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The Anti-bacterial Effect of Colloidal silver on *Streptococcus pyogenes* and

*Staphylococcus aureus* in vitro

A mini dissertation presented to the
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As partial fulfilment for the Master’s degree in Technology: Homoeopathy
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DECLARATION

I, Velenkosini Queen Mabunda, declare that this dissertation is my own work. It is being submitted for the degree of Master of Technology in Homeopathy at the University of Johannesburg. It has not been submitted to any other institution for the purpose of obtaining a qualification

__________________________

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ABSTRACT

Streptococcus pyogenes (S. pyogenes) and Staphylococcus aureus (S. aureus) are responsible for various infections in humans, including pharyngitis, tonsillitis, cellulitis, and many others. These bacterial infections have been successfully treated with antibiotics in the past years; however, many bacterial strains have become resistant to antibiotic treatment, resulting in increased mortality rates. Complementary medicine may be alternative treatment for bacterial resistant infections. Colloidal silver, an alternative treatment was studied against S. pyogenes and S. aureus in vitro.

The aim of this study was to investigate the antibacterial effect of 18 and 20 ppm colloidal silver on Streptococcus pyogenes and Staphylococcus aureus using the microdilution minimum inhibitory concentration method. The hypothesis was that colloidal silver would exhibit in vitro antibacterial activity on S. aureus and S. pyogenes. The method of choice for this study was the microdilution method. The disc diffusion method was used for trial run and spectrophotometric method was used for confirmatory and statistical purposes. All experimental procedures were completed in triplicate to eliminate laboratory errors. The experiments were conducted on S. aureus and S. pyogenes single colony units obtained from Davies Diagnostics. Pure cultures were then subcultured aseptically every 24 hours for the duration of the experiment. Mueller-Hinton agar was used as media. Four paper discs were used and each disc was impregnated with 18 ppm, 20 ppm colloidal silver, control groups (cefepime and distilled water) respectively. These discs were placed on agar plates, which had been inoculated with S. aureus and S. pyogenes respectively. Following 24 hours of incubation, the Iodonitrotetrazolium chloride (INT) dye was added to the plates and zones of inhibition were observed, measured and recorded within minutes. The 96 well microdilution method was used to determine the minimum inhibitory concentration of the 18 and 20 ppm colloidal silver. Results were visually inspected after adding the INT dye following 24 hours of incubation.

The results obtained from both the agar disc dilution method and the microdilution method indicated that both concentrations of the test compounds inhibited bacterial growth on S. pyogenes, but not on S. aureus. The noticeable colour changes (from a clear colour to purple)
indicated bacterial growth, and the unchanged clear colour indicated the inhibitory action of the test compounds, as illustrated in chapter 4.
ACKNOWLEDGMENTS

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Dr. T. Tsele-Tebakang  Supervisor
Professor T. G. Barnard  Co-supervisor
Riahaanah Paulse  Laboratory technician
# TABLE OF CONTENTS

1

1.1 The problem statement 1

1.2 Aim 2

1.3 Objectives 2

1.4 Hypothesis 2

1.5 Null hypothesis 2

3

LITERATURE REVIEW

2.1 Streptococcus pyogenes

2.1.1 Morphology and structure

2.1.2 Pathogenesis and pathology

2.1.3 *Streptococcus pyogenes* infections

2.2 Staphylococcus aureus

2.2.1 Morphology and structure

2.2.2 *Staphylococcal aureus* infections

2.3 Antibiotic therapy

2.3.1 Introduction to antibiotic therapy

2.3.2 History of antibiotics

2.3.3 Cefepime hydrochloride monohydrate

2.4 Antibiotic resistance

2.4.1 Inappropriate antibiotic usage

2.4.2 Decreased antibiotic compliance

2.4.3 Bacterial gene mutation

2.4.4 Biofilm formation

2.5 Natural antibiotic resistance

2.6 Complementary medicine

2.5.3 Colloidal silver
2.5.3.2.1 Colloidal silver

2.6 Related studies

3.1 Materials
3.1.1 Stock cultures
3.1.2 Media, buffers and consumables
3.1.3 Conventional medication - Cefepime
3.1.4 Complementary medication - Colloidal silver

3.2 Methods
3.2.1 Aseptic techniques
3.2.2 Growth and maintenance of bacteria
3.2.3 Preparation of media
3.2.4 Preparation of the iodonitrotetrazolium chloride solution
3.2.5 Disc diffusion method
3.2.6 Microdilution method
3.2.7 Ethical considerations

4.1 Agar disc diffusion method
4.1.1 Antibiotic resistance
4.1.2 The concentration of colloidal silver
4.1.3 The choice of method
4.1.4 The shape and stabilization of compound

4.2 Microdilution method

5.1 Conclusions
5.2 Recommendations
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Microscope photo of Gram stained <em>S. pyogenes</em> cells illustrating the typical cell shape</td>
<td>3</td>
</tr>
<tr>
<td>2.2</td>
<td>The visual illustration of the heart</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td><em>S. aureus</em> virulence factors</td>
<td>9</td>
</tr>
<tr>
<td>2.4</td>
<td>Typical visual illustration of the blood</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>The general structure of penicillin</td>
<td>16</td>
</tr>
<tr>
<td>4.1</td>
<td>Agar plates showing typical results obtained for the colloidal silver</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Solutions against <em>S. aureus</em> (i) and <em>S. pyogenes</em> (ii) as well as the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bacterial lawns using the agar disc diffusion method.</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>The typical microdilution method results of the 96 well plate layout</td>
<td></td>
</tr>
<tr>
<td></td>
<td>used to test the colloidal silver compounds against <em>S. aureus</em> and *S.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pyogenes.</td>
<td></td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Safe levels of colloidal silver ingestion in ml/kg daily</td>
</tr>
<tr>
<td>3.1</td>
<td>Summary of media, reagents and chemicals used for the experimental work.</td>
</tr>
<tr>
<td>4.1</td>
<td>Summary of results obtained for the three experiments testing the two compounds (18 ppm and 20 ppm) using the agar disc diffusion method.</td>
</tr>
<tr>
<td>4.2</td>
<td>Summary of serially diluted test compound concentrations in each well before and after addition of bacterial strains.</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

1.1 The problem statement

S. pyogenes and S. aureus are both Gram-positive bacteria which are responsible for various communicable infections. The infections caused by S. pyogenes include pharyngitis, streptococcal tonsillitis, impetigo, cellulitis and pyoderma (Ahmad et al., 2014). Infection by S. aureus manifests in individuals with compromised immune systems, causing scalded skin syndrome, abscesses, nosocomial pneumonia, meningitis, osteomyelitis and toxic shock syndrome (Stevens et al., 2002).

Antibiotics have been used effectively for treatment of bacterial infections; however they have now become less effective as more and more bacterial strains are becoming drug-resistant (WHO, 2015). Bacteria not only became drug resistant as consequence of mutation, but also acquired genes conferring resistance to antibiotics (Martínez, 2012). There is also decreased compliance with the use of antibiotics and this is partly due to associated side effects (Ventola, 2015); which include skin rash, haemorrhaging, nausea, vomiting, headaches, and candidiasis (Bhullar et al., 2012).

Due to the many challenges with antibiotic use, including easily accessible non-prescription antibiotics, drug resistant bacterial strains and inadequate access to proper treatment due to the unavailability of antibiotics (WHO, 2015; Tangcharoensathien et al, 2018), most people are turning to complementary and alternative treatment for bacterial infections (Wolsko et al., 2002). Colloidal silver is one of the treatments used as an alternative for bacterial infections. There are no known adverse effects from the use of colloidal silver; however, prolonged use can cause a permanent blue appearance (Owen, 2013). Colloidal silver does not interact with other medications or herbs; therefore can be taken safely with other drugs (Iroha et al., 2007). No research is known
to have been done specifically on colloidal silver 18 & 20 ppm against *S. aureus* and *S. pyogenes*.

1.2 Aim

The aim of the study was to investigate the anti-bacterial effect of colloidal silver on *Streptococcus pyogenes* and *Staphylococcus aureus* using the microdilution minimum inhibitory concentration (MIC) method.

1.3 Objectives

The objective of this study was to determine whether or not colloidal silver displays anti-bacterial properties and also determine the concentration at which the treatment is most effective. The findings will create an opportunity for more research to be conducted in this field, and it add value to the knowledge of complementary and alternative medicine.

1.4 Hypothesis

Both 18 and 20 ppm concentrations of colloidal silver solutions exhibit an antibacterial activity against *S. pyogenes* and *S. aureus in vitro*.

1.5 Null hypothesis

Colloidal silver solution in 18 and 20 ppm concentrations does not exhibit antibacterial activity against *S. pyogenes* and *S. aureus in vitro*. 
CHAPTER TWO

LITERATURE REVIEW

2.1 Streptococcus pyogenes
2.1.1 Morphology and structure

*Streptococcus pyogenes* (S. pyogenes) is also known as Group A β-haemolytic Streptococcus (GAS), belonging to Lancefield serogroup A as it displays an antigen A on its cell wall (Ahmad *et al.*, 2014). Being a β-haemolytic bacterium, it produces a toxin called streptolysin, which forms a zone of haemolysis on blood agar; indicating its ability to destroy red blood cells (Reese, 2016). *S. pyogenes* is a Gram-positive anaerobic bacterium which is immotile. It is a non-spore-forming coccus of about 0.5-1.2 µm in size, spherical in shape and usually grows in pairs or chains (Todar, 2012).

It has a very complex and chemically diverse cell wall, composed of lipoteichoic acid, M proteins and hyaluronic acid, which contribute to its virulence. The outermost capsule of this bacterium is made up of hyaluronic acid which resembles the hosts’ connective tissue; this allows it to go unrecognized as foreign by the host (Fiedler *et al.*, 2015).

The horizontal gene transfer (HGT) and prophage integration, which are commonly found in *S. pyogenes* genome give them plasticity and genomic variation (Fiedler *et al.*, 2015; Ibrahim *et al.*, 2016). These confer more virulence and resistance, they also change the regulation of existing genes. The *emm* gene, which encodes the M protein contains conserved, semi-conserved, as well as hypervariable regions. It is used as an epidemiological marker for GAS (Ibrahim *et al.*, 2016).
Figure 2.1 Microscope photo of Gram stained *S. pyogenes* cells illustrating the typical cell shape (NHS, 2016).

2.1.2 Pathogenesis and pathology

Infection by *S. pyogenes* is highly contagious and it spreads through direct contact with skin or fluid of an infected individual and for this reason it is classified as an infectious disease (Stephenson, 2011). It is also possible to carry the bacteria without showing any symptoms but still be contagious. *S. pyogenes* is the major cause of upper respiratory tract infections (Camara *et al.* 2013).

Streptococcal infections range from being localized to invasive. Localized infections can include tonsillitis and pharyngitis. Invasive infections can be dangerous as they are generalized and may involve the blood stream; these may include infections like necrotizing fasciitis, sepsis or streptococcal toxic shock syndrome (Ibrahim *et al.*, 2016).

GAS is usually found in pharyngeal mucosa and tonsils, these are primary reservoirs which are responsible for growth and transmission of the bacteria. Nasal-associated lymphoid tissue (NALT) is a human tonsil homologue which is involved in antigen uptake for mucosal immunity (Kunisawa *et al.*, 2008). T helper 17 cells and secretary antibodies are important elements in combating mucosal infections and can be influenced in NALT. The response of the T helper 17 cells is influenced by intranasal *S. pyogenes* infection or by immunization with a protein that inhabits the cell wall of the bacterium, known as the SrtA (Chen *et al.*, 2016).
The immune system can react against various microbes and antigens. Under normal circumstances, it doesn't react against the host's own antigenic substances; this is known as immunological tolerance (Abbas et al., 2014). The manner in which the immune system responds is dependent on antigen route, host ability to capture antigen, cellular availability and prior exposure to antigen (Newson, 2015). Normal oral flora, mucosal cilia, as well as secretory IgA all play a role in non-specific host defense against upper respiratory tract infections. Suppressed immune systems due to chronic illness, old age and therapy (such as chemotherapy) are predisposed to infections (National Institution of Health (NIH), 2016).

Over many years, β-haemolytic streptococci were recognized as intracellular pathogens that could invade human cells. *S. pyogenes* can survive in human host cells and remain impenetrable to antibiotic therapy (Ryan & Juncosa, 2016). Bacteria must come into contact with the host’s extracellular matrix (ECM) in order to cause an infection. The first contact with ECM proteins is established by specific adhesins (Courtney et al., 2002). Streptococci colonize the host cells, bacteria may also multiply extracellularly to form small colonies and develop biofilm-like structures that isolate and protect them from host defences (Ryan & Juncosa, 2016).

### 2.1.3 *Streptococcus pyogenes* infections

GAS infections have been linked with increased rates of mortality in poorly developed countries, while it has declined greatly in well-developed countries (Carapetis et al., 2005).

Since 1980, there has been an increase in reports of cases of acute rheumatic fever, streptococcal pharyngitis and scarlet fever (Efstratiou, 2000). In 2005, it has been estimated that the number of people suffering from severe GAS infections was 18.1 million and a further 1.78 million new cases reported each year of which about 517 000 lives are claimed yearly (Ralph & Carapetis, 2013).

Towards the end of the 20th century, there has been a decline in the incidence and severity of streptococcal infections due to the use of antibiotics. In the past 15 years, the incidence of streptococcal infections peaked again in a more severe manner; this is attributed to the resistance that the bacterial strains have developed over antibiotics (Luca-Harari et al, 2009).
2.1.3.1 Streptococcal pharyngitis

Streptococcal pharyngitis is the inflammation of the pharynx due to infection with GAS (Center of Diseases Control and prevention (CDC), 2016).

2.1.3.1.1 Anatomy

Pharynx is a fibromuscular tube lined with squamous epithelium (Levy et al., 2015). It is located posterior to oral and nasal cavities and posterior to the larynx. Pharynx extends from the base of the skull to the inferior border of the cricoid cartilage (Swenson, 2008). It is divisible into nasopharynx, oropharynx and laryngopharynx (Ejaima & Sittelnissa, 2016).

The nasopharynx is regarded as the posterior part of the nasal cavity. The oropharynx extends inferiorly from the soft palate to the superior border of epiglottis (Swenson, 2008). The inferior part of the pharynx is known as the laryngopharynx. It extends from the epiglottis to the cricoid cartilage and becomes continuous with the oesophagus (Swenson, 2008; Levy et al., 2015).

2.1.3.1.2 Physiology

The pharynx forms a part of the digestive and respiratory systems. It is a channel for deglutition and respiration (Swenson, 2008). Deglutition has four phases, namely: ingestion phase, oral phase, pharyngeal phase and oesophageal phase. Pharyngeal phase consist of gravity, pharynx elevation over food particles (bolus), tongue movements and contraction of constrictor muscle; allowing for swallowing to occur (Levy et al., 2015).

2.1.3.1.3 Signs and symptoms

Pharyngitis due to S. pyogenes is often characterized by sore throat, dysphagia, swollen glands and redness of the throat (Carrillo-Marquez, 2016). Concomitant symptoms may include a headache, abdominal pain, nausea and vomiting; this is usually witnessed in children. On physical examination, pharyngeal erythema, tonsillar enlargement, palatal petechiae and anterior cervical lymphadenopathy may be observed (CDC, 2016).

2.1.3.1.4 Diagnosis and treatment
Clinical examination and case history alone are not enough to diagnose streptococcal pharyngitis as other bacterial and viral infections may cause similar symptoms. A rapid antigen detection test or a throat culture can be used to confirm diagnosis (CDC, 2016; Kalra et al., 2016).

A challenge in treating pharyngitis is the existence of the carrier state where one carries the bacteria with no apparent symptoms (CDC, 2016). The failure of bacteria to mount antibody response results in carriage rather than infection. Carriage is said to be benign and harmless to host as it contributes to neither infection nor autoimmune disorders, but it is an important potential source of infection to surrounding uninfected individuals (Ralph & Carapetis, 2012).

Streptococcal pharyngitis is usually treated with antibiotics such as penicillin, amoxicillin and erythromycin. If left untreated, it can lead to serious complications such as rheumatic heart disease (RHD) (Carrillo-Marquez, 2016).

2.1.3.1.5 Prevalence

Globally, streptococcal pharyngitis accounts for 15-30% of pharyngitis in children and 5-15% in adults (CDC, 2016). Approximately 37% of streptococcal pharyngitis occurs in children aged 5 to 15 years and lower (about 24%) in children under the age of 5 years. It is more prevalent in late winter and early spring (Kalra et al., 2016).

In the United States of America, streptococcal pharyngitis accounts for about 15 million doctor visits per year. Twenty to thirty percent of children are infected, and a much lower percentage of streptococcal pharyngitis occurs in adults (Shulman et al., 2012).

A review of school-based studies done on streptococcal carriage in lower-socio economic areas in Soweto, South Africa, revealed that there is a carriage prevalence of 5.2% of Group A β-haemolytic Streptococcus (GAS) in primary school children. Carriage rates are 1.62% and 16.8% in black participants from traditional and urban communities respectively. Urban white participants have a carriage prevalence of 3.4%. There is 32.2%-45.5% prevalence of GAS pharyngitis in patients presenting with sore throat in South Africa (Engel, 2013).

2.1.3.2 Tonsillitis
Tonsillitis is an acute infection affecting the palatine tonsils (Hallberg, 2011). Tonsillitis is contagious and is caused by various viruses and bacteria, GAS being the most common cause (Pietrangelo & Nall, 2016).

There are three manifestations of tonsillitis; acute, which has a rapid onset and intensified symptoms; subacute tonsillitis, which has a gradual onset and less intensified symptoms; chronic tonsillitis, which is persistent with intermittent symptoms (Stevens, 2002).

2.1.3.2.1 Anatomy

Tonsils are two lumps of lymphoid tissue on either side of the mouth (Harding, 2016). They are located at the back of throat at the entrance of the upper aerodigestive tract (Geißler et al., 2017). Tonsils form a part of the Waldeyer’s ring, which is a collection of lymphoid tissue arranged in a ring form (Hallberg, 2011; Geißler et al., 2017). The Waldeyer’s ring consists superiorly of adenoids, laterally of palatine tonsils, inferiorly of the lymphoid nodules located at the posterior third of the tongue (Hallberg, 2011).

2.1.3.2.2 Physiology

Tonsils are a part of the first line of defense against foreign pathogens attacking the immune system (Chen et al., 2016). They consist of B and T lymphocytes. There are three types of T lymphocytes; they include cytotoxic T cells, helper T cells and regulatory T cells (Hallberg, 2011). Lymphocytes are white blood cells which protect the body against various pathogens/antigens (Sampson, 2017). The role of B lymphocytes is to recognize antigens and activate the production of antibodies involved in the destruction of antigens. Cytotoxic T cells foreign antigens. Helper T cells activate the immune response of B and other T cells. Regulatory T cells suppress the immune system and prevent autoimmune disorders and by downregulating excessive response against self-antigens (Chen, 2005).

2.1.3.2.3 Signs and symptoms

Generally, tonsillitis is characterized by a sore throat and dysphagia. A pain radiating from throat to the ears is also very common. It may be accompanied by fever, headache, malaise and cervical adenopathy (Sasaki, 2016). Tonsils usually appear red
and swollen and a purulent exudate may also form over the tonsils (Doerr, 2015; Sasaki, 2016).

In acute tonsillitis there is a sudden severe sore throat, fever, dysphagia, hypertrophy and erythema of tonsils as well as enlarged lymph nodes. Subacute tonsillitis lasts for the duration of 3 weeks to 3 months and it may include slightly enlarged tonsils, halitosis, mild to moderate sore throat and enlarged lymph nodes. Chronic tonsillitis is characterized by mildly red and pitted tonsils, non-tender lymph nodes with an intermittent sore throat (Stevens, 2002; Harding, 2011).

Complications of tonsillitis are classified into suppurative and non-suppurative. Suppurative sequelae of tonsillitis include peritonsillar and pharyngeal abscess. Non-suppurative sequelae may include rheumatic fever and glomerulonephritis (Campisi & Tewfik, 2003).

2.1.3.2.4 Diagnosis and treatment

Health history and physical examination are the basis of the diagnosis of tonsillitis; however, further investigates are helpful for confirmation of diagnosis and treatment (Stevens, 2002). The isolation of the bacteria from tonsils is important in the management of streptococcal tonsillitis; this is because antibiotic use requires knowledge of the specific aetiology, therefore, a throat swab culture is performed for diagnosis (Sasaki, 2016). A full blood count can also assist in determining whether or not tonsillitis is due to bacterial or viral infection (Stevens, 2002).

Acetaminophen and ibuprofen are used to control pain. Penicillin and amoxicillin are primarily used for treatment of tonsillitis. Cephalosporins and macrolides are given when a patient is allergic to penicillin (Doerr, 2015). Tonsillectomy, a procedure done to remove tonsils (Harding, 2016), is highly recommended if patients with a history of 3-5 bacterial tonsillitis within 3-5 years, 6 or more episodes of tonsillitis in a year, tonsillitis unresponsive to antibiotics, and sleep apnoea due to infection (Stevens, 2002; Doerr, 2015).

2.1.3.2.5 Prevalence

Tonsillitis is common in children between 5-1 years of age and adults between the ages of 15 and 25 years. It accounts for 120 out of 2000 doctor visits in the United Kingdom.
and across Europe. Those at risk of streptococcal tonsillitis are the immunosuppressed patients and patients with a personal or family history of tonsillitis (Tidy, 2014).

2.1.3.3 Scarlet fever

Scarlet fever is a bacterial infection caused by *S.pyogenes*. It usually follows or occurs in association with streptococcal pharyngitis (Holden *et al.*, 2015). It is highly contagious and is transmitted through direct contact with an infected individual. The disease can be latent for 2-5 days before clinical manifestations; this is known as the incubation period (CDC, 2016).

2.1.3.3.1 Signs and symptoms

In its early stages, the clinical features are vague and can easily be confused with other infectious diseases (Holden *et al.*, 2015). These features include a sore throat, fever, dysphagia, malaise, vomiting, nausea, lymphadenopathy, rash and headache. A diagnostic feature is a dark red tongue, also known as a strawberry tongue (CDC, 2016). A diffuse, red rash develops approximately the second day post infection. The rash is usually non-itching. It spreads from the torso and continues proximally to the limbs, sparing the nose, palms and soles (Bush, 2016). Infected individuals may also present with a flushed face, except for the area around the mouth, this is known as circumoral pallor (Holden *et al.*, 2015).

2.1.3.3.2 Diagnosis and treatment

This condition is diagnosed based on the clinical presentation; however, a rapid streptococcal test can also be done to confirm diagnosis (PubMed Health, 2014).

Antibiotics such as penicillin, tetracycline and chloramphenicol, are used for the treatment of scarlet fever. They prevent one from spreading the infection; however, they may be associated with side effects. Possible side effects include nausea, vomiting, skin rash and diarrhoea. Paracetamol is used to manage pain and fever (Gerber *et al.*, 2009). Holden *et al* (2015) mentions that to date, no penicillin-resistant isolates of GAS have been identified.

2.1.3.3.3 Prevalence
Although uncommon today, outbreaks still occur, affecting mostly children (Bush, 2016). Scarlet fever is more prevalent in children, mostly 5-12 year olds. It is estimated that 1 out of every 4 individuals have had scarlet fever before in the United States of America (PubMed Health, 2014).

In the year 2011 in Beijing, the scarlet fever monthly incidence rates peaked by 2.9-6.7 higher than the years 2006-2010 (Yang et al., 2013).

Although scarlet fever is rare in South Africa and surrounding native countries, it is frequent during winter and is more prevalent in areas with colder climates like Cape Town (Parsons, 2016).

2.1.3.4 Rheumatic fever
Rheumatic fever is sequelae of untreated streptococcal throat infection. It causes the antibodies to act against self (autoimmune disease); therefore it is regarded as a serious condition (Weatherspoon, 2015). An autoimmune disease is a condition in which the immune system attacks its own body cells due to inappropriate recognition of foreign cells (Watson, 2015).

Acute rheumatic fever (ARF) typically follows 2-3 weeks post streptococcal pharyngitis. It has rheumatologic, neurologic and cardiac manifestations (Meador, 2017). Acute rheumatic fever is nonsuppurative and immune-mediated (Beaudoin, 2015). Rheumatic heart disease (RHD) is a chronic cardiac disease resulting as a consequence of recurrent episodes of acute rheumatic fever (Katzenellenbogen et al., 2017). It results in permanent cardiac valve damage, putting one at risk to develop cardiac conditions like endocarditis, congestive heart failure and stoke (Beaudoin, 2015).

As the ultimate complication is heart disease, below is more information about the heart, ARF and RHD.

2.1.3.4.1 Anatomy
The heart is a muscular organ located in the thorax, medial to the lungs and posterior to the sternum (The Heart Institute (THI), 2016). It is enclosed within the pericardium, which is a double layered fibrous sac, including the visceral and parietal layers (Peebles
et al., 2011). The heart consists of two upper chambers, the right and left atria, and two lower chambers, the right and left ventricles; these are separated by the septum and heart valves (THI, 2016). Within the heart are atrioventricular valves: tricuspid valve on the right, and mitral valve on the left; and semilunar valves: pulmonic valve between pulmonic artery and right ventricle, and the aortic valve between the aorta and the left ventricle (Katz, 2011). Below is a visual presentation of the heart anatomy.

![Figure 2.2: The visual illustration of the heart anatomy (Pearson Education Inc, 2004)](image)

2.1.3.4.2 Physiology

The heart functions as a pump, supplying blood to the organs, tissues and cells in the body (THI, 2016). In the cardiac cycle, the ventricles contract in a systole phase and relax in a diastole phase to produce a single heart beat (Klabunde, 2016).

In the circulatory system of the heart, the blood flows from upper and lower parts of the body through the great veins (superior and inferior vena cava, as shown in figure 2.2) to the right atrium and passively fills the right ventricle. Atrial contraction increases ventricular filling by 20% (Klabunde, 2016; Hall, 2016). The tricuspid valve opens in atrial contraction and closes in atrial relaxation to prevent backflow of blood to atrium (THI, 2016). During the right ventricular systole, the pulmonary valve opens as blood is ejected through pulmonary arteries into the lungs for oxygenation (Hall, 2016).

Oxygenated blood leaves the lung through pulmonic veins into the left atrium. When the left atrium contracts, it pushes open the mitral valve, filling the left ventricle. As with the
right side of the heart, the left ventricle contracts after filling, causing the aortic valve to open. Blood then flows through the aorta and supplies the body (Whitlock, 2018).

2.1.3.4.2 Signs and symptoms

Symptoms of rheumatic fever may include painless skin nodules, chest pain, palpitations, fatigue, abdominal pains, sore joints, migratory arthritis, fever, jerky and uncontrollable movements of the limbs (Johnson & Murrell, 2012).

Symptoms of RHD include dyspnoea, fatigue, arrhythmia, chest pain, fainting, as well symptoms of rheumatic fever, including fever, muscle ache and joint pain. Damaged heart valves are common on investigation (WHO, 2016).

2.1.3.4.3 Diagnosis and treatment

Diagnosis for ARF is based on Jones criteria, which includes the major criteria and minor criteria. Either two major criteria or one major and two minor criteria, with history of strep throat are adequate to diagnose ARF (Webb et al., 2015).

The major criteria includes: carditis, Sydenham’s chorea, subcutaneous nodules, arthritis and erythema marginatum. The minor criteria includes: fever, arthralgia, first degree heart block, increased acute phase reactants and evidence of prior GAS infection (Webb et al., 2015; Rana & Kumar, 2016).

Throat swab culture, streptococcal antibody test or a rapid antigen detection test can be used to diagnose present or recent history of streptococcal infection (Gerber et al., 2017). Echocardiogram and electrocardiogram can be used to diagnose rheumatic heart disease (Rana & Kumar, 2016).

Penicillin is usually given as treatment together with anti-inflammatory and anti-convulsants (WHO, 2016). Cephalosporins, including clindamycin, azithromycin and clarithromycin can be administered to individuals who are allergic to penicillin (Gerber et al., 2017).
For individuals with RHD, prednisone is given for 2-6 weeks, depending on the severity of symptoms. Towards the end of prednisone therapy, salicylates can be given to prevent rebound of carditis (Chin, 2017).

2.1.3.4.4 Prevalence

Acute rheumatic fever (ARF) as well as RHD remain important causes of cardiovascular disease, affecting mostly children and young adults (Mirabel et al., 2014). In the United States, ARF is no longer a notifiable disease. Its incidence rates have greatly declined since the late 20th century to 0.04-0.06 cases per 1000 children (Beaudoin et al., 2015). In Western countries, RHD has almost been eradicated; however, it is still a major concern in developing countries (Mirabel et al., 2014).

Acute rheumatic fever (ARF) cases have been reported in large numbers since the 12th and 13th centuries. With time, these incidences reduced in number prior to penicillin discovery. The reduction is attributed to improved housing, sanitization and a better service delivery in health care (Stevens, 2002; Mirabel et al, 2014).

South Africa has the highest global prevalence of RHD, but the incidence of ARF is reported to be low, this could be due to substantial underdiagnosis of ARF. The prevalence is 5.7 per 1 000 in children between the ages 5-14 years (Ralph & Carapetis, 2012). Highest incidence rates are reported in slum dwellers, followed by rural populations then urban populations. Poor hygiene, overcrowding and lack of access to good health greatly increase incidence rates (Mirabel et al., 2014; Ralph & Carapetis, 2012).

2.2 Staphylococcus aureus
2.2.1 Morphology and structure

S. aureus is a gram-positive bacterium, spherical in shape and it occurs in grape-like clusters. It is about 1 µm in diameter (Foster, 1996). It forms a large yellow coloured colony on rich medium and it is haemolytic on blood agar, indicating that it can destroy red blood cells (Todar, 2012). S. aureus express a fibrin/fibrinogen binding protein, which helps attachment to blood clots and injured tissue (Foster, 1991). Receptors which promote attachment to collagen are associated with osteomyelitis and arthritis (Foster, 1996). Its surface proteins also promote attachment to host proteins that form
on the ECM of the epithelium and endothelium (Todar, 2012). The genome of this bacteria is about 2.8 mega-based pairs long with 2 600 open reading frames. Staphylococci form a coherent group at the genus level (Foster, 1996).

*S. aureus* has an ability to attach to extracellular matrix proteins, platelets and fibrin. Its virulence is determined by plasmids and staphylococcus cassette chromosome (Easmon & Adlam, 1983), as shown in figure 2.3. The bacteria secrete surface associated adhesins, endotoxins, exoenzymes and capsular polysaccharides. These numerous secretions as well the bacterial capsule are responsible for increased virulence (Tong *et al.*, 2015).

![Figure 2.3 S. aureus virulence factors (Rao, 2011).](image)

*S. aureus* has always been more prevalent in hospitals and it was initially treated effectively with methicillin. It has now become resistant to the drug (Dormanesh *et al.*, 2015). Research studies show that the methicillin resistant *Staphylococcus aureus* (MRSA) has increased in number and about 50% of the strains affecting humans are resistant to drugs (Kluytmans *et al.*, 1997). Its resistance is accounted for by a transposon, a DNA segment that can move into a new position within the same or a different chromosome (Dormanesh *et al.*, 2015); this also makes the bacterium resistant to disinfectants. The gene capable for its resistance is known as *mecA* (Sousa & Lencastre, 2004).
This bacterium is one of the main causes of a variety of suppurative infections (Naidoo et al., 2013). Common infections due to *S. aureus* include boils, styes, furuncles, pneumonia, mastitis, meningitis, urinary tract infections, osteomyelitis, endocarditis and toxic shock syndrome (Todar, 2012; Sousa & Lencastre, 2004). Humans are a reservoir of the bacteria. Asymptomatic colonization of *S. aureus* is more common than infection (Kluytmans et al., 1997). The bacteria usually inhabit the nasopharynx, skin or perineum. It is transmitted by direct contact to an infected carrier (Chambers, 2001).

Infection is more prevalent in drug users, diabetics, young children, immunocompromised patients and people with long standing intravascular catheters (Bush & Perez, 2016). Although staphylococcal infections are frequent, they usually remain at the portal of entry. This may be a hair follicle, upper respiratory tract, broken skin or wound. Complications arise when the bacteria enters the blood stream, resulting in septicaemia; which can be fatal (Todar, 2012; Chambers, 2001).

### 2.2.2 Staphylococcal *aureus* infections

According to Schaumburg et al. (2014) about 3.28 cases per 1 000 hospital admissions in South Africa are due to *S. aureus* infection annually. In Mozambique, 178 cases per 100 000 hospital admissions are due to infection by *S. aureus* compared to the United States of America, which has about 2.3 cases per 100 000 hospital admissions yearly.

Asia is one of the many regions with the highest incidence of MRSA and has increasing vancomycin-resistant *S. aureus* (VRSA). MRSA is mostly prevalent in health care facilities and its incidence varies with each country. The majority of the hospital associated bacterial strains from various countries are of the same genotype, this suggests international dissemination of a few health care associated clones (Chen & Huang, 2014).

Dramowski et al. (2017) documented the infectious disease exposures and outbreaks in Africa. It is recorded that 4 outbreaks of MRSA occurred between 2012 and 2015. Between 2012 and 2013, 24 neonates were affected. These outbreaks included sepsis, conjunctivitis and skin and soft tissue infections. The screening which was carried out identified MRSA carriage in 16 out of 140 neonates. The on-going transmission of these
drug resistant bacterial strains suggested hospital staff screening as well, and it was found that 2 out of 208 staff were carriers.

Eibach et al. (2017) obtained nasal swabs from children below the age of 15 years on hospital admission at the Agogo Presbyterian Hospital. This was done from April 2014 until January 2015. *S. aureus* isolates were identified by their antibacterial susceptibility and the presence of toxic shock syndrome toxin-1 (TSST). One hundred and twenty children out of 544 were colonized with the bacteria, the carriage rates increasing during rainy seasons. About 2% of the bacterial isolates were MRSA and 13% were resistant to 3 or more antimicrobials. This study concludes that the carriage of *S. aureus* among Ghanaian children is dependent on age, sex and seasonality.

There is an estimated 30% of humans who are nasal carriers or *S. aureus*; however, the carriage rate varies with each geographic location, season, age, as well as sex. Dutch children have shown a low carriage rate during the first year of life, becoming stable at 20-30% and increasing to 40-50% between the ages of 6-12 years. Studies conducted on adults in West and Central Africa have shown ranging carriage rates from 21% in Ghana, to 29% in Gabon and 36% carriage rate in Senegal (Eibach et al., 2017).

Below are the different types of *S. aureus* infections discussed in detail.

2.2.2.1 Cutaneous abscess

This is a localized pus collection in the skin. It results due to infection by various pathogens, *S. aureus* being one of them (Bush & Perez, 2016).

2.2.2.1.1 Anatomy

The skin is made up of the epidermis, the dermis, and the hypodermis (Habif, 2016). The epidermis is the outermost layer (Hoffman, 2014), which is 50-100 µm thick (WHO, 2009) and is made up of squamous epithelium. The innermost layer of the epidermis is made up of a row of columnar basal cells (Habif, 2016). The most superficial layer of the epidermis is called *stratum corneum* (WHO, 2009).
The dermis follows beneath epidermis and it is 1-2 µm thick (WHO, 2009). It consists of the connective tissue, hair follicles and sweat glands (Hoffman, 2014). The dermis is made up of three types of connective tissue: the collagen, elastic tissue and reticular fibers (Habif, 2016). The connective tissue is a collection of tissues that maintains the form of the body, providing internal support and cohesion (Fawcett, 2017).

The hypodermis, also known as the subcutaneous layer is the innermost layer of skin (Hoffman, 2014). It is 1-2 µm thick (WHO, 2009) and it contains fibroblasts, adipose tissue, connective tissue, nerves and blood vessels (Kita, 2018).

2.2.2.1.2 Physiology

The function of the *stratum corneum* is to reduce water loss by maintaining water and electrolyte balance (WHO, 2009; Page, 2018). The skin is involved in thermoregulation, a process in which the body maintains its core internal temperature of 37°C - 37.8°C (Page, 2018). This is achieved through perspiration/sweating and vasodilation upon sensation of extensive heat. When the body temperature is falls below normal, the skin regulates the temperature through vasoconstriction (Holland, 2016).

The skin functions as a mechanical and physical barrier against microbial infections and trauma (Page, 2018; James, 2015). The primary defence against microbial infections, *S. aureus* in particular, is the neutrophil response. The neutrophils migrate to the site of infection, together with microphages to combat the infection. In compromised immunity, the bacteria can enter and evade the response of neutrophils and macrophages by blocking chemotaxis of leukocytes, hiding away from host antibodies and from detection via polysaccharides capsules or by forming a biofilm (Tong *et al.*, 2015).

The skin is also important in the synthesis of vitamin D by absorbing ultraviolet B radiation and converting cutaneous 7-dehydrocholesterol to previtamin D₃, which is converted to vitamin D₃ in the body (NIH, 2016).

2.2.2.1.3 Signs and Symptoms

Cutaneous abscess is characterized by pus accumulation within the dermis, inflammation and induration, as a result, a skin lesion forms (Mansour *et al.*, 2016). It is painful, tender, and usually erythematous (Dhar, 2017).
If an abscess is severe, it could be septic, therefore must be treated with medication and not only surgically drained (Mansour et al., 2016).

2.2.2.1.4 Diagnosis and treatment

Cutaneous abscessed is diagnosed by physical examination. A culture from pus is recommended to identify MRSA (Dhar, 2017).

Trimethoprim-sulfa-methoxazole, clindamycin and tetracyclines are the most common antibiotics used to treat abscess (Singer & Talan, 2014); however, conditions within the abscess like low pH, increased bacterial load, and debris, may limit the efficacy of treatment. (Stearne et al., 2001). Besides the conditions mentioned prior, antibiotics have been less effective due to bacterial strains that have been become resistant to treatment (Moran et al., 2006).

2.2.3.2 Staphylococcus scalded skin syndrome (SSSS)

This is an exfoliative disease of the skin that expands rapidly. The causative bacteria (S. aureus) produce epidermolytic toxins (Bush & Perez, 2016) which spread through the blood stream, causing erythema, blistering and scalding of the skin (Cribier et al., 1994). The toxins target the protein desmoglein I in the epidermis. Please refer to section 2.2.3.1.1 and 2.2.3.1.2 for anatomy and physiology of the skin respectively.

2.2.3.2.1 Signs and symptoms

Symptoms include the exfoliation of the skin, followed by erythematous cellulitis. Red blisters with a scalded appearance develop on the surface of the skin. When the blisters rupture, it leaves a burn like appearance (Mishra et al., 2016). This condition can be severe if a patient has existing poor renal function. Children are mostly affected as they secrete exfoliates through kidneys. The skin may become tender and itch, fever and diarrhea and dehydration may also present (Su et al., 2016).

2.2.3.2.2 Diagnosis and treatment

The skin appearance of burn like epidermal exfoliation is diagnostic of SSSS. Skin biopsy and a culture test may be performed for confirmation. Blood, urine, throat or skin sample may be used for culture (Mishra et al., 2016; Handler & Schwartz, 2014).
Fluid and electrolyte replacement is compulsory in cases of dehydration. Antibiotic therapy is intravenously administered during hospitalization. Oral antibiotics are given 6-7 days post hospitalization, when the patient has been discharged (Mishra et al., 2016). Penicillase-resistant antistaphylococcal antibiotics are given intravenously almost immediately. Vancomycin or linezolid are given in areas high prevalence of mrsa or in patients who are not responding to initial treatment. (Dhar, 2017). Paracetamol is used in the treatment of pain and fever when necessary. Fusidic acid or mupirocin can be added to gel dressings and applied topically to weeping burn like skin (Johnson, 2004; Patel, 2004).

2.2.3.2.3 Prevalence

SSSS has the highest incidence rates in infants; however, this does not exclude children and adults (Bush & Perez, 2016). Neonates and children below the age of 5 years are at higher risks of SSSS. The immunocompromised patients and individuals with renal failure are also at risk of SSSS (Arbuthnott, 2013). This condition is not very common globally. The first outbreak was noticed in Ireland (Mishra et al., 2016).

2.2.3.2 Nosocomial pneumonia

This refers to pneumonia (a bacterial or viral lung infection) contracted in a hospital. It is also known as hospital acquired pneumonia (HAP). Nosocomial pneumonia usually develops two days post hospital admission (Nseir et al., 2002). The common bacteria responsible for this condition are Klebsiella pneumonia, Escherichia coli, non-enterobacteriaceae bacteria and S. aureus.

2.2.3.2.1 Anatomy

The lungs are a pair of air-filled organs situated in the thorax (Hoffman, 2014). The mediastinum separates the two pulmonary cavities, right and left. These bilateral compartments contain the lungs and pleura (Roberts & Weinhaus, 2005). Each lung is enclosed in a pleural sac, which is made up of the visceral pleura, which covers the lung surfaces, and the parietal pleura, which lines the pulmonary cavities (Moore et al., 2013).

Both right and left lungs are divided into lobes, 3 in the right (upper, middle and lower lobes) and 2 in the left (upper and lower lobes) (Tidy, 2015). The functional subunits of lungs are called segments, and are closely related to the segmental bronchi, branches
of the main bronchi which derive from the bifurcation of the trachea (Celis, 2017). The right lung comprises 10 segments and the left lung comprises 8 segments (Moore et al., 2013). From segmental bronchi are bronchioles which end in alveoli, which are small air sacs (Tidy, 2015).

2.2.3.2.2 Physiology

The main function of the lungs is to transmit oxygen to the rest of the body during inhalation and to rid the body of carbon dioxide (CO$_2$) during expiration (Tidy, 2015). In the interstitium, a thin layer of cells between the alveoli, the exchange of oxygen and waste product occurs (Hoffman, 2014). The lungs are also responsible for maintaining the pH of the blood controlling the amount of CO$_2$ in the body. The conversion of angiotensin I to angiotensin II for the regulation of blood pressure occurs in the lungs (Moore et al., 2013; Tidy, 2015).

2.2.3.2.3 Signs and symptoms

Clinical manifestations of nosocomial pneumonia include fever, malaise, chills, dyspnoea, cough, chest pain and rigor. In a ventilated patient, pneumonia manifests as worsening hypoxemia and increased tracheal secretions (Sethi, 2017).

2.2.3.2.4 Diagnosis and treatment

Patient history and chest x-ray are used for diagnosis; however, blood culture and sputum culture are important for diagnosis of causative pathogen and are useful in treatment selection can also be useful (Sethi, 2017; Brusch, 2017).

Treatment is given in accordance with the results of the investigated microbiological cultures. Vancomycin or linezolid is recommended in the presence of MRSA. For methicillin susceptible Staphylococcus aureus, piperacillin-tazobactam, cefepime, levofoxacin or meropenem is recommended (Brusch, 2017). Antibiotics such as moxifloxan, amoxicillin, or macrolides are used to treat HAP (Tong et al., 2015).

2.2.3.2.5 Prevalence

Mortality rates range from 25-50% despite antibiotic therapy (Tong et al., 2015). The risk factors include cigarette smoking, chronic pulmonary disease, diabetes, low body mass index, high gastric pH, previous antibiotic history, previous history of pneumonia,
renal insufficiency and chronic hepatic disorder. Hospitalization and surgery, including endotracheal intubation increase the risks of the infection (Sethi, 2017; Wooten & Winston, 2013). During the 1918 pandemic, nosocomial pneumonia it was implicated as a devastating complication of influenza until it was documented even after the pandemic, as it remained in the absence of preceding influenza (Chickering & Park, 1919).

2.2.3.3 Osteomyelitis

Osteomyelitis is a bacterial infection of the bone. It consists of various inflammatory and destructive bone disorders caused by microbial infection (Schmitt, 2017). The bacteria invade osteoblasts, leading to inflammation, necrosis and destruction of bone at the site of infection. *S. aureus* is the most common cause of chronic osteomyelitis (Lu *et al.*, 2016).

Acute osteomyelitis refers to recent bone infection by haematogenous spread, occurring predominantly in children. It develops within 2 weeks post infection. Subacute or chronic osteomyelitis is bone infection that develops secondary to bone and surrounding bone tissue injury in adults. Subacute osteomyelitis manifests within a month and chronic osteomyelitis after a few months post infection (Carek *et al.*, 2001).

Vertebral osteomyelitis involves the end plates of adjacent vertebrae and from here, the infection can spread directly into the disc space (Corrah *et al.*, 2011).

2.2.3.3.1 Anatomy

Bones forms a major part of the skeletal system. It is a rigid tissue composed of collagen and calcium phosphate (Whedon & Heaney, 2017). Collagen is a protein and it provides a soft framework. Calcium phosphate is a mineral which strengthens and hardens the bone framework (NIH, 2015). There is a cortical bone and trabecular bone. The cortical bone or the cortex is dense and compact and it makes up the exterior smooth layer. The trabecular bone is the inner spongy and honeycomb-like layer (NIH, 2015). Within the trabecular, are blood vessels and marrow (Whedon & Heaney, 2017).

The bones are organized into different shapes and sizes, suitable for their functions. There are 5 classifications of bones, including: long bones, short bones, flat bones, Irregular bones and pneumatic bones (Peate & Nair, 2017). Long bones have an
elongated diaphysis and an expanded end, known as epiphysis. Short bones contain no shaft and are usually cuboidal in shape; these form the carpal and tarsal bones. Flat bones resemble shallow plates and they form boundaries for cavities. Irregular bones are irregularly shaped bones like those of the vertebra, pelvis and base of the skull. Pneumatic bones have airspaces within and therefore lighter than other bones, examples of which are the maxilla, sphenoid, or ethmoid (Singh, 2009).

Osteoblasts, osteoclasts, osteocytes and mesenchymal stem cells are the 4 types of cells found in the bone (Whedon & Heaney, 2017).

2.2.3.3.2 Physiology

Bone is responsible for calcium and phosphate equilibrium. Calcium plays a key role in neuromuscular function, interneuronal transmission, cell membrane integrity and permeability, as well as in blood coagulation (Whedon & Heaney, 2017). The regulation of calcium and phosphate is through the feedback mechanisms of parathyroid hormone (PTH), calcitonin (CT) and vitamin D.

The release of PTH is stimulated by low blood calcium levels. PTH stimulates the bone to release calcium into the bloodstream and signals the kidney to increase tubular reabsorption of calcium and excretion of phosphorus (Liess & Staros, 2014). PTH also stimulates the kidneys to convert vitamin D to calcitriol, which in the small intestines increases the absorption of dietary calcium and phosphorus (Poole & Reeve, 2005). When low levels of calcium are detected in the blood, both calcitriol and PTH work in conjunction to stimulate the maturity of osteoclasts, is a catabolic agent that enhances the release of calcium and phosphorus from bone into the bloodstream (Whedon & Heaney, 2017). During hypercalcaemia, PTH and calcitriol stimulate osteoblastic activity, resulting in bone formation and mineralization as calcium and phosphorus are reabsorbed by the bone (Peate &. Nair, 2017). Calcitonin interacts directly on osteoclasts by binding to their receptor sites and inhibiting osteoclastic activity (Whedon & Heaney, 2017).

Bones form the skeleton which provides structural support for the rest of the body. The bones of the skeleton also permits motion and protect internal organs (Clarke, 2008).
The marrow in the bone is an environment for haematopoiesis, the synthesis of blood cells (Taichman, 2005).

2.2.3.3.3 Signs and symptoms

The general signs and symptoms of osteomyelitis include fever, bone pain, inflammation, warmth and erythema of affected area. Infection can occur following injury/trauma or by haematogenous spread (Bush, 2016).

Animal models have demonstrated that healthy bone is highly resistant to infection. Direct trauma or large bacterial inoculum is needed to cause bone infection (Tong et al., 2015).

Clinical manifestations of acute osteomyelitis include inflammation, fever, irritability and lethargy. On physical examination, there is tenderness over the affected bone and also decreased range of movement (Carek et al., 2001).

Subacute and chronic osteomyelitis usually present with localized bone pain, erythema and drainage around the site of infection (Tong et al., 2015). The cardinal signs include a draining sinus tract, instability, bone deformity, impaired vasculature around the affected area and decreased range of motion (Carek et al., 2001).

Vertebral osteomyelitis is characterized by back pain, localized percussion tenderness and fever. If the nerves are compressed, the patient will experience weakness, tingling and a sensation of paralysis. Paralysis or neurological dysfunction may later develop (Vaccaro & Schroender, 2015).

Complications arising from osteomyelitis include recurring osteomyelitis and gangrene (NHS, 2014). The recurrence may be due to underlying conditions such as poor blood circulation and a weak immune system. Gangrene can occur if blood supply is dramatically reduced; the consequence may be amputation (Tice et al., 2003).

2.2.3.3.4 Diagnosis and treatment

Diagnosis is based on clinical findings, case history and physical examination, as well as laboratory tests. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels may be increased. Full blood count may reveal leukocytosis (Carek et al., 2001).
Blood culture is important for diagnosis of the causative agent. Radiographic studies may reveal bone destruction (Carek et al., 2001; Abernethy & Carty, 1997).

Treatment is determined by the type of infection. Acute osteomyelitis is treated with antibiotics such as nafcillin, oxacillin, vancomycin, ceftazidime or cefepime. Chronic osteomyelitis is treated with ampicillin, piperacillin or vancomycin. Surgery may be necessary in cases of paravertebral or epidural abscesses (Schmitt, 2017; Bush, 2016).

2.2.3.3.5 Prevalence

Certain conditions like diabetes, rheumatoid arthritis, HIV or any other condition affecting the immune system can increase risks of osteomyelitis (Prieto-Pérez et al., 2014; Hatzenbuehler et al., 2011). There are higher chances of osteomyelitis occurring again if you have had it before (NHS, 2014).

In the United States, the overall prevalence is 1 case in 5 000 children. Neonatal prevalence is 1 in a 1 000 cases. The prevalence of osteomyelitis due to trauma is 16%, increasing to 30-40% in diabetic individuals. Vertebral osteomyelitis is rare that in occurs in 2.4 cases per 100 000 people (King & Taylor, 2017).

In South Africa, the mortality rates related to osteomyelitis have declined from 20-30% to less than 10% due to improved health care treatment. Morbidity occurs in cases of delayed diagnosis (Stellenbosch University, 2008).

2.2.3.4 Meningitis

Meningitis is inflammation of the meninges, pia and the arachnoid mater, that surround the brain and spinal cord. S. aureus is one of many pathogens which cause meningitis (Gantz, 2005).

S. aureus meningitis is mainly nosocomial and occurs predominantly post neurosurgery. It can be acquired in a community based setting where there is an association with predisposing factors including endocarditis, compromised immunity and injection drug use (Brouwer et al., 2010). Meningitis due to S. aureus may arise either by haematogenous spread from a site of infection that is not located in the central nervous system (CNS) or as a result of neurosurgery (Teh & Slavin, 2012). The primary source
of haematogenous *S. aureus* is infective endocarditis (IE), pneumonia or skin and soft tissue infections (Tong *et al*., 2015).

### 2.2.3.4.1 Anatomy

The meninges are layers of connective tissue surrounding the brain and the spinal cord. They are composed of 3 layers known as the dura mater, arachnoid mater, and pia mater (Bailey, 2017).

The dura mater is the outer layer of the meninges. It is made up of the periosteal layer, which attaches the dura mater to the cranium, and the meningeal layer, which makes up the inner layer of the dura mater (Bailey, 2017). Between the meningeal and periosteal layers are dural venous sinuses. These sinuses function as veins to drain blood from the brain to the internal jugular vein, where it flows back to the heart (Aghoghovwia & Chaves, 2017).

The arachnoid mater forms the middle layer of the meninges. It serves to connect the dura mater to the pia mater (Bailey, 2017). It has a web-like appearance and is connected to the pia mater by fibrous extensions. The space through the extensions makes up the subarachnoid space and allows for the passage of blood vessels and nerves (Aghoghovwia & Chaves, 2017). The membrane of the arachnoid mater consists of projections called the arachnoid granulations. These granulations drain the cerebrospinal fluid (CSF) from the subarachnoid space into the dural venous sinuses (Aghoghovwia & Chaves, 2017; McMinn, 2014).

The pia mater is the inner layer of the meninges and it is in direct contact the brain and spinal cord. The pia mater has numerous blood vessels to supply the nervous tissue. It contains the choroid plexus, which is a network of capillaries and specialized ciliated epithelial tissue that produce CSF (McMinn, 2014; Bailey, 2017).

### 2.2.3.4.2 Physiology

The main functions of the meninges include providing protection for the brain and spinal cord from mechanical injury, supplying the cranium and the cerebral hemispheres with blood and also permitting the flow of CSF (Samuel, 2017). CSF serves as a mechanical barrier against shock and provides lubrication between the surrounding cranial bones and the brain and spinal cord (Bailey, 2017).
2.2.3.4.3 Signs and symptoms

The clinical manifestations of meningitis include fever, severe headache, altered mental state and meningismus (Brouwer et al., 2010).

2.2.3.4.4 Diagnosis and treatment

Meningitis is diagnosed by analysis and culture of cerebrospinal fluid (Brouwer et al., 2010). If CFS findings are negative for infectious meningitis, images studies such as magnetic resonance imaging (MRI) or computed tomography scan, should be done to identify the focal site of abnormality for biopsy (Greenlee, 2017). Treatment is dependent on the cause of infection, the type of infection (community acquired of nosocomial meningitis) and the age group (Greenlee, 2017). Ampicillin, aminoglycoside or cefotaxime is usually given to neonates. A broad spectrum cephalosporins and vancomycin are administered to children and infants. The same is given for adults, but with the addition on ampicillin (Brouwer et al., 2010).

2.2.3.4.5 Prevalence

*S. aureus* is a rare cause of bacterial meningitis and it accounts for 4.9 to 6.4 % of cases (Tong et al., 2015). Global mortality rates for nosocomial *S. aureus* meningitis have been reported to be 14%, and 50-60% for community acquired *S. aureus* meningitis in 2009 (Brouwer et al., 2010). Mortality rates of haematogenous meningitis are about 43-50% higher than that of post–surgical meningitis (Tong et al., 2015).

2.2.3.6 Toxic shock syndrome

Toxic shock syndrome (TSS) is potentially fatal and it is due to staphylococcal bacterial exotoxin, although other bacteria may cause it, like *S. pyogenes* (Bush & Perez, 2016). It is classified into menstrual and non-menstrual TSS (Schlievert et al., 2010). Menstrual TSS is associated with the use of tampons by women previously healthy (Davis et al., 1980). Non-menstrual TSS usually occurs in children and in women who are not menstruating. It is associated with other bacterial infections, such as pneumonia (Reingold et al., 1982). TSS toxin 1 (TSST-1) is a superantigen responsible for mTSS. Vaginal colonization with TSST-1 producing *S. aureus* predisposes one to develop TSS. Although the pathogenesis of mTSS is not fully understood, studies have revealed that
tampons can be colonized with, and transmit *S. aureus* throughout the bloodstream during menstruation (Schlievert *et al*., 2010).

### 2.2.3.6.1 Anatomy

Blood is a combination of plasma, cell fragment called formed elements and cells circulating throughout the body via blood vessels (Nordqvist, 2017).

Blood plasma consists of 90% water and 8% proteins including albumin, alpha and beta globulins, gamma globulins, fibrinogen and prothrombin proteins (CliffsNotes, 2018). It also contains waste products like urea, uric acid and bilirubin, nutrients which are absorbed from the digestive tract, electrolytes and respiratory gases, including oxygen and CO$_2$ (CliffsNotes, 2018; Hoffman, 2014). Formed elements constitute the red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes) (Nordqvist, 2017). There are 5 types of leukocytes, these include: neutrophils, eosinophils, basophils, lymphocytes and monocytes (CliffsNotes, 2018). Refer to figure 2.4 below for a clearer view on blood constituents.

![Figure 2.4 Typical visual illustration of the blood (WebMD, 2014).](image)

### 2.2.3.6.2 Physiology

Erythrocytes serve to transport oxygen and CO$_2$ to and from the lungs to supply tissues and organs (Nordqvist, 2107). Leukocytes function to protect the body against foreign pathogens causing infection. Each type has a specific function (Hoffman, 2014). Neutrophils arrive first at the site of infection and engulf bacteria through phagocytosis.
(CliffsNotes, 2018). Eosinophils are activated during parasitic infection and allergic reactions and they phagocytise the complexes formed by antibodies on antigens. Basophils are similar in action to eosinophils, but they are only found in connective tissue (Hoffman, 2014). The B lymphocytes and T lymphocytes are cells involved in the body’s immune system. Monocytes mature to become macrophages which microbes and debris (CliffsNotes, 2018). Thrombocytes are actively involved in clotting. They release enzymes that active haemostasis, which is the stoppage of haemorrhage (Nordqvist, 2107).

2.2.3.6.3 Signs and symptoms

TSS is characterized by sudden fever, hypotension, diarrhoea, nausea, vomiting, rash, headache and muscle ache (Higuera, 2016). This condition can cause systematic damage and multi-organ failure. Desquamation of palms and soles occur 1-2 weeks after onset. When the nervous system is involved, the patient may be disorientated and have an altered state of consciousness (Chung & Hudacek, 2016).

2.2.3.6.4 Diagnosis and treatment

In order for a patient to be confirmed to have staphylococcal toxic shock syndrome, the case must meet all of the following diagnostic criteria: fever exceeding 38.9°C, hypotension/shock with systolic blood pressure of less than 90mm Hg despite fluid administration, diffuse macula erythematous rash followed by desquamation, and the involvement of at least three abnormally functioning organ systems (Tong et al., 2015). Urine dipstick can be done for traces of infection. Blood culture is taken to confirm infection and the microbe causing infection. Full blood count may reveal thrombocytopenia (Higuera, 2016).

Treatment of TSS includes identifying and removing the cause of the infection (tampon or wound), antibiotic administrations and surgery (Low, 2013). As TSS is a medical emergency, hospitalization is important (Higuera, 2016). Antibiotics such as clindamycin, vancomycin or daptomycin are given for the treatment of TSS. In cases of severe TSS, intravenous administration of immunoglobulin is necessary (Bush & Perez, 2016).

2.2.3.6.5 Prevalence
Menstrual TSS (mTSS) has been linked with the use of superabsorbent tampons, resulting in an annual infection rate of 13.7% per 100,000 menstruating women in the 1980s. After superabsorbent tampons were removed from the market, the rate of infection drastically decreased (Sharma et al., 2018; Tong et al., 2015).

2.3 Antibiotic therapy

2.3.1 Introduction to antibiotic therapy

The conventional treatment for management and control of bacterial infections is the use of antibiotics (Aminov, 2010). Antibiotics are either bactericidal or bacteriostatic in action. The suffixes ‘cidal’ means to kill and ‘static’ to inhibit; therefore antibiotics can either destroy bacteria or inhibit bacterial growth (Rollins, 2000). They also lessen the time that the infected individual becomes contagious. Antibiotics prevent complications arising from untreated infections. They relieve discomfort associated with infection and speed up healing (Levison, 2016).

2.3.2 History of antibiotics

2.3.1.2 The discovery of sulphonamides

A medical scientist: Paul Ehrlich’s goal was to discover a drug that could target the disease-causing microbes but not the host (Gaynes, 2011). This was based on the observation of selective microbial staining that aniline and other synthetic dyes exerted. In 1904 Ehrlich began a screening program to find a drug against syphilis (Aminov, 2010), a sexually transmitted disease caused by Treponema pallidum. Syphilis was considered incurable at that time (Morris, 2016). Paul, with his team, started synthesizing organoarsenic derivatives of atoxyl, which are amino derivatives of phenylarsonic acid whose amine group is in the 4th position in chemistry, and had them tested in syphilis-infected rabbits (Gaynes, 2011).

In 1909 Paul and his team discovered a 6th compound in the 600th trial which cured the syphilis-infected rabbits and the drug was numbered 606 (Gaynes, 2011). The drug was marketed under the name Salvarsan by Hoechst (Mahoney et al., 1943). The pharmaceutical industry adopted the systematic screening approach which Paul used,
leading to the discovery of a variety of antimicrobial drugs including the sulfonmaide
drugs like sulfonamidochrysoidine (Aminov, 2010).

2.3.2.2 The discovery of penicillin

Bacteriologist Alexander Fleming discovered penicillin in September 3rd 1928 (Gaynes,
2011). He first discovered the antimicrobial properties of mould when he noticed that
there was no bacterial growth in the zones around fungus on agar plates (Fleming,
1929). After 12 years of his initial observation, Fleming involved chemists in resolving
problems with purification and stability of Penicillium (Aminov, 2010), but there was
progress in isolating penicillin as a therapeutic substance. In 1940, the Oxford team
published a paper on the purification of penicillin quantities adequate for clinical testing
(Gaynes, 2011).

The protocol which the oxford team used led to the mass production and distribution of
penicillin in the year 1945. Fleming introduced a screening method using inhibition
zones in bacterial lawns on an agar medium plate and it became widely used as a
screening method for antibiotic-producing microorganisms (Aminov, 2010).

Penicillin is derived from moulds of Penicillium and is obtained by extraction of
submerged cultures grown in a medium. The nucleus of penicillin is 6-aminopenicillanic
acid. This nucleus consists of a thiazolidine ring that is attached to a β-lactam group.
The ring carries a secondary amino group (Bentley, 2004). The structure of penicillin
contains a substituent which determines the main antibacterial and pharmacological
properties of the drug (Waxman & Strominger, 1983).

Figure 2.5 The general structure of penicillin (TutorVista, 2018).

2.3.2.3 Antibiotic therapy for S. pyogenes
There is a wide range of antibiotics available for the treatment of Streptococcal infections. Some of the antibiotics used for treating streptococcal infections include levofoxacin, moxifloxacin, cefuroxime, vancomycin, tetracycline, azithromycin, pefloxacin, cephalexin, cefepime ciprofloxacin, erythromycin, amoxicillin, and penicillin (Bush & Perez, 2016).

2.3.2.4 Antibiotic therapy for \textit{S. aureus}

\textit{S. aureus} is treated with antibiotics such as methicillin, nafcillin, oxacillin, dicloxacillin, flucloxacillin (Crossely, 2009). Linezolid is the first antibiotic that has showed to have broad \textit{in-vitro} activity against antibiotic-resistant Gram-positive bacteria. It prevents the formation of the 70S initiation complex, therefore inhibiting bacterial protein synthesis (Stevens \textit{et al.}, 2002).

2.3.3 \textbf{Cefepime hydrochloride monohydrate}

Cefepime is a fourth generation cephalosporin. It is used as a broad-spectrum antibiotic with an efficacious activity against most gram-negative and gram-positive bacteria. Cefepime is resistant to hydrolysis by common plasmid and/or chromosomally-mediated \(\beta\)-lactamases (Shah \textit{et al.}, 2016). It is proven to be effective in the treatment of a variety of infections, including pneumonia, urinary tract infections, skin and soft tissue infections, intra-abdominal infections, as well as febrile neutropenia (Schlecht, 2015).

Cefepime may have adverse effects such as allergic reactions, muscle twitching, or dizziness; and should therefore be used with caution (Thompson & Jacobs, 1993). Cefepime has been shown to have good activity against both gram positive and gram negative bacteria (Okomo\textit{to et al.}, 1994).

2.3.3.1 Mechanism of action

Cefepime is a \(\beta\)-lactam and it inhibits the biosynthesis of the bacterial cell wall by attaching to penicillin-binding-proteins (PBPs), and disrupts the final transpeptidation step of peptidoglycan formation (Meroueh \textit{et al.}, 2006). Bacteria that are exposed to a concentration of cefepime higher than their microdilution inhibitory concentration (MIC) break down due to the inhibition of PBPs and the progressing activity of the cell wall autolytic enzymes (Endimiani \textit{et al.}, 2008).
The methylpyrrolidinium group of this antimicrobial agent confers a zwitterionic charge that promotes bactericidal activity by fast penetration through the spores in the outer membrane of bacteria (Nikaido et al., 2008).

2.3.3.2 Clinical management and dosage

Cefepime is usually given intravenously or intramuscularly in doses of 0.5 to 2 g. The intravenous dose is diluted in 50/100 ml of a compatible intravenous fluid. For moderately severe infections such as pneumonia, 1 to 2 g of cefepime is administered twice daily for 10 days. For mild to moderate urinary tract infections, 0.5 to 2 g is given intravenously twice a day for 7 to 10 days. For moderate and severe skin and soft tissue infections due to S. aureus or S. pyogenes, 2 g of cefepime is given every 12 hours for 10 days. Intra-abdominal infections require 2 g to be administered twice daily for a minimum of 7 days (Endimiani et al., 2008).

2.3.3.3 Pharmacokinetics and metabolism

The concentration and distribution of cefepime in a patient is dependent on the kind of infection as well as the overall health condition the patient (Endimiani et al., 2008). In people with normal serum creatinine levels, the mean plasma cefepime concentration after 12 hours from the initial dose is about 3.2 mg/l and the mean half-life is 2.5 hours; however, the plasma drug concentration varies for each person (Lipman et al., 1999). The average volume of distribution of cefepime is 18.0 to 20.1 mg/l. Serum protein binding is about 20%. The total body clearance ranges between 122-136 ml/min and the mean renal clearance is 105 ml/min (Sampol, et al., 2000; Endimiani et al., 2008).

Cefepime is excreted by the kidneys as the unchanged active drug (Endimiani et al., 2008; Van der Auwera & Santella, 1993). Urinary recovery of the unchanged drug is 80% of the administered drug. In a low dose (0.25 g) given intravenously, the concentration of cefepime in urine is 190 mg/l during the 2 hours post administration (Barbhaiya et al., 1990). This concentration decreases to 90 mg/l after 8 hours of administration. Ten to twenty percent of a dose of cefepime is metabolized to N-methylpyrrolidine (NMP) and is then converted to N-oxide (NMP-N-oxide) (Van der Auwera & Santella, 1993).
2.3.3.4 Toxicity and adverse effects

Like most antibiotic therapies, cefepime is associated with side effects in sensitive individuals. This may lead to decreased compliance, thus promoting drug resistant bacterial strains (Bhullar et al., 2012). The most common adverse effects from the use of cefepime include diarrhoea, skin rash, bleeding, nausea, vomiting, headaches, and candidiasis (Endimiani et al., 2008; Thompson & Jacobs, 1993).

Cefepime has been linked to a higher mortality rate due to toxicity (Yahav et al., 2007). Studies have attributed this to the drugs’ ability to supress the inhibitory neurotransmission via a concentration-dependent modulation of the gamma amino-butyric acid (GABA) receptors (Sugimoto et al., 2003). Drug-related neurological signs and symptoms such as encephalopathy, decreased consciousness, hallucinations, cognitive disturbances, confusion, myoclonia and seizures have been reported in patients with severe kidney dysfunction (Lamoth et al., 2010).

Toxicity occurs mostly in patients with renal disease as creatinine clearance is impaired. This also interferes with the clearance of cefepime (Barbhaiya et al., 1990; Tam et al., 2003). It is recommended by the manufacturer of the drug to adjust the dose if the glomerular filtration rate (GFR) is below 50 l/min (Tam et al., 2003); however dose adjustments becomes difficult for patients with life threatening infections as they require maximal doses of the drug (Lamoth et al., 2010).

2.4 Antibiotic resistance

Antibiotic resistance occurs when bacteria change the way they respond to antibiotics by resisting the effect of the drugs (CDC, 2017). It has become a global challenge in the health care systems as it leads to higher medical costs, prolonged hospital stays and increased mortality rates (WHO, 2015; Michael et al., 2014). Main factors contributing to antibiotic resistance include the bacteria’s ability to change its structure, as well as the overuse and misuse of antibiotics. The pharmaceutical industry’s lack of new drug developments also contributes to this global challenge (Ventola, 2015).
Health Organization (WHO) fact sheet, (October 2015), it is mentioned that the challenge with antibiotic resistance is threatening the achievements of modern medicine and that this may be a post-antibiotic era in which even common infections can be fatal (WHO, 2015).

Some of the bacterial strains which are already resistant to antibiotics include Mycobacterium tuberculosis, Neisseria gonorrhoeae, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, S. pyogenes and some species of Enterobacter, Salmonella and Shigella. S. aureus became resistant to methicillin two years after it was introduced in 1960 (Nathan & Cars, 2014; CDC, 2013).

Multi drug resistant (MDR) bacterial strains are present in every continent and in nearly all hospitals worldwide. There is a variation in the incidence of these strains and the infections they cause. MDR bacteria are responsible for about two-thirds of all health care associated infections (Bax & Griffin, 2012).

According to Aminov (2010), there are approximately 25 000 patients who die from infections with MDR strains in the European Union annually, while more than 63 000 patients in the United States die annually from hospital acquired bacterial infections.

There has been a widespread antimicrobial resistance in GAS (Bozdogan et al., 2003) and this has been linked to the unnecessary usage of the antibiotic (Betriu et al., 2002). Recent reports also show that tetracycline has become less effective in the treatment of streptococcal infections (Ray et al., 2016).

S. aureus is the major disease causing agent of most hospital infections. Methicillin has been the drug of choice against the bacteria, but recent studies have revealed that about 45-50% of the strains that clinically manifest in human are now resistant to methicillin (Dormanesh et al., 2015). This is due to inappropriate antibiotic usage, decreased antibiotic compliance, biofilm formation, and the mutation of bacterial strains (Ventola, 2015).

2.4.1 Inappropriate antibiotic usage

World Health Organization (2015) stated that resistance develops more rapidly through the overuse and misuse of antibiotics (WHO, 2015). Inappropriate antibiotic prescribing
by health care contributes to emergence of drug resistant strains (Tangcharoensathien et al., 2018); this could be due to the lack of awareness on limited use of restricted antibiotic classes (Chaves et al., 2014), and antibiotic prescription prior or in absence of culture tests for sensitivity (Tangcharoensathien et al., 2018).

The patients’ lack of knowledge on, and demand for antibiotics play a role in antibiotic resistance (García et al., 2011). The prevalence of non-prescription antibiotics poses a challenge as consumers use the easily accessible over the counter antibiotics even for viral infections (Larson et al., 2003).

Foodborne transmission of drug resistant bacterial strains from animals to people is increasing as agricultural sectors continue to inappropriately use antibiotics in animals (WHO, 2015). A study carried out in 25 European countries showed that 44.3% of veterinarians seldom perform laboratory sensitivity tests for bacterial diagnosis before prescription (De Briyne et al., 2013). Most farmers have limited knowledge and understand of antibiotic action and indication, leading to increased antibiotic misuse and overuse (Om & McLaws, 2016).

2.4.2 Decreased antibiotic compliance

Decreased antibiotic compliance is partly due to associated side effects (Ventola, 2015); which include skin rash, haemorrhaging, nausea, vomiting, headaches, and candidiasis (Bhullar, et al., 2012).

Poverty is a major factor contributing to decreased compliance to treatment. Limited resources is directly proportional to access to proper health care; including medication and information, thus leading to decreased compliance to treatment (WorldBank, 2014).

2.4.3 Bacterial gene mutation

Mutation is heritable changes in the DNA sequence. These genetic changes may, or may not alter the phenotype of organism (Snyder & Champess, 2003). Some antibiotics inhibit bacterial growth; however, bacterial cells that have stopped growing are not sensitive to bacteriostatic agents. This allows the non-growing mutants to survive (Habibi Najafi & Pezeshki, 2013). Subinhibitory concentrations of antibiotics provoke changes in cellular transcriptions and trigger different cellular responses in species of bacteria (Gutierrez et al., 2013). Low concentrations of antibiotics also induce genetic
transformability as well as the expression of virulence genes such as toxins and adhesins in *Escherichia coli* and *Staphylococcus aureus*, thus facilitating antibiotic resistance through horizontal gene transfer (Doss *et al.*, 1993).

### 2.4.4 Biofilm formation

Biofilms are multicellular complexes held together by an extracellular matrix produced by bacteria (López *et al.*, 2010). These complexes allow bacterial cells to grow in multicellular aggregates, which are encased within the extracellular matrix (Hall-Stoodley & Stoodley, 2009). Biofilms can form in natural, medical devices or industrial settings and they contribute to bacteria becoming resistant to antimicrobials (López *et al.*, 2010).

### 2.5 Natural antibiotic resistance

This is known as intrinsic resistance, which is the innate ability of bacteria to resist the effect of antibiotics through certain morphological characteristics (Walsh, 2000). Soon after the introduction of antibiotics, bacterial strains developed resistance, not only through gene mutations in the target of antibiotics, but by acquiring genes conferring antimicrobial resistance (Martínez, 2012). Natural ecosystems, including the human gastro-intestinal tract, contain various elements that can confer resistance upon transfer to a new host (Sommer *et al.*, 2009). The gene conferring resistance in a human pathogen does not confer resistance in its original host (Martínez, 2012). The gene conferring resistance evolved in their original host to function differently than resisting antimicrobial activity in natural ecosystems; that is supported by the findings that several proteins involved in bacterial physiology contribute to intrinsic resistance to antibiotics (Laskaris *et al.*, 2010; Linares *et al.*, 2010).

### 2.6 Complementary medicine

Complementary medicine (CM) refers to groups of healing and diagnostics disciplines that are separate from conventional health care (Barnes & Bloom, 2008). According to the Therapeutic Goods Administration (TGA) of Australia (2018), CM is also known as traditional or alternative medicine, and it includes the use of vitamin, mineral, herbal, aromatherapy and homoeopathic products (TAG, 2018). The Medicines Control Council
(MCC), now South African Health Products Regulatory Authority (SAHPRA), defines CM as any substance that originates from plants, animals or minerals; used, manufactured of sold to treat disease or supplement health and include Health Supplements or Discipline-Specific medicines, which are; used according to identified disciplines including: Aromatherapy, Ayurveda, Homeopathy, Traditional Chinese Medicine, Unani Tibb and Western Herbal Medicine (SAHPRA, 2018). CM may also be defined by to include professional practices such as Chinese Medicine and Acupuncture, Homeopathy, Ayurveda, Chiropractic, Naturopathy, Osteopathy, Phytotherapy, Therapeutic Aromatherapy, Unani-Tibb, Therapeutic Massage Therapy, and Reflexology (HPASA, 2013).

Most people using CM seek to improve general health and wellbeing, and also to relieve symptoms associated with chronic ailments and ailments caused by use of conventional medicine (Wolsko et al., 2002).

2.5.3 Colloidal silver

Colloidal silver is a suspension of metallic silver nanoparticles in a colloidal base which deactivates enzymes responsible for bacterial replication and metabolism (Iroha et al., 2007). To form colloidal silver, the concentration of silver in the form of nanoparticles must be over 50% of the total silver content, this includes both ionic silver and silver nanoparticles (Silver colloids, 2015).

It is formed by electrolysis; a process by which electric current is passed through a substance to produce a chemical change. A pure silver element; which is attached to simple molecules of proteins, is suspended into distilled water to form silver colloid nanoparticles (Sun, 2013).

2.5.3.1 Different types of silver

2.5.3.2.1 Colloidal silver

Colloidal silver is made up of metallic silver nanoparticles suspended in distilled water. Colloidal silver nanoparticles are complete and do not react with other elements (Silver colloids, 2015).
2.5.3.2.2 Ionic silver

Ionic silver consists of silver ions and water, and does not contain silver particles (Silver colloids, 2015). The ionic form of silver is highly reactive and it combines with other chlorides in the body to form silver chloride (Peters, 2013).

2.5.3.2.3 Metallic silver

Metallic silver refers to silver particles found in colloidal silver (Peters, 2013). These particles are in a complete atomic structure and therefore do not react with other elements in the body (Silver colloids, 2015).

2.5.3.2 Colloidal silver uses as treatment

Between the years 1900 and 1940, silver was primarily used as an antibiotic in medical practice by a few people. Ancient physicians prepared silver medicines in their offices and they were either administered orally or injected (Richards, 2016).

Colloidal silver is not a product of any CM disciplines, but it is widely used for its anti-bacterial and anti-viral properties by CM practitioners, including homeopaths and phytotherapists. Its use dates back to the times before the discovery of penicillin and other conventional antibiotics (Kheybari, 2010).

There are various silver-based preparations available for effective management of infections, including topical cream and gel, as well as liquid for oral use. Topical colloidal silver preparations (10-30 ppm) or silver coated implants have been recommended for preventive and therapeutic purposes against biofilm formation (Dharmshaktu et al., 2016). The active ions which are released by silver bind and exert an inhibitory effect over various bacterial cells. The sulphydryl group and nucleic acid are sites of action where ions bind (Chaloupka et al., 2010).

2.5.3.3 Safety of colloidal silver

2.5.3.3.1 Manufacturers

In 1999, the United States Food and Drug Administration (FDA) issued a final rule to declare that all over the counter (OTC) ingredients containing colloidal silver for either
internal or external use are not recognized as safe and effective due to lack of substantial scientific evidence supporting the claims that it works (FDA, 1999).

Smith. (2010) mentions that there are only 3 reputable manufacturers of colloidal silver in South Africa, including Phuza health, Silverlab and Biosil; however, their products have not been registered with the South African Medicines Control Council, therefore the safety of colloidal silver remains unknown.

Silverlab manufactures its products in accordance with the Good Manufacturing Practices (GMP) and International Organization for Standardization (ISO) 9001 standards and principles. GMP refers to standards of manufacturing that guarantees reproducibility of products to set specifications (WHO, 2018; Jadhav, 2013). ISO refers to the standard specifications for management of the manufacturer (Jadhav, 2013).

2.5.3.3.2 Upper safe limits

According to Smith (2010), a man weighing 70 kg can use 1388 litres of 18 ppm ionized silver in his life time; this would lead the quarter or halfway mark of developing argyria.

The Silver Safety Council has developed a formula for determining maximum safety daily dosages of colloidal silver. In the formula, body weight is multiplied by 12 and the total is divided by the concentration of colloidal silver used in ppm, this will give the total number of colloidal silver drops one can take daily without developing argyria (Barwick, 2013).

The environmental protection agency (EPA) integrated risk information system (IRIS) listed the safety information of silver. Safety is assessed in oral reference dose (RfD), which is based on the assumption that all substances have a threshold in which toxicity is likely to occurs (Pollack, 2017; Watson et al., 2009). RfD is measured in units of ml/kg-day (Pollack, 2017). Table 2.1 below shows the safe doses (ml/teaspoon) of colloidal silver 10 ppm per bodyweight (kg/lbs) daily.

2.5.3.3.3 Argyria

Argyria is a rare condition characterized by a permanent bluish discolouration of the skin. It is associated with prolonged ingestion of silver compounds (White et al., 2003; Owen, 2013). Generalized argyria occurs as a result of increased serum silver levels
following prolonged ingestion of various forms of silver. Generally, argyria requires oral doses of 6 g or intravenous administration of 1 g of silver daily (Chang et al., 2006).

The typical signs and symptoms include blue skin pigmentation, which intensifies in sunlight, bluish discolouration of the mucous membranes, the hair may have a metallic appearance. Silver particles may accumulate in and affect internal vital organs such as liver, kidneys and spleen (Cho et al., 2008; Molina-Hernandez et al, 2015).

Table 2.1 Safe level of colloidal silver ingestion in ml/kg daily

<table>
<thead>
<tr>
<th>Bodyweight</th>
<th>RfD’</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg</td>
<td>lbs</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>20</td>
<td>44.1</td>
</tr>
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<td>286.6</td>
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<td>140</td>
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<tr>
<td>150</td>
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<tr>
<td>160</td>
<td>352.7</td>
</tr>
<tr>
<td>170</td>
<td>374.8</td>
</tr>
<tr>
<td>180</td>
<td>396.8</td>
</tr>
</tbody>
</table>

2.6 Related studies

Kheybari et al (2010) carried out a study to evaluate the synthesis and antimicrobial effects of silver nanoparticles produced by a chemical reduction method. Two forms of colloidal silver nanoparticles were prepared using the one-step synthetic method with Ethylene glycol and glucose used as reducing agents. The antimicrobial effect of the silver nanoparticles was determined using the minimum inhibitory concentration. Bacterial strains used in this study were S. aureus, Staphylococcus epidermidis, Escherichia coli and Pseudomonas aeruginosa. The results showed that the silver nanoparticles in colloidal solution have high antibacterial activity against both gram positive and gram negative bacteria.
A research project on the antibacterial activity of colloidal silver nanoparticles was conducted by Lkhagvajav et al. (2011). Colloidal silver nanoparticles were prepared by a sol-gel method and they were tested on various strains of bacteria and fungi. MIC was determined using the broth microdilution method. Results showed that the silver nanoparticles have an inhibitory activity on the growth and multiplication of *Escherichia coli*, *S. aureus*, *Candida albicans*, *Bacillus subtilis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Ihora et al. (2007) used the agar dilution method to investigate the antibacterial effect of colloidal silver 5 ppm and 20 ppm against *E. coli* and *S. aureus*. They used the nutrient broth and the nutrient agar as media and the results demonstrated that the bacteria tests are susceptible to colloidal silver.

Conception et al. (2007) compared the antimicrobial potency of colloidal silver (10 ppm, 20 ppm and 30 ppm) with the antibiotic eye drops against *Escherichia coli*, *S. aureus*, *Staphylococcus epidermis*, and *Bacillus subtilis* using the antibacterial-activity testing (ABAT) and Kirby Bauer methods. The Kirby Bauer disc diffusion method demonstrated that 30 ppm colloidal silver had antibacterial effect against the bacteria used, with the zones of inhibition greater than that of the antibiotics tested.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Stock cultures

Reference strains of *Streptococcus pyogenes* (ATCC 12384) and *Staphylococcus aureus* (ATCC Baa-1026) were purchased from Davies Diagnostics in a lyophilised form. The strains were stored at 4-5°C and reconstituted in sterile saline water as required.

3.1.2 Media, buffers and consumables

A summary of all the media, buffers, chemicals and consumables used are summarised in Table 3.1 below. All reagents were used as received and used without any further purification or modification.

Table 3.1 Summary of media, reagents and chemicals used for the experimental work.

<table>
<thead>
<tr>
<th>Description</th>
<th>Catalogue number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsensitized tubes</td>
<td>69285</td>
<td>Biomerieux</td>
</tr>
<tr>
<td>Saline solution</td>
<td>V1204</td>
<td>Biomerieux</td>
</tr>
<tr>
<td>Mueller Hinton agar</td>
<td>CM0337B</td>
<td>Laboratory Specialities (PTY) LTD T/A Thermo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fisher Scientific (Oxoid)</td>
</tr>
<tr>
<td>Petri dish 90mm</td>
<td>1C097</td>
<td>Clinical Science Diagnostics</td>
</tr>
<tr>
<td>96-well microplate with lid</td>
<td>734-2097</td>
<td>Monitoring &amp; Control Laboratories (Nunc)</td>
</tr>
<tr>
<td>Swabs PS cotton in PP tube sterile</td>
<td>300261</td>
<td>Merck Chemicals (Deltalab)</td>
</tr>
<tr>
<td>P-Iodonitrotetrazolium violet</td>
<td>I8377</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Neomycin sulfate hydrate</td>
<td>J61499.14</td>
<td>Monitoring &amp; Control Laboratories</td>
</tr>
<tr>
<td>Cefepime hydrochloride monohydrate</td>
<td>J66237.03</td>
<td>Monitoring &amp; Control Laboratories</td>
</tr>
<tr>
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<td>Laboratory Specialities (PTY) LTD T/A Thermo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fisher Scientific (Oxoid)</td>
</tr>
<tr>
<td>Inoculating loops, 10ul</td>
<td>612-9354</td>
<td>Monitoring &amp; Control Laboratories (VWR)</td>
</tr>
</tbody>
</table>
3.1.3 Conventional medication - Cefepime

Cefepime hydrochloride monohydrate was used as positive control against both the \textit{S. aureus} and \textit{S. pyogenes} strains during the experiments.

3.1.4 Complementary medication - Colloidal silver

Two different concentrations of colloidal silver (18 ppm and 20 ppm) were used during the experiments. The 18 ppm colloidal silver (Silver lab) contains ionic colloidal silver in deionized purified water. The 20 ppm colloidal silver (BIO-SIL) contains ISO 3696 distilled water and fine silver. The treatment was stored below 25°C away from direct sunlight. The concentrations of colloidal silver used were chosen based on the fact that the reputable manufacturers, Silverlab and Bio-sil, only produce 18 and 20 ppm respectively.

3.2 Methods

3.2.1 Aseptic techniques

Aseptic techniques were adhered too, to ensure that no contamination takes place, thus ensuring reliability of results.

3.2.2 Growth and maintenance of bacteria

The stock culture arrived freeze dried in a vial/container and needed to be hydrated before use. The unopened vial was taken out of the storage and allowed to equilibrate to room temperature. The vial was opened and sterile forceps used to remove one pellet of bacteria without removing the desiccant. Immediately, the vial was recapped and returned to the storage of 4-5°C. The pellet of bacteria was inoculated into a sterile flask containing 100 ml saline solution. A sterile swab was used to crush the pellet and it was heavily saturated in the hydrated suspension.
The saturated swab was used to inoculate the Mueller-Hinton agar culture plate, with the swab streaked over one-third of the plate. Inoculations were placed in an incubator for 24 hours at 35°C.

The streak plate method was used to subculture \textit{S. pyogenes} and \textit{S. aureus} onto Mueller-Hinton agar plates. Fresh cultures were prepared for each experiment.

A sterile cotton swab was used to transfer bacteria onto agar plates for subculturing. Bacteria were spread onto plates in a zigzag pattern using sterile plastic inoculating loops. A new loop was used between each step, and the used swabs and loops were discarded for safe disposal. The agar plates were then incubated for 24 hours at 35°C and used to prepare the bacterial suspension required for the experiments.

\subsection*{3.2.3 Preparation of media}

Mueller-Hinton broth was used as media in the study. It consists of beef infusion from casein acid hydrolysate and starch.

To prepare the media, 21 g of the powder was suspended in 1 000 ml distilled water. This suspension was heated to boil to dissolve the medium. For sterilization, the solution was autoclaved at 121°C for 15 minutes. The media was cooled to about 50°C and poured into sterile plastic petri dishes. After solidification of the agar, the plates were stored at 4°C.

\subsection*{3.2.4 Preparation of the Iodonitrotetrazolium chloride solution}

A 0.4 mg/ml working solution of the Iodonitrotetrazolium chloride (INT) was prepared using sterile water and stored at 4°C until needed. The solution was used in the sections below.

\subsection*{3.2.5 Disc diffusion method}

This method was used to determine if the solutions had any antibacterial properties. Bacterial strains were prepared and grown as described in section 3.2.2 and used to create the bacterial lawns on the Mueller-Hinton agar plates. The bacterial lawns were created by transferring colonies from the agar plate with subcultured bacteria into the test tube containing saline. The bacterial suspension was adjusted with saline to reach
a solution correlating to a 0.5 McFarland standard. Saline from the test tube was used for adjustments where necessary. A sterile cotton swab was immersed in this suspension and the total area of the agar plate covered with the bacterial suspension.

Sterile discs were placed onto the media with sterile forceps according to pre-designed grid to allow the placement of multiple discs on each plate. Following this, 20 µl of each test solution, blank control (sterile water added), solution control (saline solution) and positive control (Cefepime).

These plates were incubated for 24 hours at 35°C. Following incubation, the plates were taken out and placed on a sterile working surface. To assist in the visualization of the bacterial-inhibition, an agarose overlay containing INT (final electron acceptor) was added onto the plates. The INT overlay was prepared by mixing it with 1% (w/v) agarose solution (prepared in Tris-Acetic-EDTA buffer, pH 8.3), to a final INT concentration 0.2 mg/ml. The plates were incubated at 37°C until a bright pink or dark pink colour developed, indicating bacterial growth, i.e. no bacterial inhibition.

3.2.6 Microdilution method

Microdilution method was used as an antibiotic susceptibility test to determine the sensitivity of the bacteria (S. aureus and S. pyogenes) against colloidal silver. The bacterial suspension used for this method, corresponding to a 0.5 McFarland standard, was prepared as described above. The 96 well plates were divided into two halves to accommodate the two bacteria used.

The first half (1-6) was used for S. aureus and the second half (7-12) was for S. pyogenes.

All wells were filled with 100 µl Mueller Hinton broth. Fifty microliter of either test compound, cefepime (positive control), media (media control to test sterility), water (negative or growth control) were added to the first well in each row. The compounds added were serially diluted in the next well by transferring 50 µl from the first well, mixing properly, and by transferring 50 µl into the next well. This was continued to the last well (6th well) and the last 50 µl removed was discarded. Lastly, 50 µl of the required bacterial suspension was added to each of the wells, excluding the media control wells that received 50 µl saline solution.
The plates were then closed and incubated for 24 hours at 35°C.

Following incubation, 100 µl of the INT solution was added to each well and incubated for 30 minutes at 35°C. The development of a bright pink or dark pink colour indicated bacterial growth, i.e. no bacterial inhibition.

**3.2.7 Ethical considerations**

3.2.7.1 Experimental study approval

The research study was carried out after permission was granted by The Faculty Academic Ethics Committee, as shown in Annexure A. The experiments were done at the University of Johannesburg’s Water and Health Research Centre, Doornfontein Campus, under supervision of a qualified laboratory technician and with permission granted (Annexure B).

3.2.7.2 Laboratory protection and safety

Upon entering the laboratory, hygiene measures, like washing hands, cleaning working area surfaces and sterilizing instruments with ethanol, were taken.

A laboratory coat, hand gloves and body covering clothing and shoes were worn during laboratory experiments for protection of self from accidental spills.

Instrumentation and material used were handled away from self and laboratory technician for protection. Inflammable substances were used with caution.

All laboratory instruments used during experiments were washed at the end of each experiment. Disposables were discarded in relevant bins provided by the laboratory technician after use.

3.2.7.3 Procedure, reliability and validity measures

Aseptic techniques were adhered too, as discussed in section 3.2.1. The experimental methods are discussed in section 3.2.1 to 3.2.6 above.

Only proper functioning instruments and laboratory utensils were used. Instruments and utensils, as well as working surface were sterilized with ethanol. Proper functioning
laboratory incubators and refrigerators were used to ensure there were no unwanted alterations to bacteria.

Cefepime hydrochloride monohydrate, McFarland standard, as well as the INT salt was obtained from Sigma-Aldrich; which is well known and trusted to abide by rules and regulations set for quality control. The experiment was supervised by a qualified laboratory technician to ensure that everything was done correctly.

Agar disc diffusion method and microdilution methods were both used to ensure reliability of results.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Agar disc diffusion method

The agar disc diffusion method, also known as the Kirby Bauer disc diffusion method, was used to test if the two compounds could inhibit bacterial growth for both *S. aureus* and *S. pyogenes*. The results were visually inspected, and zones of inhibition were measured. Examples of typical results obtained for both bacteria is shown in Figures 4.1.

![Figure 4.1](image)

Figure 4.1  Agar plates showing typical results obtained for the colloidal silver solutions against *S. pyogenes* (i) and *S. aureus* (ii), as well as bacterial lawns using the agar disc diffusion method.

The quarters in figure 4.1 represent the 18 ppm colloidal silver solution (A), 20 ppm colloidal silver solution (B), Cefepime control (C) and distilled water control (D). By visual inspection of the result images in figure 4.1, both concentrations of colloidal silver inhibited the bacterial growth of *S. pyogenes*, but not that of *S. aureus*; Cefepime control antibiotic exhibited an inhibitory effect on both bacteria used; and distilled water control showed no effect against both *S. pyogenes* and *S. aureus*.

A summary of the results obtained from the three repeats experiments of the agar disc diffusion method is shown in Table 4.1 below. The zones of inhibition were measured...
(mm) and recorded for each colloidal silver compound (18 and 20 ppm) against \textit{S. aureus} and \textit{S. pyogenes} for the three repeat experiments done.

### Table 4.1 Summary of results obtained for the three experiments testing the two compounds (18 ppm and 20 ppm) using the agar disc diffusion method.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Controls</th>
<th>18 ppm Colloidal silver</th>
<th>20 ppm Colloidal silver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Zone (mm)</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>0 mm</td>
<td>12 mm</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Streptococcus pyogenes}</td>
<td>0 mm</td>
<td>12 mm</td>
<td>+</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Resistant to the test compound; \textsuperscript{2}Sensitive to the test compound

As expected the positive antibiotic control (Cefepime) inhibited the growth of both bacteria as a clear zone of inhibition was observed in quadrants C of figures 4.1 (i) and (i). The results showed that the \textit{S. pyogenes} strain was sensitive to the two test compounds used, as seen in figure 4.1 (i), quadrants A and B. Both 18 and 20 ppm had an inhibitory effect on \textit{S. pyogenes}, with 9 and 8 mm zones of inhibition respectively, as shown in table 4.1. Neither concentration of the test compound was effective on \textit{S. aureus} as no zone of inhibition was observed in table 4.1 and figure 4.1, indicating bacterial growth.

\textit{S. aureus} was resistant to colloidal silver. Various factors can influence the results obtained. This can include the method used, the concentration, the shape and stabilization of colloidal silver used, as well as the natural or acquired resistance to the compound tested.

#### 4.1.1 Antibiotic resistance

\textit{S. aureus} is more virulent than \textit{S. pyogenes} (Acharya, 2015), and it was resistant to both concentrations of colloidal silver used, as seen in table 4.1 and quadrants A and B of figure 4.1 (i). It could be that it may have adapted to the experimental environment.
and developed resistance. Genetic mutation of the strain could have occurred, resulting in resistance.

Gutierrez et al. (2013) mentioned that Subinhibitory concentrations of antimicrobials may provoke cellular changes in bacteria. Both 18 and 20 ppm colloidal silver concentrations might have been too low that they caused *S. aureus* to change its structure to adapt to the environment and develop resistance to colloidal silver.

Doss et al. (1993) further confirms that low concentrations of antibiotics also induce genetic transformation as well as the expression of virulence genes, thus exhibiting resistance to antimicrobials.

### 4.1.2 The concentration of colloidal silver.

The concentration of colloidal silver used could have been too low to exhibit an effect on *S. aureus*. Unlike *S. pyogenes*, *S. aureus* contains a catalase enzyme and it is not fastidious, as it does not need enriched media to grow (Acharya, 2015). *S. aureus* is more virulent than *S. pyogenes*, therefore, higher concentrations of colloidal silver could exhibit an inhibitory effect. The study published by Concepcion et al. (2007) showed that with their colloidal solver mixtures only higher concentrations of colloidal silver (30 ppm) were effective against *S. aureus*, suggesting that the two compounds tested in this study may have had too low a concentration of the active compound. Colloidal silver 18 ppm and 20 ppm were chosen based on the availability by manufacturing companies (Silverlab and Biosil), and on the fact that no studies are known to have been done using these concentrations.

Ihora et al (2007) conducted a similar study using 5 ppm and 20 ppm of colloidal silver against *E. coli* and *S. aureus* with the agar diffusion method and showed that the bacterial strains tested were susceptible to the treatment. Conversely, a study published in the *Journal of Wound Care* demonstrated that a colloidal silver solution at concentrations ranging from 22 ppm and 403 to 413 ppm, showed no effect against various bacteria, including *S. aureus* and *S. pyogenes in vitro* (Ahmed et al., 2013). This may be that different reference strains, or even clinical strains have different virulence factors (Bax & Griffin, 2012), yielding different experimental results.

### 4.1.3 The choice of method
Concepcion et al. (2007) showed that the choice of method is also important. The team evaluated the antimicrobial activity of colloidal silver (10 ppm, 20 ppm, and 30 ppm) compared with ophthalmic antibiotics including trobamycin, lomefloxin, ampicillin, and moxifloxacin. These were tested against Escherichia coli, S. aureus, Staphylococcus epidermis, and Bacillus subtilis using the antibacterial-activity testing (ABAT) and Kirby Bauer methods. The Kirby Bauer disc diffusion method (agar disc diffusion method) demonstrated that the bacteria tested was sensitive to 30 ppm colloidal silver, with the zone of inhibition more pronounced than that of trobamycin and ampicillin against S. aureus. No zone of inhibition was noted for all three concentrations of colloidal silver on all bacteria for the ABAT method, highlighting the need for using the correct methods.

In this study, agar disc diffusion method was used with 18 and 20 ppm colloidal silver. Concepcion et al. (2007) in their study found only 30 ppm to be effective in inhibiting bacterial growth. This again indicates the importance of the concentration used (section 4.1.2) as well as the use of the correct method.

**4.1.4 The shape and stabilization of compound**

The shape and stabilization of the colloidal silver, when prepared e.g. as nanoparticles (AgNPs), could also have an impact on the bacterial growth and resistance. This was studied using bacterial biofilms of S. aureus, methicillin-resistant S. aureus, and Pseudomonas aeruginosa; bacterial biofilms are responsible for various bacterial infections. AgNPs exhibited an antibiofilm activity against the bacteria (Richter et al., 2017).

Similarly, Matthew & Kuriakose studied the antimicrobial properties of AgNPs encapsulated bovine serum albumin. The AgNPs stabilized by bovine serum albumin showed antibacterial activity against S. aureus, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumonia (Matthew & Kuriakose, 2012).

**4.2 Microdilution method**

This method was used for confirmatory purposes as results were obtained with agar disc diffusion method. Figure 4.2 below shows the typical 96 well plate layout used to test the compounds using the microdilution method. The dilutions, stains and well
composition is shown in the figure. The 18 ppm colloidal silver solution is shown as C1 and 20 ppm colloidal silver solution as C2.

Wells that changed colour from clear to purple after the addition of the INP showed growth and thus no inhibition of bacterial growth by treatment compounds. The wells with an unchanged clear colour after addition of test compound show bacterial inhibition by the compound.

**Figure 4.2** The typical microdilution method results of 96 well plate layout used to test the compounds using the microdilution method.

The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) for the compounds against the two bacterial strains. The results obtained with the microdilution method confirmed that both the 18 ppm and 20 ppm colloidal silver solution had no effect on the *S. aureus* strain as the colour turned from clear to purple; however, the colloidal silver 20ppm demonstrated an inhibitory effect of *S. pyogenes* as the colour remained.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The results demonstrated that *S. pyogenes* growth was inhibited by both the 18 ppm and 20 ppm colloidal silver solutions tested and which was confirmed by agar disc diffusion method and microdilution method. None of the colloidal silver solutions had any inhibitory effect on the *S. aureus* strain used. As discussed in section 4.1.1 and 4.1.2, either the *S. aureus* strain could have developed resistance to the colloidal silver solutions, or the colloidal silver solution may have had too low concentration of the active compound to be effective against this strain.

In conclusion, colloidal silver (18 ppm and 20 ppm) have an anti-bacterial effect on *S. pyogenes*, but not on *S. aureus*. This research has added to the developing volume of information to better understand the antibacterial effect and efficiency of colloidal silver mixtures.

5.2 Recommendations

Further research needs to be conducted to determine whether or not the obtained results are conclusive.

Researchers should conduct various susceptibility techniques including the broth dilution method, agar dilution method and ABAT. These techniques should be used as comparing techniques.
Research should be conducted on a various bacterial strains employing a wider range of colloidal silver concentrations. The results will reveal the most effective colloidal silver concentration as well as the susceptible bacterial strain.

Repeat studies on *S. pyogenes* using various susceptibility techniques and colloidal silver concentrations to confirm the inhibitory antibacterial of colloidal silver.

Colloidal silver nanoparticles have demonstrated antibiofilm activity against various bacteria. More research should be invested in studying the effect of colloidal silver solutions against bacterial biofilms. This may help in improving the treatment of bacterial infections, especially since we are encountering challenges with antibiotic resistant strains.
CHAPTER SIX

REFERENCES


Chaves, N.J., Cheng, A.C., Runnegar, N., Kirschner, J., Lee, T., Buisng, K.(2014). Analysis of knowledge and attitude surveys to identify barriers and enablers of
appropriate antimicrobial prescribing in three Australian tertiary hospitals. *Internal Medicine Journal*, 44(6), pp. 568-574.


ANNEXURES

Annexure A

Permission by The Faculty Academic Ethics Committee
Annexure B

Permission by the Water and Health Research Centre