men than in women.

Risk factors for GAS infections

Faster transmission of GAS is stimulated by several factors. In the 20th century, most developed countries contained the spread of these infections. This was attributed to improved living conditions (Ralph & Carapetis 2012; May, Bowen & Carapetis 2016), however, this was not the case for the developing countries and the Indigenous populations in developed countries. Poor housing conditions characterised by dampness, poor ventilation and lack of temperature control, continue to exist among these communities and hence encourage the transmission of these infections (Ralph & Carapetis 2012; Liu et al. 2014; Oliver et al. 2017; Liu et al. 2018; Baker et al. 2019; Francis et al.

2019). According to a descriptive study carried out by Oliver et al. (2017) in New Zealand which involved interviewing 55 cases of acute rheumatic fever, respondents confirmed that cold, damp houses increased transmission of infection.

Overcrowding especially at household level, in military camps, in enclosed social places and other institutions, is the decisive environmental factor for the spread of GAS infections (Tartof et al. 2010; Mearkle et al. 2017; Baker et al. 2019; Bennett et al. 2019; Cannon et al. 2019). Due to overcrowding, coughing or sneezing from one infected person in the family or crowd can easily infect others (Walker et al. 2014). Since these bacteria are believed to survive on dry surfaces and materials for up to 6.5 months, there is increased likelihood of their transmission in overcrowded settings (Kramer, Schwebke & Krampf 2006).

Contamination in hospitals is also a risk factor that the review highlighted and needs to be addressed if infection at hospital level is to be prevented or controlled. This contamination ranges from shared hospital equipment (Beaudoin et al. 2014; Sosa 2016; Sharma et al. 2019), surroundings like curtains, furniture, walls and floors (Mahida et al. 2014; Winter 2014; Teatero et al. 2018; Dickinson et al. 2019) and devices or implants (Iwata & Iwase 2017; Baker et al. 2019; Olp, Chamales & Schmiedecke 2020). Contamination from health care workers due to poor infection control practices can also occur (Beaudoin et al. 2014; Ahmed et al. 2018; Dickinson et al. 2019; Nanduri et al. 2019).

Substandard infection control practices, including errors in equipment sterilisation, lack of cleaning and disinfection of shared hospital equipment, lack of proper use of personal protective equipment, poor waste management and disposal and poor wound care practices, are also major contributors to the transmission of GAS infections in hospitals (Beaudoin et al. 2014; Ahmed et al. 2018; Nanduri et al. 2019; Sharma et al. 2019). According to a study by Mahida et al. (2014) in an Ear Nose and Throat ward in 2014 in the United Kingdom, ward curtains sampled and tested for GAS during an outbreak in a hospital showed that ten out of thirty-four curtains tested positive for GAS.

This review also showed that cross infection by asymptomatic health care workers to elderly residents in care homes, especially those who work in more than one care home, is also very common (Deutscher et al. 2011b; Cummins et al. 2012; Ibrahim et al. 2016). Cross infection, by health workers colonised with GAS, to patients, was also reported to occur in hospitals (Qing-Zeng et al. 2013; Beaudoin et al. 2014).

Exposure to asymptomatic persons or cases of GAS infections can also occur at the household level (Lamden 2011; Verstraelen et al. 2011). Such exposure is high in overcrowded households as discussed earlier. In addition, limited household resources, such as those of washing and laundry, contribute to an increase in bacterial load on the skin of household members or objects in the house, resulting in increased transmission. Moreover, sharing bedding and personal items like towels is also

a predisposing factor for transmission of GAS infections (Lee, Cowling & Lau 2017; Zhang et al. 2017; Baker et al. 2019).

According to this review, high numbers of social contacts, which is a key environmental factor for GAS transmission, and very common in schools, hospitals and other enclosed social places increases the chances of transmission of GAS infections (Bono-Neri 2017; Chen et al. 2017; Baker et al. 2019). Exposure to asymptomatic persons in such settings and in overcrowded homes also increases the transmission of these infections, especially among the most vulnerable populations (Mearkle et al. 2017).

Personal hygiene and hand hygiene are key to control and prevention of communicable diseases like the common cold, diphtheria, rubella and GAS infections (Checchi 2009; Winter 2014; Dauby et al. 2019). Poor personal and hand hygiene have been proven to be risk factors for GAS infections in all age groups (Athey et al. 2016; Hancock-Allen et al. 2016; Kobayashi et al. 2016; Chen et al. 2017; Adebanjo et al. 2018; Teatero et al. 2018). However, school going children, especially boys have been reported to be highly susceptible: this has been attributed to lower hygiene standards among the boys (Lee, Cowling & Lau 2017). Poor hygiene practices, like infrequent tooth brushing, among people living in homeless shelters, also contributes to increased risk of GAS spread among these population groups (Adebanjo et al. 2018).

Inability to afford quality health care is also a driving factor for the continued existence and transmission of many infections including GAS infections. Most disadvantaged populations have very low incomes therefore are unable to afford good health insurance plans hence persistent health problems (Tartof et al. 2010; Allen et al. 2011; Adebanjo et al. 2018; Francis et al. 2019).

This review also identified broken skin as a risk factor for GAS transmission. Healthy skin provides a barrier of protection against infections (Whitehead, Smith & Nourse 2011; Anderson et al. 2016; Teatero et al. 2018). When broken, it provides a good growth environment for GAS hence high chances of infection (Siemens et al. 2016; Adebanjo et al. 2018; Baker et al. 2019; Dauby et al. 2019).

Environmental tobacco smoke exposure and long-term exposure to severe air pollutants, factors highlighted in this review, compromise the immune system thus exposing people to infections including GAS infections (Hodgins et al. 2015; Liu et al. 2018; Baker et al. 2019).

This review showed that GAS bacteria exists in more than 250 gene types and mutates rapidly. This has led to minimal cross immunity within communities hence increased risk of transmission (Lynskey, Lawrenson & Sriskandan 2011; Ikebe et al. 2015; Liu et al. 2018; Tyrrell et al. 2018). Multi drug resistance to antibiotic treatment for GAS infections, a factor that leads to treatment failure in some communities, has influenced the persistence of these infections in the affected populations

(Lynskey, Lawrenson & Sriskandan 2011; Walker et al. 2014; Lee, Crowling & Lau 2017; Brennan-Krohn, Ozonoff & Sandora 2018).

Knowledge gaps on proper diagnosis and management of GAS infections among health workers still exist (Dooling et al. 2013; Smit, Nyquist & Todd 2013; Karaky et al. 2014; Mathan, Erkart & Houlding 2017; Di Muzio et al. 2020). A study carried out in Italy in 2017-2018 on paediatricians' knowledge on the diagnosis and management of GAS infections showed that only 8% of 6160 paediatricians understood the diagnosis and treatment guidelines on GAS diagnosis and management and therefore were able to adhere to the guidelines. Only half or fewer of the questions asked were answered by almost half (40.8%) of the paediatricians (Di Muzio, d'Angelo et al. 2020).

Seasonal variation was also reported as a factor that influenced the transmission of GAS infections. High cases were seen to be reported during winter and early spring months (Yang et al. 2013; Oppegaard, Mylvaganam & Kittang 2015; Tyrrell et al. 2018; Barth et al. 2019; Oliver et al. 2019a).

GAS biofilms can also form on human tissues especially in necrotising soft tissue infections (Siemens et al. 2016). It is therefore necessary for clinicians to consider this when administering treatment to avoid further complications or treatment failure that can lead to death.

Alcohol and intravenous drug use are also risk factors for GAS infections (Athey et al. 2016; Cornick et al. 2017; Kwiatkowska et al. 2018; Teatero et al. 2018; Tyrrell et al. 2018) as has been reported in many studies involving people in homeless shelters (Teatero et al. 2018). However, not all GAS cases found in populations which inject drugs or use alcohol are directly linked to these factors. An outbreak investigation carried out in England among GAS cases who were injecting drug users, alcoholics or both, revealed that transmission of these infections was not associated with alcohol or drug use (Bundle et al. 2017), however, very few studies have explored this factor and this therefore presents an area for future research.

Prevention and control measures/strategies

Strategies aiming to prevent or treat GAS infections should be feasible, accessible and affordable especially in low resource settings (Ralph & Carapetis 2012). Prevention and control of GAS infections has been approached from public and environmental health and clinical perspectives, however, most of the intervention programs available focus more on clinical intervention and there is limited data on possible infection prevention strategies in the community (Turner et al. 2017). The available public health strategies focus on minimising transmission and protection of the people most vulnerable to GAS infections in all areas with increased potential for infection. Primary preventive strategies are also necessary since they prevent irreversible health conditions that may arise from complications due to GAS infections (Kumar et al. 2014; Nakauyaca et al. 2019): these strategies include epidemiological investigations and improved surveillance systems (Hamilton, Stevens &

Bryant 2013; Engelthaler et al. 2016; May, Bowen & Carapetis 2016; Turner et al. 2016; Zhang et al. 2017; Liu et al. 2018; Oliver et al. 2019b; Sharma et al. 2019), improved quality of housing (Tartof et al. 2010; May, Bowen & Carapetis 2016; Steer et al. 2016; Francis et al. 2019), good hand hygiene which includes regular proper hand washing with soap and water, or use of alcohol hand rub (Sosa 2016; Zhang et al. 2017; Ahmed et al. 2018; Dickinson et al. 2019; Francis et al. 2019; Hammond-Collins et al. 2019; Nanduri et al. 2019) and avoiding overcrowding (Shetty et al. 2014; Cannon et al. 2019). Improved personal hygiene is also key in controlling transmission especially in boys who tend to be more at risk than girls (Lee, Cowling & Lau 2017). Limited or no sharing of personal items like towels and even bedding should be encouraged to reduce the spread of GAS infections (Zhang et al. 2017; Dauby et al. 2019). Sharing of items that could be contaminated with saliva, such as water bottles, drinking glasses, utensils, etc. should also be avoided (Adebanjo et al. 2018).

Environmental sanitation should be maintained in all social places, schools, hospitals, residential care homes and all areas considered risky transmission zones (Cummins et al. 2012; Saavedra-Campos et al. 2017; Zhang et al. 2017; Dickinson et al. 2019). These bacteria have been reported to be susceptible to moist heat of 121^{o}C for at least 15 minutes and dry heat of 170^{o}C for at least 1 hour. In addition, the bacteria are also susceptible to 1% sodium hypochlorite, 4% formaldehyde, 2% glutaraldehyde, 70% ethanol, 70% propanol, 2% peracetic acid, 3-6% hydrogen peroxide and 16% iodine (Block 2001; Winter 2014). Therefore, disinfection and sterilisation of high touch or potentially contaminated surfaces using the specifications above can control transmission (Mahida et al. 2018; Nanduri et al. 2019). Constant disinfection and cleaning of shared equipment, especially in hospitals, should be encouraged (Dooling et al. 2013; Ahmed et al. 2018). This review also highlights decontamination and thorough cleaning of curtains and communal facilities, such as bathrooms and toilets, as key measures in prevention of infections such as GAS infections (Winter 2014; Dickinson et al. 2019). Curtains in high risk areas such as hospital settings should also be changed frequently: once a month in high risk areas and in low risk areas twice a year (Mahida et al. 2014, Winter 2014). Placing handwashing basins or hand sanitisers close to where curtains are opened or closed could help prevent cross contamination from contaminated hands (Mahida et al. 2014). Hospitals should also consider using disposable curtains or plastic screens instead of washable curtains (Mahida et al. 2014, Winter 2014).

On the other hand, medical personnel should adhere to infection control practices when handling patients in hospitals. This review emphasised proper use of personal protective equipment, aseptic management of wounds, and proper disposal of medical waste (Al-ajmi et al. 2012; Cho & Fernando 2013; Beaudoin et al. 2014; Chalker et al. 2016; Ahmed et al. 2018; Dickinson et al. 2019; Palladino et al. 2019; Sharma et al. 2019; Watts et al. 2019; Dohoo et al. 2020) as some of the infection control practices. Medical practitioners are also required to adhere to diagnosis and treatment guidelines to effectively control GAS infections (Smith et al. 2012; Mathan, Erkart & Houlding 2017; Di Muzio et al. 2020) as seen in the reviewed articles. This review also advocates for proper management of co-

infections such as influenza and diabetes mellitus (Yip et al. 2016; Saavedra-Campos et al. 2017; Hupp, Kallstrom & Myers 2018; Oliver et al. 2019a) and patients who have undergone surgical interventions such as tonsillectomy and other similar operations (Banigo et al. 2018; Hupp, Kallstrom & Myers 2018) so as to reduce risk of development of GAS infections.

Screening of health workers, asymptomatic cases including social contacts and family members of infected persons, and post exposure prophylaxis for vulnerable groups is encouraged (Hamilton, Stevens & Bryant 2013; Cohen et al. 2019; Dauby et al. 2019; Dickinson et al. 2019; Hammond-Collins et al. 2019; Nanduri et al. 2019; Oliver et al. 2019b; Rottenstreich et al. 2019). Successful screening should include both the throat and peritoneal sites (Shinar et al. 2016; Mahida et al. 2018). However, lack of official guidelines concerning the prevention of secondary disease using contact prophylaxis remains a challenge in many countries (Oliver et al. 2019b). In addition, chemoprophylaxis can sometimes be ineffective especially in controlling outbreaks due to the introduction of new strains as a result of the mutating nature of GAS (Nanduri et al. 2019). In addition, jurisdictional variation in chemoprophylaxis recommendations also exists in different countries and even states within the same country (Oliver et al. 2017; Oliver et al. 2019b).

Health education for health care providers, patients and communities is key for prevention of GAS infections (Smith et al. 2012; Shetty, Mills & Eggleton 2014; Bridges 2015, Engelthaler et al. 2016; Bono-Neri 2017; Gray et al. 2017; Mearkle et al. 2017; Dickinson et al. 2019; Hammond-Collins et al. 2019). This should include messages that encourage people to cover coughs or sneezes with a tissue or a forearm, which is effective in prevention of most infections transmitted through respiratory droplet (CDC 2009; Hammond-Collins et al. 2019). Messages on proper health seeking behaviour should also be emphasised since this also helps to reduce disease spread through treatment success (Allen et al. 2011; Shakoor et al. 2017; Hammond-Collins et al. 2019). Capacity building of health workers on GAS infections and their control, treatment guidelines and infection prevention practices, which include aseptic wound care, should be undertaken (Ralph & Carapetis 2012; Bridges 2015, Gray et al. 2017; May, Bowen & Carapetis 2016): this will help to reduce existing knowledge gaps in GAS prevention and management. Health care workers will also be able to provide targeted advice to clients and hence, break the transmission chain (Hancock-Allen et al. 2016; Ibrahim et al. 2016; Paulson et al. 2016; Brennan-Krohn, Ozonoff & Sandora 2018; Rößler et al. 2018; Di Muzio et al. 2020). After training is completed, facilities should try to avoid high staff turnover which is likely to contribute to knowledge gaps in the control and management of GAS infections (Dooling et al. 2013).

This review also advocated for strengthened sustainable programs that aim to reduce preventable medical conditions among the vulnerable populations. These programs include HIV prevention, malnutrition prevention, and skin disease prevention (Hamilton, Stevens & Bryant 2013; Liu et al.

2015; Anderson et al. 2016; Langley et al. 2016; Seale et al. 2016; Zhang et al. 2017; Adebanjo et al. 2018) among other programs.

Cannon et al. (2019) and Francis et al. (2019) argue that exchange in residential areas for the population at most risk for GAS infections, especially children, should be minimised if not avoided. Physical activity, which helps to boost immune systems should be encouraged for all populations with a special focus on children (Liu et al. 2015; Zhang et al. 2017).

People working in high risk areas or sick children are encouraged to stay at home once they are diagnosed with GAS infections until they finish their medication and are well (Lamden 2011; Al-ajmi et al. 2012; Qing-Zeng et al. 2013; Kobayashi et al. 2016). This review also highlighted isolation of patients or residents infected with GAS in hospitals or care homes as a prevention measure for further GAS transmission (Deutscher et al. 2011b; Inkster et al. 2012).

Clinical intervention, such as early accurate diagnosis and treatment as an effective preventive measure for GAS infections, was highly advocated for (Di Pierro et al. 2013; Lu et al. 2013; Hernandez & Wolk 2015; Hikone et al. 2015; Chalker et al. 2016; Chen et al. 2016; Paulson et al. 2016; Mearkle et al. 2017; Liu et al. 2018; Wang et al. 2019; Dohoo et al. 2020). The use of a recommended antibiotic regimen to treat GAS infections is believed to shorten the duration of symptoms and therefore reduces the likelihood of transmission to family members, classmates, and other close contacts. It also prevents the development of complications, including acute rheumatic fever (Cunningham 2000; Krishna et al. 2014; Nakauyaca et al. 2019). According to an outbreak investigation carried out in a military camp in Canada between December 2016 and April 2017, reluctance to seek medical care, and low compliance with antibiotics, were reported as factors that hindered treatment success which led to the increased spread of GAS infection (Hammond-Collins et al. 2019), patients are therefore advised to adhere to the treatment advice to avoid treatment failure and therefore reduce disease spread (Giraldez-Garcia et al. 2011; Abd El-Ghany et al. 2015; Mathan, Erkart & Houlding 2017; Di Pierro 2019; Hammond-Collins et al. 2019). In addition, early and improved treatment of skin infections and burns (Cunningham 2000; May, Bowen & Carapetis 2016; Adebanjo et al. 2018; Dauby et al. 2019) has been encouraged as a prevention measure.

Support of antisepsis measures during delivery and neonatal cord care (Seale et al. 2016) is also important in GAS transmission control among birthing women and neonates as seen in the reviewed articles.

This review also advocated for aggressive management of clients by health care workers, especially those with underlying medical conditions and those with medical implants, intrauterine devices or other implants (Laatiris et al. 2012; Cho & Fernando 2013; Hodgins et al. 2015; Follmann et al. 2016; Dohoo et al. 2020; Krughoff et al. 2020). Management of patients without underlying medical conditions according to set standards and guidelines should also be practised at hospital level to

prevent GAS transmission and complications as a result of GAS infections (Anderson 2014; Shetty, Mills & Eggleton 2014; Bura et al. 2017; Ahmed et al. 2018; Di Muzio et al. 2020).

Use of non-antibiotics for treatment of GAS infections was also mentioned in one of the reviewed articles as a prevention measure that could be considered (Di Pierro et al. 2013). This would reduce antibiotic resistance leading to treatment failure which is one of the factors favouring transmission of GAS infections (Lee, Cowling & Lau 2017; Brennan-Krohn, Ozonoff & Sandora 2018; Rivera-Hernandez et al. 2020), however, very few studies have been done on non-antibiotic treatment of GAS and therefore this is an area that needs to be explored further.

Improved detection methods for GAS, like whole genome sequencing enables early detection of the infections leading to effective medication hence reduced transmission rate (Fittipaldi et al. 2013; Tagini et al. 2017; Turner et al. 2017; Knoderer et al. 2019; Nanduri et al. 2019). Safe injection practices should also be encouraged among people who inject drugs (Kwiatkowska et al. 2018) as reported in this review. This will prevent and control GAS transmission rates in these populations.

Governments should ensure availability of affordable and accessible health care services to all citizens (Tartof et al. 2010; May, Bowen & Carapetis 2016; Steer et al. 2016; Oliver et al. 2017; Adebanjo et al. 2018; Francis et al. 2019) to prevent and control all infections. Equitable resource distribution is key in reducing social disadvantage which highly influences GAS transmission (Ralph & Carapetis 2012).

Currently there is no licenced vaccine for the control of GAS infections (Efstratiou & Lamagni 2017; Bi et al. 2019; Oliver et al. 2019a; Rivera-Hernandez et al. 2020), although development of a vaccine is underway (WHO 2005; Waddington, Snelling & Carapetis 2014; Seth et al. 2016; Makthal et al. 2017). This process has been hindered by factors like the availability of various unique GAS serotypes, antigenic variations within the same serotype, safety concerns and lack of consensus on clinical endpoints for establishment of proof of concept (Cunningham 2000; Walker et al. 2014; Gupta et al. 2016; Seth et al. 2016; Bi et al. 2019; Vekemans et al. 2019). Studies in this review however indicate that vaccination could help reduce these diseases and therefore highly recommend its development and use (Seale et al. 2016; Chochua et al. 2017; Makthal et al. 2017; Barth et al. 2019; Dauby et al. 2019, Oliver et al. 2019b). Rivera-Hernandez et al. (2020) suggests that vaccination can reduce antibiotic use and therefore reduced antibiotic resistance. Reducing antibiotic resistance will further reduce transmission of GAS infections through increased treatment success as discussed earlier. Studies have also proposed screening for invasive GAS during pregnancy to prevent transmission to pregnant women who are a high-risk group for GAS infections (Hamilton, Stevens & Bryant 2013; Shinar et al. 2016; Alexander et al. 2018).

GAS is an important global pathogen with diverse clinical manifestations and limited epidemiological data (Kumar et al. 2014; Turner et al. 2017; Barth et al. 2019; Worthing et al. 2020). Currently, lack

of mandatory patient notification in most countries limits the ability of public health programs to effectively target, prevent and control this condition (Ralph & Carapetis 2012; Sivagnanam et al. 2015; Oliver et al. 2019a; Thielemans et al. 2020; Worthing et al. 2020). In addition, standard guidelines on management and treatment of these infections do not exist in most countries (Oliver et al. 2019b). Most studies and countries focus more on clinical management of the infections rather than prevention at community level. Since GAS diseases are not notifiable worldwide, conducting informed public health and research initiatives aiming to reduce the impact of these conditions still remains a challenge (Barth et al. 2019; Oliver et al. 2019b). Efforts to improve reporting systems, scaling up public and environmental health interventions at community level coupled with effective treatment of cases, will help reduce the transmission rate (Sanyahumbi et al. 2016; Oliver et al. 2019a; Oliver et al. 2019b, Thielemans, Oliver et al. 2020, Worthing et al. 2020). A multi-disciplinary approach in control of GAS infections will also help reinforce infection control strategies (Bard et al. 2017; Demoré et al. 2018; Kwiatkowska et al. 2018).

1.2.1.6 Conclusion

This review identified that GAS infections are responsible for morbidity and mortality worldwide, yet the infections are still ignored, however, a higher disease burden exists in developing countries and indigenous populations in developed countries. Currently, these infections have been reported in other populations in developed countries and therefore their re-emergence needs to be addressed. Though some outbreaks have been investigated, and studies done show that GAS is transmitted through respiratory droplets and contact, future research also needs to investigate foodborne GAS infections and other environments that allow GAS survival to better understand the bacteria and reduce available transmission routes. Since the incidence is higher in school going children, this condition should be considered and addressed as children of this age are still developing and such infections can affect their education and consequently their societal development in the future creating a cycle for the disease. To break this cycle, public health community-based preventive measures need to be strengthened. Making GAS diseases notifiable at the national level would help to inform public health and research initiatives aiming to reduce the impact of this condition. Governments also need to consider greater equity in distribution of resources, which will raise living standards and hence reduce the burden of communicable diseases such as GAS infections. Moreover, action should be taken to ensure all citizens access quality health care, which is essential in reducing disease burden in communities.

Table 1. 1: Synthesis of the key findings relating to GAS risk factors, modes of transmission and the prevention and control strategies found in the articles that met the inclusion and exclusion criteria (described in Figure 1.1) and were included in this review

		REFERENCES
Common areas of prevalence	Developing countries	International Commission on Microbiological Specifications for Foods 1996; Carapetis et al. 2005; Kramer, Schwebke & Kampf 2006
	Indigenous communities in developed countries	Carapetis et al. 2005; Kramer, Schwebke & Kampf 2006; Di Pierro et al. 2013
	Socially deprived communities	Shetty, Mills & Eggleton 2014; Oliver et al. 2017
Mode of transmission	Respiratory droplets and contact with infected persons or surfaces	Al-ajmi et al. 2012; Beaudoin et al. 2014; Sosa 2016; Steer et al. 2016; Zhang et al. 2017; Sharma et al. 2019
	Contaminated food	Kemble et al. 2013; Liu et al. 2014; Chen et al. 2017
Common areas of exposure to GAS infections	Schools, nurseries and kindergartens	Lee, Cowling & Lau 2017; Zhang et al. 2017; Watts et al. 2019
	Hospitals	Sharma et al. 2019; Watts et al. 2019
	Care homes	Cummins et al. 2012; Watts et al. 2019

	Military training facilities	Chen et al. 2017; Hammond-Collins et al. 2019
	Homeless shelters	Engelthaler et al. 2016; Adebanjo et al. 2018; Mosites et al. 2018; Teatero et al. 2018; Tyrrell et al. 2018
At risk groups	Children 5-15 years old	Liu et al. 2014; Steer et al. 2016; Lee, Cowling & Lau 2017; Liu et al. 2018; Baker et al. 2019; Cannon et al. 2019; Dohoo et al. 2020
	Elderly	Cummins et al. 2012; Chalker et al. 2016; Steer et al. 2016; Mearkle et al. 2017
	People with underlying medical conditions	Seale et al. 2016; Sosa 2016, Cannon et al. 2019; Dohoo et al. 2020
	Pregnant women, women with postpartum status and neonates	Deutscher et al. 2011a; Steer et al. 2016; Mearkle et al. 2017
	Gender - Boys and men	Lee et al. 2017; Liu et al. 2018
Risk factors for GAS infections	Household crowding	Carapetis et al. 2005; Tartof et al. 2010; Mearkle et al. 2017; Oliver et al. 2017; Zhang et al. 2017; Baker et al. 2019; Bennett et al. 2019
	Housing conditions - dampness, temperature, poor ventilation	Liu et al. 2014; Oliver et al. 2017; Liu et al. 2018; Baker et al. 2019

	Hospital equipment	Beaudoin et al. 2014; Sosa 2016; Sharma et al. 2019
	Neighbourhood deprivation	Bennett et al. 2019; Cannon et al. 2019
	Cross infection by health care workers	(Cohen et al. 2019; Sharma et al. 2019)
	Contaminated devices	Iwata & Iwase 2017; Baker et al. 2019; Krughoff et al. 2020
	Number of social contacts	Torres et al. 2016; Baker et al. 2019
	Exposure to asymptomatic persons	Deutscher et al. 2011b; Lamden 2011; Mearkle et al. 2017
	Limited household resources - including those of washing, teeth cleaning and laundry	Adebanjo et al. 2018; Baker et al. 2019
	Environmental tobacco smoke exposure and long-time exposure to severe air pollutants and mutating GAS gene types	Marshall et al. 2015; Tagini et al. 2017, Liu et al. 2018; Baker et al. 2019
	Exposure to biting insects and skin injuries	Lynskey, Lawrenson & Sriskandan 2011; Whitehead, Smith & Nourse 2011; Baker et al. 2019
	Contaminated surrounding and surfaces	Cummins et al. 2012; Saavedra-Campos et al. 2017,;Mosites et al. 2018; Dickinson et al. 2019
	Seasonal variation	Wu et al. 2016; Shakoor et al. 2017; You et al. 2018; Barth et al. 2019

	Poor personal and hand hygiene	Mahida et al. 2014; Winter 2014; Lee et al. 2017; Adebanjo et al. 2018
	Co-infection with other infections like influenza	Hodgins et al. 2015; You et al. 2018; Thielemans et al. 2020
	Inability to afford quality health care	Tartof et al. 2010; Allen et al. 2011; Torres et al. 2016
Prevention and control measures/strategies	Early diagnosis and treatment	Di Pierro, et al. 2013; Lu et al. 2013; Hernandez & Wolk 2015; Chalker et al. 2016; Chen et al. 2016; Sosa 2016; Lee et al. 2017; Mearkle et al. 2017; Liu et al. 2018; Sharma et al. 2019; Vekemans et al. 2019; Dohoo et al. 2020
	Infection control in hospitals	Al-ajmi et al. 2012; Cho & Fernando 2013; Beaudoin et al. 2014; Chalker et al. 2016; Palladino et al. 2019; Sharma et al. 2019; Watts et al. 2019; Dohoo et al. 2020
	Epidemiological investigations/ improved surveillance systems	Hamilton et al. 2013; Engelthaler et al. 2016; May, Bowen & carapetis 2016; Turner et al. 2016; Zhang et al. 2017; Liu et al. 2018; Sharma et al. 2019
	Affordable and accessible health care services	Tartof et al. 2010; May, Bowen & Carapetis

		2016; Oliver et al. 2017; Rottenstreich et al. 2019
	Improved quality of housing	Tartof et al. 2010; May, Bowen & Carapetis 2016; Steer et al. 2016
	Health education for health care providers and patients/community	Shetty et al. 2014; Engelthaler et al. 2016; Mearkle et al. 2017
	Screening of health care workers, asymptomatic cases and post exposure prophylaxis for vulnerable groups	Hamilton, Stevens & Bryant 2013; Cohen et al. 2019; Rottenstreich et al. 2019
	Environmental sanitation	Cummins et al. 2012; Zhang et al. 2017
	Proper handwashing	Sosa 2016; Zhang et al. 2017
	Avoid overcrowding	Shetty, Mills & Eggleton 2014; Cannon et al. 2019)
	Reduced malnutrition and HIV infection	(Seale et al. 2016; Linder et al. 2017; Zhang et al. 2017
	Improved personal hygiene and avoid sharing of personal items	Lee, Cowling & Lau 2017; Zhang et al. 2017
	Improved microbial detection method for GAS	Rottenstreich et al. 2019; Sharma et al. 2019
	Supporting antisepsis measures at delivery and in neonatal cord care, and wound care.	Kobayashi et al. 2016; May, Bowen & Carapetis 2016; Seale et al. 2016; Hupp, Kallstrom & Myers 2018; Mosites et al. 2018

	Capacity building of health care workers.	Bura et al. 2017; Mathan, Erkart & Houlding 2017; Mosites et al. 2018; Di Muzio et al. 2020
	Reduced movement of children to new areas	Cannon et al. 2019; Francis et al. 2019
	Encourage physical activity for children	Liu et al. 2015; Zhang et al. 2017
Suggested future prevention and control strategies	Vaccination	Seale et al. 2016; Chochua et al. 2017; Makthal et al. 2017
	Screening for iGAS during pregnancy	Hamilton, Stevens & Bryant 2013; Shinar et al. 2016; Alexander et al. 2018

1.3 GAS in Australia

GAS affects remote and rural communities in developed countries like Australia. GAS infections are endemic in these populations (Cannon et al. 2019, p.5; Harris et al. 2011, p. 1220; Seth et al. 2016, p. 2955).

In Australia, records indicate that GAS infections and their sequalae among the Indigenous Australians continue to persist at equal or higher levels when compared with cases in developing countries (May, Bowen & Carapetis 2016, p. 201). Tropical regions of northern Queensland and the Northern Territory, the only areas that report GAS infections as notifiable diseases, have the highest reported rates. The incidence rate in Indigenous populations ranges from 23.9 cases per 100,000 population to 82.5 cases per 100,000 population and of 4.7 cases per 100,000 population to 10.3 cases per 100,000 population in non-Indigenous populations (Boyd et al. 2016, p. 1022). Additionally, the incidence of acute rheumatic fever, an invasive GAS infection, is estimated to be 194 cases per 100,000 people per year among Indigenous Australian children aged 5-14 years, which is one of the highest documented rates internationally (Francis et al. 2019, p. 288). This has been linked to the higher proportion of Indigenous people in northern Australia who experience high levels of socioeconomic disadvantage and household crowding (Boyd et al, 2016, pp. 1022-1023; May, Bowen & Carapetis 2016, p. 201-02). According to Baker et al. (2019, pp. 9-10) and Bennett

et al. (2019, p. 3), low socioeconomic status characterised by overcrowding and high social contact are key environmental factors for the transmission of the disease. GAS is most prevalent in children, the elderly, immunocompromised and pregnant women (Harris et al. 2011, p. 1221; Oliver et al. 2019a, pp. 5-6; Hamilton, Stevens, & Bryant, 2013, p. 875), however, the incidence of GAS infections peaks in childhood (Oliver et al. 2019a, pp. 3-4). Transmission occurs most in schools, care homes and hospitals (Vasant et al. 2019, pp. 1-2; Oliver et al. 2019b, pp. 6-7).
GAS infections are not nationally notifiable in Australia (Francis et al. 2019, p. 288). Moreover, there are no national Australian guidelines regarding the prevention of secondary iGAS disease. In addition, jurisdictional variation in chemoprophylaxis recommendations also exists across Australia (Oliver et al. 2019b, pp.6-8).

1.4 Prevention and control of GAS transmission

Control of this disease has been impacted by limited knowledge on its prevention and control among affected populations, mismanagement of patients due to lack of standard guidelines on GAS diseases management, and lack of targeted prevention strategies due to limited epidemiological data, among other factors, as seen in the systematic review (Baker et al. 2019, p. 8; May, Bowen & Carapetis 2016, p. 203).

To reduce and prevent transmission at community level, proper hand hygiene, proper infection control practices, effective cleaning of high touch surfaces, coupled with reduced overcrowding among other intervention measures should be adhered to (Zhang et al. 2017, p. 4; Inkster et al. 2012, p. 42; Dooling et al. 2013, p. 1565; Mahida et al. 2014, p. 144; Sarangi & Rowsell 1995, pp. 163-64).

Hand hygiene, when effectively used, can reduce disease transmission leading to reduced morbidity and mortality rates (Sickbert-Bennett et al. 2016, pp. 1629-30; Aiello et al. 2008, pp. 1378-79; Pittet 2005, p. 185). According to Ostrowsky and Rosenthal (2018, pp. 1, 3), hand washing is one of the most important and effective measures that can be used to control *S. pyogenes* infections. Hand hygiene can be achieved by either proper hand washing with soap and safe running water or by the use of hand sanitisers (Katz 2004, p. 460).

Ideally, hand washing with soap and water should not be substituted with the use of hand sanitisers since hand washing is able to remove soil, dirt, chemicals and grease that cannot be removed by hand rubs (Bjerke 2004, p. 296, CDC 2020b; Todd et al. 2010, p. 2137), moreover, the presence of dirt, chemicals and grease influences the effectiveness of hand sanitisers (Simonne 2005, p. 2). Additionally, hand sanitisers should not be used where there is a high infection situation or when washing is convenient, however, according to the World Health Organization (2020) there is poor compliance to basic hand washing with soap and water. This compliance rate among health care

workers does not exceed 40%. The WHO therefore recommends the use of hand sanitisers, whose compliance rate is higher than that of hand washing. Hand sanitisers have also been recommended particularly in situations where people are out and about and cannot access hand washing facilities. Hand sanitisers are safe and effective when properly used. Alcoholic compounds used as hand sanitisers kill twice the number of microorganisms compared with those removed with medicated soap in 30 seconds (Widmer 2000, p. 139). The use of hand sanitisers is therefore effective, economical and saves time when compared with normal handwashing.

This research aimed to determine the effectiveness of hand sanitisers available in a local supermarket in the destruction of GAS. The findings of this project will help public and environmental health practitioners give informed advice to patients, communities and health care workers on effective GAS prevention using hand hygiene, specifically by use of hand sanitisers. Communities will also be able to make informed decisions on which hand hygiene method they can suitably use in preventing the spread of GAS infections and other respiratory infections spread through respiratory droplets, ingestion and contact with infected persons.

1.5 Research questions, aims and objectives

1.5.1 Research questions

- Who is at most risk of getting group A *Streptococcus* infections?
- What are the risk factors associated with Group A *Streptococcus* transmission?
- How are Group A *Streptococcus* infections prevented and controlled?
- How effective are some of the available hand sanitisers in destruction of Group A *Streptococcus?*

1.5.2 Aims

To better understand Group A *Streptococcus* bacterium, its transmission risk factors and potential control strategies.

1.5.3 Objectives

To review literature on Group A *Streptococcus* using a systematic review method to determine the transmission, at risk groups, common areas of transmission risk factors for transmission and available prevention and control strategies for GAS infections.
To carry out laboratory analysis of hand sanitisers available in the market to determine their effectiveness in the destruction of Group A *Streptococcus* bacteria.

In summary, this chapter highlighted that GAS, or *S. pyogenes,* is a gram-positive non-motile and non-spore forming bacteria majorly transmitted by respiratory droplets. However, it can also be transmitted by contact with infected skins sores and contaminated food and equipment. GAS infects 18.1 million people worldwide and results in 500,000 deaths each year. In Australia, the incidence of these infections is high in Indigenous populations. These infections are prevalent in low socioeconomic areas, and children, the immunocompromised, and the elderly are at greatest risk of getting infected. High social contact and overcrowding are some of the major risk factors for the infections. The prevention and control measures for GAS infections currently in place put emphasis on clinical management rather than public and environmental health control. In order to ensure prevention and control is achieved, governments should consider making GAS infections notifiable nationally to inform public health and research initiatives. Stakeholders should also emphasise prevention and control of GAS infections using public and environmental health strategies. This includes ensuring communities and health care workers educate others and adhere to infection prevention and control practices. One of the infection control practices includes hand hygiene techniques. Hand hygiene can be achieved by hand washing with soap or by use of hand sanitisers. This study therefore aimed to review literature on GAS and determine the effectiveness of locally available hand sanitisers in the destruction of GAS bacteria using laboratory culture method.

The next chapter discusses the materials and methods used in this study in the laboratory. The materials and methods for the literature review are discussed earlier in this chapter, specifically, in 1.2.1.3.

2.0 MATERIALS AND METHODS

This chapter presents the sample collection procedures, including biosafety information and details of the samples collected for testing. It also discusses the culturing techniques used and describes the positive and negative controls for the study. The culturing methodology, which includes determination of bacterial growth, sample testing, determination of the limit of detection and minimisation of potential culture inhibitors of the study is also presented. The purpose of this study was to determine the effectiveness of locally available hand sanitisers in the destruction of GAS bacteria by assessing them in the laboratory using a standardised test approach.

2.1 Sample collection

2.1.1 Biosafety information

Permission was sought from Flinders University Institution Biosafety Committee (IBC) to work with *S. pyogenes* which is a PC2 (Physical Containment Level 2) microorganism. This is because *S. pyogenes* is transmissible by the respiratory route (WHO 2005; Zhang et al. 2017, p. 1) and working with it is a significant risk to humans (Kimman, Smit & Klein 2008, p. 409). Since the study was part of ongoing work in the Environmental Health laboratories, it was undertaken under an approval notice granted on 18th June 2020, (Approval number 2020-19) as seen in Appendix A2. Training on the recommended approach on handling PC2 microorganisms was also undertaken (Appendix A1).

2.1.2 Sample collection

Samples to be tested were obtained from a local supermarket in August 2020. This is one of the sampling techniques used in sample collection (Marshall 1996, p. 522). A total of five samples were obtained. Four samples were hand sanitisers, and one sample was a dish washing liquid that was used as a comparison sample. The details of the samples collected are as follows:

Sample 1: Name: Uniquely Natural Hand Sanitising Gel, 125 mL (made by Uniquely Natural, Unit 2-3/20 Ellemsea Circuit, Lonsdale, South Australia)
Ingredients; Alcohol Denat (alcohol denatured), purified water, hydroxypropyl guar and citric acid
Batch number and expiry date not indicated on the product

Figure 2. 1: Sample 1 hand sanitiser (Uniquely Natural Hand Sanitising Gel)

Sample 2: Name: Travel Hand sanitizer, 50 mL (Made in China, Distributed by PJ SAS Trading Pty Ltd, 70A Orange Grove Road, Liverpool, NSW 2170, Sydney, Australia; Phone (02)96025444
Ingredients: Alcohol, aqua, carbomer, DMDM hydantoin, triethanolamine
Batch number. MN0315
Expiry date: 15/03/2020

Figure 2. 2: Sample 2 hand sanitiser (Travel Hand sanitizer)

Sample 3: Dettol, 50 mL (Made in Thailand, Reckitt Bencksler, Sydney, NSW, Australia, Auckland New Zealand; Phone Aust: 1800022046; NZ 0800403030)
Ingredients: Alcohol Denat (alcohol denatured), water, PEG/PPG-17/6 copolymer, propylene glycol, acrylates/C10-30 Alkyl acrylate cross polymer, tetrahydroxy propyl ethylenediamine, fragrance and limonene
Batch number. EB90 080520
Expiry date: 800522

Figure 2. 3: Sample 3 hand sanitiser (Dettol®)

Sample 4: ZC Hand Sanitizer Gel, 50 mL (Manufacturer not indicated)

Ingredients: Ethyl alcohol 75%

Batch number. Not indicated

Expiry date: 2023-03

Figure 2. 4: Sample 4 hand sanitiser (ZC Hand sanitizer gel)

Comparison sample: Ultra Palmolive dish washing liquid, 400 mL (Made in Australia, Colgate-Palmolive Pty Ltd 345 George Street, Sydney, 2000)

Ingredients: anionic and non-ionic surfactants

Batch number: 0043AU

Expiry date:2020-11

Figure 2. 5: Dish washing liquid (Ultra Palmolive dish washing liquid)

2.2 Microbiological culture of samples

A stock sample of *S. pyogenes* was obtained from the Biology Department at Flinders University and was used to make the working culture used in the study.

2.2.1 Negative controls

Sterile water plus the hand sanitiser minus the *S. pyogenes* was cultured and incubated on Todd-Hewitt broth (THB) plus bacteriological agar (BA) as per the manufacturer's instructions (Oxoid Australia 2020a; Oxoid Australia 2020b).

2.2.2 Positive controls

S. pyogenes and sterile water minus the sanitisers was cultured and incubated as per the manufacturer's instructions (Oxoid Australia 2020a).

2.2.3 Culture methodology

All procedures were carried out using existing bacteriological culture methods (Gera & McIver 2013, pp. 1-2). In each test, a positive control, a negative control and the sample being tested were undertaken in triplicate.

2.2.3.1 Bacterial growth and viable cell count analysis

Growth rate for *S. pyogenes* was determined by estimating the number of bacteria in a culture grown at 37°C. Single *S. pyogenes* colonies from the working culture were grown overnight in 100 mL THB broth at 37°C. This was done in triplicate. The turbidity of the culture was determined by measuring

the optical density at 600 nm (OD_{600}) every 2 hours for 14 hours using the Shimadzu UV-1800 UV spectrophotometer. Maximum growth was achieved at 14 hours. One mL of the culture was put into cuvettes (40 x 4 x 45) mm and measured using the spectrophotometer to determine the optical density expressed as Absorbance Units (AU) (Oxoid Australia 2020a; Gera & McIver 2013, p. 3).

Growth curves were constructed by plotting the OD_{600} values against time. Data were expressed as the mean and standard deviation from the three independent experiments (Roobthaisong et al. 2017, p.3/22).

To calculate the number of colony forming units (CFU/mL) at a given time, the turbidity of the culture was adjusted to 0.4 AU. To adjust turbidity, the culture was centrifuged at 4,000 rpm (revolutions per minute) for five minutes. The supernatant was then discarded. Sterile water was added, and the cells resuspended again in the centrifuge until a sample of 0.4 AU was achieved. One millilitre of the neat sample was serially diluted by a factor of 10^{-1} to 10^{-10}. One hundred microlitres from each tube was plated onto sterile culture plates with THB/BA. Plating was done in triplicate for each dilution made. The plates were incubated for 24 hours after which the colonies were counted manually.

All agars and broths used were autoclaved at 121^{o}C for 15 minutes. They were then allowed to cool by placing in a water bath at 50^{o}C for 10-15 minutes before use (CABRI Consortium 2013).

2.2.3.2 Testing efficiency of the sanitiser

Sanitisers were tested according to the “BS EN 1276:2009 Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas” standards, with a number of changes. In summary, 1 mL of the (0.4 AU at OD_{600}) sample of *S. pyogenes* cells was added to 9 mL of hand sanitiser sample and mixed gently for 30 seconds. Contact time of 30-60 seconds for the sanitiser and cells was allowed as per the standards (BS EN 1276:2019). One hundred microlitres of the mixture were plated on THB/BA and spread evenly using a sterile spreader (Sanders 2012, p. 4). The same procedure was repeated with 10 µL of the mixture. The plates were sealed with parafilm® to prevent air and contaminants from getting onto them during incubation. The plates were then incubated for 20-24 hours at 37^{o}C. Colonies were counted after incubation.

2.2.4 Limit of detection

To determine the limit of detection, a set of serial dilutions of the culture sample was run. The initial concentration after culture was 0.98 AU. This was adjusted to 0.4 AU and 10 x 1:10 serial dilutions performed. The density of bacteria in the dilutions was determined by plating 100 µL of each dilution and plates with 30-300 colonies considered for counting (Matthews, Kniel & Montville 2017, p. 74). This count was expressed as CFU per mL to estimate the number of bacteria in the initial suspension.

This allowed for the limit of detection of *S. pyogenes* concentration in the broth culture to be determined.

2.2.5 Maximising cell growth and detection

To maximise detection of *S. pyogenes*, the recommended culture media (THB), which is suitable for the growth of *S. pyogenes*, anaerobic conditions and incubation temperature of 37^{o}C were maintained (Spellerberg & Brandt 2016, p. 2). This also minimised any potential inhibitors.

In summary, this chapter highlighted how biosafety clearance for the study was sought from Flinders University Biosafety Committee and biosafety training was undertaken, as presented in Appendix A1 and A2. Five samples were used in the study; four hand sanitisers and one dishwashing liquid, were purchased from a local supermarket. A stock sample of *S. pyogenes* for the study was obtained from the Biology Department at Flinders University and working samples prepared at the Environmental Health laboratory of the same institution. Broth cultures were then prepared from working samples and the effectiveness of the purchased samples on GAS destruction was tested using the prepared cultured broth as per the standards (BS EN 1276:2019).

The next chapter discusses the results of the experimental work undertaken.

3.0 RESULTS

This chapter presents the results of the tests undertaken on the hand sanitisers and dishwashing liquid, including how the growth curve of *S. pyogenes* was determined. The results showing the effectiveness on the destruction of GAS bacteria are also presented.

3.1 Growth curve determination

After the broth cultures were incubated, the readings of the OD_{600} were taken and recorded as shown in Table 3.1. A growth curve was then drawn to determine the time when the *S. pyogenes* cells growth rate was highest and when there was minimum or no growth.

Table 3. 1: Optical density of *S. pyogenes* cells against time during incubation of broth culture

Time (hours)	Optical density at 600nm (OD_{600}) expressed in Absorbance units (AU)				
	Culture 1	Culture 2	Culture 3	Mean	Standard deviation
0	0	0	0.0001	0.00003	0.00000577
2	0.005	0.004	0.005	0.005	0.00057735
4	0.08	0.01	0.072	0.054	0.03831449
6	0.39	0.199	0.394	0.326	0.11144655
8	0.815	0.446	0.73	0.664	0.19323647
10	1.018	0.887	0.988	0.964	0.06863187
12	0.962	0.956	0.918	0.945	0.02386071
14	0.99	0.969	0.982	0.980	0.01059874

The highest growth rate was achieved after 7 hours of incubation with an optical density of approximately 0.5 AU as seen in Figure 3.1.

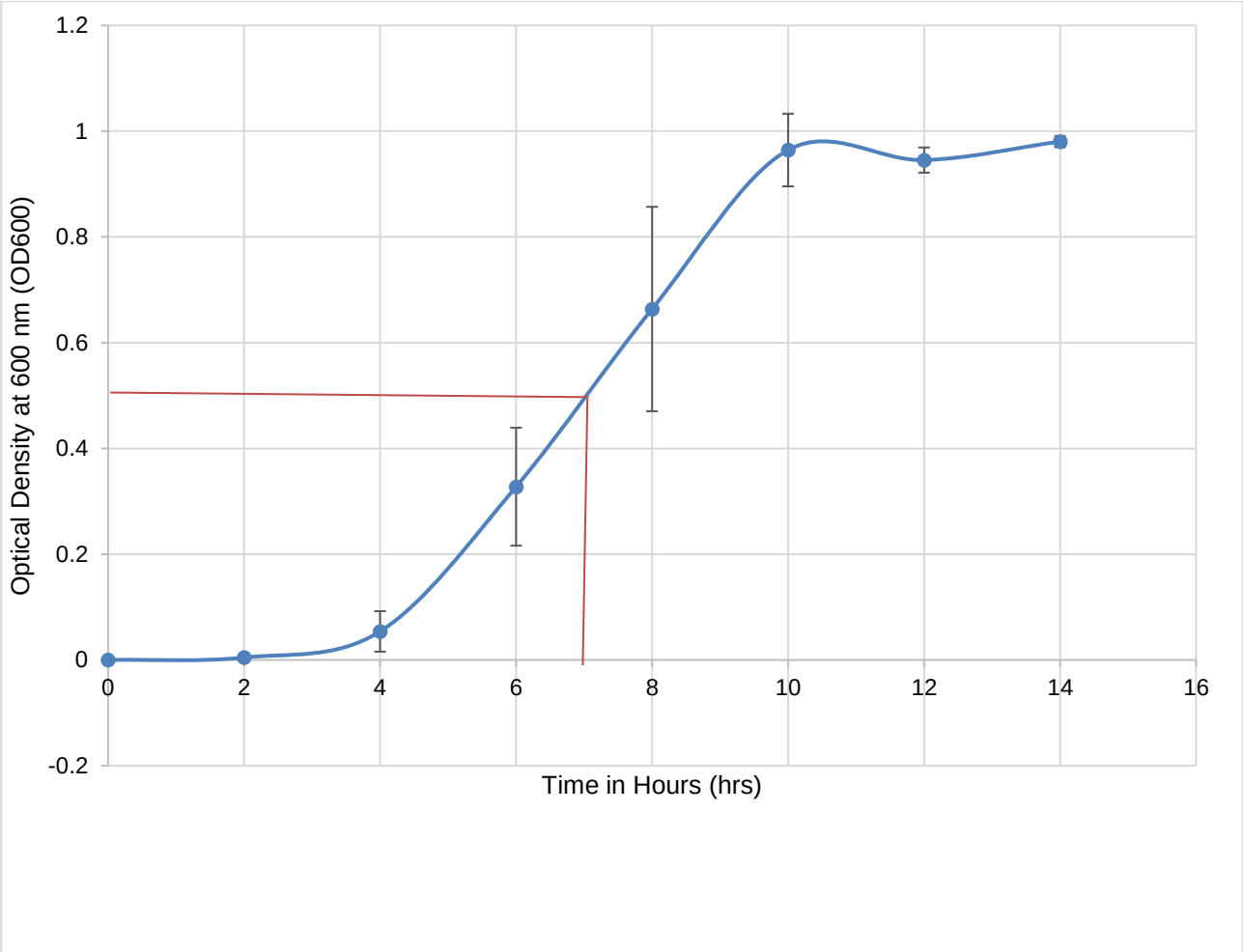
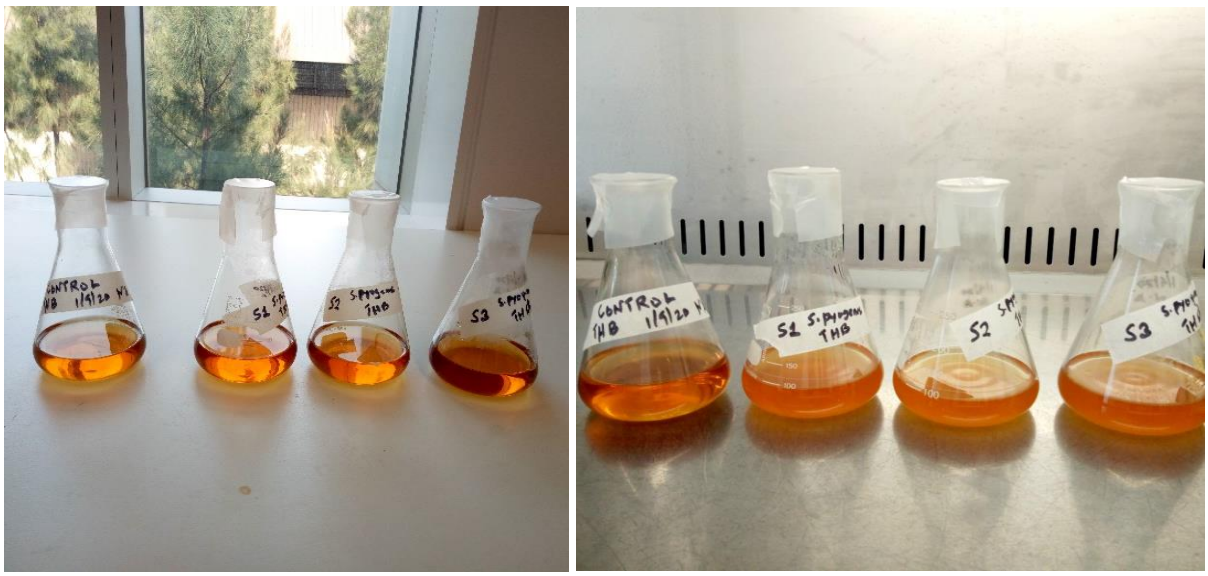


Figure 3. 1: Optical density at 600nm for the growth of *S. pyogenes* cells over time



(a) **(b)**

Figure 3. 2: Broth cultures before incubation (a) and after incubation (b). Colour change is seen in volumetric flasks labelled S1, S2 and S3. There is no observable change in the control broth

3.2 Positive controls

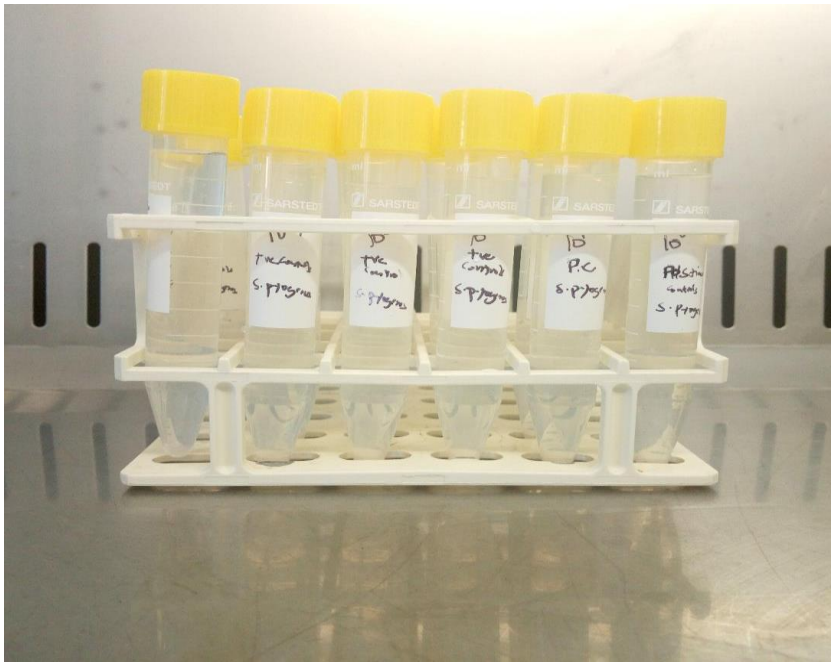


Figure 3. 3: Serial dilutions of *S. pyogenes* cells from 10^{-1} to 10^{-10}

Growth of *S. pyogenes* cells from the dilutions was done in triplicate. After 24 hours of incubation, *S pyogenes* colonies were on the plate cultures of the initial sample and those serially diluted up to 10^{-6}. No growth was present on plates cultured from the *S. pyogenes* cells serially diluted at 10^{-7} to 10^{-10}.

The results of the cultures *S. pyogenes* cells after serial dilution are summarised in Table 3.2.

Table 3. 2: Number of colonies present on the plates with THB/BA after incubation for 24 hours

Dilution	No. of colonies present on the plate (100 µL)	Concentration (cells/mL)
10^{0}	TNTC (Too numerous to count)	-
10^{-1}	TNTC	-
10^{-2}	TNTC	-
10^{-3}	TNTC	-
10^{-4}	284	2.84×10^{7}
10^{-5}	33.67	3.4×10^{7}
10^{-6}	<30	-

10^{-7}	0	0
10^{-8}	0	0
10^{-9}	0	0
10^{-10}	0	0

Note: The number of colonies is the mean and standard deviations of the total colonies counted from the three plates incubated for each dilution. Only plates with 30-300 colonies were counted. Counts outside this range were excluded because they may give erroneous indications of the actual bacterial composition of the sample. Plates with more than 300 colonies were recorded as TNTC (Too Numerous to Count) (Maturin & Peeler 2001, p. 2; O'Toole 2016, p. 3127).

After counting the colonies, the *S. pyogenes* cell concentration used for this study was 3.4 x 10^7 CFU/mL.

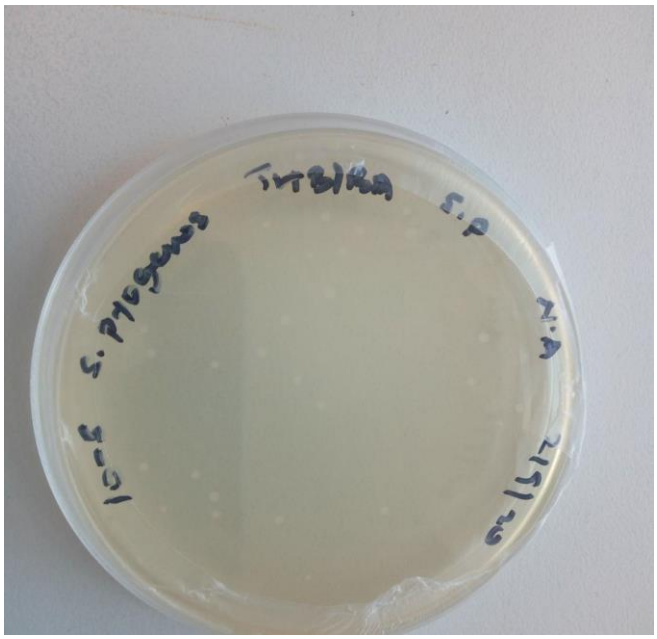


Figure 3. 4: *S. pyogenes* colonies grown on THB/BA drawn from mixture diluted at 10^{-5}

3.3 Negative controls

No growth was seen on the cultured plates.

3.4 Hand sanitiser tests

Log reduction conveys how effective a product is in reducing pathogens. The greater log reduction, the more effective the product is at killing disease-causing pathogens (BS EN 1276:2019). The log

reduction calculations were done based on the “Log and Percent Reductions in Microbiology and Antimicrobial Testing 2015” formula below.

log reduction = $\log_{10}$ (A÷B)

Or

log reduction = $\log_{10}$ (A) – $\log_{10}$ (B)

Where:

A = Number of viable microorganisms before treatment

B = Number of viable microorganisms after treatment

To determine percentage reduction;

$$\text{Percent reduction} = \frac{(A - B) \times 100}{A}$$

The log reductions for the different samples tested in this study were as follows:

Sample 1 – No growth was seen on the incubated plates with the test mixture. This means that the number of viable microorganisms after treatment was zero (0 CFU/mL). The sample’s log reduction was 7 log (log 7).

Sample 2 – The plate had TNTC number of colonies after treatment and incubation. Serial dilutions were further undertaken to determine the exact log reduction of the hand sanitiser.

Table 3. 3: The number of colonies on culture plates after serial dilutions of the mixture of sample 2 hand sanitiser and *S. pyogenes* cells

Dilutions of sample 2 (hand sanitiser) and *S. pyogenes* cells	**Concentration (cells/mL)**
10^{0}	TNTC
10^{-1}	TNTC
10^{-2}	TNTC
10^{-3}	145
10^{-4}	12
10^{-5}	1
10^{-6}	0
10^{-7}	0

10^{-8}	0
10^{-9}	0
10^{-10}	0

After serial dilution and incubation of the mixture of hand sanitiser and *S. pyogenes* cells, a log reduction of log 1 was found. Similarity of colony distribution was seen between culture plates of positive controls diluted at 10^{-4} and hand sanitiser and cells mixture culture plates diluted at 10^{-3}. These findings were calculated as shown below.

log reduction = $\log_{10}$ (A) – $\log_{10}$ (B)

$\log_{10}(2.84 \times 10^{7})$ –$\log_{10}$ (1.46×10^{6}) CFU/mL

1.38×10^{1} CFU/mL

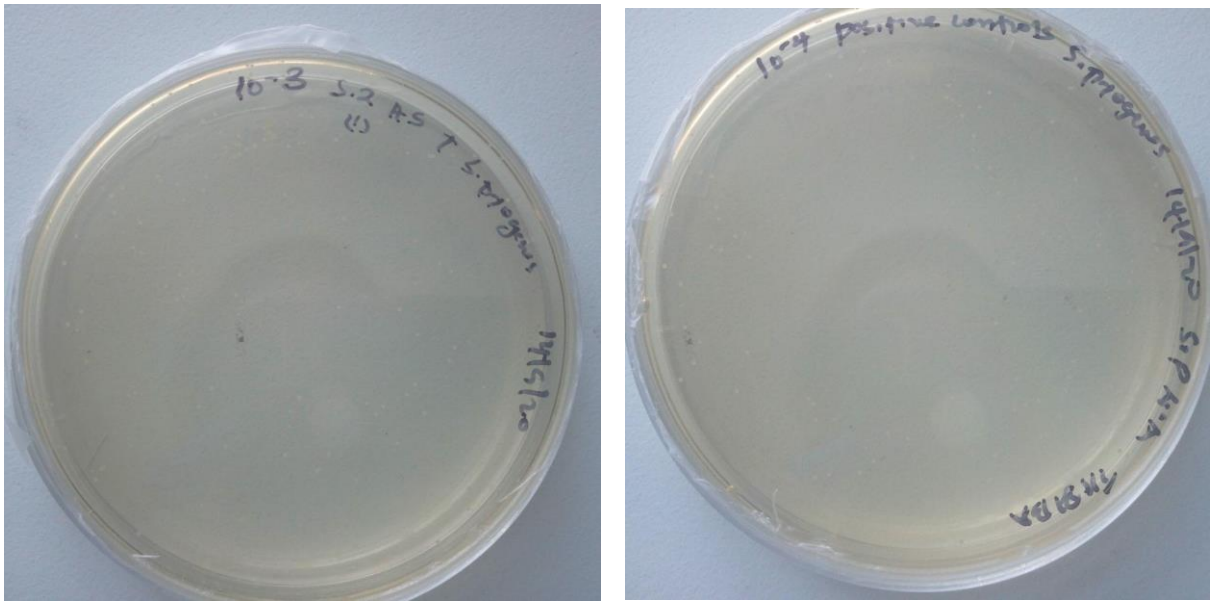


Figure 3. 5: Distribution of colonies in positive controls (10^{-4}) and sample 2 test (10^{-3})

Sample 3 and 4: After treatment and incubation of the test mixture, no growth was seen on the incubated plates. The samples recorded a log reduction of 7 log.

In the comparison sample no growth was seen on the incubated plates with the test mixture. The expected log reduction for this sample was ≥ 3 log (hand washes) as per the standards used. The sample's log reduction was 7 log.

Table 3. 4: Expected log reduction, log reduction and percentage reduction achieved by the tested samples (BS EN 1276:2019; Log and Percent Reductions in Microbiology and Antimicrobial Testing: 2015).

Sample	Expected log reduction (log)	Log reduction achieved (log)	Percentage reduction
Hand sanitisers			
1	≥ 5	7	99.99999%
2	≥ 5	1	90%
3	≥ 5	7	99.99999%
4	≥ 5	7	99.99999%
Comparison sample – Dish washing liquid			
1	≥ 3 (hand washes)	7	99.99999%

In summary, the highest growth rate of *S. pyogenes* was achieved at 7 hours after incubation. The turbidity of the cells at this time was approximately 0.5 AU. Maximum growth was achieved at 14 hours after incubation and the turbidity was 0.98 AU. This turbidity was later adjusted to 0.4 AU as required by the standards (BS EN 1276:2019), serially diluted and 100 µL incubated on culture plates to determine the cell concentration for the study, which was found to be 3.4×10^7 CFU/mL. Three out of four hand sanitisers caused a log reduction of log 7 on the test organism, *S. pyogenes*. The other hand sanitiser caused a log reduction of log 1. The dishwashing liquid which was the comparison sample also recorded a log reduction of log 7.

The next chapter discusses hand sanitisers and the results of this study in detail.

4.0 DISCUSSION

This chapter presents basic information about hand sanitisers, and the factors that affect their effectiveness in the destruction of microorganisms. It also compares alcohol-free and alcohol-based hand sanitisers, presenting their advantages and limitations. General limitations of using hand sanitisers are also discussed. The chapter concludes by highlighting the general limitations of the entire study.

In Australia, GAS infections are endemic in remote and rural communities which are mostly inhabited by Indigenous Australians (May, Bowen & Carapetis 2016, p. 201). These communities are characterised by overcrowding, high social contact and poor personal and hand hygiene, factors that favour the transmission of these infections (Boyd et al, 2016, pp. 1022-23; May, Bowen & Carapetis 2016, p. 201-02). This is discussed in detail in the systematic review above carried out for this study (Chapter 1.3).

In order to prevent and control the spread of GAS infections, several prevention and control measures including hand hygiene can be put in place as discussed earlier (Chapter 1.2.1.5). Community engagement is key to successful implementation of hand hygiene programs. Behavioural change aimed at promoting good hand hygiene practices should be encouraged and strengthened (Jumaa 2005, p. 10; Francis et al. 2019, p. 292). People need to be educated and informed about proper hand washing and proper application of hand sanitisers in order to achieve standard hand hygiene (Greenaway et al. 2018, p. 200-01). In a study carried out by Babeluk et al. (2014, pp. 4-5) on healthy volunteers in a community setting, there was reduced bacterial load on the hands of the volunteers after education in the correct use of hand rubs. This demonstrates the value of education in improving hand hygiene.

This study explored the effectiveness of the use of hand sanitisers, a hand hygiene method used in the control and prevention of GAS transmission. Hand sanitisers were purchased and tested in the laboratory to determine their effectiveness in the destruction of *S. pyogenes* bacteria as discussed in the methodology of this study (Chapter 2.0).

4.1 Prevention and control of GAS transmission using hand sanitisers

Hand sanitisers, also called hand antiseptic, or hand rubs, are foam, gel, cream, spray, wipes or liquid agents applied to the hands for the purpose of removing disease-causing organisms (Todd et al. 2010, p. 2130; WHO 2006, p. 9). Hand sanitisers are classified as either alcohol–based or alcohol-free depending on the active ingredient used. Alcohol-based products contain between 60 and 95 percent alcohol, usually in the form of ethanol, isopropanol or *n*-propanol while alcohol-

free sanitiser makes use of chemicals like quaternary ammonium and benzalkonium chloride which have antiseptic properties (Gold & Avva 2018; Jain et al. 2016, p. 429; Jing et al. 2020, p. 3). The alcohol and chemicals are important in ensuring the effectiveness of hand sanitisers in killing microorganisms.

Hand sanitisers were first used in the 1980s (Block 2001, p 21; Todd et al. 2010, p. 2129). Nowadays hand sanitisers are preferred in many circumstances because they are easy to use, can be placed at any point in a room and do not require complicated plumbing systems to manage waste like the handwashing facilities. Moreover, they have a wide antimicrobial spectrum, act rapidly, spread easily without friction which damages skin and as they evaporate rapidly they don't need drying facilities (Jumaa 2005, p.6; Widmer 2000, p. 141-42). Additionally, the hand washing process consumes more time compared to the hand sanitiser process. Handwashing takes almost double (40-60 secs) the time used to carry out hand hygiene using hand rub (20-30 secs) (WHO 2009a). According to the World Health Organization (2020), poor compliance to basic hand washing with soap and water still exists. The WHO therefore recommends the use of hand sanitisers whose compliance rate is higher than that of hand washing.

According to the findings of a study carried out by Larson et al. (2005, p. 382), compliance to hand hygiene by use of hand sanitisers among health care workers in an intensive care unit was high compared to normal hand washing. Despite the high compliance, it is important to note that hand sanitisers do not remove soil, dirt, blood, chemicals and grease, they are also less effective where the microbial load is very high (Bjerke 2004, p.296, CDC 2020b; Todd et al. 2010, p. 2137). In such situations, use of both hand hygiene methods is recommended.

Hand sanitisers work by destroying pathogens through breaking apart their cells and proteins from the inside therefore reducing their viability (Simonne 2005, p. 3; Golin, Choi & Ghahary 2020, p. 1064). Their effectiveness depends on several factors. These factors include but are not limited to, the ingredients, quantity applied, frequency of use, duration of exposure, and the susceptibility of the microorganisms present on the hand to the hand sanitiser (Liu et al. 2010a, p. 398; Suchomel et al. 2012, p. 331; Jing et al. 2020, p.17). For maximum anti-microbial effect to be achieved, sufficient quantities (2.5-3 mL or half a teaspoon) of hand sanitiser should be applied to the hands (Jing et al 2020, p. 7; Gold & Avva 2018). The application process should also be as per the recommended steps identified by WHO. The WHO (2009a) recommends eight steps to effective hand sanitiser application: application of the product in a cupped hand, equivalent to volumes described above, sufficient to cover all hand surfaces. This is followed by rubbing hands palm to palm and right palm over left dorsum with interlaced fingers and vice versa. The next step involves rubbing palm to palm with fingers interlaced then rubbing backs of

fingers to opposing palms with fingers interlocked. Rotational rubbing of left thumb clasped in right palm and vice versa is then done. Lastly, rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa is done. Once dry, the hands are safe. The entire process should take 20-30 seconds.

4.1.1 Alcohol-based versus alcohol-free hand sanitisers

Both alcohol-based and alcohol-free hand sanitisers provide the same protection against microorganisms. For alcohol-free hand sanitisers, enough quantities of the recommended chemical are required for them to be effective. Alcohol-free hand sanitisers are non-flammable and therefore ideal for use in most settings including places with high risk of fire, like hotels (Jing et al. 2020, p. 3). The low concentrations of benzalkonium, the most important ingredient of alcohol-free hand sanitisers, also make them less toxic and therefore ideal for all population groups including young children and the very elderly (Jing et al. 2020, p.3). These products have also been found to provide continued protection after the solution has dried up unlike the alcohol-based ones whose antimicrobial effect ends once the product has dried up (Gold & Avva 2018). Due to the fact that they contain no alcohol, they can be used across all communities including communities where religion restricts the use of alcohol or alcohol-based products (WHO 2009b), however, alcohol-free hand sanitisers are not widely used since they are costly compared with alcohol-based ones (Gold & Avva 2018).

Alcohol-based sanitisers on the other hand are commonly used simply because they are cost effective and easily available (Larson et al. 2005, p. 382). Alcohols have a wide antimicrobial spectrum including gram-positive bacteria, gram-negative bacteria, mycobacteria, fungi and some enveloped viruses, but poor activity against bacterial spores, oocysts and some non-enveloped viruses (Jing et al 2020, p. 3; Golin, Choi & Ghahary 2020, p. 1064). Hand hygiene with the use of hand sanitisers can therefore be recommended for use in the control of GAS infections in different communities since GAS is susceptible to alcohol.

Alcohol content in alcohol-based hand sanitisers also determines the antimicrobial effect of those products. According to Gold and Avva (2018), hand sanitisers with an alcohol content less than 60% are ineffective in killing microorganisms because they contain high water content which favours microbial growth and survival. Additionally, hand sanitisers with an alcohol content higher than 95% are equally ineffective because they don't contain water which is essential in the protein denaturing process. Alcohol-based hand sanitisers are also self-drying unlike alcohol-free hand sanitisers which do not dry on hands as effectively as alcohol-based ones. They can also be easily made or manufactured compared with alcohol-free ones (WHO 2020, pp. 1-9), nevertheless, they are highly flammable due to the high level of alcohol in them and therefore

pose a risk of fire. They are also toxic when ingested and can be easily misused by people with alcohol dependence trying to seek the desired effect (Archer et al. 2007, pp. 1154-55; WHO 2009b). According to WHO (2009b), use of alcohol-based hand sanitisers is prohibited by some religions and there can therefore be challenges in implementing their use in some communities.

Generally, use of hand sanitisers requires less time than hand washing (WHO 2009a, Larson et al. 2005, p. 382). Hand sanitisers are also gentler on skin and cause less skin irritation and dryness than frequent soap and water washes (Larson et al. 2005, p. 381; Babeluk et al. 2014, pp. 4). Moreover, they act quickly to kill microorganisms on hands. Hand sanitisers are also more accessible than sinks, and are portable, hence have been recommended for use in enclosed group settings like classrooms, staff rooms, and offices (Jumaa 2005, p. 6; Widmer 2000, p. 141-42). This promotes periodic hand hygiene with minimal movement therefore improving performance in such settings. Studies show that adequate hand hygiene can actually reduce child absenteeism from school due to a variety of infections (Willmott et al. 2016, p. 48). A study conducted by Prazuck et al. (2010, p. 997) on the relationship of compliance of primary school children to hand hygiene and absenteeism of children from school due to gastroenteritis, showed a reduction of absenteeism when hand sanitisers were used effectively. Working days lost by parents due to caring for sick children were also reduced.

4.1.2 Effectiveness of tested hand sanitiser samples and comparison sample

The process of testing hand sanitiser began by preparation of broth cultures to be used for the entire study as discussed in the methodology (Chapter 2.2.3.1). Maximum growth rate for *S. pyogenes* occurs between 14-20 hours depending on the incubation conditions (Gera & McIver 2013, p. 12; Savic & McShan 2012, p. 1429). It took 14 hours for maximum growth to be achieved in the liquid cultures in this study, a time limit within the findings of other studies. The cell concentration in the broth was then adjusted from 0.980 AU to 0.4 AU as required by the standards (BS EN 1276:2019). Sanitisers were then applied to the *S. pyogenes* cells and each culture done in triplicate. Positive and negative controls were included in the study as discussed in the methodology.

Agar plates were prepared by pouring 10-15 mL of autoclaved agar (THB/BA) on the plates and allowed to set (Sanders 2012, p. 4). The temperature of the agar was maintained at 50-70^{o}C prior to pouring (Maturin & Peeler 2001, p. 6; Sanders 2012, p. 4).

Aseptic techniques during the entire process must be adhered to for experimental success to be achieved (Garcia 2010; Gera & McIver 2013, p. 3). Aseptic techniques applied during this study

included working close to a lit bunsen burner, sterilisation of working tops with 70% alcohol before use and sterilisation of equipment before use. Moreover, the agar plates were kept closed whenever possible and the spreader and other equipment that comes into contact with cultures or media were effectively sterilised. Breathing on the plates was also avoided since breath can also be a source of contamination during plating.

For maximum growth to be achieved, the lids of the plates were sealed using parafilm® to prevent release of CO_2 during growth and protect the plates from any external contamination. Sealing the plates can also allow the culture to be placed in a non-CO_2 or ambient air incubator (Gera & McIver 2013, p. 3).

Test conditions for efficiency of hand sanitisers against *S. pyogenes* were also adhered to as required by the standards (BS EN 1276: 2019). They included maintaining contact time of 30-60 seconds and incubation temperature of 37^{o}C. Effectiveness of the tested samples was then expressed in terms of log reduction. Log reduction is the effectiveness of a disinfectant, hand sanitiser or hand washes in reducing the pathogens (Ochwoto et al. 2017, p. 2). This is a mathematical term that is used to express the relative number of living microbes that are eliminated by disinfection or sanitisation. Log reduction stands for a 10-fold (one decimal) or 90% reduction in numbers of live bacteria. When testing the effectiveness of hand sanitisers, a log reduction of 1 is low and therefore shows that the hand sanitiser's antimicrobial effect is equally low. The higher the log reduction, the more effective the hand sanitiser's antimicrobial effect. The standards require log reduction for hand rubs to be ≥5 log (BS EN 1276:2019).

The results of this study showed that three out of four samples of hand sanitiser tested recorded a log reduction of log 7. This means they met the required minimum requirements of an effective hand sanitiser and are therefore fit for use in the control of GAS transmission due to their effectiveness in the destruction of these bacteria. The other sample recorded a log reduction of log 1. This is below the required minimum requirement of ≥5 log reduction stated in the standards which means that the hand sanitiser is ineffective in killing GAS bacteria and is therefore not suitable for use in the control of GAS transmission.

From the results of this study, it is evident that most (75%) of the sample hand sanitisers tested and available in the market are effective in reducing *S. pyogenes* and could be recommended for use as a preventive measure for transmission of diseases associated with the bacteria. However, since the study involved very few samples, testing of more samples needs to be done to quantify this finding.

Results of the dishwashing liquid, the comparison sample, also showed a log reduction of log 7. Dish washing liquids are not disinfectants and have no specified standards for testing their efficiency against microbial reduction (Holah & Hall 2006, p. 533), however, most researchers compare their effectiveness with that of other hand washes when determining their log reduction rate. In this study, the log reduction of this sample was compared against that of hand washes. The standards BS EN 1276:2019 require a minimum log reduction ≥3 log for these kinds of products. The results of the dish washing sample tested showed that the dishwashing liquid was effective in killing *S. pyogenes,* this therefore means that some dish washing liquids or hand washes could be recommended for use in the prevention of GAS transmission. Again, since the study was done on a small scale more testing needs to be done to quantify these results.

4.1.3 Limitations of using hand sanitisers for hand hygiene

The use of hand sanitisers also has its own limitations. Some brands of hand sanitisers are not made as per the recommended standards and sometimes do not live up to their marketing claims (Jain et al. 2016, p. 425). In this study, sample 2 failed the test despite being marketed as being able to kill 99.9% germs. Registering a log reduction of log 1, which is equivalent to 90% microbial reduction rate, is in contradiction with the marketing claims of the 99.9% microbial reduction rate written on the product. The brand however did not state the alcohol content in the ingredients which made it difficult to conclude whether it was the alcohol content level that contributed to ineffectiveness or other factors, since GAS seems to be susceptible to alcohol as seen from the results of other sanitisers tested in this study and the available literature.

Hand sanitisers are also costly compared with detergents and other hand washes (Suchomel et al. 2012, p. 329) meaning they can only be afforded by individuals or communities that are more affluent. This cost implication can also lead to economical use of the hand sanitisers, which ends up compromising their effectiveness (Suchomel et al. 2012, p. 331; Osei-Asare et al. 2020, pp. I,6).

Since the study showed effectiveness of detergents in destruction of GAS bacteria, their use in hand hygiene targeting reduced transmission of GAS can be recommended. Detergents are often affordable, cheaper than hand sanitisers, easy to use and have both immediate and sustained activity on microorganisms compared with hand sanitisers which only have immediate activity on microorganisms (Jain et al. 2016, p. 429; Gold & Avva 2018).

4.1.4 Limitations of the study

A small sample size was used in this study. Four samples of hand sanitiser and one comparison sample only were tested due to time constraints. Further studies are therefore recommended to explore the findings of this study in greater depth.

The study was also carried out with the assumption that the test was done on clean hands with *S. pyogenes* bacteria only. Interfering substances and the neutralisation of residual microbial activity, steps in mimicking ideal situations in the laboratory (BS EN 1276: 2019) were not done. In most situations, hands carry more than one type of microorganism, and dirt, depending on different environments. When these elements are factored in the results of the effectiveness of the samples could be a different.

In summary, hand sanitisers are foam, gel, cream, spray, wipes or liquid agents applied to the hands for the purpose of removing disease-causing organisms and are classified as either alcohol free or alcohol based. They are preferentially used due to the high compliance rates by users, portability, less time consuming and less cost involved in installation. Their effectiveness depends on factors like the ingredients used in their manufacture, quantity applied, frequency of use, duration of exposure, and the susceptibility of the microorganisms present on the hand to the hand sanitiser. Both types of hand sanitiser offer the same protection to users however alcohol-based hand sanitisers are preferred since they are cheap, easily available locally and easy and cheap to manufacture. Although alcohol-free ones are costly, they are preferred in settings susceptible to fires and also best for use by children due to their low toxicity levels. From the results of this study, alcohol-based hand sanitisers (3 samples) were seen to be effective in destruction of GAS bacteria except for one which was ineffective. The comparison sample also showed effectiveness in GAS destruction. Hand sanitisers could therefore be recommended for use against transmission of GAS bacteria, however, more studies need to be undertaken to quantify this. Putting in mind that hand sanitisers can be costly and some brands fail to meet their marketing standards, as seen by the sample that was ineffective in this study, advocacy for their use should be carefully done in low socioeconomic settings where GAS infections are more prevalent.

The next chapter presents a conclusion to this study and suggestions for future work.

5.0 CONCLUSION

Hand hygiene programs are the most important infection control measure in enclosed group settings and other social places and have potentially large public health and economic implications. The design, implementation and analysis of these programs should be carried out with care and commitment. The microbial numbers on hands, even if they appear clean, cannot be improved if hand hygiene is performed incorrectly, or with an ineffective product. Hand sanitisers are not cleaning agents and are not meant to be a replacement for soap and water, but used as a complementary habit. Hand sanitisers are most effective when used in conjunction with diligent handwashing.

The results of this study demonstrate that hand sanitisers can be used to prevent GAS transmission at the community level. However, hand washing with soap and water remains the best option since it is safe, affordable and can clean soiled hands which is particularly important with children. Normally, the hands of children, who are highly susceptible to GAS infections, are soiled due to their high physical activity. Hands of the homeless population, which is also a group at most risk of getting GAS infections, also fall into this category. Therefore, hand washing is the best hand hygiene technique. Additionally, since these infections are highly prevalent in communities of low socioeconomic status, use of hand sanitisers may face challenges with proper use due to the economic cost involved and the ineffectiveness of some brands against killing GAS bacteria.

5.1 Future work

Hand sanitisers, when effectively used, have been seen to reduce disease transmission and to lower morbidity and mortality rates. The results of this study show that most hand sanitisers are effective in killing GAS pathogens and are therefore suitable for preventing transmission. However, conclusive recommendations cannot be made from the results of this study since the sample size of hand sanitisers tested was very small. Testing a wider variety of hand sanitisers needs to be done to conclusively recommend this hand hygiene technique.

Some hand sanitisers in the market do not live up to their marketing claims. As seen in this study, one sample tested failed to effectively kill *S. pyogenes* cells even though it was on the market, marketed as effective in microbial destruction and being used by consumers. This therefore demonstrates the need to carry out further investigation on the sanitisers that fail the test to determine why they are not effective. This would include measuring the alcohol content in the hand sanitiser and determining the other ingredients of the product.

High touch surfaces are also potential places where *S. pyogenes* pathogens can be picked up. It would therefore be informative to swab surfaces in communities to determine the presence and microbial numbers of GAS on such surfaces in both socially advantaged and disadvantaged communities. It will also be informative to swab surfaces before and after cleaning to determine the effectiveness of detergents or wipes on the destruction of the pathogens. Swabs can also be collected from the hands of the people at most risk to determine numbers of GAS microorganisms on human hands before and after the use of different brands of hand sanitisers to further assess their effectiveness.

Since the effectiveness of a disease control intervention measure is gauged by reduction of morbidity and mortality cases (Mathur 2011, p. 611; Sickbert-Bennett et al. 2016, pp. 1629-30; Aiello et al. 2008, pp. 1378-79; Pittet 2005, p. 185), future work involving comparison of morbidity due to GAS before and after implementation of hand hygiene using hand sanitisers or a combination of both hand hygiene techniques in populations which are more susceptible to GAS infections also needs to be urgently undertaken especially as these infections are increasing.

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APPENDICES

A1 Biosafety training certificate

Flinders
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Flinders University Institutional Biosafety Committee

Certificate of Completion

This is to certify that

Nelly Janira Avire

satisfied the

Biosafety Training requirements of Flinders University, South Australia

for working with genetically modified organisms, microbiologicals of risk group 2, and working in a physical containment facility

2020

Professor Melissa Brown
Chairperson
Institutional Biosafety Committee
Flinders University

A2 Biosafety committee approval for the project No. 2020-19

Flinders University
Institutional Biosafety Committee

Research Development & Support
Union Building

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Adelaide SA 5001

Telephone +61 8 7221 8353
ibcadmin@flinders.edu.au

23rd June 2020

Dr Harriet Whiley
College of Science and Engineering

Dear Dr Whiley,

Notice of Approval: 2020-19, 'Survival of *Streptococcus pyogenes* on surfaces', PC2 Microbiological

I am pleased to inform you that your application for a microbiological dealing, as above and attached, was approved by the Flinders University Institutional Biosafety Committee (IBC) on the 18th June 2020.

In addition to compliance with all conditions outlined in your approved application and risk assessment, the following specific conditions relating to your approval must be adhered to.

Approval is under the following conditions:

- The approval period ends on the 18th June 2025.
- The project must be undertaken in the PC2 facilities listed in the application. The IBC must be notified prior to any change in facilities used.

Your attention is drawn to the following requirements of *Australian/New Zealand Standard 2243.3* and University Policy:

- All biohazard waste must be disposed of as per the guidelines in Section 16 of the Flinders Biosafety Manual, and using appropriate disinfection or sterilisation techniques outlined in Appendix F of *AS/NZS 2243.3*.
- Any spill or unintentional release of risk group 2 microorganisms outside of a PC facility must be reported to the IBC Chairperson or IBC Executive Officer as soon as reasonably practicable. Contact details are available on the Flinders University biosafety website: https://staff.flinders.edu.au/research/integrity/biosafety#contacts
- All work health and safety incidents must be reported to the University via the FlinSafe portal: https://flinsafeportal.flinders.edu.au/FlindersEcPortal/
- It is necessary to apply to the IBC if any changes to the approved dealings are required. For amendments relating to facilities or personnel, please email ibcadmin@flinders.edu.au. For further information about making changes to approved projects, please refer to the website at https://staff.flinders.edu.au/research/integrity/biosafety/applying

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If you need further information or assistance regarding this notice please contact the IBC Executive Officer (ibcadmin@flinders.edu.au; ph. 7221 8353).

Yours sincerely

Prof Melissa Brown
Chairperson
Institutional Biosafety Committee

cc Mae White

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