ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF DIABETIC FOOT ULCERS IN SOUTH AFRICAN PUBLIC HOSPITALS

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Declaration

I, Maxine Turner (née Grose), declare that this dissertation is my own work. It is being submitted in fulfillment for the degree of Master of Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Maxine Turner (née Grose) **Date**

Abstract

Diabetic foot syndrome is defined as the presence of a diabetic foot ulcer (DFU) associated with neuropathy, peripheral artery disease and infection. While the use of antimicrobials in the treatment of DFUs remains a mainstay, choice of antimicrobial remains problematic due to the presence of polymicrobial infections. The aim of this study was to determine antimicrobial susceptibility patterns of micro-organisms isolated from DFU patients visiting selected Gauteng public healthcare settings and treatment practices, in order to suggest a clinically effective treatment protocol.

Microbial sample swabs of 45 patients with DFUs were taken and a structured questionnaire was administered to the patients. A review of each patient's file was completed to assess treatment practices. Each sample swab was spread onto blood agar plates and thereafter, pathogens were isolated. The antimicrobial susceptibility patterns of all isolated pathogens were determined using the disc diffusion test. Pathogens were grouped according to macromorphological characteristics as well as susceptibility patterns and were then identified.

A total of 445 pathogens were isolated and susceptibility testing was done. The most effective antimicrobial was found to be gentamicin, followed by ciprofloxacin. Amoxicillin/ clavulanic acid is the first line treatment according to standard treatment guidelines and a large number of pathogens were resistant to this antibiotic. The total pathogen variety isolated from wound samples was 17. The most commonly isolated pathogens were *Proteus mirabilis*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Based on data obtained from the structured questionnaire and file review, the use of preventative measures among patients is poor with 48.9% of patients reporting that they did not make use of any preventative measures. The most commonly used preventative measure was crutches. The most frequently occurring concurrent medical conditions were hypertension followed by dyslipidaemia and peripheral neuropathy. Polypharmacy among the patient study was found to be prevalent with 55% of the population prescribed five or more medications. Potential medication interactions were examined and a total of 210 interactions were documented.

The data obtained through patient questionnaires emphasises the need to encourage patient education and preventive measures for the management of DFUs. Globally, causative pathogens and corresponding susceptibility patterns differ from country to country and the same is demonstrated in this study. These findings demonstrate the need to reassess the standard treatment guidelines and base treatment plans on local epidemiological data. This study provides valuable data on common causative pathogens in DFU infections as well as the resistance patterns of these pathogens forming a baseline with which to base future DFU treatment plans.

Dedication

I dedicate this thesis to my parents, Derek and Laura Grose, who without, this and everything else would not have been possible.

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List of publications and presentations arising from this study

Presentations (Appendix A)

1. MJ Grose, S Leigh-de Rapper, SF van Vuuren. Diabetic foot ulcers: Is it time to readdress the standard treatment guidelines? South Africa. The 43rd Annual APSSA Conference on Pharmaceutical Sciences, 21-23 August 2022, Rhodes University, Makhanda. [podium presentation, **winner of the Young Scientist competition in the pharmacy practice category**].

2. MJ Grose, S Leigh-de Rapper, SF van Vuuren. Susceptibility patterns and treatment practices of diabetic foot ulcers in selected South African public hospitals. South Africa. Faculty of Health Sciences Research Day, 15 September 2022, University of the Witwatersrand, Johannesburg. [poster presentation, **winner of the best student poster presentation in the clinical sciences and therapeutics for health category**].

Publications

1. MJ Grose, S Leigh-de Rapper, SF van Vuuren. The antimicrobial susceptibility patterns of diabetic foot ulcers in the South African public healthcare sector.
   Publication submitted to the South African Medical Journal in January 2023.

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List of abbreviations

AIDS	Acquired immunodeficiency virus
AMC	Amoxicillin/ clavulanic acid
BD	Twice daily
CFU/ml	Colony forming units per millilitre
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CN	Gentamicin
COVID-19	Coronavirus disease of 2019
CVD	Cardiovascular disease
DA	Clindamycin
DFU	Diabetic foot ulcer
eGFR	Estimated glomerular filtration rate
FCA	Fluconazole
FQ	Fluoroquinolone
GNR	Gram-negative rods
GPC	Gram-positive cocci
HIV	Human immunodeficiency virus
HJH	Helen Joseph Hospital
HREC	Human Research Ethics Committee
IDF	International Diabetes Federation
IDSA	Infectious Diseases Society of America
IMI	Intramuscular injection

IV	Intravenous
IWGDF	International Working Group on the Diabetic Foot
mm	Millimetres
MRSA	Methicillin resistant *Staphylococcus aureus*
PAD	Peripheral artery disease
PEDIS	Perfusion status, ulcer extent and depth, infection status, and foot sensation
SAASP	South African Antibiotic Stewardship Program
SAMF	South African Medicines Formulary
SINBAD	Site, ischemia, neuropathy, bacterial infection, area, depth
STAT	Immediately
STG	Standard Treatment Guidelines
TCC	Total contact cast
TDS	Three times daily
T/S	Trimethoprim/ sulfamethoxazole
VA	Vancomycin
WHO	World Health Organisation
WHS	Wound Healing Society
ZOI	Zone of inhibition

Chapter 1: Introduction

1.1. Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder, associated with hyperglycaemia that occurs as a result of a defect in insulin action, insulin secretion or a combination of both (Bastaki, 2005; Kerner and Brückel, 2014). According to the International Diabetes Federation (IDF), as of 2021, an estimated 537 million adults are living with diabetes worldwide (IDF, 2021). Globally, countries are facing an increasing prevalence of DM, regardless of their differences in economic status, demographic and/or epidemiological status (Animaw and Seyoum, 2017).
Type 1 diabetes is characterized by pancreatic β-cell destruction which is the result of an auto-immune process, often leading to a total lack of insulin (Baynes, 2015). Type 2 diabetes is more prevalent than type 1 diabetes and is often described as a lifestyle disease (Olokoba *et al.*, 2012). Type 2 diabetes occurs due to impaired insulin secretion due to the dysfunction of pancreatic β-cells, as well as impaired insulin action through insulin resistance (Baynes, 2015). Type 2 diabetes generally develops in those patients with a genetic predisposition as well as known risk factors with the most prominent risk factor being abdominal obesity (Blair, 2016).

1.2. Risk factors associated with the development of DM

While type 1 diabetes is attributed to an auto-immune process, type 2 diabetes is a multifactorial disease that occurs as a result of a combination of environmental and genetic risk factors (Bi *et al.*, 2012; Baynes, 2015; Bellou *et al.*, 2018). Lifestyle factors play an important role in the development of type 2 diabetes and include excess caloric intake which results in obesity, decreased physical activity, alcohol consumption and tobacco smoking (Bi *et al.*, 2012; Bellou *et al.*, 2018). A high caloric intake with inadequate dietary fibre content is a universally recognised risk factor and contributes to an increased accumulation of visceral and abdominal fat (Altobelli *et al.*, 2020). A diet that is high in glycaemic load and trans-fat intake is associated with an increased risk of developing type 2 diabetes, while a diet that is higher in cereal fibre and polyunsaturated fat is associated with a decreased risk of type 2 diabetes development (Bi *et al.*, 2012).

Physical inactivity contributes to the development of obesity and DM (Eaton and Eaton, 2017). Moreover, both strength and endurance training has been shown to improve insulin sensitivity through various mechanisms (Eaton and Eaton, 2017). Excessive alcohol consumption contributes to the development of obesity, impairs liver function and often results in pancreatitis, all of which play a role in the development of type 2 diabetes (Bi *et al.,* 2012). Active cigarette smoking is associated with an increased risk of developing type 2 diabetes with current smokers having a 45% increased risk of developing this disorder (Bi *et al.,* 2012; Yuan and Larsson, 2019). Smoking is associated with the development of central obesity, inflammation, insulin resistance and hyperglycaemia and thus increases the risk of developing type 2 DM (Bellou *et al.,* 2018). Individuals that present with either type 1 or type 2 diabetes are at risk of developing multiple complications which if not controlled, have the potential to reduce the patient's quality of life (van Netten *et al.,* 2018).

1.3. Complications of diabetes

Complications among patients presenting with both type 1 and type 2 diabetes are common and are a significant cause of morbidity and mortality (Papatheodorou *et al.,* 2018). Diabetes associated complications are classified as either microvascular or macrovascular (Papatheodorou *et al.,* 2018). Microvascular complications occur due to damage of the small blood vessels and include retinopathy, nephropathy and neuropathy; while macrovascular complications arise due to damage of the large blood vessels; which include cardiovascular disease, stroke, and peripheral artery disease (PAD) (Fowler, 2008; Papatheodorou *et al.,* 2018). Complications that do not fit into the aforementioned categories include dental disease, increased susceptibility to infection as well as diabetic foot syndrome (Papatheodorou *et al.,* 2018).

Diabetic foot syndrome is the most common cause of hospitalisation and lower limb amputation (Volmer-Thole and Lobmann, 2016; Papatheodorou *et al.,* 2018). A foot ulcer is defined as a full-thickness wound below the ankle on a weight-bearing or exposed surface that involves at least the epidermis layer and part of the dermis layer of the skin (Shin *et al.,* 2020; van Netten *et al.,* 2020). A diabetic foot ulcer (DFU) is a foot ulcer in a person with diagnosed DM and typically presents alongside neuropathy or peripheral artery disease in the lower extremity (van Netten *et al.,* 2020). Neuropathic DFUs often result in the formation of open

wounds, the primary risk factor for the development of infections of the foot (Lavery *et al.*, 2006; Chastain *et al.*, 2019). It is estimated that over 50% of DFUs become infected and in the diabetic patient, which increases the risk of amputation and mortality (Hobizal and Wukich, 2012; Chastain *et al.*, 2019).

1.4.The pathophysiology of DFUs

1.4.1 Neuropathy

The pathogenesis of DFUs (Figure 1.1) is multifactorial, involving neuropathic, vascular and immune system components, all of which arise due to the original hyperglycaemic state attributable to chronic and poorly controlled DM (Aumiller and Dollahite, 2015). Hyperglycaemia results in oxidative stress on nerve cells, leading to neuropathy and nerve dysfunction (Clayton and Elasy, 2009). Nerve dysfunction may lead to a loss of function of sweat glands as well as a reduced ability to moisturize the skin of the foot, leading to dry skin and epidermal cracks (Clayton and Elasy, 2009; Aumiller and Dollahite, 2015). Nerve dysfunction may also result in muscle weakness and imbalance leading to anatomical deformities such as hammered or clawed toes. This may result in subsequent abnormal loading on the foot which contributes towards the development of DFUs (Hobizal and Wukich, 2012; Aumiller and Dollahite, 2015). Furthermore, sensory neuropathy results in the loss of protective sensation which exacerbates the development of the DFU, as the patient is unable to detect the repetitive trauma to the foot (Clayton and Elasy, 2009; Aumiller and Dollahite, 2015; Chastain *et al.*, 2019). Patients with altered pain response are also vulnerable to trauma caused by ill-fitting footwear (Hobizal and Wukich, 2012).

1.4.2. Vascular changes

Vascular changes that contribute towards the development of DFUs correspond with hyperglycaemia-induced changes in the peripheral arteries of the foot. These include; endothelial injury, hyperlipidaemia, increased platelet viscosity and subsequently, the development of atherosclerosis (Aumiller and Dollahite, 2015; Bandyk, 2018).

Peripheral artery disease (Figure 1.2), is defined as an obstructive atherosclerotic vascular disease that results in impaired circulation in one or more extremities and contributes to DFU development and non-healing (van Netten *et al.*, 2020). The presence of peripheral artery

disease has been identified in 30% of DFUs and as a result, increases the risk of non-healing DFUs, infection and amputation, thereby making peripheral artery disease a significant contributor in the pathogenesis of DFUs (Dinh and Veves, 2005; Hinchliffe *et al.*, 2020).

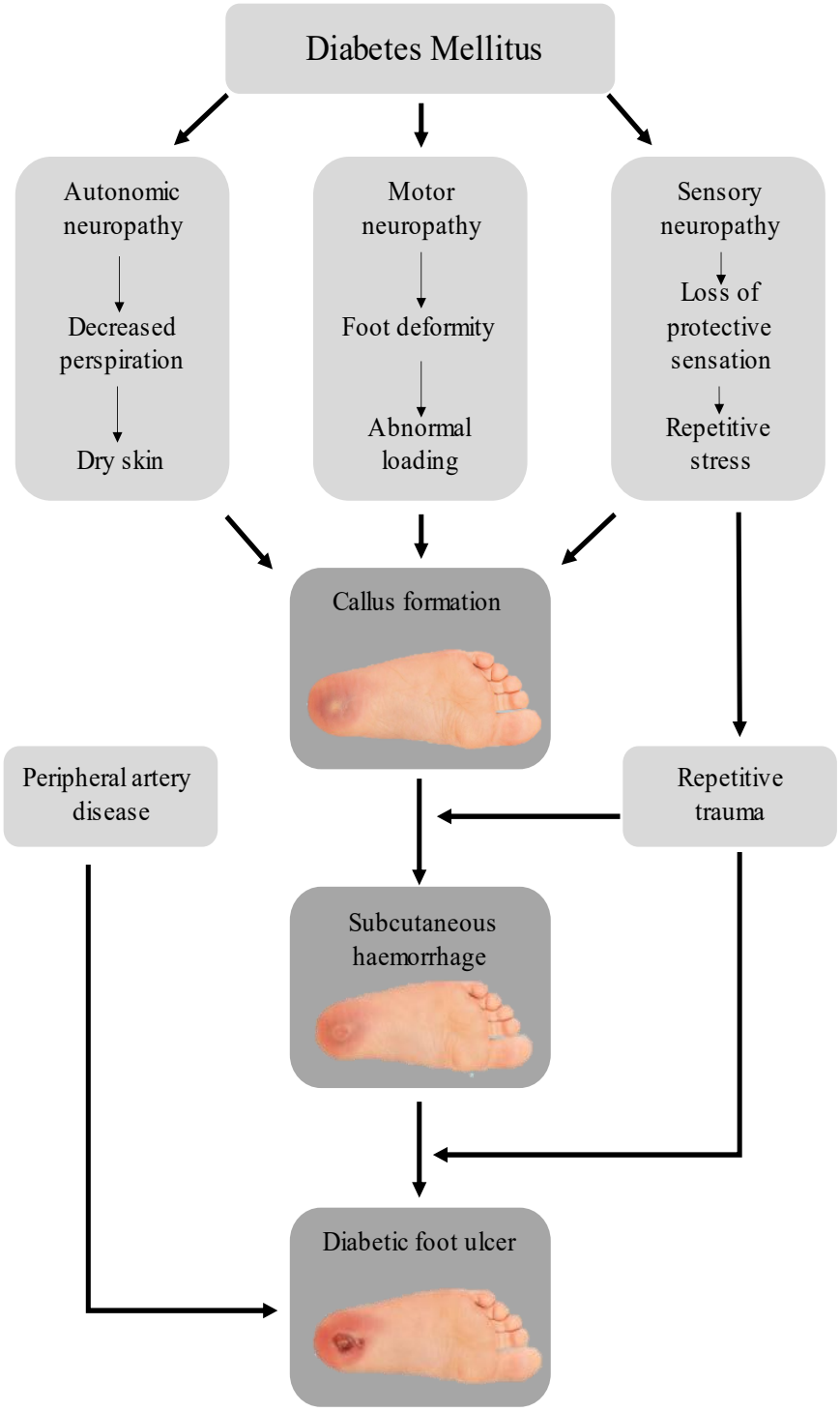


Figure 1.1: The pathogenesis of diabetic foot ulcers. Adapted from Armstrong *et al.* (2017) and Orthotics (2021).

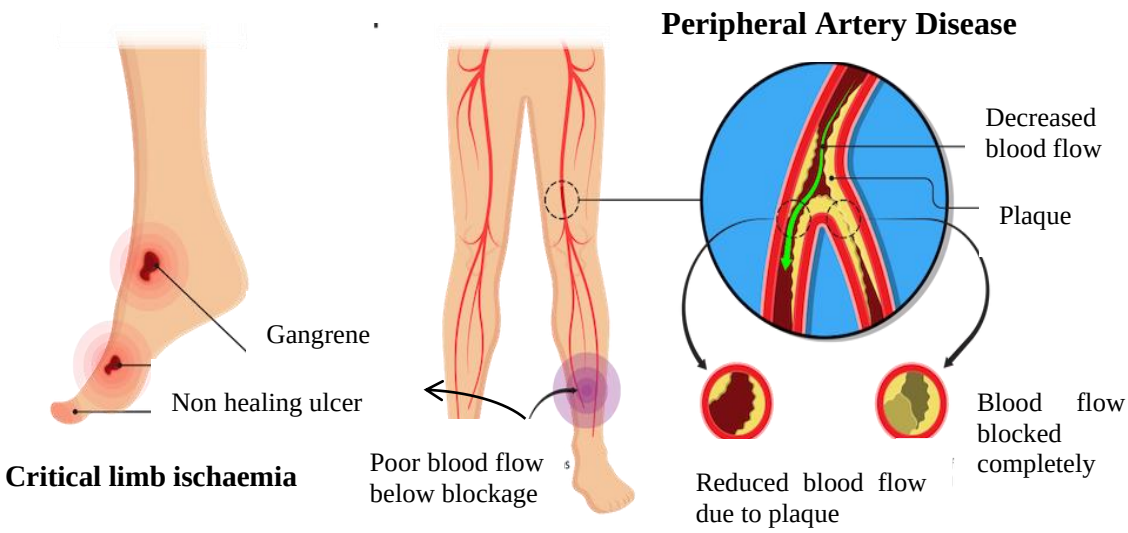


Figure 1.2: The role of peripheral artery disease and diabetic foot ulcers (Mayo Clinic, 2021).

Peripheral artery disease in the diabetic patient is characterized by impairment of macro- as well as microcirculation, the latter of which is unique to the diabetic patient (Dinh and Veves, 2005). Microcirculatory changes decrease the elastic ability of the capillary walls thus limiting vasodilation and impairing wound healing function. Furthermore, the decreased elastic ability of the capillary walls results in the thickening of the basement membrane of these vessels. This may hinder the normal exchange of nutrients thereby reducing the ability of the diabetic patient to ward off infection (Rayman *et al.*, 1986; Dinh and Veves, 2005; Chao and Cheing, 2009).

1.5. Epidemiology of DFUs

It has been estimated that of those diagnosed with diabetes, 32% will develop DFUs within their lifetime (Lazzarini *et al.*, 2018). A systematic review and meta-analysis on the global epidemiology of diabetic foot ulceration found that the worldwide prevalence was 6.3% (Zhang *et al.*, 2017). North America had the highest prevalence of DFU at 13% and in Africa, at 7.2%, which is higher than both Asia (5.5%) and Europe (5.1%) (Zhang *et al.*, 2017; Raghav *et al.*, 2018).

In South Africa, data indicates that the prevalence of DFUs was estimated to be between 5.4 - 6% (Rotchford and Rotchford, 2002; Zhang *et al.*, 2017). The annual incidence of DFUs in those patients with established neuropathy is higher than in those without neuropathy

(Edmonds *et al.,* 2021). A study undertaken in South Africa in 2009, found that diabetes and subsequent diabetic foot syndrome were responsible for 2 000 new amputations, annually (Bertram *et al.,* 2013). A study undertaken South Africa in Cape Town found that among the 243 black African participants, diabetic foot complications were present in one third of the cohort (Levitt et al., 1997). A more recent study found that 28% of diabetic patients presenting to primary healthcare facilities in South Africa had developed DFUs (Mothabeng *et al.,* 2022).

1.6. Risk factors contributing towards DFU development

According to a systematic review examining the risk factors associated with the development of foot complications in the diabetic patient, insulin use has shown an association to the development of DFU, however, this could be explained by the fact that insulin therapy is often employed later in the progression of DM (Vibha *et al.,* 2018). While age has not been shown to impact on the risk of developing a DFU, a longer duration of living with diabetes, increases the risk of developing a DFU (Rossboth *et al.,* 2021).

The prevalence of DFUs is higher among male patients which is thought to occur due to the higher incidence of peripheral neuropathy in this cohort (Bruun *et al.,* 2013; Zhang *et al.,* 2017; Sorber and Abularrage, 2021). Furthermore, increased DFU presence in males has been suggested to occur as a result of female patients often experiencing less severe neuropathy, increased joint mobility, lower foot pressure and a greater tendency towards self-care routines in comparison to men, who are also more likely to be exposed to trauma (Kateel *et al.,* 2018). In populations considered high risk for the inadequate accessibility to healthcare services, there is also a higher risk for the development of DFUs (Sorber and Abularrage, 2021).

In those patients with low socio-economic status, DFUs are found to be more common (Hokkam, 2009, Vibha *et al.,* 2018). This could be due to multiple reasons including; lack of awareness, poor health seeking behaviours as well as the inability to afford DFU treatment (Vibha *et al.,* 2018). Patients who smoke are also more prone to developing DFUs than those who do not (Vibha *et al.,* 2018, Rossboth *et al.,* 2021). Smoking is an established risk factor for the development of peripheral artery disease which in turn is an important risk factor for the development of DFU and thus smoking is hypothesised to exaggerate the pathogenesis of DFUs (Vibha *et al.,* 2018).

In South Africa, the human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDs) epidemic is still a reality with The Joint United Nations Programme on HIV/AIDS reporting that in 2019, 7 500 000 children and adults were diagnosed as HIV positive. Diabetes and HIV both result in the increased susceptibility to infection by means of a reduced immune system (Cohen *et al.,* 2010). The use of antiretroviral therapy, and in particular the use of non-nucleoside reverse transcriptase inhibitors like stavudine and lamivudine, have been known to result in metabolic syndrome as a side effect, thus promoting the onset of diabetes or worsening existing DM (Tien *et al.,* 2008; Cohen *et al.,* 2010). In South Africa, lamivudine forms part of the first line treatment regimen for HIV positive individuals which means that there is a greater risk of developing diabetes in this cohort. Alongside metabolic syndrome, antiretroviral therapy also results in the development of peripheral neuropathy and thus compounds the risk of developing DFUs in the HIV positive, diabetic patient (Cohen *et al.,* 2010).

1.7. The classification of DFUs

The classification of diabetic foot ulcers enables a logical and standardised approach to treatment and allows for ease of communication between healthcare professionals (Frykberg, 2002; Game, 2016). In terms of the classification of DFUs, there have been multiple wound classification systems created, based on parameters that include: the extent of infection, ischemia, neuropathy, depth of the ulcer as well as its location (Frykberg, 2002). The large number of proposed DFU classification systems suggest that there are no systems applicable for standardised worldwide use (Monteiro-Soares *et al.,* 2020).

The most widely used classification system for DFUs is the Wagner ulcer classification system (Table 1.1) and this is due to the fact that it is simple and timely to use (Game, 2016). The system uses six grades to classify DFUs with the first three grades based on the depth of the ulcer (Game, 2016). Thereafter, the Wagner classification system takes into account the presence of osteomyelitis or gangrene as well as the degree of tissue necrosis (Frykberg, 2002). However, this classification system does not address the parameters of peripheral artery disease, infection and does not differentiate between gangrene caused by infection or ischemia (Frykberg, 2002; Game, 2016; Vera-Cruz *et al.,* 2020). Due to this, the Wagner system has been deemed too simplistic to provide prognostic information for individualized patient care (Monteiro-Soares *et al.,* 2020).

Table 1.1: Wagner Ulcer Classification System (Wagner, 1987; Frykberg, 2002).

Grade	Lesion
0	No open lesions; deformity or cellulitis may be present
1	Superficial ulcer; partial or full thickness
2	Ulcer extension to ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis
3	Deep ulcer with abscess, osteomyelitis, or joint sepsis
4	Gangrene localized to portion of forefoot or heel
5	Extensive gangrenous involvement of the entire foot

The most universal classification system appears to be the SINBAD (Site, Ischemia, Neuropathy, Bacterial Infection, Area and Depth) system as it is appropriate for clinical care in terms of descriptive purposes, as well as scoring and prognostic purposes (Game, 2016). The SINBAD system can also be used in the setting of an audit as it allows for the classification of all lesions and is simple enough to be used in daily practice (Game, 2016). In an attempt to provide recommendations on the use of classifications for DFUs, the IWGDF suggests the use of the SINBAD system (Table 1.2).

Table 1.2: The SINBAD system (Monteiro-Soares *et al.,* 2020).

Category	Definition	Score
Site	Forefoot	0
	Mid-foot and hind-foot	1
Ischaemia	Pedal blood flow intact: at least one palpable pulse	0
	Clinical evidence of reduced pedal blood flow	1
Neuropathy	Protective sensation intact	0
	Protective sensation lost	1
Bacterial infection	None	0
	Present	1
Area	Ulcer <1 cm^2	0
	Ulcer ≥ 1 cm^2	1
Depth	Ulcer confined to skin and subcutaneous tissue	0
	Ulcer reaching muscle, tendon or deeper	1
Total possible score		**6**

This system uses a scoring system to grade the site, area, depth, sepsis, arteriopathy, and denervation of the DFU as either zero or one where the maximum score obtained can be six (Monteiro-Soares *et al.,* 2020). According to the IWGDF, the SINBAD system is simple and efficient as no special equipment is required for clinical examination and it contains the

necessary information that may be needed for future specialised treatment (Monteiro-Soares *et al.,* 2020). While the SINBAD system is an appropriate resource for poor healthcare settings and has been validated to measure ulcer healing, as well as amputation prediction; it is important that the individual clinical descriptors be used when communicating with other healthcare professionals as just a numerical score provides very little information about the DFU (Monteiro-Soares *et al.,* 2020).

For the purposes of research, it has been suggested that the most appropriate classification system is the PEDIS system (Perfusion status, ulcer Extent and Depth, Infection status, and foot Sensation) as this system is very detailed, but is not necessarily appropriate for the classification of every lesion (Game, 2016). The PEDIS system was developed as a descriptive classification for use in research and not for use in diagnostic and prognostic purposes, it is thus not used as a scoring system (Monteiro-Soares *et al.,* 2020). When classifying an infected diabetic foot ulcer, the IWGDF recommend using the International Working Group on the Diabetic Foot and Infectious Diseases Society of America system (IWGDF/IDSA) (Table 1.3) in order to characterise and guide infection management (Monteiro-Soares *et al.,* 2020). The system comprises four grades of infection severity and was originally developed as part of the PEDIS classification system (Game, 2016; Monteiro-Soares *et al.,* 2020). The IWGDF/IDSA classification system has been validated as a strong predictor of the need for hospitalisation as well as for both major and minor amputation (Lavery *et al.,* 2007; Monteiro-Soares *et al.,* 2015). The IWGDF/IDSA classification system consists of four grades of severity of diabetic foot infection. A critique of the IWGDF/IDSA classification system is its complexity and limited application to different populations (Monteiro-Soares *et al.,* 2020).

Table 1.3: The IWGDF/IDSA classification system (Monteiro-Soares *et al.,* 2020).

PEDIS grade	Infection severity	Clinical manifestations
1	Uninfected	Wound lacking purulence or any manifestations of inflammation
2	Mild	Presence of more than two manifestations of inflammation (purulence, erythema, tenderness, warmth, or induration), but any cellulitis/erythema extends ≤2 cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness

3	Moderate	Infection in a patient who is systemically well and metabolically stable but which has more than one of the following characteristics: cellulitis extending >2 cm, lymphangitic streaking, spread beneath the superficial fascia, deep-tissue abscess, gangrene, and involvement of muscle, tendon, joint, or bone
4	Severe	Infection in a patient with systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leucocytosis, acidosis, severe hyperglycaemia, or azotaemia)

In South Africa, treatment in the public health sector is based on the Standard Treatment Guidelines (STG) and incorporates medicines listed in the Essential Drugs List. Furthermore, the South African Antibiotic Stewardship Program (SAASP) also provides guidelines on antibiotic prescribing. Although the IWGDF recommend the use of the SINBAD system and IWGDF/IDSA classification system to classify DFUs and infections, guidelines specific to South Africa suggest classifying DFUs as mild, moderate or severe (Table 1.4) (Wasserman *et al.,* 2015). When comparing the IWGDF/IDSA classification system to the South African classification system, some similarities can be seen, however, the IWGDF/IDSA classification system is more complex in its description of the DFU infection which may allow for the more accurate classification of the DFU and thus the employment of more effective treatment practices.

Table 1.4: Classification of DFUs in the South African context suggested by SAASP (Wasserman *et al.,* 2015).

Classification	Description
Mild	Limited to skin/superficial subcutaneous tissue <2 cm beyond ulcer margin
Moderate	Cellulitis >2 cm, deep fascial involvement, gangrene, abscess, osteomyelitis
Severe	Systemic complications

In comparison to the SINBAD system of classification, the South African system of DFU classification is simplified and lacks important factors such as the presence of ischaemia and neuropathy. Furthermore, when compared to the Wagner classification system, the South African classification system groups a wide spectrum of aspects of diabetic foot syndrome into a single classification. In other words, the moderate classification of DFU encompasses

cellulitis, gangrene and osteomyelitis all of which differ in severity. The Wagner system classifies cellulitis, gangrene and osteomyelitis in separated groups, grades two, three and four respectively, allowing for more specific classification of the DFU and thus better targeted care. With this in mind, according to Mr. Lucus Breedt (personal communication), the National Executive Secretary of the Podiatry Association of South Africa, the Wagner system of classification is more commonly used by podiatrists in the South African public healthcare sector.

1.8. The reoccurrence of DFUs

The impact that DFUs have on the quality of life of the patient is immense. Patients with DFUs have been found to suffer from hindered mobility, depression, social isolation as well as consequent fatigue (Vileikyte, 2001). Besides a reduced quality of life, patients with DFU also experience adverse economic effects, with many people being unable to work as well as having the burden of the additional costs of treatment (Vileikyte, 2001).

Reoccurrence of a DFU, even after it has healed fully is not uncommon (Armstrong *et al.,* 2017). A review of 19 studies concluded that around 40% of patients have a recurrence of DFUs within one year after ulcer healing; and 60% of patients within three years (Armstrong et al., 2017). Risk factors for the reoccurrence of DFUs include: the male gender, smoking, chronic disease status as well as past DFUs, the presence of plantar ulcers, peripheral artery disease and neuropathy (Armstrong *et al.,* 2017; Huang *et al.,* 2019). Of these risk factors, one of the strongest predictors of reoccurrence is a history of previous DFU as well as sign of skin damage like the presence of a callus or blistering (Crawford *et al.,* 2015; Armstrong et al., 2017). According to Gazzaruso *et al.,* (2021), renal function plays an important role in DFU healing. Impaired renal function, defined as an estimated glomerular filtration rate (eGFR) of <60 ml/L strongly predicts negative outcomes including a greater probability of DFU reoccurrence and minor amputation (Gazzaruso *et al.,* 2021).

A study undertaken in Egypt reported a reoccurrence rate of 61.3% in patients and determined that the most prominent risk factors for reoccurrence were smoking, poor glycaemic control, neuropathy, peripheral artery disease and previous plantar ulcers (Khalifa, 2018). These findings correspond to those determined by Armstrong *et al.,* (2017) as well as Huang *et al.,*

(2019). A similar study in the United States of America found that reoccurrence rates for DFUs one year after initial healing was 30.6% and at three years was 64.6% (Hicks *et al.,* 2020). Another study in Saudi Arabia found a DFU reoccurrence rate of 58.5% during its two-year study period (Tabanjeh *et al.,* 2020). The high reoccurrence rates demonstrated worldwide needs to be addressed by means of reducing the risk factors as reoccurring DFUs account for significant hospital admission, disability, increased medical costs and higher mortality rates (Khalifa, 2018).

Diabetic foot ulcers that become infected are a significant cause of lower extremity amputation (Costa *et al.,* 2017). Lower extremity amputation is divided into minor and major amputation where minor amputation is defined as the surgical removal of bones and soft tissue below the ankle joint and major amputation occurs at or proximal to the ankle, above or below the knee (Rathnayake *et al.,* 2020). In the diabetic patient, the risk of lower limb amputation is 15 times higher than in patients who are not diabetic (Yazdanpanah *et al.,* 2015). In the South African province of Kwa-Zulu Natal there are approximately 1.4 million diabetic patients who access public healthcare, and approximately 2 400 amputations are performed annually (Naidoo and Ennion, 2019). A five-year audit of the amputations that occurred in one Kwa-Zulu Natal hospital found that 53.1% of amputations were due to DM (Manickum *et al.,* 2019). A more recent study in Kwa-Zulu Natal found that 53.7% of amputations were attributable to DM (Khan *et al.,* 2020). The high rate of amputation in Kwa-Zulu Natal corresponds to the findings of another study undertaken in 2018 whereby patients presented to rural healthcare facilities with advanced stages of DFU severity, requiring amputations as a result (Cheddie *et al.,* 2018). A study undertaken in the Gauteng province found that 1 565 DFU related amputations occurred over a period of 30 months (Ntuli and Letswalo, 2021). These findings highlight the seriousness of the consequences of DFUs and the need to reduce their prevalence and improve treatment protocols.

1.9. Diabetic foot ulcer infections

Infection of a DFU occurs when a virulent micro-organism invades the host and ultimately results in local tissue damage (Noor *et al.,* 2015). In the pathogenesis of DFUs, autonomic neuropathy results in the inability of the skin to remain moisturised which in turn results in the overlying skin becoming vulnerable to breaks and subsequent infection (Noor *et al.,* 2015).

Furthermore, the presence of neuropathy, ischaemia and immunosuppression promotes the development of infection that ascends from the DFU (Costa *et al.,* 2017). Impaired host defenses that occur due to hyperglycemia include leukocyte dysfunction and structural changes to macrophages which impairs their function (Dinh and Veves, 2005; Hobizal and Wukich, 2012). As a result of these changes, a prolonged inflammatory state is created and normal wound healing time is prolonged (Hobizal and Wukich, 2012; Aumiller and Dollahite, 2015). Diabetic foot ulcer infections are caused by a range of pathogenic micro-organisms and it is important to identify the most common causative pathogens in order to treat the patient effectively.

The micro-organisms that cause infection in diabetic foot ulcers depend on patient foot hygiene as well as pathogen-specific resistance patterns (Malepati *et al.,* 2017). Differing patterns of causative pathogen type in various regions of the world highlight the importance of carrying out research specific to geographical location in order to provide the most appropriate treatment (Chastain *et al.,* 2019). Diabetic foot infections, resulting from diabetic foot ulcers, are generally polymicrobial in nature consisting of Gram-positive, Gram-negative, and anaerobic bacteria. In some cases, fungal pathogens are prevalent (Miyan *et al.,* 2017; Chastain *et al.,* 2019; Sadeghpour Heravi *et al.,* 2019). Diabetic foot infections that are acute in nature or of a shorter duration appear to present with simpler bacteriological profiles (Sadeghpour Heravi *et al.,* 2019). Chronic ulceration and subsequent infection of DFUs is often due to polymicrobial colonisation which occurs as a result of a weakened immune system and increased frequency of ineffective treatment (Sadeghpour Heravi *et al.,* 2019).

Gram-positive bacteria implicated in diabetic foot ulcers and infections may comprise of *Streptococcus, Staphylococcus as* well as *Enterococcus* species (Sadeghpour Heravi *et al.,* 2019). The most common causative pathogen in DFUs is *Staphylococcus aureus* (Chastain *et al.,* 2019; Pitocco *et al.,* 2019; Sadeghpour Heravi *et al.,* 2019). Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a mainstay in skin and soft tissue infections and more so in the feet of diabetic patients (Chastain *et al.,* 2019). A study in India demonstrated that 19% of bacteria isolated from DFUs was identified as MRSA (Malepati *et al.,* 2017). In Italy, a review of 765 diabetic foot infections concluded that 27% of infections were attributed to *S. aureus* and of those infections, 59% were methicillin-resistant (Pitocco *et al.,* 2019). A review of causative pathogens of diabetic foot infection in Korea found that *S. aureus* was the

most common with prevalence ranging from 26% to 46% (Kwon and Armstrong, 2018). Similar results were obtained in Pakistan where *S. aureus* was the most frequent (21%) Gram-positive isolated (Miyan *et al.*, 2017). In Scotland, 96% of isolates were identified as Gram-positive organisms with *S. aureus* being the most common organism (Macdonald *et al.*, 2020). While MRSA is a common causative pathogen in DFUs, it is not the only pathogen responsible for causing infection.

A study undertaken in India identified *Enterococci* as the most frequent cause of diabetic foot infection and of these isolates, 71% were determined to be resistant to vancomycin (Shahi and Kumar, 2016). A similar study undertaken in Mexico found that following *S. aureus, Enterococci* were the most frequently isolated group of micro-organisms and 51% of the strains isolated were resistant to vancomycin (Sánchez-Sánchez *et al.*, 2017). The most commonly identified *Enterococci* in DFUs include *Enterococcus faecalis* (Semedo-Lemsaddek *et al.*, 2016; Sadeghpour Heravi *et al.*, 2019).

While it seems that Gram-positive organisms, in particular, *S. aureus* are responsible for the majority of infections that occur as a result of DFUs, it is not the case in all parts of the world. In Western countries and countries like Korea, it is true that Gram-positive organisms are more commonly implicated as the causative organisms of DFUs, however, in developing countries on continents with warmer climates like Asia and Africa, Gram-negative organisms are more prevalent (Lipsky *et al.*, 2016; Kwon and Armstrong, 2018; Chastain *et al.*, 2019). This may be due to differing lifestyle factors of different populations across the globe which include; footwear practices and personal hygiene (Lipsky *et al.*, 2016; Kwon and Armstrong, 2018). Among the Gram-negative bacilli, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus* spp. are the most common causative pathogens (Lipsky *et al.*, 2012). A study undertaken in India found that Gram-negative bacilli were isolated in 65.1% of DFUs making them more prevalent than Gram-positive cocci (39. 8%) (Shanmugam *et al.*, 2013). Of these bacilli, *Pseudomonas* spp. and *E. coli* were the most common (Shanmugam *et al.*, 2013). A similar study in India reported that 76% of causative organisms in DFUs were Gram-negative organisms with *P. aeruginosa* being the predominant pathogen (27%), followed by *K. pneumoniae* (22%) (Mehta *et al.*, 2014). In Kenya, DFUs and subsequent infection are most commonly caused by *S. aureus* and *E. coli*, and it was established that 65% of infection causing pathogens were Gram-negative (Mutonga *et al.*, 2019).

Anaerobic infections are predominantly seen in diabetic foot ulcers that are deeper and chronic in nature, and are mostly linked to the presence of ischemia, necrosis, gangrene, or foul odour (Kwon and Armstrong, 2018). Anaerobes are almost always seen in polymicrobial infections and are present alongside aerobic bacteria (Al Benwan *et al.*, 2012). Anaerobes that have been identified in diabetic foot infections include species of *Peptostreptococcus*, *Veilonella*, and *Bacteroides* (Al Benwan *et al.*, 2012; Pitocco *et al.*, 2019). In a study undertaken in Singapore, it was found that the predominant anaerobic isolates were *Peptostreptococcus* sp. (46%) and *Bacteroides fragilis* (19%) (Ng *et al.*, 2008). When it comes to understanding the prevalence of anaerobic organisms in DFUs there is limited data which may be attributed to the complex methodology required to ensure adequate sampling, sample transportation and culture conditions (Ng *et al.*, 2008).

Much like anaerobes, fungal pathogens and their role in diabetic foot ulcers are not frequently studied (Saseedharan *et al.*, 2018). Although evidence exists for fungal infection in diabetic foot ulcers, clinicians and surgeons treat these ulcers as if they could only be as a result of bacterial infection, employing antibacterial agents and not antifungal agents as a result of insufficient literature available (Ozturk *et al.*, 2019). Predisposing factors such as high glucose levels, vascular insufficiency and neuropathy allow conditions for the colonisation of pathogenic fungi (Raiesi *et al.*, 2018). The most frequently isolated fungal pathogen in DFUs is *Candida albicans* followed by *Aspergillus* sp. According to Raiesi *et al.* (2018), more than 80% of patients that present with DFUs had a history of trauma or burns. This is significant in that trauma and burns coupled together with impaired leucocyte function are hypothesised risk factors for the development of fungal infection (Raiesi *et al.*, 2018). Furthermore, the severity of fungal infection corresponds to the degree of immunosuppression experienced by the patient. For example; *C. albicans*, the most common cause of DFU fungal infections occurs in those with modest degrees of immunosuppression; whereas *Aspergillus* sp., the second most common occurring pathogenic fungal micro-organism in DFUs tends to occur in patients with an intermediate degree of immunosuppression (Raiesi *et al.*, 2018). A study undertaken in India revealed that the prevalence of fungi in diabetic wounds was 27% (Chellan *et al.*, 2010). Of these fungal isolates, 77% were identified as *Candida* sp. with the most common species being *Candida parapsilosis* (26%) (Chellan *et al.*, 2010). Another study found that 19% of its participants presented with fungal infection of the DFU, *Candida* sp. accounted for 26% of these infections, followed secondly by *Aspergillus* sp. which accounted for 21% of fungal

infections (Raza and Anurshetru, 2017). The presence of a fungal infection that is not treated will delay healing time (Chellan *et al.,* 2010).

1.10. Treatment options, practices and guidelines for infectious DFUs

Initially, treatment of diabetic foot ulcers includes debridement, offloading methods as well as local wound care (Del Core *et al.,* 2018). Debridement of an ulcer makes it acute in nature, decreases biofilm burden and reduces pressure, all of which are essential for ulcer healing (Del Core *et al.,* 2018). International guidelines like the Wound Healing Society (WHS) guidelines suggest that if infection is suspected in the DFU, cultures should be performed before the administration of antibiotics (Lipsky *et al*., 2012; Lavery *et al*., 2016). Empiric treatment is guided by the severity of the infection and changed to directed therapy as soon as culture results are obtained (Del Core *et al.,* 2018).

In the South African public healthcare sector, the treatment of DFUs is based on guidelines set out in the STG and the Essential Drugs List as financial restrictions and lack of resources prevent healthcare workers from obtaining cultures before empiric treatment is prescribed (Abahamye, 2016). These guidelines are developed using evidence-based medicine and are based on global susceptibility patterns.

In terms of treatment practices in the case of mild infection, the clinician's choice of oral empiric antibiotics should be directed at aerobic Gram-positive cocci and aerobic *Streptococci* while considering recent patient antibiotic use as well as local susceptibility patterns (Lipsky, 2007; Del Core *et al.,* 2018; Lipsky *et al.,* 2020). Various regimens for the directed treatment of DFUs and infection exist (Table 1.5) and the choice of regimen should be based on culture results (Lipsky *et al*., 2012; Lavery *et al*., 2016).

According to the STG, patients at a public hospital in South Africa presenting with DFUs are treated with amoxicillin/clavulanic acid taken orally and those with severe infections are treated with amoxicillin/clavulanic acid given intravenously. For those patients allergic to penicillin, clindamycin taken orally and gentamicin given intravenously is recommended (National Department of Health, South Africa, 2020). Like the STG, the South African Antibiotic Stewardship Program (SAASP) recommend amoxicillin/clavulanic acid as the

mainstay in DFU treatment. In addition to this, SAASP also recommend the use of clindamycin and ciprofloxacin in the case of penicillin allergy. It should be noted that while this regimen is relatively broad-spectrum, it is not based on recent resistance patterns specific to South Africa and it does not include coverage for fungal infection. When compared to the suggested empirical regimens outlined in Table 1.5, although closely aligned with suggested guidelines, South African guidelines do not account for nosocomial or MRSA infections or the recent exposure to antibiotics. Furthermore, there is no consideration for the treatment of chronic DFUs given in the STG. The limited treatment options suggest a "one size fits all" approach when it comes to treating DFUs in the South African public healthcare sector. This may result in poor treatment outcomes and higher reoccurrence rates.

1.11. Antimicrobial resistance patterns of infections DFUs

Worldwide, empiric therapy regimens for DFU's include cephalexin, amoxicillin/clavulanic acid and clindamycin (Chastain *et al.,* 2019). In those patients with risk factors predisposing to *P. aeruginosa* infection, which include puncture wounds, exposure to contaminated water sources and warm climates such as that found in South Africa, empiric therapy addressing this pathogen may be of benefit. Furthermore, antibiotics with extended spectrum of activity may be of benefit in the case of increased prevalence of MRSA and Gram-negative bacteria (Chastain *et al.,* 2019). A study carried out in India, where *S. aureus* and *P. aeruginosa* were the most common causative pathogens of DFU, concluded that doxycycline was the best candidate for empirical coverage of Gram-positive organisms and that meropenem was the most appropriate choice for Gram-negative organisms (Sekhar *et al.*, 2018). In Iran it was found that penicillin was the least effective antimicrobial and resistance to ciprofloxacin and ceftriaxone was high with 66% and 63% resistance, respectively (Najari *et al.*, 2019). In Egypt, it was found that most isolates were susceptible to amikacin (Hassan *et al.,* 2019). The varying results of resistance patterns and subsequent antimicrobial susceptibility in different regions of the world indicate the importance of carrying out antimicrobial susceptibility testing in specific regions.

Table 1.5: Suggested antibiotic treatment guidelines for DFU infection treatment.

Likely causative pathogens	Additional considerations	IWGDF Guidelines*	South African Standard Treatment Guidelines (STG)	South African Antimicrobial Stewardship Program (SAASP)	Comments
Aerobic GPC	Acute, low-risk of MRSA	Penicillin's; first-generation cephalosporins	Amoxicillin/clavulanic acid, oral, 875/125 mg every 12 hours for 10 days.	Flucloxacillin 500mg orally every 6 hours for 7-14 days	STG and SAASP guidelines aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
Aerobic GPC	Penicillin allergy	Clindamycin; FQ; T/S; macrolide; doxycycline	Clindamycin, orally, 150-450 mg every 8 hours	Clindamycin 450 mg orally every 8 hours	STG and SAASP guidelines aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
MRSA	Healthcare-associated infection; high local prevalence of MRSA	Co-trimoxazole; doxycycline; clindamycin; glycopeptide; linezolid; daptomycin; T/S; FQ	Not specified	Not specified	No alignment to international guidelines. Lack of resistance data to constitute antibiotic alternatives.
GPC and GNR	Recent exposure to antibiotics	Amoxicillin/clavulanic acid; second- or third-generation cephalosporin; FQ	Not specified	Not specified	No alignment to international guidelines. Lack of resistance data to constitute antibiotic alternatives.

Likely causative pathogens	Additional considerations	IWGDF Guidelines*	South African Standard Treatment Guidelines (STG)	South African Antimicrobial Stewardship Program (SAASP)	Comments
GPC and GNR	Chronic; No complicating features; recent exposure to antibiotics	Amoxicillin/clavulanic acid; second- or third-generation cephalosporin; ertapenem; FQ	Not specified	Amoxicillin/clavulanic acid, oral, 875/125 mg every 12 hours for 10 days.	SAASP guidelines aligned to international guidelines however, there is a lack of resistance data to constitute antibiotic alternatives.
GNR (*Pseudomonas)*	Macerated ulcer and warm climate	Amoxicillin/clavulanic acid; penicillin + ceftazidime; penicillin + ciprofloxacin; group 2 carbapenem	Not specified	Not specified	No alignment to international guidelines. Lack of resistance data to constitute antibiotic alternatives.
GPC, GNR and obligate anaerobes	Necrotic, gangrenous ischaemic limb; foul odour	Clindamycin (± FQ); metronidazole (+ FQ); ticarcillin/clavulanate or piperacillin/tazobactam; second/third gen cephalosporin + clindamycin or metronidazole	Amoxicillin/clavulanic acid, IV, 1.2 g every 8 hours or clindamycin, oral, 150–450 mg every 8 hours and gentamicin, IV, 6 mg/kg daily	Amoxicillin/clavulanic acid, IV, 1.2 g every 8 hours or clindamycin 600 mg iv every 8 hours and ciprofloxacin 400 mg iv every 8 hours	STG and SAASP guidelines aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.

* - Lipsky, 2007; Lipsky *et al.*, 2016; GPC- Gram-positive cocci; MRSA – methicillin resistant *Staphylococcus aureus*; GNR – Gram-negative rods; T/S - trimethoprim/sulfamethoxazole; FQ – Fluoroquinolone; group 1 carbapenem – ertapenem; group 2 carbapenem - imipenem, meropenem, doripenem

Multidrug resistant micro-organisms are common causative pathogens in DFUs (Kandemir *et al.,* 2007; Henig *et al.,* 2020). The increased frequency of these pathogens is partly due to frequent hospital admission and exposure to healthcare settings, inappropriate antibiotic prescribing practices and the chronic nature of the wound (Kandemir *et al.,* 2007; Henig *et al.,* 2020). In 2014, a study undertaken in China found that 53% of organisms isolated were multidrug resistant (Ji *et al.,* 2014). More recently, a study found that 60% of the patients admitted to a Detroit medical centre in the USA had diabetic foot infections involving at least one multidrug resistant pathogen, and in one quarter of these cases, more than one multidrug resistant organism was reported (Henig *et al.,* 2020).

Table 1.6 provides on overview of the bacteriology and susceptibility patterns found in some studies on DFUs worldwide. Research indicates that *S. aureus* is one of the most common causative pathogens for infection in DFUs followed by *P. aeruginosa,* the latter of which is not accounted for in the South African treatment guidelines due to a lack of empirical data. Of the antibiotics reported to be most successful in treating DFUs, vancomycin and imipenem are most often used (Amjad *et al.,* 2017; Giridhar and Kalavathi, 2018; Kagwa *et al.,* 2018; Hassan *et al.,* 2019; Najari *et al.,* 2019; Goh *et al.,* 2020). In one study, amoxicillin/clavulanic acid was the most successful in treating DFUs (Ogba *et al.,* 2019), however, in two different studies it was shown to have the highest rates of resistance (Amjad *et al.,* 2017; Kagwa *et al.,* 2018). In 2021, a review of 112 research papers found that the most common pathogens found in DFUs were *S. aureus, P. aeruginosa* and *E. coli* (Macdonald *et al.,* 2021). It is important to note that the majority of antibiotics that show increased levels of resistance are beta-lactam antibiotics (Amjad *et al.,* 2017; Sánchez-Sánchez *et al.,* 2017; Giridhar and Kalavathi, 2018; Kagwa *et al.,* 2018; Sekhar *et al.,* 2018; Hassan *et al.,* 2019; Najari *et al.,* 2019; Ogba *et al.,* 2019; Goh *et al.,* 2020). This suggests that the beta-lactam class of antibiotics should be avoided in empiric treatment or a delay in treatment should occur until culture results indicate that they are appropriate. In South Africa, amoxicillin/ clavulanic acid, a beta-lactam antibiotic, is the mainstay of DFU infection treatment.

Table 1.6: Overview of the bacteriology and susceptibility patterns of DFUs worldwide.

Location	Most common causative pathogens	Treatment exhibiting the best results	Treatment exhibiting the most resistance	Author
India	*Pseudomonas* spp; *E. coli; S. aureus*	Meropenem, vancomycin, linezolid, imipenem	Cephalosporins, penicillin, cefazolin	Priyadarshini Shanmugam, 2013
Cameroon	*Proteus* spp; *E. coli; S. aureus*	Ciprofloxacin, imipenem	Ampicillin, oxacillin	Akwah *et al.*, 2015
Pakistan	*S. aureus; E. coli*	Vancomycin, imipenem	Cephalosporins, amoxicillin/clavulanic acid	Amjad *et al.*, 2017
Pakistan	*S. aureus; E. coli; K. pneumoniae*	Vancomycin, imipenem	Ciprofloxacin, cefuroxime	Miyan, *et al.*, 2017
Mexico	*S. aureus; Enterobacter* spp	Levofloxacin, cefalotin, amikacin	Penicillin, dicloxacillin	Sánchez-Sánchez *et al.*, 2017
India	*S. aureus; P. aeruginosa*	Vancomycin, linezolid, carbapenems	Penicillin, cephalosporins	Giridhar and Kalavathi, 2018
Kenya	*S. aureus; Proteus* spp	Imipenem, piperacillin/tazobactam, ciprofloxacin	Amoxicillin/clavulanic acid, clindamycin	Kagwa *et al.*, 2018
India	*S. aureus; P. aeruginosa*	Doxycycline, linezolid, meropenem	Ampicillin, cefazolin	Sekhar *et al.*, 2018
India	*P. aeruginosa; K. pneumoniae, S. aureus*	Vancomycin, tetracycline, imipenem, amikacin	Ampicillin, benzyl penicillin	Shareef *et al.*, 2018

Location	Most common causative pathogens	Treatment exhibiting the best results	Treatment exhibiting the most resistance	Author
Egypt	*S. aureus; K. pneumoniae; C. albicans*	Imipenem, amikacin	Ceftazidime, erythromycin, oxacillin	Hassan *et al.*, 2019
Guyana	*P. aeruginosa; E. coli; S. aureus*	Imipenem, amikacin	Ciprofloxacin; erythromycin	Kurup and Ansari, 2019
Iran	*Staphylococcus* spp.*; E. coli*	Ampicillin, vancomycin, imipenem	Penicillin; ciprofloxacin; ceftriaxone	Najari *et al.*, 2019
Nigeria	*S. aureus; P. aeruginosa*	Erythromycin, amoxicillin/clavulanic acid	Ampicillin, co-trimoxole	Ogba *et al.*, 2019
India	*S. aureus; P. aeruginosa; E. coli, K. pneumoniae*	Vancomycin, linezolid, imipenem	Ceftriaxone, amoxicillin/clavulanic acid	Otta *et al.*, 2019
Malaysia	*P. aeruginosa; S. aureus*	Vancomycin, imipenem, amikacin	Penicillin, tetracycline, ciprofloxacin	Goh *et al.*, 2020
Global review	*S. aureus; P. aeruginosa; E. coli*	Not specified	Not specified	Macdonald *et al.*, 2021

In the studies carried out on the African continent; in Nigeria, Kenya and Egypt, the results differ greatly. In Kenya, *S. aureus* and *Proteus* sp. were the most commonly identified micro-organisms, which were most susceptible to imipenem, piperacillin/tazobactam and ciprofloxacin and were most resistant to amoxicillin/clavulanic acid and clindamycin (Kagwa *et al.,* 2018). However, in Nigeria where the most common causative pathogens were found to be *S. aureus* and *P. aeruginosa,* the best treatment results were seen with erythromycin and amoxicillin/clavulanic acid (Ogba *et al.,* 2019). This was different once again in Egypt where erythromycin was found to be one of the antimicrobials with the most resistance (Hassan *et al.,* 2019).

These results highlight the varying causative pathogens in infections in DFUs as well as the varying susceptibility rates and thus highlights the importance of carrying out local susceptibility studies to ensure that the most appropriate treatment regimen is prescribed. No study investigating the causative pathogens of DFUs exists in the South African context where treatment is guided by global trends, highlighting the importance of this study.

1.12. The management of non-infectious DFUs

In order to prevent the development of diabetic foot infections and thus reduce the need for antimicrobial treatment and subsequent amputation, the IWGDF recommend that patients who are risk of developing DFUs should be educated in foot self-care and self-protective behaviours (Schaper *et al.,* 2020). Education about preventing DFUs should include emphasis on daily foot inspection as well as the correct process to follow should a change in the appearance or temperature of foot be identified (Schaper *et al.,* 2020). Those patients at risk should understand the consequences of the loss of protective sensation, the importance of proper foot, nail and skin care as well as the most appropriate choice of footwear (Mayfield *et al.,* 2004). The following are the recommended preventative measures that patients should adhere to in order to reduce the incidence of ulceration (Mayfield *et al.,* 2004; Schaper *et al.,* 2020);

- Avoid walking barefoot, in socks without footwear, or in thin-soled slippers.
- Avoid wearing tight shoes and shoes with rough edges and uneven seams.
- Wear socks or stockings without seams. If this is not possible, wear the seams on the outside of the foot (inside out).

- Avoid wearing tight socks and stockings (compression stockings) unless advised by the healthcare professional.
- Wash feet daily in water that is at a temperature below 37°C and make sure that feet are completely dry.
- Do not warm feet by means of a heater or hot water bottle.
- Do not use chemical agents or plasters to remove corns and calluses without the recommendation by a healthcare professional.
- Use emollients to moisturise dry skin (avoid using emollients in between toes).
- Toenails should be cut straight across in order reduce the occurrence of in-grown nails.
- If new shoes require breaking in, do so gradually in order to avoid the formation of blisters.
- In the case of visual difficulties or if the patient is unable to examine the foot themselves, help should be enlisted from other people like family members.

In the diabetic patient, wearing inappropriate footwear or walking barefoot may result in foot trauma and subsequent ulceration (Schaper *et al.,* 2020). Appropriate footwear is footwear that fits, protects and accommodates the shape of the feet (van Netten *et al.,* 2018). The use of therapeutic footwear is associated with a decrease in the risk of DFU formation or the recurrence of DFUs in patients who already have developed peripheral neuropathy (Jorgetto *et al.,* 2019). Although patients who have not lost protective sensation can select properly fitted shoes from retailers, it is suggested that patients with neuropathy and subsequent loss of protective sensation or PAD should be cautious when selecting, or being fitted with footwear. This is especially true for those with foot deformities or a history of previous DFU (van Netten *et al.,* 2018). While no controlled studies exist on the specific role of footwear to prevent a non-plantar foot ulcer, poorly fitted footwear have been noted as a cause of non-plantar foot ulceration. This suggests that properly fitting footwear may reduce ulcer incidences (Bus *et al*., 2016).

In Africa, the use of protective footwear as a preventative measure is not routinely prescribed when compared to Western countries, largely as a result of the poor socio-economic factors present in Africa (Abbas and Archibald, 2005). All healthcare providers at primary level should be able to carry out basic screening examinations as well as provide the education required in order to prevent and manage DFUs (Mayfield *et al.,* 2004; Schaper *et al.,* 2020). A study carried

out in the Tshwane district of South Africa found that both diabetes care and screening practices were sub-optimal (Webb *et al.,* 2015). Only 6% of participants had been noted to have received a foot examination within the previous year (Webb *et al.,* 2015). Another South African study determined that only 32.5% of patient's had their feet examined by a doctor or nurse and only 5.8% had their feet examined by a podiatrist (Dikeukwu and Omole, 2013). Podiatrists, who are professionals trained to assess and implement treatment for ailments of the lower limb and foot, manage and treat DFUs through various interventions and by means of patient education (Sithole and Abrahamse, 2017). In South Africa, there is also a shortage of podiatrists alongside the inadequate provision of foot care, which further affects the prevalence of DFU (Clarke and Tsubane, 2008).

1.12.1. Ambulatory aids and pressure offloading devices

In patients with established ulcers, the use of pressure offloading devices will hasten healing time. The use of offloading methods remains important even after the ulcer has healed as the risk of reoccurrence within the first twelve months is as high as 40% (Bus, 2016; van Netten *et al.,* 2018). According to the IWGDF, the most effective manner to reduce plantar pressure is through use of a total contact cast (TCC) or a removable walker (Wu *et al.,* 2008; Schaper *et al.,* 2020). These devices reduce pressure in the forefoot by up to 87% and for this reason they are preferred to devices that extend to the ankle which only reduce pressure by 40% to 60% (Bus, 2016). In the case of non-plantar ulcers, it is recommended that removable ankle-high off-loading devices, footwear modifications, toe spacers or orthoses be used based on the nature and location of the ulcer (Schaper *et al.,* 2020). Total contact casts are the gold standard of offloading methods followed by removable walkers (Wu *et al.,* 2008; Schaper *et al.,* 2020). Although the use of TCCs is recommended, a study in Columbia found that 2% of treatment centres actively made use of TCCs while removable walkers were used in 15% of treatment centres (Wu *et al.,* 2008). The lack of use of removable walkers was attributed to their high cost (Wu *et al.,* 2008). Total contact casts are not commonly used as they require specialist training in order to be safely fitted (Wu *et al.,* 2008). They are not easily removed to assess the foot and as its application impairs daily activities such as sleeping, bathing and postural stability.

1.13. Summary

In South Africa, DFUs are increasingly common with one third of diabetic patients said to develop a DFU in their lifetime (Lazzarini *et al.,* 2018). These ulcers are particularly susceptible to infection due to the nature of the diabetic patient's health state and lack of preventative measure use (Costa *et al.,* 2017). Infection of the DFU is often caused by drug resistant micro-organisms which is partly due to inappropriate antibiotic prescribing practices and the chronic nature of the wound (Kandemir *et al.,* 2007; Henig *et al.,* 2020).

Inappropriate antibiotic prescribing is hypothesised to be prevalent is South Africa as the treatment of DFUs is based on guidelines set out in the STG and the Essential Drugs List where financial restrictions and lack of resources prevent healthcare workers from obtaining cultures before empiric treatment is prescribed (Abahamye, 2016). All of these factors combined delay healing time and increase the risk of reoccurrence and amputation (Khan *et al.,* 2020). Overall, the factors described contribute towards the economic burden that DFUs place on South Africa as well as reduce the quality of life of patients immensely (Figure 1.3).

By identifying antimicrobial resistance patterns specific to South Africa, more accurate and concise guidelines can be developed thereby reducing the development of further antimicrobial resistance, improving healing times and reducing the risk of reoccurrence and amputation. This study will provide valuable information related to all aspects of DFU treatment resulting in improved patient outcomes.

1.14. Research question

What are the antimicrobial resistance patterns in diabetic foot ulcers and are the most effective treatment practices employed in the South African public healthcare sector for the management of these conditions?

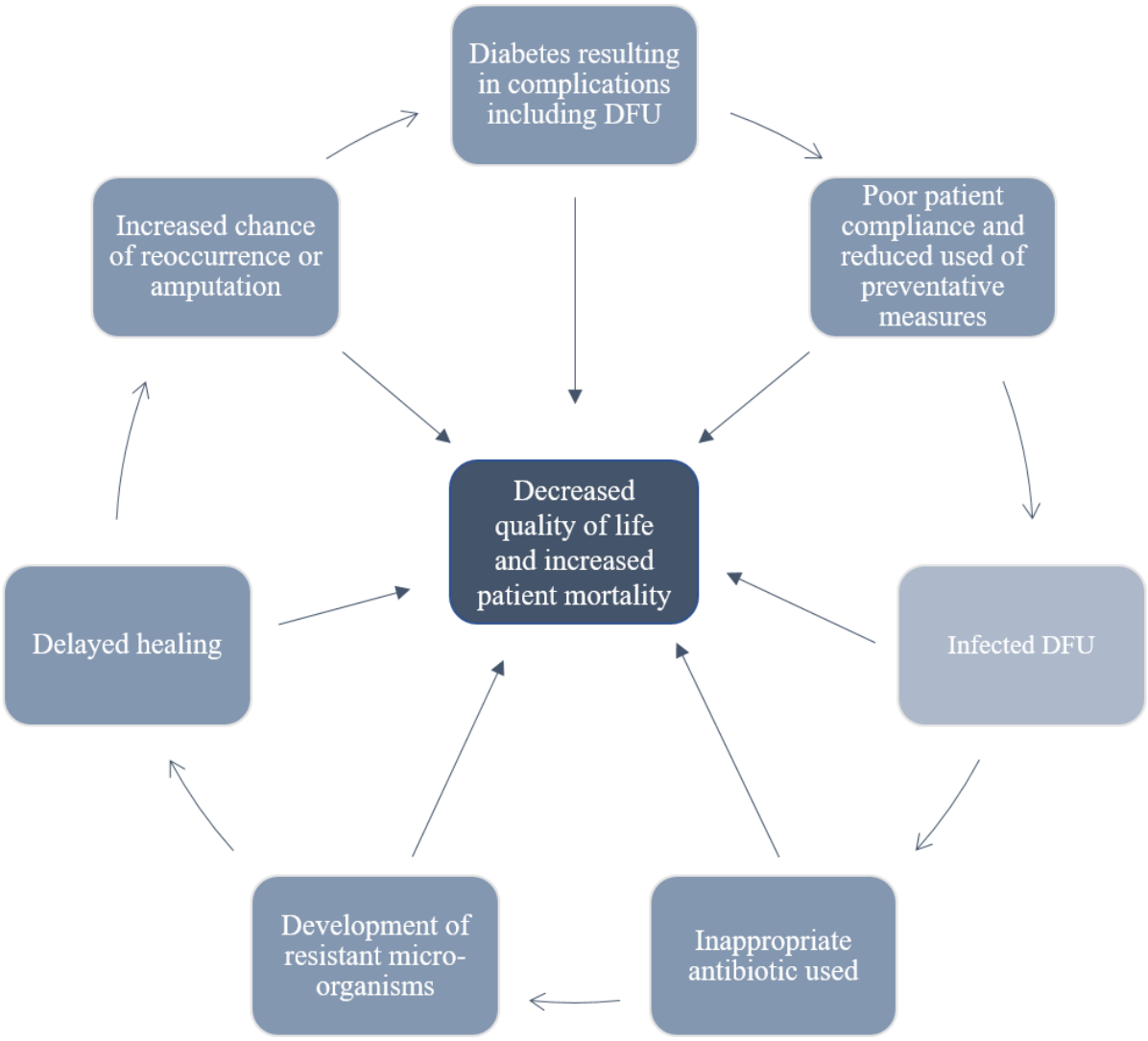


Figure 1.3 Summary of the consequences of DFUs.

1.15. Aims and objectives

This study will aim to determine the antimicrobial resistance patterns micro-organisms isolated from diabetic foot ulcers in selected Gauteng public healthcare settings, in order to suggest clinically effective treatment protocol and infection management practices. With this in mind, the following objectives were implemented;

- To obtain wound samples from DFU patients in order to identify micro-organisms implicated in these infections.
- To determine the antimicrobial resistance patterns of pathogens obtained from DFU patient samples, by performing antibiotic susceptibility testing using zone of inhibition studies.

- To determine, by means of a patient interview and review of patient records, the use of preventative measures and past treatment practices in the management of diabetic foot ulcers.
- To suggest additional recommendations to the existing local treatment guidelines for diabetic foot ulcers in the public hospitals based on the findings of the above-mentioned objectives.

1.16. Outline of the dissertation

Chapter 1 provides a detailed background, literature review and rationale for this study.

Chapter 2 provides details on the patient demographics and how they may influence the pathophysiology of the DFU. The methodology used to obtain this information is explained in detail. Further information is given as to the nature of common co-morbidities as well as the state of record keeping.

Chapter 3 provides an overview of the methodology used to undertake the microbiology component of the study. The results of the isolation, antimicrobial susceptibility testing and identification of micro-organisms are discussed in this chapters. These results were then used to generate an overall summary of the antimicrobial resistance patterns of micro-organism isolated from DFUs in the South African public healthcare sector.

Chapter 4 analyses the clinical data of the patients and includes chronic medication use, preventative measure use, ulcer severity and patient treatment history. This chapter examines past and present treatment practices and their alignment to both local and international guidelines. Finally, a holistic treatment plan is provided by including a detailed antibiogram developed from the results obtained in Chapter three.

Chapter 5 provides an overview of the entire study and how the results pertain to the objectives set out in Chapter one. The chapter includes the limitations of the study as well future recommendations and conclusion.

Chapter 2: Patient characteristics

2.1. Introduction

Knowledge and practice regarding diabetic foot ulcers (DFUs), as well as the ability to identify individuals at risk of developing a DFU, is vital in reducing complications and subsequent lower limb amputation (Pourkazemi *et al.,* 2020). According to a recent study, DFU prevention methods are insufficiently used and recognised in both research and the clinical setting (van Netten *et al.,* 2020b). It is therefore important to understand and identify patient specific characteristics and demographics that may increase the risk of DFU development in order to identify the patients at risk. By effectively identifying high risk patients, preventative measure use and patient education can be effectively implemented to curb DFU development.

This chapter aims to identify the demographics of the sample population as well as categorize factors that predispose individuals for developing a DFU based on demographic data.

2.2. Methods

2.2.1. Ethics

Ethics was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (M210431) (Appendix B). Due to a fire at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), an ethics amendment was applied for and granted in order to include Helen Joseph Hospital (HJH) as a study site (Appendix B).

Ethics approval was granted and patient confidentiality was maintained through the use of key numbers which was ascribed to all participants (for example A001, A002). The patient information leaflet (Appendix C) was given to the patient before obtaining informed consent to participate in the study. Informed consent (Appendix D) was obtained from participants before wound samples were taken. The patient was also asked if they consented to a photograph of the DFU being taken. The principal investigator of this study, unless otherwise stated, was responsible for capturing all photographic evidence included in this dissertation.

To ensure the safety of the principal investigator, all sample collections were carried out either by, or under the direct supervision of Mr. Mokoena, the consulting podiatrist from the CMJAH. Furthermore, the processing of samples was supervised by laboratory staff who possess the necessary knowledge for handling and processing potentially infectious samples. The study was carried out according to biosafety level two (BSL-2) guidelines (CDC, 2020).

2.2.2. Setting

This study was conducted at the CMJAH and HJH on patients visiting the podiatry and wound clinics. Approval of the collection site was obtained from both of these facilities (Appendix E). In collaboration with Mr. Mokoena, it was established that an average of 100 patients on average, attend the healthcare facilities on a yearly basis. A total minimum sample size of 50 patients was required for this study in order to attain a 50% attrition rate. The decreased prevalence of DFU in this setting due to the COVID-19 pandemic was considered in the sample size calculation. Participation was on the day of study enrolment and no follow-up visits were required.

2.2.3. Inclusion and exclusion criteria

All patients (female or male in gender) were selected and invited to participate in this study. Patients identified to participate had to be diabetic and present with a DFU.

Patients were excluded from participation in this study if the patient was a paediatric patient as DFUs are not commonly seen in this population group.

2.2.4. Patient selection

Patient selection was based on purposive, homogenous sampling as the goal of this research is to understand those patients who present with foot ulceration as a consequence of diabetes. Patients who met the inclusion criteria were identified as potential candidates for this study. The patients were then asked if they would like to participate in the study following provision of study information by means of a patient information sheet. Upon positive affirmation of consent to participate, the patient was asked to sign an informed consent form and the

administration of the structured questionnaire took place using a retrospective review tool (Appendix F).

2.2.5. Patient classification

Patients were classified as having type 1 or type 2 diabetes based on the patient's history obtained from the file review or interview. Once classified by diabetic type, patients were further classified by level of DFU infection. This classification is dependent on severity of the ulcer and is classified as either mild, moderate or severe as per Figure 2.1.

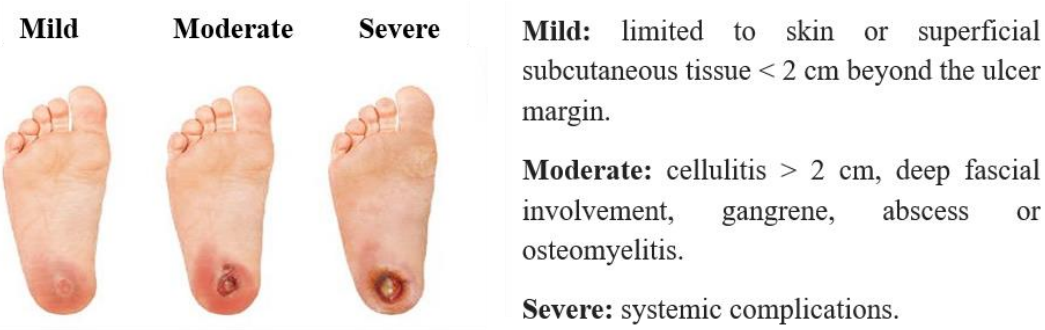


Figure 2.1: Classification of diabetic foot adapted from Diabetestreatmentguide.org (2021) and the SAASP (Wasserman *et al.*, 2015).

After this initial classification according to South African guidelines, the patients DFU was subsequently classified according to the Wagner classification (Chapter 1, Table 1.1) in order to compare the most commonly used classification system worldwide to the South African guidelines.

2.2.6. Patient record review and administration of a structured questionnaire

Participants who were selected for this study had their past records reviewed in order to determine the likelihood of previous DFU infections as well as to determine the occurrence of co-morbidities. The treatment protocol implemented was recorded alongside the number of infections treated and if sample cultures were taken. Twelve-month patient records identified the infection frequency, the causative micro-organism (if identified) and the antimicrobial used to treat the DFU. This information assisted in determining the prevalence of DFU and the prescribing patterns of antimicrobials which guided the development of the treatment policy.

The review of records took place after the patient consultation. A short, structured questionnaire was used in order to determine the use of preventative aids that had not been prescribed. The patients were interviewed in order to overcome any language or educational barriers that may have presented upon a self-administered questionnaire.

2.3. Results and discussion

Sample and data collection took place from August 2021 until July 2022. A total of 51 ulcer swabs from 45 patients were included in the study. Of these patients, six presented with two DFUs. Of the patient population, 32 (71.1%) were outpatients while the remaining 13 were inpatients mainly admitted to surgical wards. The majority of patients (73.3%) presented to HJH. This is due to the fact that many patients who would normally attend CMJAH were referred to HJH following the fire at CMJAH that caused a temporary closure of the podiatry unit.

2.3.1. Age

Upon obtaining informed consent to participate in the study, participants were asked various demographic questions in order to determine the demographic information of the patient population. Table 2.1 depicts the age groups of participants.

Table 2.1: Participant age distribution.

Age	Number of participants	Percentage
30-40 years	2	4
40-50 years	6	13
50-60 years	18	40
>60 years	19	42
Total number of patients	45	100

The results from this study indicated that the greatest rate of incidence of ulceration occurred in those over the age of 60 years (42%). A similar rate of incidence (40%) was seen in the 50-to-60-year age group. These results indicate that only 17% of DFUs were seen in patients younger than 50 years of age. The oldest participant was 80 years old and the youngest

participant was 36 years of age. The median age of participants was 59 (n=45). According to the systematic review and meta-analysis carried out by Rodrigues *et al.* (2022), increasing age is a consistent risk factor associated with DFU incidence and consequent lower limb amputation.

2.3.2. The association of gender with DFU incidence

According to a meta-analysis evaluating the influence of epidemiologic and patient behaviour–related predictors on amputation rates in diabetic patients, the male sex was found to be a significant risk factor for amputation (Shin *et al.*, 2017). Another study concluded that gender-specific characteristics may influence the development of diabetes related complications (Di Giovanni *et al.*, 2021). Figure 2.2 illustrates the incidence of DFUs according to gender.

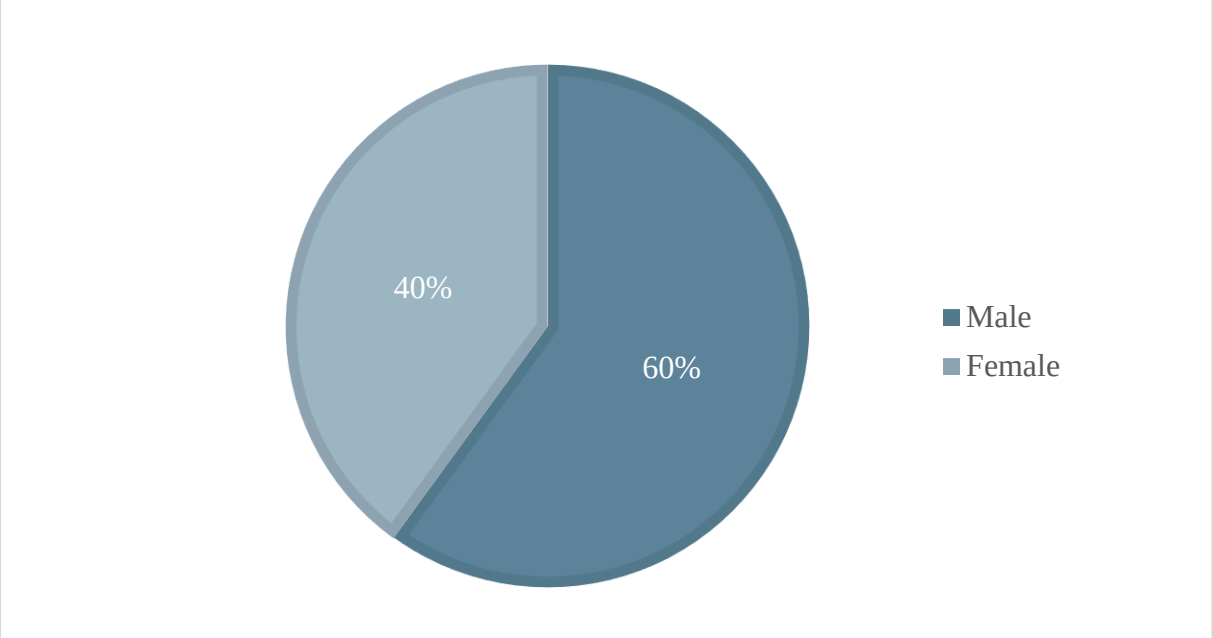


Figure 2.2: The distribution of male and female participants.

The results from this study demonstrated a higher prevalence of DFU among male participants (60%) in comparison to female participants (40%). This finding was consistent with previous studies (Krishnamurthy, 2018; Kateel *et al.*, 2018; Otta *et al.*, 2019). The prevalence of DFUs has been suggested to be greater among the male population due to an increased incidence of peripheral neuropathy and higher likelihood of exposure to trauma in comparison to females (Kateel *et al.*, 2018, Sorber and Abularrage, 2021, Rodrigues *et al.*, 2022).

2.3.3. The presence of diabetes related complications

Co-morbidities linked to the development of DFU include diabetic neuropathy, hypertension and dyslipidaemia (Rodrigues *et al.*, 2022). Figure 2.3 indicates the incidence of the most commonly seen diabetes related complications among the study participants as reported in the patient's file.

Hypertension was found to be present in 84.4% of patients making it the most common concurrent medical condition amongst this cohort. This result is consistent with another South African study where it was found that 85.7% of DFU patients presented with hypertension (Dikeukwu and Omole, 2013).

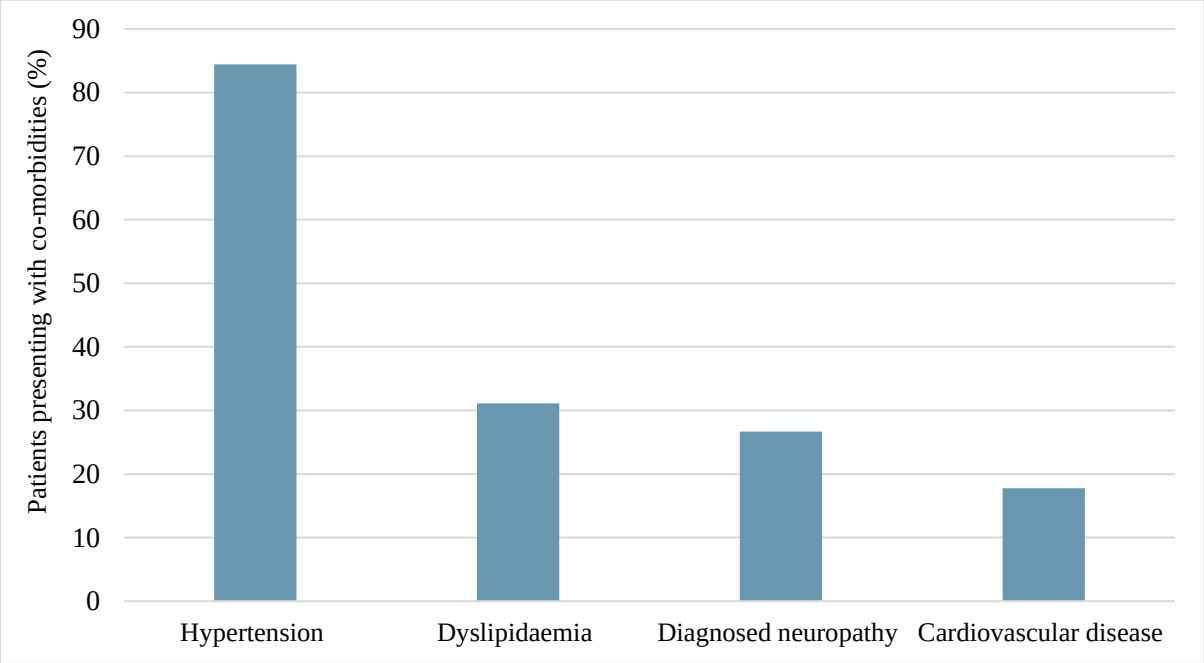


Figure 2.3: The most common diabetes related complications among participants.

Hypertension is a critical co-morbidity in patients presenting with DFUs as it results in atrial wall stiffening and thus further promotes the advancement of PAD and subsequent DFU development. The findings in this study are consistent with previous studies where 58.3-69.9% of patients presented with hypertension (Kateel *et al.*, 2018; Abdulghani *et al.*,2018; Sayiner *et al.* 2019). This result is congruent with World Health Organisation (WHO) findings where low-to-middle income countries have been determined as having a higher incidence of hypertension amongst their population (WHO, 2022). This can be seen when comparing the

prevalence of hypertension in South Africa, a low-to-middle income country (35.1%) to that of the WHO Region of the Americas, a high-income region (19%) (Rayner *et al.*, 2019; WHO. Int. 2022).

A previous diagnosis of dyslipidaemia and high cholesterol levels were determined in 31.1% of patients, according to patient files. This figure does not reflect the true incidence of dyslipidaemia amongst the study population as a number of patients were noted to be on lipid lowering treatment agents, however, no report was made of the diagnosis in the patients' file. Figure 2.4 illustrates the incidence of dyslipidaemia amongst patients and those receiving treatment. Based on those patients with diagnosed dyslipidaemia and those patients who did not have a formal diagnosis but who were receiving lipid lowering treatment, a more plausible representation of dyslipidaemia in the study population is 60%.

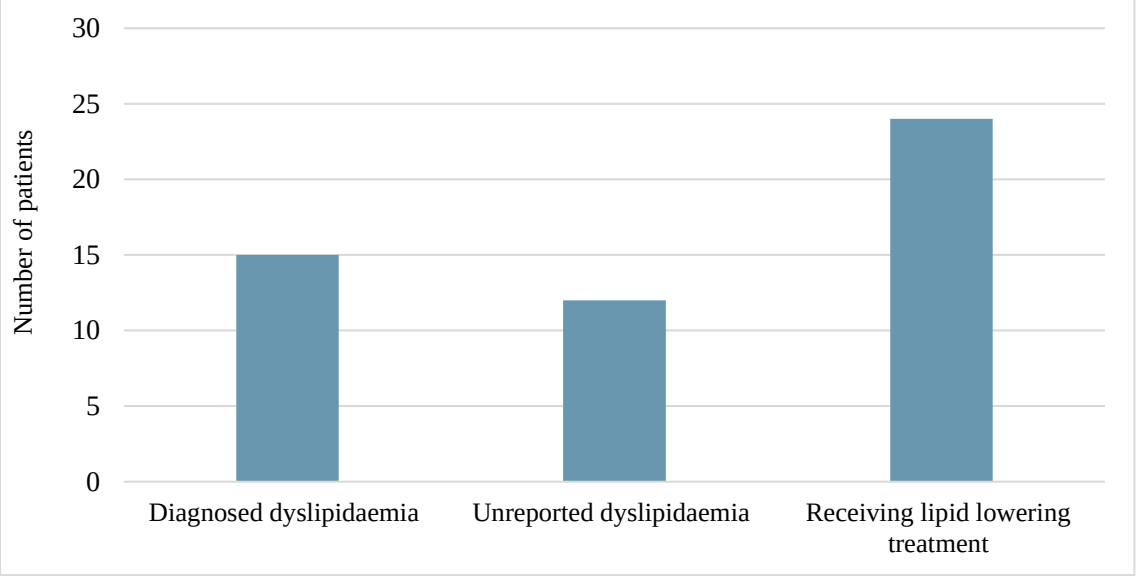


Figure 2.4: The incidence of reported and unreported dyslipidaemia as well as those receiving treatment.

A study undertaken in Egypt concluded that the presence of dyslipidaemia significantly increased the risk for DFU development (AbdAllah and Sharafeddin, 2020). In contrast, a systematic review carried out by Rossboth *et al.* (2021) concluded that while dyslipidaemia is often associated with type 2 diabetes mellitus, it is not associated with diabetic foot conditions. While conflicting reports exist about the relationship between dyslipidaemia and DFU risk, it can be said that patients presenting with dyslipidaemia are 2.23 times more likely to develop

peripheral neuropathy, a significant factor in the pathophysiology of DFUs (Khawaja *et al.*, 2018; Chastain *et al.*, 2019).

Peripheral neuropathy was noted in the patients' files in 26.7% of patients; however, upon physical examination of the patients, only 35.6% reported feeling pain and tenderness around the area of ulceration that was not interpreted as tingling, pins and needles or jabbing. This suggests that peripheral neuropathy was not appropriately diagnosed in 29% of patients. Upon review of the patient files, it was noted whether or not the patient had received a diagnosis of peripheral neuropathy as well as what treatment was prescribed, if any. The patient was also asked whether or not they felt any pain sensation in the area of the ulcer. Figure 2.5 shows the patients grouped by whether or not they had peripheral neuropathy and the status of their treatment plan. Furthermore, it indicates those patients in which peripheral neuropathy potentially remains undiagnosed and untreated.

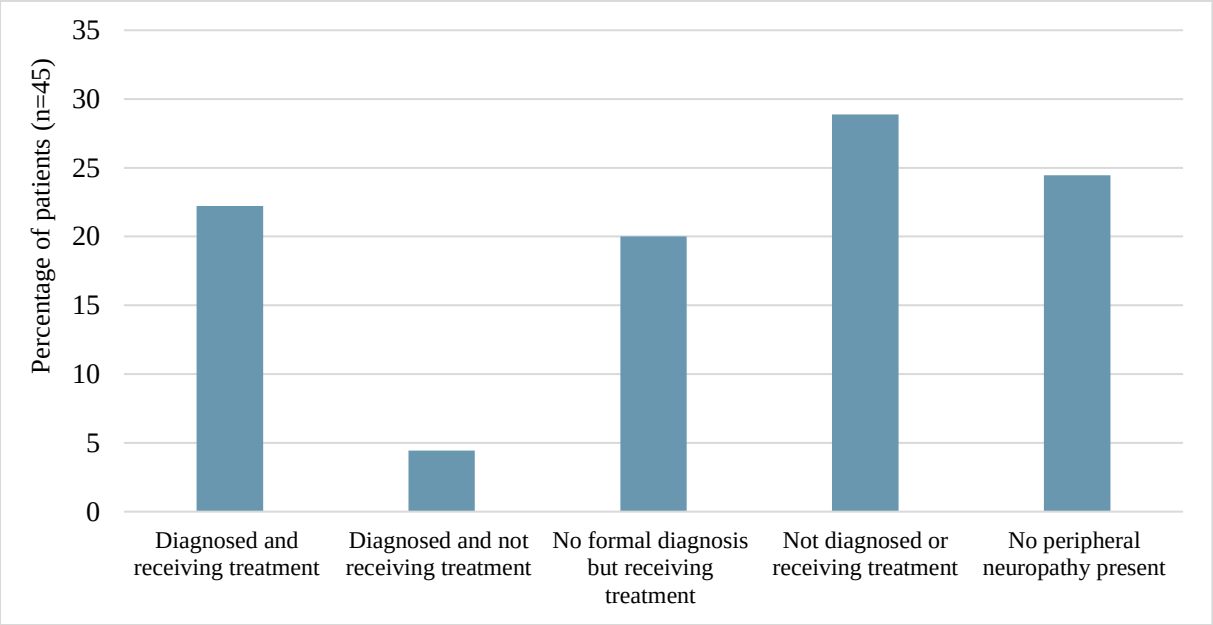


Figure 2.5: The presence of peripheral neuropathy in participants and the status of their treatment plan.

From these results, it can be deduced that the incidence of peripheral neuropathy as documented in the patients' files is 46%. From the results obtained, 29% of patients presented with signs and symptoms of peripheral neuropathy, however, they remained undiagnosed and untreated. This means that the more likely incidence of peripheral neuropathy in the study population may potentially be closer to 75%. This finding is corroborated by Almobarak *et al*. (2017) who reported the incidence of peripheral neuropathy association to DFU to be 82.1%. Sharma *et al.*

(2016) reported that 95.8% of DFU patients presented with peripheral neuropathy. Megallaa *et al.* (2019) found that 64% of their sample population had developed loss of protective sensation; while 82.2% had abnormal vibration perception threshold. Vibration perception threshold is the lowest voltage that can be detected in the foot, at least 50% of the time (Garrow and Boulton, 2006). Overall, the results suggest a high incidence of peripheral neuropathy in patients presenting with foot ulceration.

The fact that 29% of the study population sample remained undiagnosed, despite presenting with signs and symptoms of peripheral neuropathy is significant as peripheral neuropathy in the feet is responsible for the loss of protective sensation, abnormal loading, repetitive trauma and autonomic dysfunction and subsequent DFU development (Edmonds and Foster, 2004; Chastain *et al.*, 2019). A diagnosis of peripheral neuropathy is based both on quantitative tests and clinical signs, but may also occur without any reported symptoms (Hicks and Selvin, 2019). Thus, regular screening for peripheral neuropathy is important, with the American Diabetes Association recommending that screening for peripheral neuropathy in diabetic patients occur from the time of diagnosis and annually thereafter (American Diabetes Association, 2019). The results from this study suggest that peripheral neuropathy screening does not occur as often as prescribed. A possible reason for this may be due to language barriers between the patient and healthcare practitioner resulting in miscommunications during the examination process (Shaikh *et al.*, 2013). Furthermore, the shortage of podiatrists in South Africa compounds with the inadequate provision of foot care and thus diagnoses of peripheral neuropathy by podiatrists may be less frequent (Clarke and Tsubane, 2008).

An additional concern is that patients' health conditions are not effectively reported in their files and that patient record keeping is not effectively implemented and monitored. This is problematic in that medical practitioners may miss critical information about previous diagnoses, treatments and prescriptions when assessing and providing new treatment plans for patients (Marutha and Ngoepe, 2017). A study undertaken by Steyn *et al.* (2008) in public health care centres in South Africa aimed to identify healthcare provider-related determinants of diabetes and hypertension management. The study determined that co-morbid conditions, as well as special investigations concerning the progression of disease, were infrequently noted (Steyn *et al.*, 2008). The lack of complete patient health records often results in poorer quality patient care, and further adversely effects clinical research (Odekunle *et al.*, 2017).

2.4. Summary

- The prevalence of DFUs was higher with progression in patient age with the 82% of patients being over the age of 50.
- A greater majority of DFU patients identified as male in gender.
- Hypertension is the most common co-morbidity in the sample population.
- Dyslipidaemia was poorly reported in patient files with 26.7% of patients receiving lipid lowering agents without record of a diagnosis.
- Patient record keeping is not effectively implemented or monitored.

Chapter 3: Microbiology

3.1. Introduction

Antimicrobial resistance in common causative pathogens of diabetic foot ulcers (DFUs) compounds the problem of rising diabetic foot infection incidences (Chastain *et al.,* 2019). Empiric antimicrobial use is often employed in the treatment of diabetic foot infections; however, the use of inappropriate antimicrobials or low concentrations of these agents could result in the development and distribution of resistant pathogens (Hassen *et al.,* 2019). It is therefore important that antimicrobial resistance patterns, specific to geographical location, be investigated in order to prescribe the most appropriate treatment regimen.

This chapter aims to isolate, identify and determine the antimicrobial resistance patterns of pathogens taken from diabetic foot wound samples in patients seeking treatment at the study hospitals.

3.2. Methodology

The microbiological methodology outlined in this chapter includes the sample collection methodology, process sampling and micro-organism isolation. Thereafter, detail is provided on the methods used to obtain antimicrobial susceptibility patterns as well as sample identification (Figure 3.1)

3.2.1. Wound sample collection

Sample collection was carried out, during patient consultation, with the podiatrist. These interactions were conducted while adhering to the COVID-19 safety regulations of the institution. Personal protection equipment such as KN95 respirator masks and gloves were worn at all times. In this study, clinical pathogens were collected directly from the wound of each participant using the Levine swabbing method (Levine *et al*., 1976; Cross, 2014). The Levine swabbing technique is considered as current best practice in wound sample collection as it provides 91% sensitivity and 57% specificity (Angel *et al*., 2011; Cross, 2014). This

technique involves collecting a sample from a limited, clean tissue area within the wound. The sample area should consist of clean tissue, as pus or necrotic wound tissue will provide an inaccurate representation of the microbiology of the wound tissue (Cross 2014). The sample area should remain small in order to express fluid from the wound bed by means of pressure (Angel *et al*., 2011). The wounded area was swabbed with a nylon-flocked swab soaked in 150 µl of 0.9% sterile saline as it improves the efficacy of bacteria transfer from the wound (Steer *et al.*, 1996; Cross, 2014). The nylon-flocked swab was rotated over a 1 cm^2 area with enough pressure to express fluid within the wound for five seconds (Angel *et al*., 2011; Cross 2014). Following this, the swab was placed into a sealed sterile receptacle. This process was repeated a second time and the second swab was transferred into an anaerobic gas jar in order to promote the growth of anaerobic micro-organisms. The collected samples were then transported on ice to the Department of Pharmacy and Pharmacology, Division of Microbiology laboratory for further analysis and immediately processed for culture growth.

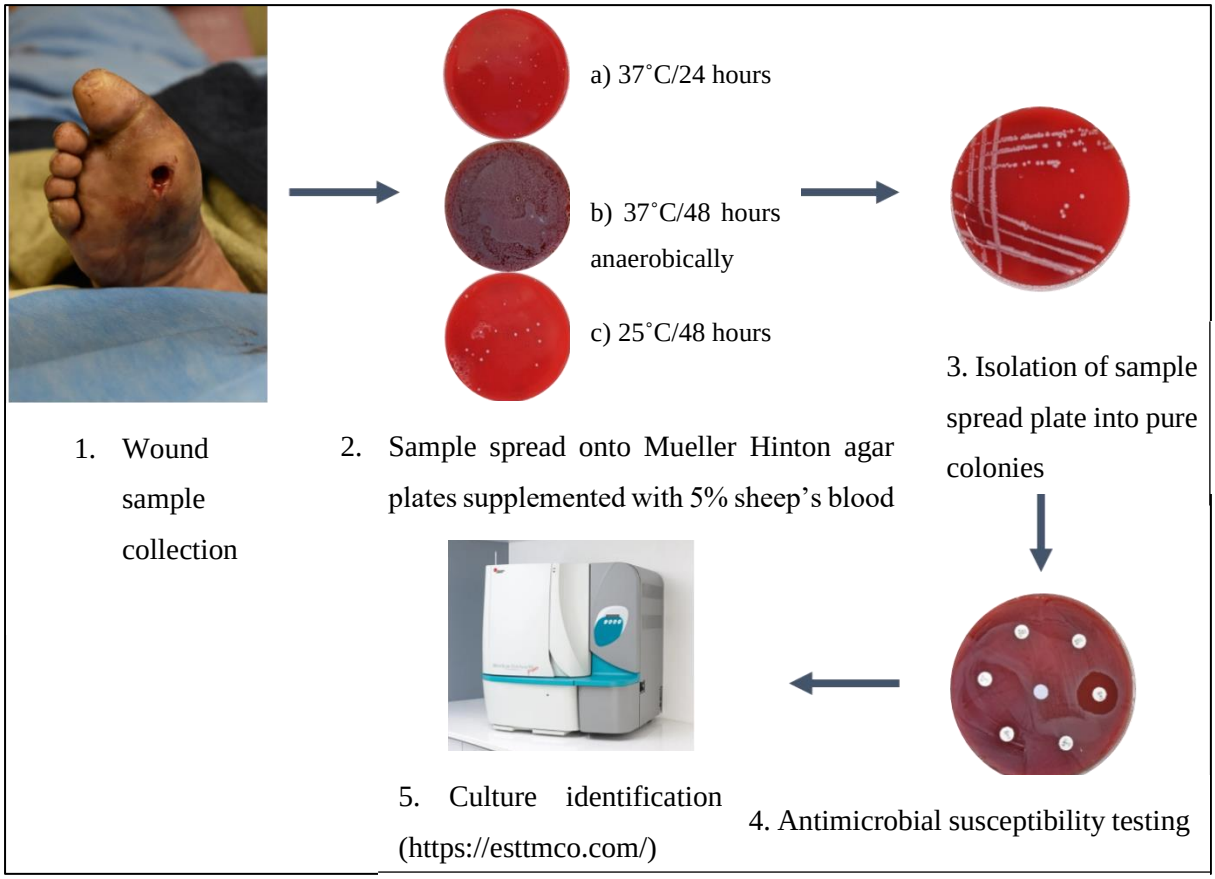


Figure 3.1. Overview of the microbiological methodology**.**

3.2.2. Preparation of Mueller Hinton agar plates

Mueller Hinton agar plates supplemented with 5% sheep blood were used in order to culture micro-organisms. This media was selected due to its nutrient-dense consistency, allowing for the growth of a wide range of microbial pathogens (Pelczar *et al.*, 1977). The Mueller Hinton agar was prepared by weighing 40 g of Mueller Hinton agar (Oxoid, ThermoFisher Scientific) to 1 L of distilled water. The mixture was autoclaved at 121°C for 30 min following which, it was allowed to cool at room temperature until the mixture reached 45°C. Next, 50 ml of sheep's blood (South African Vaccine producers; Modderfontein, South Africa) was added to the molten agar, under aseptic conditions. Quality control was managed in the laboratory by swabbing the sheep's blood onto Mueller Hinton agar, incubated at 37°C for 24 hrs and checked for microbial growth. If no growth was present, then the blood was considered sterile. The prepared media was then poured into sterile petri dishes at a volume of 15 to 20 ml per plate. The petri dishes were then sealed and left to stand in the aseptic area for 30 min to solidify. One agar plate from the batch was incubated at 37°C for 24 hrs and checked for contamination which may have occurred during the processing of making the agar plates. If there was no growth on the plate, then the batch of agar plates was considered sterile. These agar plates were then inverted and stored between 2°C and 8°C and were kept for up to 16 weeks, checking periodically for contamination, changes in the appearance of the medium to suggest contamination, haemolysis, or deterioration, before use.

3.2.3. Culturing of sample

The method used to culture the collected patient samples was adapted from Schmohl *et al.* (2012) in which the swab head is cut off from the swab stick using sterile scissors and immersed in 1 ml of a 0.9% sterile saline solution for elution of the culture by vortex (Lasec) at room temperature for 3 min at 3 200 rpm. A volume of 0.1 ml of the suspended sample was removed using sterile micropipettes and applied to the surface of Mueller Hinton agar plates supplemented with 5% sheep blood. The sample was then spread evenly on the surface using a sterile spreader.

Three Mueller Hinton agar plates, supplemented with 5% sheep blood, were used per sample. One plate was cultured at 37°C for 24 hrs to culture aerobic bacteria, a second plate was

cultured in an anaerobic incubator at 37°C for 48 hrs to culture anaerobic bacteria, and the third plate was cultured at 25°C for 48 hrs to culture yeasts as per the Clinical and Laboratory Standards Institutes (CLSI) guidelines (CLSI, 2020). This process was carried out in duplicate in order to ensure that no contamination of the samples had occurred during the spread plate process. To ensure further sterility of the blood agar plates, an additional blood agar plate was incubated with each sample batch to ensure that the blood agar plates themselves were not contaminated. The presence of ≥10 identical colonies ($\geq 10^4$ colony-forming units per millilitre (CFU/ml)) was considered as microbiological evidence of infection. If there were less than 10 bacterial colonies present, the sample was reported as having no significant growth. The sample was reported as a mixed growth if more than three bacterial colony types were identified (Lewis *et al.*, 2013).

3.2.4. Single colony culturing and colony morphology

Microbial colonies were isolated from the spread plates by means of the streak plate technique. This was undertaken as a means of attaining single colonies for further identification and antimicrobial susceptibility testing. In order to keep an accurate record of the isolates derived from the original spread plate and to ensure that the information derived was kept specific to the patient, subsequent agar plates were labelled according to the patient key and isolate number, for example, A001 I1, A001 I2, A001 I3, etc.

Micro-organisms were isolated from the spread plates that were incubated in three different conditions. Each isolate was described according to their macromorphological characteristics. Isolates were described according to size as either large (1-3 mm), small (<1 mm) and granulated or tiny (pinpoint). The pigment or colour of the isolate was noted. The shapes of the isolates were described as round, irregular, spreading, filamentous or rhizoid. The margins of the isolates were classified as entire, undulate or filiform. Finally, the elevation of the isolates was classified as either flat, raised, convex, umbonate or irregular (Breakwell *et al.*, 2007). This was done in order to identify isolates that were similar to one another to assist in grouping them at a later stage. Following the isolation and characterisation of individual isolates (Figure 3.2), antimicrobial susceptibility testing was carried out for each isolate.

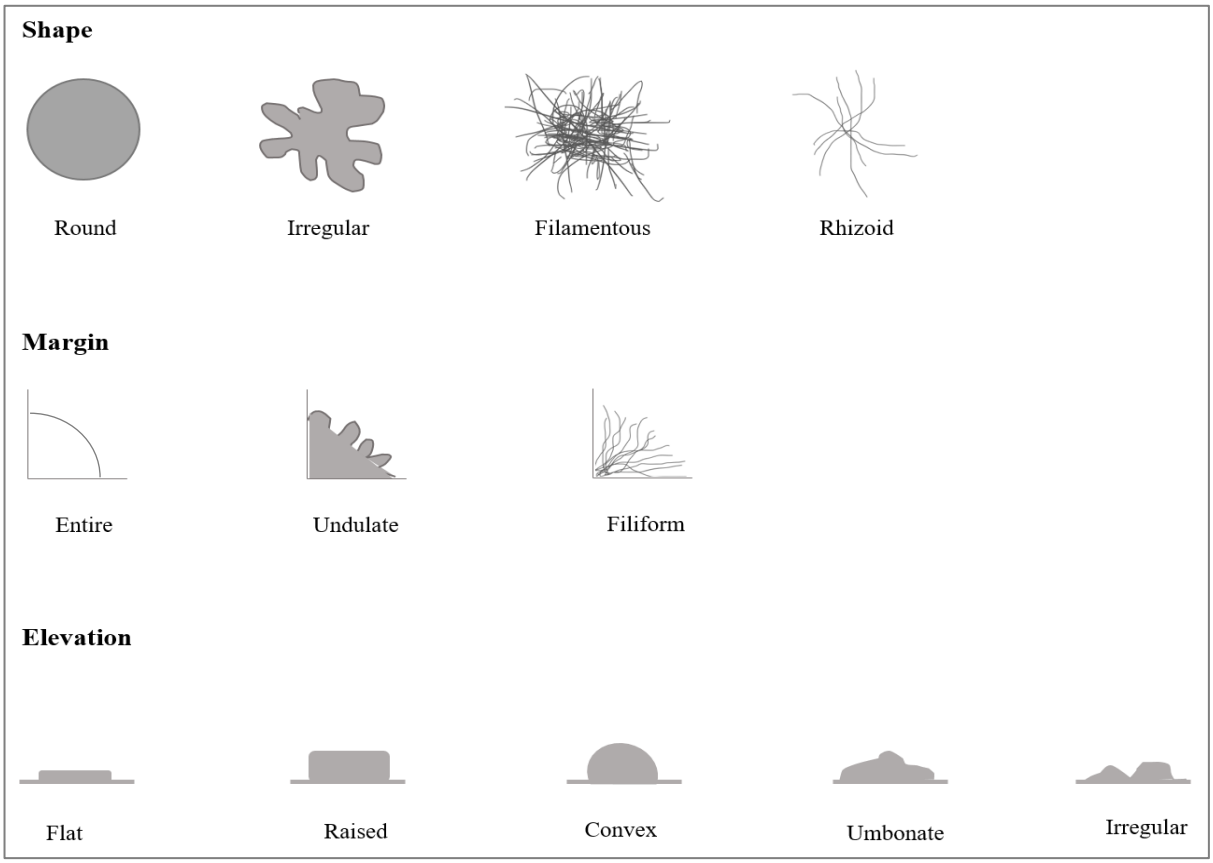


Figure 3.2. Colony macromorphological features used for identification. Image adapted from Leung and Liu (2022).

3.2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted using the disc diffusion test with zone of inhibition measurements used as a tool to obtain the results. The method was adapted from Lalitha (2004) in which the transfer of 0.1 ml of culture was applied to the surface of the Mueller Hinton agar supplemented with 5% sheep blood. Commercially prepared antibiotic disks (Oxoid, Thermo Fisher), each of which are pre-impregnated with a standard concentration of antimicrobial were placed onto the agar plate by means of an antibiotic disk dispenser (Oxoid, Thermo Fisher). The antimicrobial used included amoxicillin/clavulanic acid (3 mcg), gentamicin (10 mcg) and clindamycin (2 mcg) which are included in the South African Standard Treatment Guidelines (STG). In addition, ciprofloxacin (5 mcg), and vancomycin (5 mcg) which are the recommended antimicrobials by the South African Antibiotic Stewardship Program (SAASP) was included in the cohort of antimicrobials to account for resistant pathogens. Although this study focused on bacterial isolates and the culturing thereof, fluconazole (25 mcg) was also included as a control to account for contamination that may be

of fungal origin. A blank disk with no antimicrobial activity was used as a negative control (Figure 3.3).

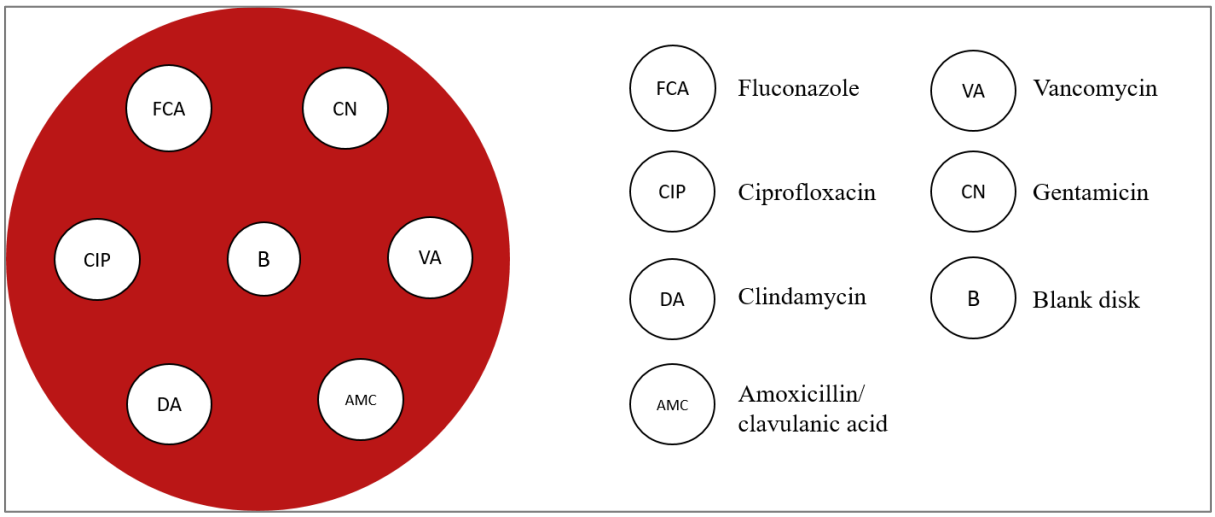


Figure 3.3: Arrangement of antimicrobial disks.

After incubation, the bacterial growth around each disk was observed and the radius measured in order to determine the zone of inhibition. The radius of each zone of inhibition (ZOI) was measured in millimetres (mm) using callipers from the centre of the antibiotic disk until the edge of the ZOI. Three varying points within the zones were considered for measurement, with the average of these recorded. These results were then compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant (CLSI, 2020). The study was done in duplicate on consecutive days.

3.2.6. Culture identification

Using both the macromorphological characteristics and antimicrobial susceptibility results, isolates were grouped together based on their appearance and susceptibility patterns. A single representative isolate from each group was then sent for identification to the National Health Laboratory Services Infection Control and Microbiology Laboratory, University of Witwatersrand. Biochemical examination which included starch hydrolysis, lipid hydrolysis, iron agar test, catalase test, indole production test and methyl red test for the confirmation of pathogens. All Gram-negative micro-organisms were identified on the MicroScan Walkaway 96 (Dade-Behring, USA) using the Microscan Rapid Negative ID Type 3 (RNID3) (Dade-Behring, USA) and API (Biomerieux, France) systems. Gram-positive micro-organisms were

identified with the MicroScan Walkaway 96 (Dade-Behring, USA)The Microscan Rapid Negative ID Type 3 is a system used to identify bacteria that do not produce significant amounts of acid or gas during fermentation. The system uses a panel of wells containing different substrates that the bacteria can use to produce specific enzymes. The system detects the presence or absence of these enzymes and compares the results to a database to identify the bacteria. The API system is a manual system that uses a panel of microtubes containing different biochemical tests to identify microorganisms. The system tests for the presence or absence of specific enzymes, metabolic pathways, and other characteristics of the microorganisms. The results are compared to a database to identify the microorganism.

Using the results generated from the antimicrobial susceptibility testing, a summary of the antimicrobial susceptibilities was developed. The percentage inhibition was calculated and compared to demonstrate antimicrobial efficacy.

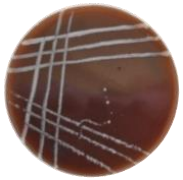
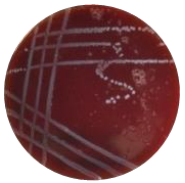
3.3. Results and discussion

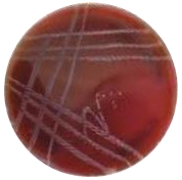
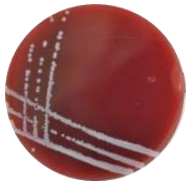
3.3.1. Microbiological characteristics

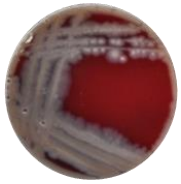
A total of 51 ulcer swabs were taken from 45 diabetic foot patients. After incubation, it was noted whether the sample was monomicrobial (single colonies) or polymicrobial (more than one varying colony). It was found that 44 samples (86.3%) resulted in polymicrobial growth and seven samples (13.7%) in monomicrobial growth. The results seen in this study are similar to those seen in a study carried out in Malaysia where 85% of patients presented with a polymicrobial infection (Goh *et al.,* 2020). In another study, polymicrobial infection was reported as occurring in 75% of patients (Al Benwan *et al.,* 2012). A similar predominance of polymicrobial infection was seen in multiple studies (Miyan *et al.,* 2017; Sannathimmappa *et al.,* 2021; Gupta *et al.,* 2022). Infections that are polymicrobial in nature are more complex to treat and are more likely to develop into chronic infections (Rhoads *et al.,* 2012; Sadeghpour Heravi *et al.,* 2019). It has been suggested the increasing presence of polymicrobial infection may be partly due to the treatment history of the patient and the manner in which the antimicrobials were used (Kathirvel *et al.,* 2018). Long term use of an antibiotic may result in future infections that are both more severe and resistant (Zubair *et al.,* 2020).

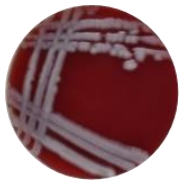
A total of 445 micro-organisms were isolated from the 51 original wound sample swabs. The average number of micro-organisms isolated per lesion was established at 2.97 ≈ 3. This number is higher than the average reported number of micro-organisms reported per lesion in other studies (Al Benwan *et al.,* 2012; Miyan *et al.,* 2017; Gupta *et al.,* 2022). This could be attributed to multiple reasons; including poor patient understanding when it comes to basic hygiene and wound care as well as a lack of access to basic amenities and general healthcare. The impact of the COVID-19 pandemic cannot be overlooked where patient adherence to wound care was drastically reduced as a result of severe lockdown restrictions (Naude, 2020).

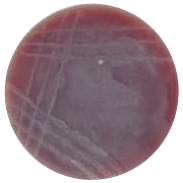
Table 3.1: A summary of the various macromorphological characteristics observed in this study.

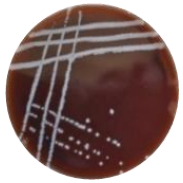
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
1		Small	White	Round	Entire	Raised	/	6
2		Small	White	Round	Entire	Raised	/	10
3		Small	White	Round	Entire	Raised	/	22
4		Small	White	Round	Entire	Raised	/	13

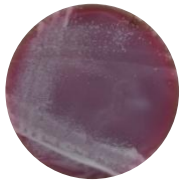
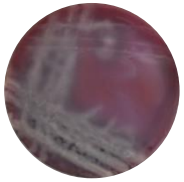
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
5		Tiny	White	Round	Entire	Flat	/	2
6		Small	Grey	Round	Entire	Flat	/	1
7		Small	Grey	Round	Entire	Raised	/	7
8		Small	Yellow	Round	Entire	Raised/ convex	Haemolytic	3

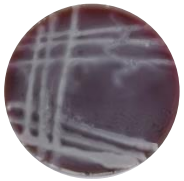
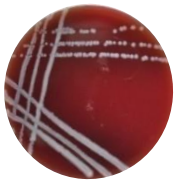
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
9		Large	Grey/ yellow	Round	Filiform	Raised	Haemolytic	55
10		Small	Grey/ green	Round	Entire	Raised	Haemolytic	1
11		Small	Grey	Round	Entire	Raised/ convex	/	14
12		Small	Grey/ yellow	Round/ irregular	Spreading	Raised	/	2

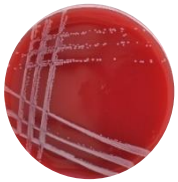
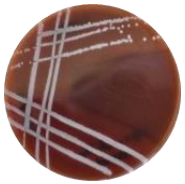
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
13		Large	Yellow	Round	Entire	Raised/ convex	/	35
14		Large	White	Filamentous	Filiform	Raised	/	1
15		Small	Grey	Round	Entire	Raised	/	1
16		Large	Grey/ yellow	Round	Entire	Raised	/	1

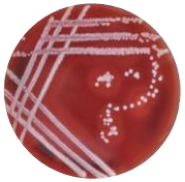
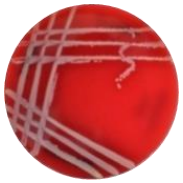
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
17		Large	Grey	Round/ irregular	Entire	Raised	/	3
18		Tiny	Grey	Spreading	Entire	Flat	Film producing	1
19		Large	Yellow	Round	Entire	Raised	/	2
20		Large	Grey/ yellow	Round	Entire	Raised	/	1

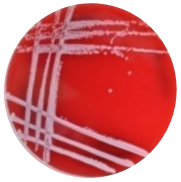
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
21		Large	Grey/ yellow	Irregular	Filiform	Raised	/	2
22		Small	Grey	Round	Entire	Flat	Haemolytic	3
23		Large	Brown/ green	Round/ spreading	Entire	Raised	Film producing	8
24		Small	Grey	Round/ spreading	Entire/ filiform	Raised	Film producing	61

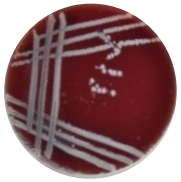
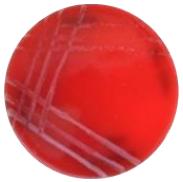
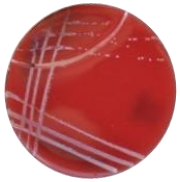
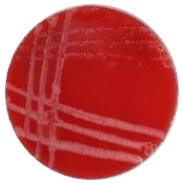
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
25		Small	Grey	Round/ spreading	Entire	Raised	Film producing	8
26		Tiny	Grey	Round/ spreading	Entire	Flat	Film producing	2
27		Small	Yellow	Round/ spreading	Entire	Flat	Haemolytic & Film producing	1
28		Tiny	Grey	Round/ spreading	Filiform	Flat	Film producing	1

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
29		Small	Grey/ yellow	Irregular/ spreading	Entire	Flat	Film producing	5
30		Small	Grey	Round	Entire	Flat	/	18
31		Small	Dark Yellow	Round	Entire	Raised	/	2
32		Tiny	Grey	Round	Entire	Flat	/	1

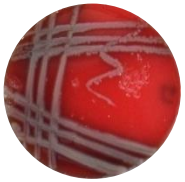
Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
33		Small	White/ grey	Round	Entire	Flat	/	2
34		Small	Yellow/ white	Round	Entire	Flat	/	7
35		Small	White	Round	Entire	Flat	Haemolytic	11
36		Small	Yellow	Round	Entire	Raised	/	17

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
37		Small	Grey	Round	Entire	Flat	Haemolytic	28
38		Small	Yellow	Round	Entire	Raised	Haemolytic	28
39		Small	Yellow	Round	Entire	Flat	Haemolytic	4
40		Large	Yellow	Round	Entire	Raised	/	1

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
41		Small	White	Round	Entire	Raised	/	3
42		Large	Yellow	Round	Entire	Raised	/	1
43		Small	White	Round	Entire	Flat	/	3
44		Tiny	Translucent	Round	Entire	Flat	/	7

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
45		Small	Yellow	Round	Entire	Raised	/	13
46		Tiny	Grey	Round	Entire	Raised	/	5
47		Tiny	White	Round	Entire	Raised	/	5
48		Tiny	Yellow	Round	Entire	Flat	/	3

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
49		Tiny	Grey	Round	Entire	Flat	/	3
50		Small	Grey/ yellow	Round	Entire	Raised	/	2
51		Tiny	Grey	Round	Entire	Flat	/	1
52		Large	Grey/ yellow	Round	Entire	Raised	/	2

Group Allocation	Graphical representation of culture	Size	Colour	Shape	Margin	Elevation	Other*	Number of isolates allocated
53		Small	Grey/ yellow	Round	Entire	Raised	/	4
54		Small	Grey/ yellow	Round	Entire	Flat	Haemolytic	2

* Additional characteristics noted

In the South African context, a shortage of podiatrists and insufficient staff training are barriers to patient care and contribute negatively towards wound progression (Allen *et al.*, 2016; Zipfel and Dembskey 2020). Table 3.1 provides a summary of the different macromorphological features observed in this study

According to Pouget *et al.* (2020), micro-organisms found in DFUs are both pathogenic and commensal. In addition, pathogens in DFUs have been shown to form biofilms, which enhance the virulence of pathogens and plays a role in the chronicity of DFUs as well as the difficulty in treating them (Pouget *et al*., 2020). The polymicrobial nature of DFUs is influenced by several microbial and host factors which include; a large variety of different micro-organisms present in the wounds (Gardner *et al*., 2013; Hitam *et al*., 2019; Pouget *et al*., 2020), wound depth allowing for an environment for complex microbiota to thrive (Gardner *et al.*, 2013; Pouget *et al*., 2020) as well as a reduced patient immune system which may increase the pathogenicity of previously low-virulence commensal bacteria including *Staphylococcus* spp. (Pouget *et al*., 2020).The average number of pathogens per lesion in this study was established at three, indicating that polymicrobial infection is a challenge faced by the South African healthcare system. In the South African context, poorer and less educated individuals have limited access to medical care and are thus less likely to seek medical intervention until the wound and subsequent infection has progressed (Mutyambizi *et al.*, 2019). It is thus important to ensure that suitable empiric treatment is employed when patients present to healthcare facilities.

3.3.2. Antimicrobial susceptibility testing

The zone of inhibition readings for each isolate can be viewed in Appendix G. Figure 3.4 illustrates a summary of the percentage of pathogens that were susceptible to the antimicrobials used and those that showed intermediate susceptibility.

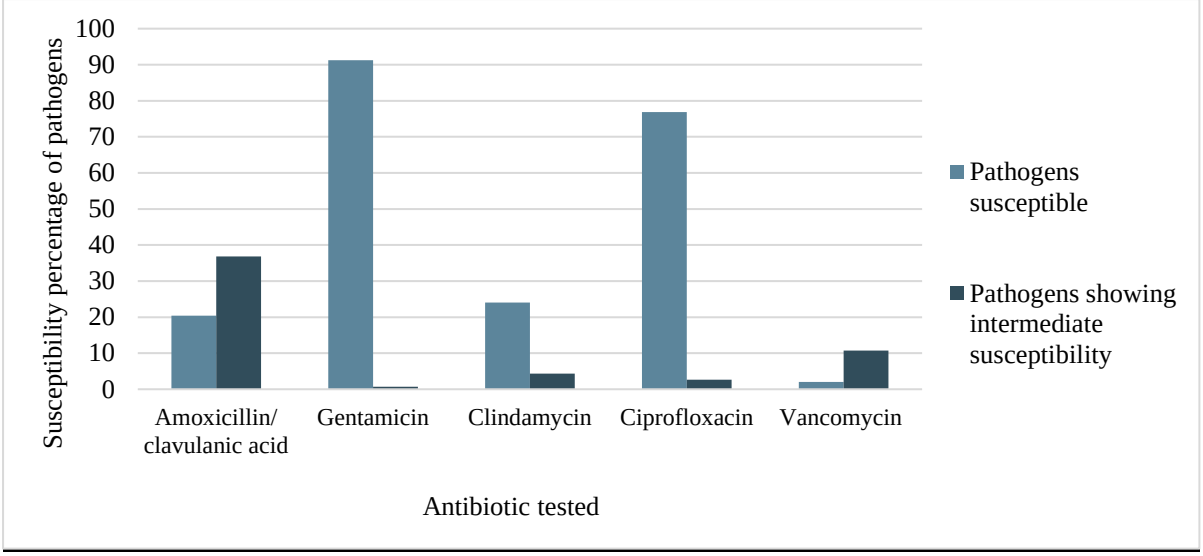


Figure 3.4: The percentage of pathogens that were susceptible and intermediately susceptible to the antimicrobials tested.

The preliminary antimicrobial susceptibility results (without having yet taken into account culture identification), display a general trend in that ciprofloxacin and gentamicin are the two best performing antimicrobials against pathogens isolated from the DFUs within this study (Figure 3.4). What is important to note from this, is that the first line treatment used in the South African public healthcare sector, amoxicillin/clavulanic acid, demonstrated poor susceptibility results against the majority of samples within this study. Similar results were seen in a study undertaken in Saudi Arabia where ciprofloxacin and gentamicin were found to be among the most sensitive antibiotics in DFU treatment (Aldawish and Alayed, 2018). In most Dutch hospitals, the empiric treatment recommended is ciprofloxacin and clindamycin (De Vries *et al.*, 2014). Another study undertaken in India found amikacin and gentamicin to be the most effective antimicrobials in DFU treatment (Singh *et al.*, 2020). Similarly, to the initial results of this study, Sani *et al.* (2019) found that both ciprofloxacin and gentamicin exhibited good susceptibility against DFU pathogens. In contrast to these studies, an investigation undertaken in Pakistan established that Gram-positive isolates showed 53.7% resistance to ciprofloxacin, while Gram-negative isolates showed 74% resistance to ciprofloxacin (Miyan *et al.*, 2017). Another study found that more than 50% of Gram-negative isolates demonstrated resistance to both ciprofloxacin and gentamicin and that most Gram-positive isolates showed partial or complete resistance to ciprofloxacin (Appapalam *et al.*, 2021). Similarly, a study in Brazil found that a large proportion *S. aureus* isolates showed resistance to ciprofloxacin (55.5%), and 43.5% of Gram-negative bacteria were resistant to ciprofloxacin (Pontes *et al.*, 2020).

3.3.3. Culture identification

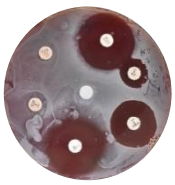
Fifty-four groups of microbial pathogens were created from the 445 micro-organisms isolated from the original DFU samples. The groups were classified based on comparable macromorphological characteristics and similar antimicrobial susceptibility patterns (Table 3.2). Following the macromorphological characterisation and the antimicrobial susceptibility testing of all 445 isolates as well as the subsequent group allocation of the isolates, a single representative isolate from each group was selected for further identification. Based on the analysis of the different groups, a total of 229 micro-organisms (51.5%) were Gram-negative micro-organisms while the remaining 216 (48.5%) were Gram-positive. Group 24 demonstrated the highest frequency of isolates (n=61), followed by groups 9 (n=55) and 13 (n=35).

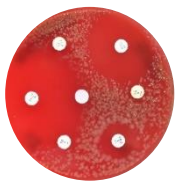
Table 3.2: Isolate groups, antibiotic susceptibility and their corresponding identification.

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
1		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Klebsiella oxytoca*
2		Gentamicin Clindamycin Vancomycin Amoxicillin/ clavulanic acid	*Enterococcus faecalis*
3		Gentamicin Ciprofloxacin Amoxicillin/ clavulanic acid Clindamycin Vancomycin	*Enterococcus faecalis*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
4		Ciprofloxacin Amoxicillin/ clavulanic acid Gentamicin Vancomycin	*Enterococcus faecalis*
5		Ciprofloxacin Amoxicillin/ clavulanic acid Vancomycin	*Enterococcus faecalis*
6		Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Enterococcus faecalis*
7		Ciprofloxacin Gentamicin	*Enterococcus faecalis*
8		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Acinetobacter baumannii*
9		Ciprofloxacin Gentamicin	*Pseudomonas aeruginosa*
10		Vancomycin	*Pseudomonas aeruginosa*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
11		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Escherichia coli*
12		Ciprofloxacin	*Escherichia coli*
13		Ciprofloxacin Gentamicin	*Escherichia coli*
14		Ciprofloxacin Gentamicin Clindamycin Vancomycin	*Escherichia coli*
15		Ciprofloxacin Amoxicillin/ clavulanic acid	*Escherichia coli*
16		Gentamicin	*Escherichia coli*
17		Ciprofloxacin Gentamicin Clindamycin Vancomycin	*Bacillus* species

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
18		Ciprofloxacin Gentamicin Vancomycin	*Bacillus* species
19		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Bacillus* species
20		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Bacillus* species
21		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Clindamycin Vancomycin	*Bacillus* species
22		Gentamicin Clindamycin Amoxicillin/ clavulanic acid Vancomycin	Coagulase negative *staphylococcus*
23		Ciprofloxacin Amoxicillin/ clavulanic acid Gentamicin	*Proteus mirabilis*
24		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Proteus mirabilis*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
25		Ciprofloxacin Gentamicin	*Proteus mirabilis*
26		Clindamycin Gentamicin Amoxicillin/clavulanic acid Ciprofloxacin Vancomycin	*Proteus mirabilis*
27		Ciprofloxacin Clindamycin Gentamicin Vancomycin	*Proteus mirabilis*
28		Vancomycin	*Proteus mirabilis*
29		Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Proteus mirabilis*
30		Vancomycin	*Corynebacterium species*
31		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Clindamycin	*Corynebacterium species*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
32		Gentamicin Amoxicillin/ clavulanic acid	*Corynebacterium species*
33		Gentamicin Vancomycin Amoxicillin/ clavulanic acid Clindamycin	*Corynebacterium striatum*
34		Clindamycin Vancomycin	*Corynebacterium striatum*
35		Ciprofloxacin Clindamycin Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Staphylococcus lugdunensis*
36		Ciprofloxacin Clindamycin Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Staphylococcus epidermidis*
37		Ciprofloxacin Clindamycin Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Staphylococcus aureus*
38		Amoxicillin/ clavulanic acid Ciprofloxacin Clindamycin Gentamicin Vancomycin	*Staphylococcus aureus*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
39		Clindamycin Ciprofloxacin Gentamicin Vancomycin	Methicillin resistant *Staphylococcus aureus*
40		Clindamycin Vancomycin Gentamicin	*Staphylococcus aureus*
41		No sensitivity observed against any of the antibiotics tested	Gentamicin/ methicillin resistant *Staphylococcus aureus*
42		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Clindamycin Vancomycin	*Staphylococcus cohnii*
43		Gentamicin Clindamycin Amoxicillin/ clavulanic acid Vancomycin Ciprofloxacin	*Staphylococcus haemolyticus*
44		Ciprofloxacin Gentamicin Clindamycin Amoxicillin/ clavulanic acid Vancomycin	*Staphylococcus intermedius*
45		Ciprofloxacin Gentamicin	*Enterobacter cloacae*

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
46		Ciprofloxacin Amoxicillin/ clavulanic acid Clindamycin Gentamicin Vancomycin	*Streptococcus anginosus*
47		Gentamicin Vancomycin Amoxicillin/ clavulanic acid Ciprofloxacin Clindamycin	*Streptococcus anginosus*
48		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Vancomycin	*Streptococcus anginosus*
49		Amoxicillin/ clavulanic acid Clindamycin Ciprofloxacin Vancomycin	Group B *Streptococcus*
50		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Clindamycin Vancomycin	*Oligella urethralis*
51		Ciprofloxacin Amoxicillin/clavulanic acid Gentamicin	*Oligella urethralis*
52		Ciprofloxacin Gentamicin Clindamycin	*Shewanella putrefacians* group

Group allocation	Susceptibility pattern	Antibiotic susceptibility patterns arranged from most susceptible (top) to least susceptible (bottom)*	Micro-organism identified
53		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid	*Citrobacter koseri*
54		Ciprofloxacin Gentamicin Amoxicillin/ clavulanic acid Clindamycin	*Citrobacter koseri*

* - Consideration was only given to those antibiotics demonstrating inhibition zones

The most frequently isolated micro-organisms identified in this study were *Proteus mirabilis* (19.3%), *Staphylococcus aureus* (13.7%), *Pseudomonas aeruginosa* (12.6%), *Enterococcus faecalis* (12.4%) and *Escherichia coli* (12.1%) (Figure 3.5). Similar results were observed in a Cameroonian study by Akwah *et al.* (2015), where *P. mirabilis* (21.6%), *E. coli* (18.8%) and *S. aureus* (17.6%) were found to be among the most predominant micro-organisms responsible for DFU infection.

Less prevalent micro-organisms isolated from DFUs in this study include coagulase-negative *Staphylococci* (7.6%) and *Corynebacterium* species (4.7%). Similar rates of infection (7.8%) with coagulase-negative *Staphylococci* were seen in a study undertaken by Mohammuddunnobi and Amin (2019). A meta-analysis examining the microbiology of diabetic foot infections found the prevalence of coagulase-negative *Staphylococci* to be 5.8% (Macdonald *et al.,* 2021). Both coagulase-negative *Staphylococci* and *Corynebacterium* spp. are normal skin commensals and have been found to be more prevalent on the skin and around diabetic foot wounds (Smith *et al.,* 2016; Patel *et al.,* 2022). Although these micro-organisms are normally found on the skin, it has been proposed that in a polymicrobial environment, such as that of a DFU, pathogenic biofilms are more likely to occur (Smith *et al.,* 2016).

Worldwide, Gram-positive micro-organisms are mostly responsible for infection of DFUs, however, in tropical and developing countries, Gram-negative micro-organisms are reported to be predominant (Sanchez *et al.,* 2022). In this study, the prevalence of Gram-negative micro-

organisms (51.5%) is only slightly greater than that of Gram-positive micro-organisms (48.5%). A study undertaken in China showed similar results with 54.1% of micro-organisms classified as Gram-negative and 45.9% as Gram-positive (Xie *et al.,* 2017). This study also emphasised the importance of knowing the antibiotic susceptibility patterns specific to regional areas as the bacterial profile of diabetic foot ulcers varied with their severity (Xie *et al.,* 2017).

Proteus mirabilis was the most common pathogen observed in this study. It is a Gram-negative, rod-shaped bacterium that is widely spread in the environment in water sources, soil, sewerage and the gastrointestinal tract of humans and animals (Drzewiecka 2016; Armbruster *et al.,* 2018; Wasfi *et al.,* 2020). *Proteus mirabilis* has been implicated in diseases of the urinary tract most commonly, but is also seen to cause disease of the gastrointestinal tract, eye, ear and skin (Wasfi *et al.,* 2020). The bacteria are well recognised for its swarming ability where cells can migrate rapidly over solid surfaces (Armbruster *et al.,* 2018; Wasfi *et al.,* 2020). An example of this phenomenon can be observed in Figure 3.6 cultured from patients A019 and A036. *Proteus* organisms that were once sensitive to antibiotics are becoming resistant by producing beta-lactamases (Hamid *et al.,* 2020). Hamid *et al.* (2020) examined the beta-lactamase production in samples taken from DFUs and found a high prevalence of multi-drug resistant *P. mirabilis,* once again highlighting the need for local resistance data.

Staphylococcus aureus was the second most commonly observed causative pathogen. Globally, this pathogen is one of the most commonly observed pathogens responsible for DFU infection (Amjad *et al.,* 2017; Miyan, *et al.,* 2017; Sánchez-Sánchez *et al.,* 2017; Giridhar and Kalavathi, 2018; Macdonald *et al.,* 2021). Its persistence, ability to adhere to the wound, and infectivity is compounded by virulence factors that include enzymes and toxins such as protease, lipases and haemolysins to name a few (Shettigar and Murali, 2020). In addition to this, *S. aureus* has also been shown to be implicated in the formation of biofilm communities (Shettigar and Murali, 2020). In this study, 55.7% of *S. aureus* isolates were methicillin-sensitive, while the remaining 44.3% were methicillin-resistant. A small percentage of isolated *S. aureus* were also identified and found to be gentamicin and methicillin-resistant. This is important knowledge to have when looking at the standard treatment of DFUs in order to account for the presence of multi-drug resistant micro-organisms when deciding on empirical treatment regimens.

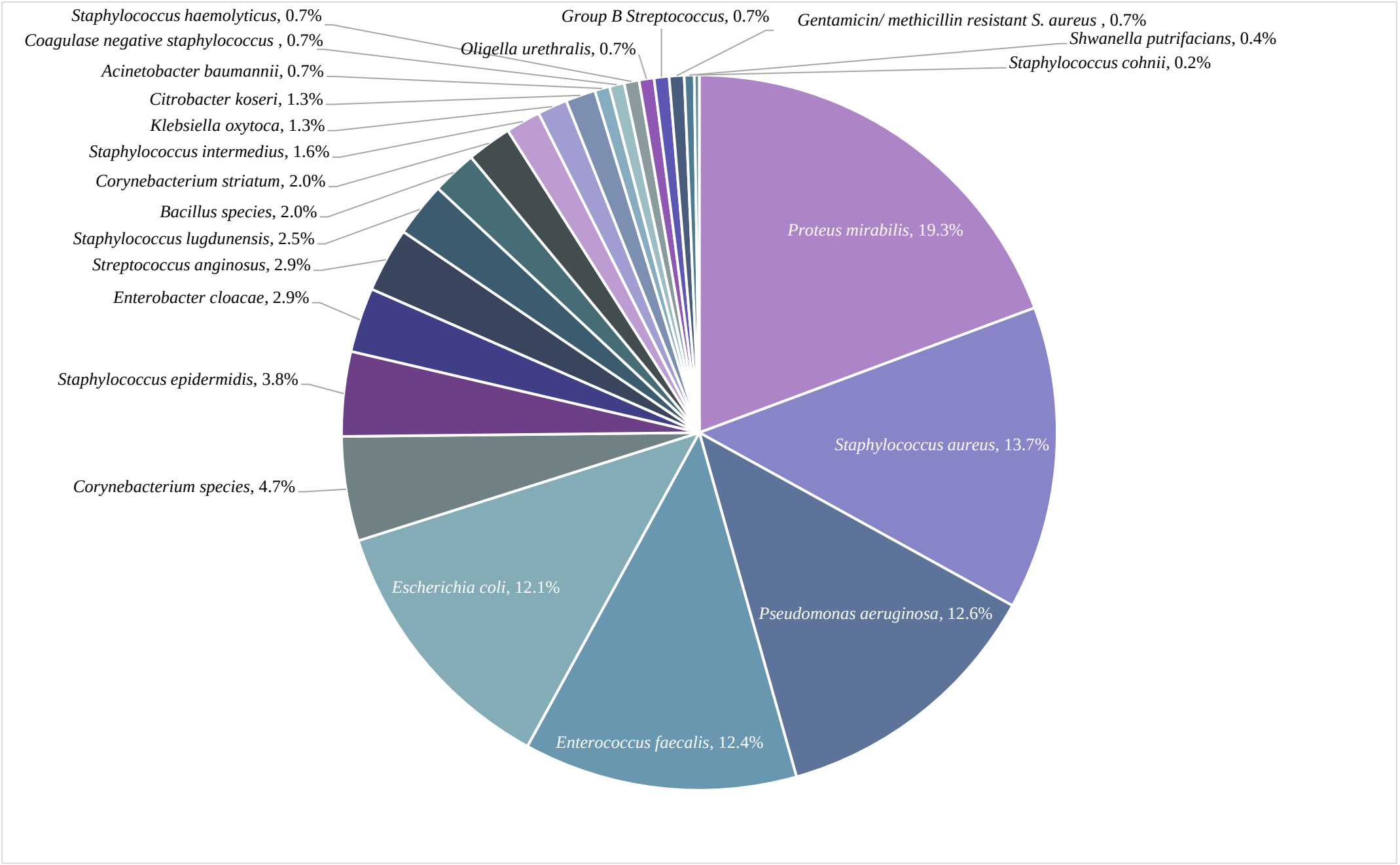


Figure 3.5: The frequency of pathogens isolated from 51 DFUs.

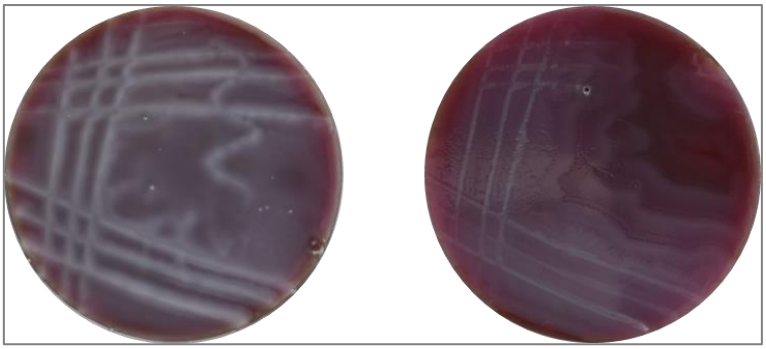


Figure 3.6: *Proteus mirabilis* isolated from patients A019 and A036 demonstrating swarming of the pathogen across the agar surface.

The third most isolated pathogen was *Pseudomonas aeruginosa*, an opportunistic Gram-negative bacterium. The bacterium is mostly associated with nosocomial infections and ventilator associated pneumonia (Pang *et al.,* 2019). The treatment of *P. aeruginosa* infection has become complicated due to antimicrobial resistance (Pang *et al.,* 2019). Resistance mechanisms include reduced membrane permeability, efflux systems, antibiotic inactivating enzymes, mutations and biofilm production (Pang *et al.,* 2019). In studies where *P. aeruginosa* was found to be a common pathogen in DFUs, the antimicrobials employed included; meropenem, vancomycin, doxycycline, imipenem and amikacin (Priyadarshini Shanmugam, 2013; Giridhar and Kalavathi, 2018; Shareef *et al.,* 2018; Kurup and Ansari, 2019; Goh *et al.,* 2020). Currently, the STG and SAASP guidelines do not account for *P. aeruginosa* infection in DFUs.

Enterococcus faecalis is known to cause bacteraemia, endocarditis, urinary tract infections and is the fourth most commonly isolated pathogen in this study (Shettigar *et al.,* 2018). *Enterococcus faecalis* is often isolated in DFUs where high resistance rates towards erythromycin, tetracycline, ciprofloxacin, and fluoroquinolones are experienced (Shettigar *et al.,* 2018; Zubair 2020). Virulence factors that make the treatment of infection with this pathogen more challenging include factors like enterococcal surface proteins as well as aggregation substances which increase the process of colonization in the host cells (Bloushy and Elbehiry, 2018). In this study, general resistance patterns were high and isolates only showed susceptibility towards gentamicin.

The fifth most commonly isolated pathogen in this study was *Escherichia coli.* The micro-organism is common worldwide and causes infection of the urinary tract, bloodstream, skin and ear (Tuem *et al.,* 2018). Antimicrobial resistance of *E. coli*, specifically in developing

countries, is reported to be a major cause of treatment failure (Tuem *et al.,* 2018). It has been shown to display differing resistance patterns among isolates from varied geographical locations (Tuem *et al.,* 2018). Worldwide, treatment regimens for *E. coli* found in DFUs include meropenem, imipenem, ciprofloxacin, vancomycin and amikacin (Amjad *et al.,* 2017; Miyan, *et al.,* 2017; Kurup and Ansari, 2019; Najari *et al.,* 2019).

Overall, when examining the commonly known virulence factors and susceptibility patterns (studied here) of the top five common causative pathogens in this study, it is clear that developing a standard treatment plan is not straightforward. The difficulty arises when trying to select a single antimicrobial treatment to adequately address the most common causative pathogens while taking into account that not every patient will present with the same polymicrobial infection. For example, patient 'x' might present with an infection caused by *P. aeruginosa* and *S. aureus* while patient 'y' might present with *E. coli* and *P. mirabilis* as causative pathogens. The difficulty arises in selecting an antimicrobial that will effectively treat both patients. However, local susceptibility data provides a good starting point in suggesting appropriate empirical antimicrobial regimens.

3.3.4. Analysis of antimicrobial susceptibility results following micro-organism identification

Using the results obtained from the antimicrobial susceptibility patterns as well as the subsequent identification of the various micro-organisms, antibiograms were developed for each micro-organism. Table 3.3 shows the overall susceptibility patterns for the identified micro-organisms. Breakpoint ranges specific to groups of pathogens were used to classify the antimicrobial activity seen as either susceptible, intermediate in susceptibility or resistant (CLSI, 2020). The first line drug (amoxicillin/clavulanic acid) in the STG was effective only against 20.4% of the micro-organisms identified within this study, with *S. aureus* accounting for 13.7% of those micro-organisms affected. Gentamicin proved to be a good treatment candidate with 91.2% of isolated micro-organisms being sensitive to the antibiotic. Gentamicin showed similar results against the top five most frequently observed pathogens.

Table 3.3: Summary of the antimicrobial susceptibility patterns for the isolated pathogens in DFUs.

Pathogen	Gram Stain	Percentage prevalence	ZOI diameter (mm)/ Antimicrobial susceptibilities											
			AMC	INT	CN	INT	DA	INT	CIP	INT	VAN	INT	FLU	INT
Proteus mirabilis	-	19.3	15	**I**	20.4	**S**	0.8	**R**	31	**S**	1.6	**R**	0	**R**
Staphylococcus aureus	+	13.7	21.8	**S**	20.4	**S**	23.2	**S**	25.8	**S**	12.8	**R**	0	**R**
Pseudomonas aeruginosa	-	12.6	0.24	**R**	16.4	**S**	0	**R**	29.2	**S**	0.62	**R**	0	**R**
Enterococcus faecalis	+	12.4	15.4	**I**	19	**S**	10.6	**R**	18.4	**R**	11	**R**	0	**R**
Escherichia coli	-	12.1	4.4	**R**	17.2	**S**	0.4	**R**	25.4	**S**	0.2	**R**	0	**R**
Corynebacterium species	+	4.7	3	**R**	4.4	**R**	1.2	**R**	3.8	**R**	14.4	**I**	0	**R**
Staphylococcus epidermidis	+	3.8	14.6	**I**	19.6	**S**	21.8	**S**	23.6	**S**	12.2	**R**	0	**R**
Enterobacter cloacae	–	2.9	1	**R**	18.8	**S**	0	**R**	27	**S**	0	**R**	0	**R**
Streptococcus anginosus	+	2.9	23.8	**S**	23.2	**S**	17.4	**I**	25.4	**S**	16.8	**I**	0	**R**
Staphylococcus lugdunensis	+	2.5	13.6	**R**	20.4	**S**	23.8	**S**	25	**S**	12.2	**R**	0	**R**
Bacillus spp.	+	2.0	8.6	**R**	20.8	**S**	9.8	**R**	26.4	**S**	11.6	**R**	0	**R**
Corynebacterium striatum	+	2.0	4.6	**R**	6.8	**R**	15	**I**	0	**R**	18.4	**S**	0	**R**
Staphylococcus intermedius	+	1.6	22.2	**S**	20	**S**	26	**S**	24.8	**S**	16	**I**	0	**R**
Klebsiella oxytoca	-	1.4	8.2	**R**	19	**S**	0	**R**	28.8	**S**	0	**R**	0	**R**
Citrobacter koseri	-	1.4	15	**I**	22.2	**S**	4.8	**R**	20.6	**R**	2.8	**R**	0	**R**
Acinetobacter baumannii	-	0.7	10.4	**R**	14.6	**I**	0	**R**	32.4	**S**	0	**R**	0	**R**
Coagulase negative *Staphylococcus*	+	0.7	22.4	**S**	26	**S**	24.4	**S**	0	**R**	11.6	**R**	0	**R**
Staphylococcus haemolyticus	+	0.7	24	**S**	23.4	**S**	26.6	**S**	17	**I**	15.4	**I**	0	**R**
Oligella urethralis	-	0.7	13.8	**R**	17	**S**	11.2	**R**	24.8	**S**	8.8	**R**	0	**R**

Pathogen	Gram Stain	Percentage prevalence	ZOI diameter (mm)/ Antimicrobial susceptibilities											
			AMC	INT	CN	INT	DA	INT	CIP	INT	VAN	INT	FLU	INT
Group B *Streptococcus*	+	0.7	21.6	**S**	0	**R**	19.6	**S**	18.6	**I**	15.4	**I**	0	**R**
Gentamicin/ methicillin resistant *S. aureus*	+	0.7	0	**R**	0	**R**	0	**R**	0	**R**	0	**R**	0	**R**
Shwanella putrifacians	-	0.5	0	**R**	23.4	**S**	16.8	**S**	31.2	**S**	0	**R**	0	**R**
Staphylococcus cohnii	+	0.2	20.8	**S**	28.6	**S**	16.4	**I**	31.2	**S**	14.8	**I**	0	**R**

Key: Interpretation as per CLSI guidelines (CLSI, 2020): **INT**: Interpretation, in bold **S:** Susceptible, **R**: Resistant, **AMC**: Amoxicillin/ clavulanic acid, **CN:** Gentamicin, **DA:** Clindamycin, **CIP:** Ciprofloxacin, **VAN:** Vancomycin, **FLU:** Fluconazole

Breakpoints: **Enterobacteriaceae (includes *E. coli, Klebsiella, Enterobacter, Citrobacter,* and *Proteus* sp.)**: AMC (S≥18; I 14-17; R≤13) CN (S≥15; I 13-14; R R≤12) DA (S≥19; I 16-18; R≤15) CIP (S≥26; I 22-25; R≤21) VAN (S≥17; I 15-16; R≤14) ***Staphylococcus* spp.:** AMC (S≥20; R≤19) CN (S≥15; I 13-14; R R≤12) DA (S≥21; I 15-20; R≤14) CIP (S≥21; I 16-20; R≤15) VAN (S≥17; I 15-16; R≤14); ***Streptococcus* spp.:** AMC (S≥20; R≤19) CN (S≥15; I 13-14; R R≤12) DA (S≥19; I 16-18; R≤15) CIP (S≥21; I 16-20; R≤15) VAN (S≥17; I 15-16; R≤14); ***Pseudomonas aeruginosa:*** AMC (S≥18; I 14-17; R≤13) CN (S≥15; I 13-14; R R≤12) DA (S≥19; I 16-18; R≤15) CIP (S≥25; I 19-24; R≤18) VAN (S≥17; I 15-16; R≤14); ***Acinetobacter* spp.:** AMC (S≥18; I 14-17; R≤13) CN (S≥15; I 13-14; R R≤12) DA (S≥19; I 16-18; R≤15) CIP (S≥21; I 16-20; R≤15) VAN (S≥17; I 15-16; R≤14); **Other Non-Enterobacteriaceae** AMC (S≥18; I 14-17; R≤13) CN (S≥15; I 13-14; R R≤12) DA (S≥19; I 16-18; R≤15) CIP (S≥26; I 22-25; R≤21) VAN (S≥17; I 15-16; R≤14)

Twenty four percent (24%) of the pathogens isolated were susceptible to Clindamycin. Similarly, to amoxicillin/ clavulanic acid, 13.7% of those pathogens that were susceptible to clindamycin were identified as *S. aureus.* Seventy-six-point eight percent (76.8%) of the pathogens isolated were susceptible to ciprofloxacin. Unlike gentamicin, only four out of the top five most frequently seen pathogens showed sensitivity towards ciprofloxacin with only *E. faecalis* demonstrating resistance. Vancomycin demonstrated the poorest results, with only *Corynebacterium striatum* demonstrating complete susceptibility. Vancomycin did, however, demonstrate intermediate activity against 10.8% of the isolates. Fluconazole did not produce any results as it is classified as an antifungal agent and only bacterial micro-organisms were isolated in this study. In this study, fluconazole was used as a control to rule out contamination that may have been of fungal origin.

International guidelines recommend that a polymicrobial infection of a DFU with both Gram-negative and Gram-positive micro-organisms be treated empirically with amoxicillin/clavulanic acid, a second- or third-generation cephalosporin or a fluoroquinolone (Lipsky, 2007; Lipsky *et al.,* 2016). Both the STG and the SAASP guidelines, recommend

amoxicillin/clavulanic acid as the mainstay in empiric antimicrobial treatment for DFUs. Our study shows that is not an appropriate choice for first line treatment as sensitivity towards this agent is only seen in 20.4% of the micro-organisms isolated. Furthermore, four of the five top most frequently seen pathogens in DFUs in this study were resistant to amoxicillin/clavulanic acid.

The presence of methicillin-resistant *S. aureus* (MRSA) cannot be discounted when evaluating the bacteriology of DFUs. International guidelines recommend that suspected infection with MRSA be treated with co-trimoxazole (trimethoprim/sulfamethoxazole), doxycycline, clindamycin, glycopeptide antibiotics, linezolid, daptomycin or a fluoroquinolone (Lipsky, 2007; Lipsky *et al.,* 2016). In this study, clindamycin showed susceptibility against all strains of *S. aureus.*

Both the STG and SAASP guidelines do not account for the presence of *Pseudomonas aeruginosa* in DFUs and thus infection with this pathogen often results in a wound of chronic nature due to its enhanced virulence and ability to produce biofilms (Srivastava and Sivashanmugam 2020). International guidelines suggest either amoxicillin/clavulanic acid, penicillin with ceftazidime, penicillin with ciprofloxacin or a group 2 carbapenem for treating a wound infected by *P. aeruginosa*. In this study, *P. aeruginosa* was sensitive only to gentamicin and ciprofloxacin while completely resistant to amoxicillin/clavulanic acid.

3.4. Summary

- A total of 445 micro-organisms were isolated with the average number of micro-organisms per lesion established as three.
- Polymicrobial infections were seen in 86.3% of DFU patients.
- The most frequently isolated micro-organisms were *P. mirabilis* (19.3%), *S. aureus* (13.7%), *P. aeruginosa* (12.6%), *E. faecalis* (12.4%) and *E. coli* (12.1%).
- Only 20.4% of the micro-organisms isolated were found to be sensitive to amoxicillin/clavulanic acid, the first line drug of choice in the STG and SAASP guidelines.
- Gentamicin was the best performing antimicrobial with 91.2% of all pathogens showing sensitivity, followed by ciprofloxacin (76.8%) for isolates obtained from the DFU in this study.

Chapter 4: Clinical Pharmacy

4.1. Introduction

The prevention of DFUs is of the greatest importance in order to provide improved patient outcomes and a reduced burden of DFUs on the South African healthcare system. The patient is the primary person responsible for foot care and thus in order to reduce the incidence of diabetic foot ulceration, it is important that the patient is made aware of risk factors and good foot care practices (Goie and Naidoo, 2016). In a resource-poor country, the most important intervention in DFU prevention is that of patient education which is both free and effective if implemented correctly (Abbas, 2020).

This chapter explains the process undertaken to identify and categorise patients, as well as examine the use of preventative measures by patients presenting with DFUs who are seeking medical intervention at the study hospitals. The previous treatment plans implemented are examined and a holistic view of the overall treatment of diabetic patients with foot ulcers is discussed. This chapter also includes a discussion on the classification of DFU severity.

4.2. Methodology

4.2.1. Validating the retrospective review tool

The retrospective review tool was adapted from a South African study which looked at resistance patterns in urinary tract infections (Jaji, 2021). Validation of the retrospective review tool in order to ensure its reliability in the context of its use was completed by means of a pilot study. Two patients from the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) podiatry unit were randomly selected. This site was used as it had been approved by both the hospital and ethics committee.

The two patients who participated in the pilot study are of an acceptable sample size as the established sample size for the study is limited. The two patients proved the reliability of the retrospective review tool by answering all the questions without needing assistance or further explanation. Conducting the pilot study led to the addition of different preventative measures

that were not previously included, for example, a walker. These changes were approved by the University of the Witwatersrand's Human Research Ethics Committee (HREC) prior to the commencement of the study.

4.2.2. Diabetic foot ulcer treatment protocol development

The development of an evidence-based protocol encompasses the determination of patients that present with risk factors for DFU development, diagnostic criteria, preventative measures and strategies used to reduce the risk of reoccurring DFUs. This information was generated from the retrospective review tool by means of a patient interview used at the time of sample collection as well as the information gathered during the review of patient files.

A treatment plan for the diagnosis and management of all diabetic foot ulcers was prepared using the results determined by the antibiotic susceptibility testing as well the results generated from the information obtained from the file review and retrospective review tool. These guidelines are intended to guide physicians in all medical specialties on the ideal dermatological treatment protocol.

4.2.3. Data analysis

Data analysis was completed using Microsoft Excel. Prevalence was measured using frequency and percentages. Relationships and correlations were determined using logistic regression. The logistic regression model was used to determine odds ratios, and thus likelihood of DFU resistance to selected antimicrobials. Since an odds ratio is a measure of association (Szumilas, 2010), its use was fitting for the nature of data that was collected in this study.

Data that was obtained from patient record reviews and the administration of the structured questionnaire was presented by means of tables and figures in order to clearly portray the use of preventative measures, the use of medication, past and present management protocols and to display correlations between the use of ambulatory aids and the frequency of infection and the link to antimicrobial resistance. Data was stored on an online drive with its access limited to the researchers and supervisor through password protection.

4.3. Results and discussion

4.3.1. Non-pharmacological management of DFUs: The use of preventative measures

In order to reduce the need for antimicrobial use and thus reduce the burden of disease and antimicrobial resistance; education on the prevention of diabetic foot ulcers is paramount (Abbas, 2020; Schaper *et al.,* 2020). Preventative measure use in this studies patient cohort was examined in order to determine whether the best treatment practices are employed in the South African public healthcare sector. Figure 4.1 provides a visual guide as to which pressure off-loading devices were seen to be used by patients in this study.

Figure 4.1: Preventative measures used by the patient cohort. Images taken from Google.

Figure 4.2. depicts the frequency of preventative, pressure off-loading devices used amongst the study population. In patients with established ulceration, the use of an offloading device has been proven to reduce healing time and improve patient outcomes (Bus, 2016; van Netten *et al.,* 2018). The most effective manner to reduce pressure on the foot is through the use of a total contact cast (TCC) or a removable walker (Wu *et al.*, 2008; Schaper *et al.*, 2020).

From the results obtained, it can be seen that the 48.9% of patients did not make use of any preventative measures. Of patients that did make use of preventative measures, the most commonly used form of pressure off-loading was through the use of crutches (26.7%). A removable walker, which is one of the most effective preventative measures, was only used by 20% of patients (Wu *et al.*, 2008; Schaper *et al.*, 2020). Specialised footwear, including footwear marketed in commercial stores for diabetic patients was only seen to be used in 6.7% of patients.

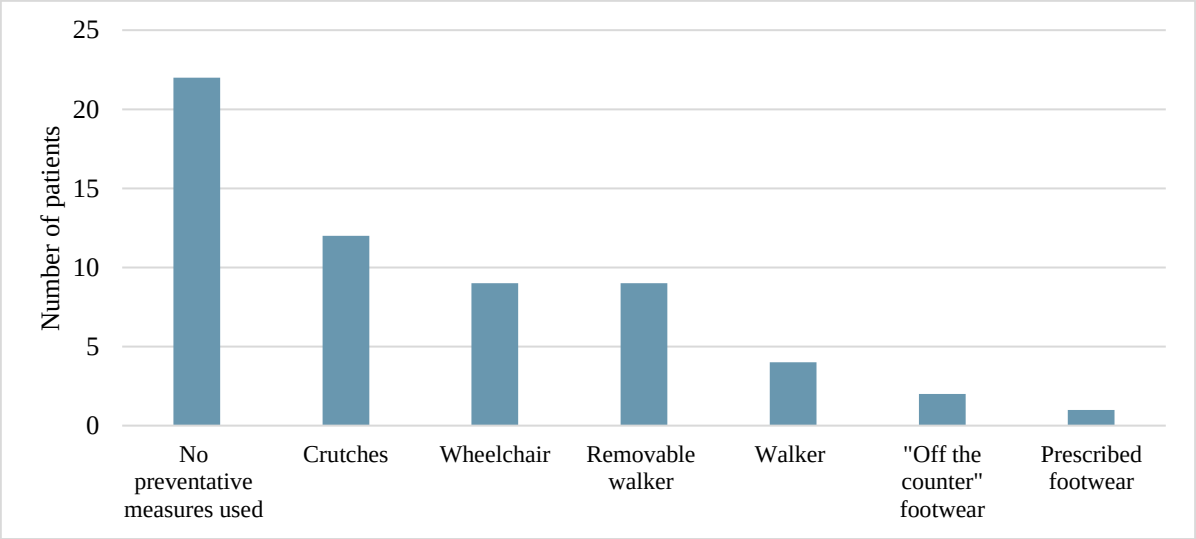


Figure 4.2: The frequency of preventative measure use among the patient cohort.

In the South African context, the STG calls for the education of patients regarding foot care, however, according to a study undertaken in a regional hospital in Durban, KwaZulu-Natal, South Africa, 90% of diabetic patients had not received any education of diabetic foot disease (Goie and Naidoo, 2016; National Department of Health, South Africa, 2020). In addition to this, Goie and Naidoo (2016) found that only 22.2% of DFU patients reported that they had personally examined their feet, and that moreover, these patients only examined their feet after developing a problem. This further indicates a lack of understanding arising from a dearth of education pertaining to the importance of foot care in the diabetic patient. A similar study in Malaysia reported that 58% of patients did not have sufficient knowledge regarding foot care, and 61.8% of patients had poor foot care practices (Muhammad-Lutfi *et al.*, 2014). A study undertaken in India found that general foot care practice was poor with many patients found to walk barefoot. Furthermore, 67.7% of patients did not check their footwear for foreign objects before putting them on and 54.8% of patients did not participate in regular foot care activities

such as cutting their toenails (Selvakumar and Shah, 2016). A more recent study undertaken in Brazil found that 65.5% of participants had little knowledge regarding preventative measure use (Sousa *et al.,* 2020). In the South African healthcare context, patients initially present to a primary healthcare clinic. Healthcare professionals at primary healthcare clinics are responsible for the screening of diseases and referral to higher levels of healthcare should the need arise (Ntuli *et al.,* 2018). It has been suggested that diabetic foot assessments and preventative measure implementation may be overlooked on account of the healthcare professionals stationed at these clinics being overworked and understaffed (Ntuli *et al.,* 2018). A lack of knowledge and patient understanding with regard to general foot care practices will ultimately negatively affect the progression and subsequent healing of DFUs.

In a review of off-loading devices for DFU treatment and prevention it was hypothesised that barriers to using off-loading devices may include; the clinician's belief that a knee-high device is not effective in the patient's everyday life, as well as the notion that most patients presenting with a DFU would be contra-indicated for the use of knee-high offloading devices due to mild ischaemia and infection (Lazzarini and Jarl, 2021). The latter reason has been disproven with guidelines indicating that mild infection and ischaemia are not contra-indications for non-removable knee-high devices, and moderate ischemia infections are not contra-indications for removable knee-high devices (Bus *et al.,* 2020; Lazzarini and Jarl, 2021). The review also found that patients did not make use of these devices due to reduced mobility and balance, a lack of motivation as well as the weight of the devices themselves (Lazzarini and Jarl, 2021). In the South African context, where the public health sector operates in a constrained resource environment, affordability may be another significant barrier in the prescribing and use or preventive off-loading devices (Wilkinson *et al.,* 2021).

Infected DFUs are a significant cause of lower extremity amputation and as such, the prevention of DFU formation and subsequent infection is important (Costa *et al.,* 2017). The results from the study regarding preventative measure use was used to determine correlation between preventative measure use, or lack thereof, and ulcer severity. Table 4.1 compares the severity of the DFU, according to the Wagner classification, to the use of preventative measures.

Table 4.1: The correlation between ulcer severity and preventative measure use.

Wagner grading	Most frequent preventative measure used	Number of DFU patients	Average number of preventative methods used per patient	Number of patients not using preventative measures
1	Removable walker (25%); Walker (16.7%); Wheelchair (16.7%)	12	0.7	7
2	Crutches (41.2%); Wheelchair (29.4%)	16	1.1	5
3	Removable walker (28.6%); Walker, wheelchair, crutches, specialised footwear (14.3% each)	9	0.8	6
4	Crutches (60%); Removable walker (20%); Wheelchair (20%)	7	0.6	3
5	No preventative measures used	1	0	1

From the results observed in this study, it can be seen that the most commonly used method of pressure off-loading in all grades of ulcer severity is through the use of crutches which were used by 32.4% of patients, overall. With the exceptions of those patients classified as Wagner stage 2, the average number of preventative measure use is less than one in all stages of ulcer severity. The only instance where crutches are not the most frequently used off-loading measure is in those patients with Wagner stage 3 ulcers. In those instances, removable walkers are seen to be more frequently used.

Risk factors for lower extremity amputation include the increasing severity of ulceration, previous lower extremity amputation, peripheral artery disease, as well as the presence of a walking disability (Ferreira *et al.*, 2018). Figure 4.3 examines the correlation between ulcer severity and the incidence of previous lower extremity amputation.

As a general trend, when ulcer severity increases, the likelihood of previous lower extremity amputation, as a result of a DFU, increases. This is concerning because patients who had undergone previous lower extremity amputation as a result of previous foot ulceration are presenting with DFUs with higher grades of severity. This infers that consequent patient education, or a lack thereof, as well as previous DFU experience by the patient, does not aid in the prevention of DFU reoccurrence and additional amputation.

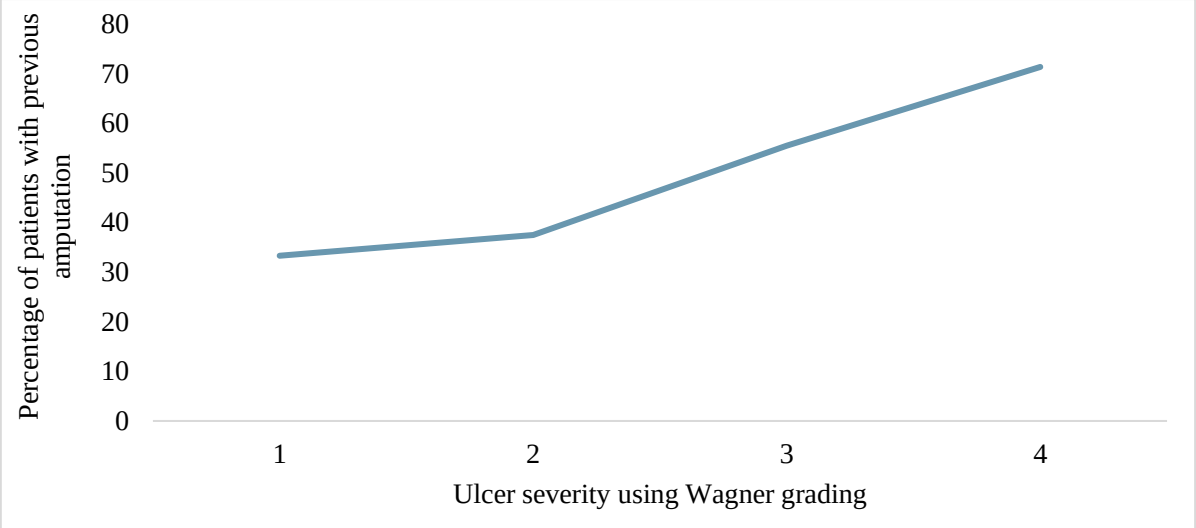


Figure 4.3: Percentage of patients with previous lower extremity amputation as a result of diabetic foot ulceration compared to current ulcer severity grading.

These findings were corroborated by a study undertaken in 14 European healthcare centres where a high ulcer reoccurrence rate was documented despite regular follow-ups and continued patient education (Dubský *et al.*, 2013). Emphasis on the importance of effective patient education is thus highlighted with various reviews highlighting its crucial role in the prevention of DFUs and reducing their social and economic burden to society (Ibrahim, 2018; Rigato *et al.*, 2018).

4.3.2. Pharmacological management and treatment practices of DFUs

The classification of DFUs allows for a standardised approach to treatment as well as ease of communication between healthcare professionals (Frykberg, 2002; Game, 2016). In South Africa, the South African Antimicrobial Stewardship Program (SAASP) suggests that the classification of DFUs be in accordance with Table 1.4 (Chapter 1). Figure 4.4 shows an illustrative comparison between the two classification systems applied to this study's sample population. The circles represent DFUs classified according to the SAASP guidelines while the rectangles represent the same DFUs classified according to the Wagner ulcer classification system.

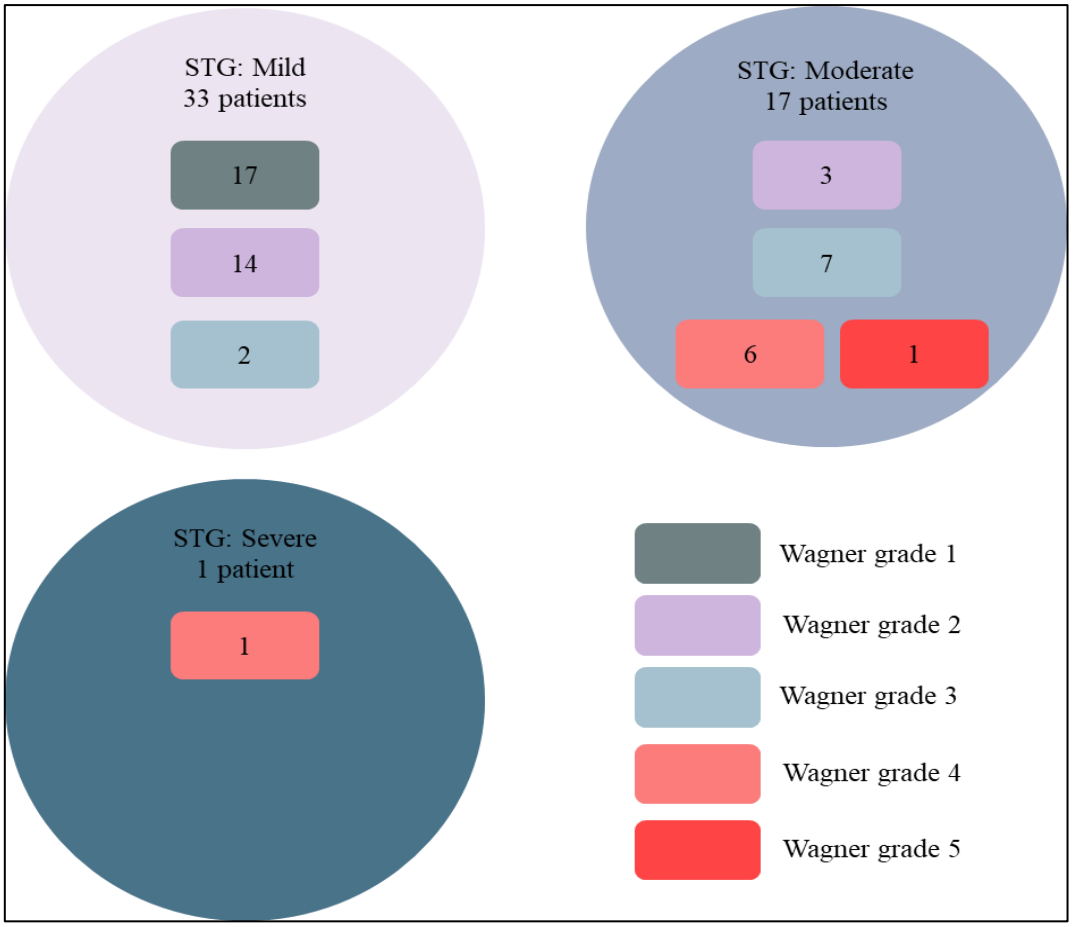
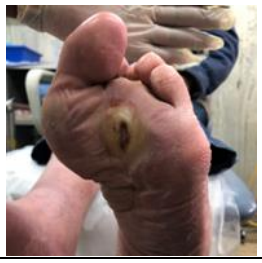
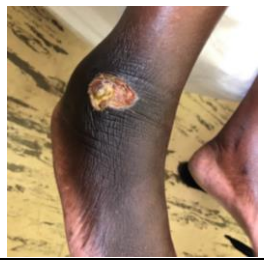
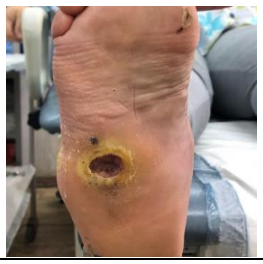
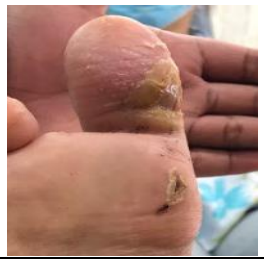
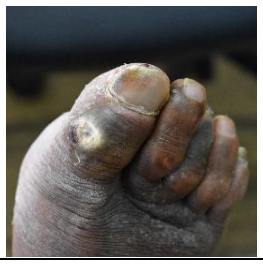


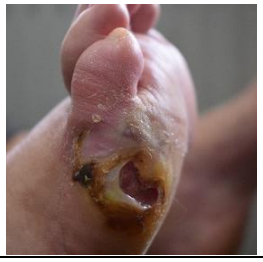
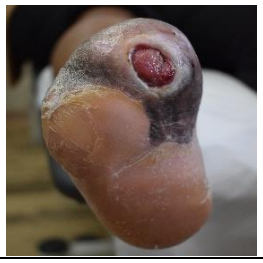
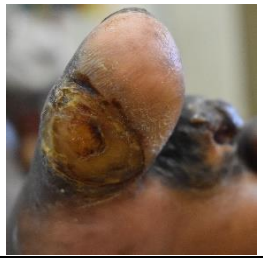
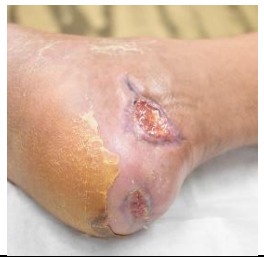
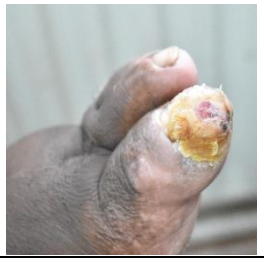
Figure 4.4: Comparison between the SAASP guideline and Wagner ulcer classification system.

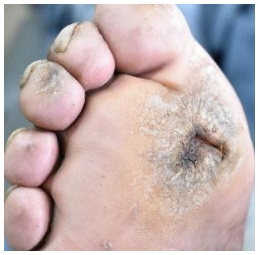
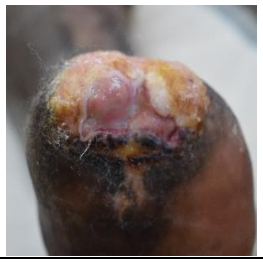
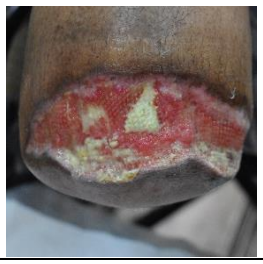
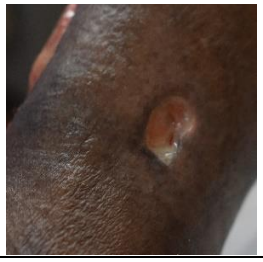
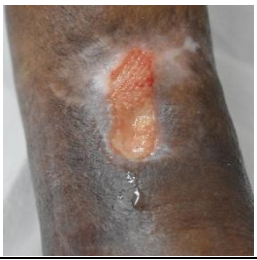
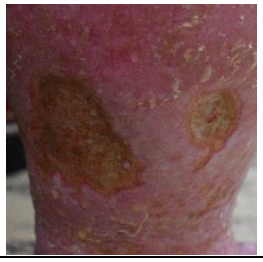
Table 4.2 provides a patient specific comparison with photographic reference to each ulcer. When comparing the two classification systems, it can be seen that the systems are very different. The SAASP guidelines for ulcer classification are not as specific when compared to the Wagner classification system. For example, the SAASP 'mild' classification includes DFUs from Wagner grades 1, 2 and 3. This suggests that the Wagner classification system is more descriptive than the SAASP guideline and allows for more selective categorisation of different ulcers based on their severity. This means that patients presenting in the South African public healthcare sector with varying degrees of ulcer severity are treated in the same broad manner with very little difference in treatment between degrees of severity. It is also interesting to observe is the overlay between the two systems whereby some grade 2 and 3 DFUs were either classified as mild and others were classified as moderate. The use of the SAASP guideline in conjunction with the STG and Essential Drug List results in the treatment plans of Wagner grade 1 through 5 DFUs, not differing to account for increasing severity. To put it simply, patients presenting in the early stages of ulceration will be treated with the same

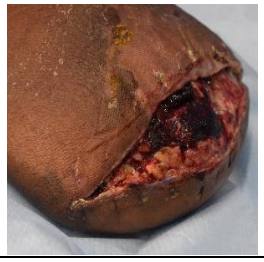
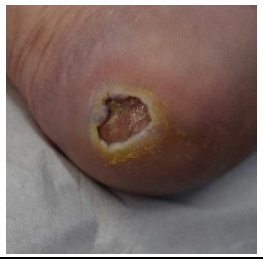
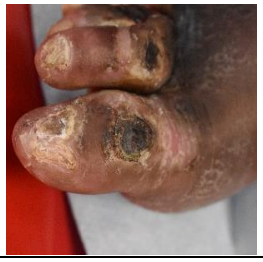
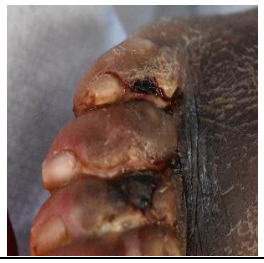
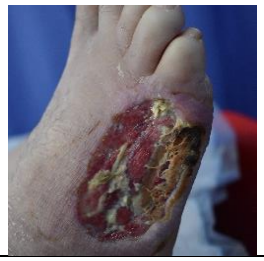
protocol as those presenting with gangrene and systemic infection. This is not an effective treatment strategy in that more severe ulcers are not targeted with appropriate empirical agents. This only potentiates the problem of antimicrobial resistance and therefore, it is important to consider changing South African guidelines to those which are more capable of discerning DFUs of different severity and subsequent treatment plans.

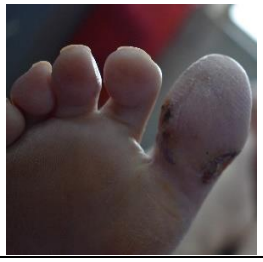
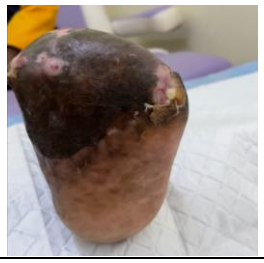
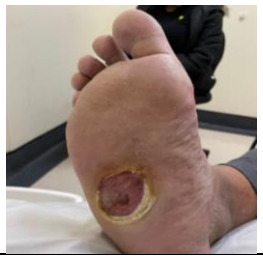
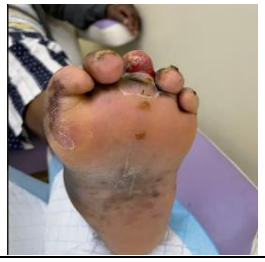
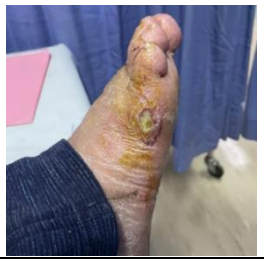
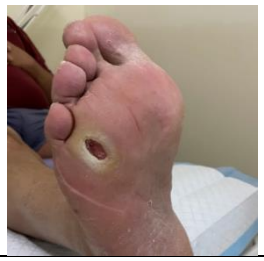
Table 4.2. Reference and comparison between local and international ulcer grading systems in relation to patient clinical wound appearance and file.

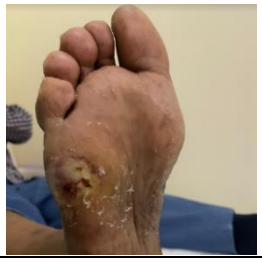
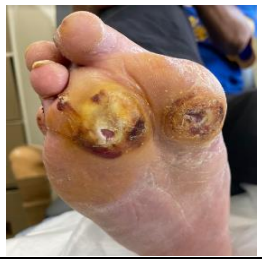
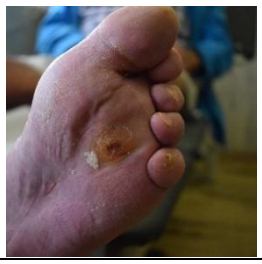
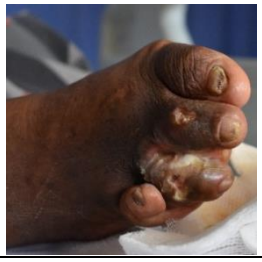
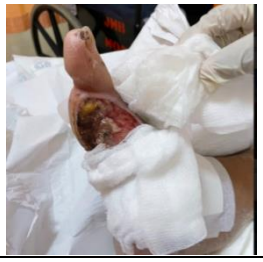
Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A001		Moderate	Grade 2	No
A002		Moderate	Grade 2	No
A003		Mild	Grade 2	Yes
A003**		Mild	Grade 1	Yes
A004		Mild	Grade 1	No

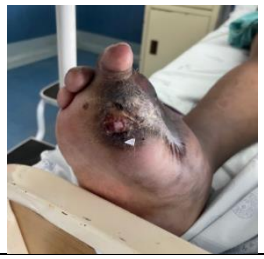
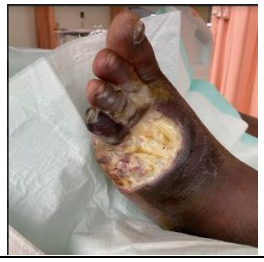
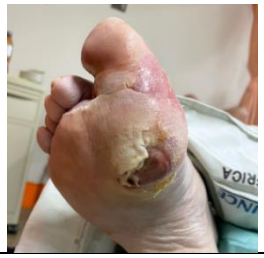
Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A005		Mild	Grade 2	No
A006		Moderate	Grade 2	Yes
A007		Mild	Grade 1	Yes
A007**		Moderate	Grade 4	Yes
A008		Mild	Grade 2	No
A009		Mild	Grade 3	No

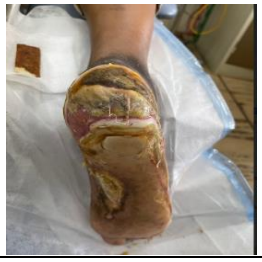
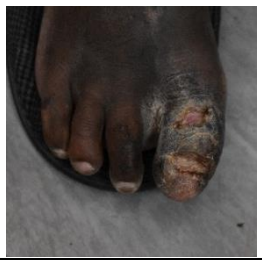
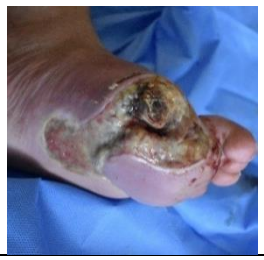
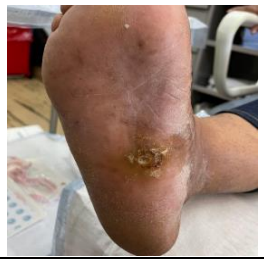
Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A009**		Mild	Grade 1	No
A010		Moderate	Grade 3	Yes
A011		Mild	Grade 2	Yes
A012		Mild	Grade 2	No
A012**		Mild	Grade 1	No
A013		Mild	Grade 2	Yes

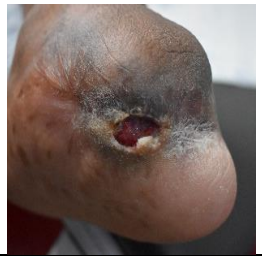
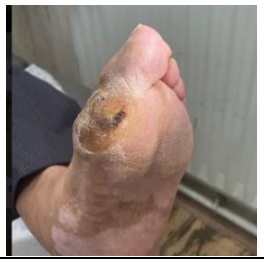
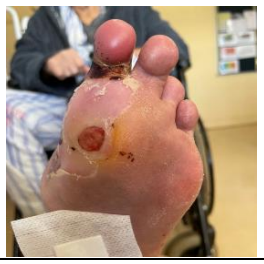
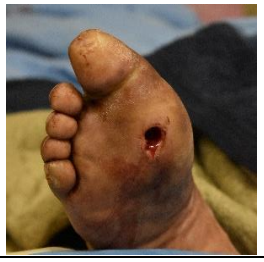
Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A014		Moderate	Grade 3	Yes
A015		Moderate	Grade 2	No
A016		Moderate	Grade 3	No
A017		Mild	Grade 2	No
A017**		Moderate	Grade 4	No
A018		Moderate	Grade 3	No

Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A018**		Mild	Grade 1	No
A019		Moderate	Grade 4	Yes
A020		Mild	Grade 2	No
A021		Mild	Grade 1	No
A022		Mild	Grade 1	Yes
A023		Moderate	Grade 1	No

Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A024		Mild	Grade 1	No
A025		Mild	Grade 1	Yes
A026		Mild	Grade 1	No
A027		Moderate	Grade 4	Yes
A028		Mild	Grade 2	No
A029		Severe	Grade 4	Yes

Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A030		Moderate	Grade 4	No
A031		Mild	Grade 3	Yes
A032		Moderate	Grade 4	Yes
A033		Moderate	Grade 5	No
A034		Moderate	Grade 3	No
A035		Mild	Grade 2	Yes

Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A036		Mild	Grade 2	No
A037		Moderate	Grade 3	Yes
A038		Mild	Grade 1	Yes
A039		Moderate	Grade 3	Yes
A040		Mild	Grade 1	No
A041		Mild	Grade 2	No

Patient code	Foot ulcer*	Severity (South African STG)	Wagner classification	Previous amputation
A042		Mild	Grade 2	Yes
A043		Mild	Grade 1	No
A044		Mild	Grade 1	Yes
A045		Mild	Grade 2	No

* All patients provided signed consent to having a photograph of their wound taken and used provided that patient confidentiality was maintained; ** Patients that presented with a second DFU.

In this study, the majority of patients presented with grade 1 DFUs (33.3%) and grade 2 DFUs (33.3%). Grade 3 ulcers were seen in 17.6% of patients while grade 4 ulcers were seen in 13.7% of patients. Only one patient presented with a grade 5 ulcer. A study undertaken in Nigeria observed the most common ulcer severity as grade 4 (36.9%), followed by grade 3 (26.2%) and grade 2 (17%) (Ugwu *et al.,* 2019). Another study undertaken in India found the most common ulcer severity as grade 4 (34%), followed by grade 2 (22%) and grade 1 (18%) (Mehraj and Shah, 2018). A possible explanation as to why fewer ulcers of higher severity were observed in this study is because patients were often admitted at casualty and transferred straight to surgical wards without any further consultations from other healthcare professionals like podiatrists. From here, many patients underwent amputations and as such were excluded

from the study. This is concerning as patients are not given the opportunity to undergo less aggressive methods of treatment and are subjected to a poorer quality of life due to the loss of a limb. A study evaluating the management of DFUs found that half of DFU amputations can be prevented with proper treatment (Weledji and Fokam, 2014). The reason as to why this may happen in the South African public healthcare sector is due to the inability of the recommended treatment to effectively treat the ulcers, hence another reason as to why the standard treatment guidelines need to be readdressed. When reviewing current and previous treatment plans, the use of alternative antimicrobials, as suggested by the international guidelines (Chapter 1, Table 1.5), besides amoxicillin/ clavulanic acid was not commonly seen. Table 4.3 describes the treatment protocols observed in the study population.

Due to missing files, and new patients with no file history, as well as files that were lost on account of the fire that occurred at CMJAH, seven patients (15.6%) did not have comprehensive records of treatment protocols for previous or current DFUs. From the information available from the remaining 38 patient files, 48 treatment protocols were recorded. Of these, 47.9% of the treatment protocols complied to those set out in the STG and SAASP guidelines. Only 14.6% were partially compliant, 33.3% were not compliant and the remaining 4.2% of the protocols could not be assessed for compliancy due to a lack of record keeping or unrelated foot conditions (onychomycosis) being treated. In the case of patient A028, the treatment protocol for a past occurrence of onychomycosis was included in the data set, as the antimicrobial effect of the treatment may have influenced the healing of the DFU itself, should a fungal pathogen have colonised the wound. While the majority of patients were treated in a manner that adhered to the STG and SAASP guidelines, the repeated treatment plans observed, and the number of patients with chronic wounds, suggest that these guidelines do not cover the most commonly isolated pathogens, as discussed in Chapter 3.

It was further found that 60.4% of the treatment protocols implemented complied partially to international guidelines. A lack of local susceptibility data prevented further compliance to international guidelines due a lack of antibiotic alternatives provided. A study undertaken in India found that the prescribing patterns of empirical antibiotic regimens did not comply with the antibiotic policy of the study hospital (Wasnik *et al.*, 2019). In contrast, an Australian study determined that 83% of patients received empiric antibiotic treatment that complied with National guidelines (Hand *et al.,* 2019). Similarly, to this study, a study carried out in Poland

suggested that although they found good adherence to local prescribing guidelines, the guidelines themselves did not cover the most common pathogens responsible for DFU infection (Kruszewska *et al.*, 2021).

Patient A003 initially presented to the Charlotte Maxeke Johannesburg Academic Hospitals (CMJAH) Podiatry unit in March 2021 with a DFU. No antimicrobial treatment plan was documented in their file history. The patient returned in August of the same year and the ulcer had still not healed, with the formation of a second ulcer evident. At this consultation, the patient was prescribed amoxicillin/clavulanic acid in accordance with the STG. Similarly, patient A010 who presented to the Helen Joseph (HJH) Podiatry clinic was treated for the same ulcer on two different occasions, six months apart. What is concerning in the case of patient A010 is that the treatment plan was not adjusted from amoxicillin/clavulanic acid six months after initial treatment, despite the antimicrobial having failed to successfully treat the DFU. In 2020, patient A025 was treated with amoxicillin/clavulanic acid as well as clindamycin due to a DFU. According to this patients file, empiric treatment was unsuccessful and that patient underwent amputation surgery. In 2020, patient A025 presented to the HJH Podiatry clinic with a recurrent DFU, the patient was once again treated with amoxicillin/clavulanic acid. This phenomenon was observed in multiple patients who had previous DFU incidents reported in their files.

In addition, no culture assays were completed for these patients and it appeared that no review of previous treatments was accounted for before prescribing a new treatment regimen. These patients' cases point out missed opportunities in improving their outcomes in that very little was done in order to address why the antimicrobial therapy was not effective. Furthermore, by continuing to make use of an antimicrobial shown to be ineffective in the past treatment of the patient, further promotes the antimicrobial resistance to this agent.

Only two patients had samples collected for microbiological testing and culture identification. The subsequent treatment protocols implemented for these patients accounted for two of the three protocols found to be non-compliant with local guidelines but compliant to international guidelines. By carrying out culture assays, targeted therapy was made possible and the patients were given the best treatment plan available to them. This potentially resulted in the wound becoming less severe and amputation was hypothetically avoided. This further demonstrates

the need for local epidemiological data in order to improve local guidelines and provide more appropriate treatment protocols. In the study carried out in India, it was found that 44% of patients had undergone culture and sensitivity testing within 48 hours of being admitted (Wasnik *et al.,* 2019). An important difference between the study undertaken in India and the current study is that 72.6% of patients in the Indian study were switched to definite therapy after sensitivity tests were carried out, while only 4.4% of this study's sample population appeared to be initiated on definite antibiotic therapy. An additional comparison of each patient's treatment protocol and comment on the status of the patient can be found in appendix H.

4.3.3. Additional factors influencing the management of DFUs

4.3.3.1. Concurrent medical conditions

Patients with diabetes have a higher risk of developing co-morbid conditions that include cardiovascular disease (CVD), hypertension, and renal disease (Papatheodorou *et al.,* 2018; Nowakowska *et al.,* 2019). Diabetic patients are also at a greater risk for developing depression, chronic obstructive pulmonary disease and thyroid gland dysfunction (Nowakowska *et al.,* 2019). It is important to be aware of the chronic, concurrent conditions that patients present with, as it may affect ulcer development, healing time and the chronicity of the DFU. For example, patients presenting with elevated blood glucose and blood pressure can result in the narrowing of blood vessels and subsequent delayed healing time due to reduced circulation (Kee *et al.,* 2019). Table 4.4 indicates the frequency of all concurrent conditions present in the study population.

A study undertaken in Spain found that the rate of incidence of hypertension was 71.9%, while hyperlipidaemia was seen in 60% of patients (Mata-Cases *et al.,* 2019). Another study demonstrated similar rates of hypertension (82.1%) and hyperlipidaemia (77.2%) (Iglay *et al.,* 2016). Cardiovascular disease is a common co-morbidity seen in diabetic patients (Papatheodorou *et al.,* 2018; Nowakowska *et al.,* 2019).

Table 4.3: Comparison of treatment protocols recorded in patient files to South African and International guidelines.

Patient code	Date of current and previous treatments	Culture collected	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A001	21/04/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	C	PC
A002	07/03/2021	No	No	Mupirocin (topical)	NC	NC
A003	12/03/2021 20/08/2021	No No	No No	No Systemic antibiotics (not specified) then Amoxicillin/ clavulanic acid (1000 mg)	NC C	NC PC
A004	20/08/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	C	PC
A005	06/08/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	C	PC
A006	*	*	*	*	*	*
A007	01/04/2019 02/09/2019 30/07/2021	No No No	No No No	No Cloxacillin Amoxicillin/ clavulanic acid (1000 mg)	NC NC C	NC NC PC
A008	23/08/2021	No	No	Amoxicillin/ clavulanic acid 1.2 g IV followed by Amoxicillin/ clavulanic acid orally (1000 mg)	C	PC
A009	21/06/2021	No	No	No	NC	NC
A010	17/02/2020	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD	C	PC
	11/08/2020	No	No	Amoxicillin/ clavulanic acid 1.2 g IV for 7 days followed by Amoxicillin/ clavulanic acid orally (1000 mg) daily for 3 days	C	PC

Patient code	Date of current and previous treatments	Culture collected	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A011	13/07/2021	No	No	Amoxicillin/ clavulanic acid 1.2 g IV TDS and clindamycin (600 mg) IV	C	PC
A012	03/06/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD; Metronidazole (400 mg) BD	PC	PC
A013	13/09/2021	No	No	No	NC	NC
A014	*	*	*	*	*	*
A015	09/2021	No	No	No	NC	NC
A016	*	*	*	*	*	*
A017	07/12/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD	C	PC
A018	16/11/2021	No	No	Amoxicillin/ clavulanic acid (250 mg) TDS	C	PC
A019	**	**	**	**	**	**
A020	20/12/2021	No	No	Clarithromycin (500 mg) BD	NC	NC
A021	2021/2	No	No	No	NC	NC
A022	25/12/2021 - 31/12/2021	Unknown	Unknown	Yes - Iv antibiotics	Unknown	Unknown

Patient code	Date of current and previous treatments	Culture collected	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A023	***	***	***	***	***	***
A024	***	***	***	***	***	***
A025	7/02/2022 29/12/2020	No No	No No	Amoxicillin/ clavulanic acid (500 mg) daily Clindamycin IV (600 mg) 8 hourly; Amoxicillin/ clavulanic acid IV 1.2 g 8 hourly for 10 days	C PC	PC PC
A026	**	**	**	**	**	**
A027	03/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly and then azithromycin (500 mg) orally for 8 days	PC	PC
A028	22/02/2022 24/03/2022	No No	No No	Clotrimazole (20 mg) BD, Fluconazole (200 mg) weekly (treatment regimen for onychomycosis) Amoxicillin/ clavulanic acid (1000 mg) BD for 7 days and Mupirocin	N/A PC	N/A PC
A029	08/03/2022	Yes	E. Coli	Piperacillin/ Tazobactam (4.5 g) IV 6 hourly Ertapenem (1 g) IVI daily for 7 days	NC	C
A030	07/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly and then amoxicillin/clavulanic acid (600 mg) IV 8 hourly	C	PC
A031	07/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly	C	PC
A032	07/03/2022	No	No	No	NC	NC

Patient code	Date of current and previous treatments	Culture collected	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A033	13/03/2022	Yes (blood and pus culture)	*Streptococcus agalactiae*, *Peptostreptococcus anaerobis*; Gram (+) diplococci and Gram (+) cocci in chains	Piperacillin/ Tazobactam (4.5 g) IV 6 hourly	NC	C
A034	09/03/2022	No	No	Amoxicillin/clavulanic acid (1000 mg) orally TDS	C	PC
A035	03/03/2022	No	No	No	NC	NC
A036	18/03/2022	No	No	Amoxicillin/ clavulanic acid (1000 mg) orally BD	C	PC
A037	13/03/2022	No	No	Amoxicillin/ clavulanic acid (1000 mg) orally BD	C	PC
A038	26/02/2021 12/03/2022	No No	No No	Amoxicillin/ clavulanic acid 1.2 g IV TDS Ceftriaxone (250 mg) IMI STAT, azithromycin (1 g) orally daily, amoxicillin/ clavulanic acid (1000 mg) orally BD, metronidazole 1 g orally STAT	C PC	PC C
A039	09/02/2022 21/03/2022	No No	No No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly	C C	PC PC
A040	22/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly Then Amoxicillin/ clavulanic acid (1000 mg) orally BD; Mupirocin	PC	PC

Patient code	Date of current and previous treatments	Culture collected	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A041	01/04/2022	No	No	Clindamycin IV (600 mg) BD; Amoxicillin/ clavulanic acid IV (600 mg) 8 hourly	PC	PC
A042	16/07/2016	No	No	No evidence of antibiotic use recorded in file.	NC	NC
	10/06/2022	No	No	No evidence of antibiotic use recorded in file.	NC	NC
A043	10/06/2022	No	No	No evidence of antibiotic use recorded in file	NC	NC
A044	23/08/2021	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hourly Then Amoxicillin/ clavulanic acid (1000 mg) orally	C	PC
	17/06/2022	No	No	BD Amoxicillin/ clavulanic acid 1.2 g IVI TDS	C	PC
A045	21/06/2022	No	No	Amoxicillin/ clavulanic acid (1000 mg) orally TDS	C	PC

Key: C – compliant; **PC** – partially compliant; **NC –** non-compliant; ***** - patient file unaccounted for; **- new patient, no file history; *** - new file created and previous file lost due to CMJAH fire thus no file history; **IV** – intravenously; **BD –** twice daily; **TDS** – three times daily; **IMI** – intramuscular injection; **STAT** – immediately

Table 4.4: The frequency of chronic, co-morbid conditions as noted in patient files.

Concurrent condition	Number of patients presenting with the condition (%)
Hypertension	38 (84.4)
Dyslipidaemia	15 (33.3)
Peripheral neuropathy	12 (26.7)
Cardiovascular disease (Including heart failure, atrial fibrillation, angina and dilated cardiomyopathy)	8 (17.8)
Metabolic syndrome	5 (11.1)
Gout	3 (6.7)
Hypothyroidism	3 (6.7)
Renal disease	2 (4.4)
Asthma	2 (4.4)
Chronic obstructive pulmonary disorder	2 (4.4)
Eczema	2 (4.4)
Visual impairment	2 (4.4)
Gastro-oesophageal reflux disorder/ peptic ulcers	2 (4.4)
Depression/ generalised anxiety disorder	2 (4.4)
Myasthenia gravis	1 (2.2)
Chronic kidney stones	1 (2.2)
Venous insufficiency	1 (2.2)
Rheumatoid arthritis	1 (2.2)
Iron deficiency anaemia	1 (2.2)

In the present study, 17.8% of patients presented with some form of CVD. Similar rates of CVD were seen in other studies with 23% and 21.6% of patients presenting with CVD, respectively (Mata-Cases *et al.,* 2019; Iglay *et al.,* 2016). It has been proposed that diabetic foot ulceration may be potentiated by the presence of CVD and renal insufficiency (Saluja *et al.,* 2020). Furthermore, it has been established that patients presenting with DFUs have a greater risk of all-cause mortality (the rate of death due to all causes of death for a population) when compared to those without DFUs (Brownrigg *et al.,* 2012). This excess risk is potentially attributed to more frequent CVD and fatal cerebrovascular disease in those patients presenting with DFUs (Saluja *et al.,* 2020).

The rate of co-morbid gout in this study population was 6.7%. A study undertaken in the UK found that 10.1% of diabetic patients presented with gout, which was a significantly greater prevalence than in patients without type 2 diabetes (Collier *et al.,* 2016). The prevalence of co-

morbid gout is increasing. It is hypothesised that this is due to an increase in dietary sucrose, metabolised to fructose which when further metabolised results in an increase in uric acid (Guariguata *et al.*, 2014; Collier *et al.*, 2016). Gout is an important diagnosis to be observant for in the diabetic patient, as it is a diagnosis that is frequently missed or the symptoms attributable to gout are misdiagnosed as pertaining to a DFU (Antoun *et al.*, 2020). Hypothyroidism in the diabetic patient is a known clinical observation with rates of occurrence being 5-15 times greater than in the non-diabetic population (Maskey *et al.*, 2015; Kalra *et al.*, 2019). Co-morbid hypothyroidism prevalence was found to be 4.1% in a study undertaken in Nepal (Maskey *et al.*, 2015). A review undertaken in 2019 suggests that the prevalence of hypothyroidism among diabetic patients ranges from 9.9–48% (Kalra *et al.*, 2019). Similar rates of hypothyroidism were seen in this study (6.7%). Hypothyroidism has been shown to have a negative effect on wound healing and therefore is a condition of concern when treating DFUs (Ekmektzoglou and Zografos, 2006). Regular screening for symptoms of both gout and hypothyroidism should be included in a holistic treatment plan for diabetic and DFU patients in order to ensure the best treatment possible.

In this study, 93.3% of patients presented with at least one additional chronic condition besides diabetes mellitus, while 80% presented with at least two concurrent chronic conditions and 40% presented with more than four co-morbidities. The highest number of concurrent conditions reported was eight; while the most frequently seen number of chronic conditions was two (Figure 4.5).

Similar results were seen in a study undertaken in Spain where 82% of patients presented with more than two co-morbidities and 31% with more than four co-morbidities (Mata-Cases *et al.*, 2019). Iglay *et al.* (2016), reported in their study of 1 389 016 participants that 97.5% of patients had at least one co-morbid condition and that 88.5% presented with at least two co-morbidities.

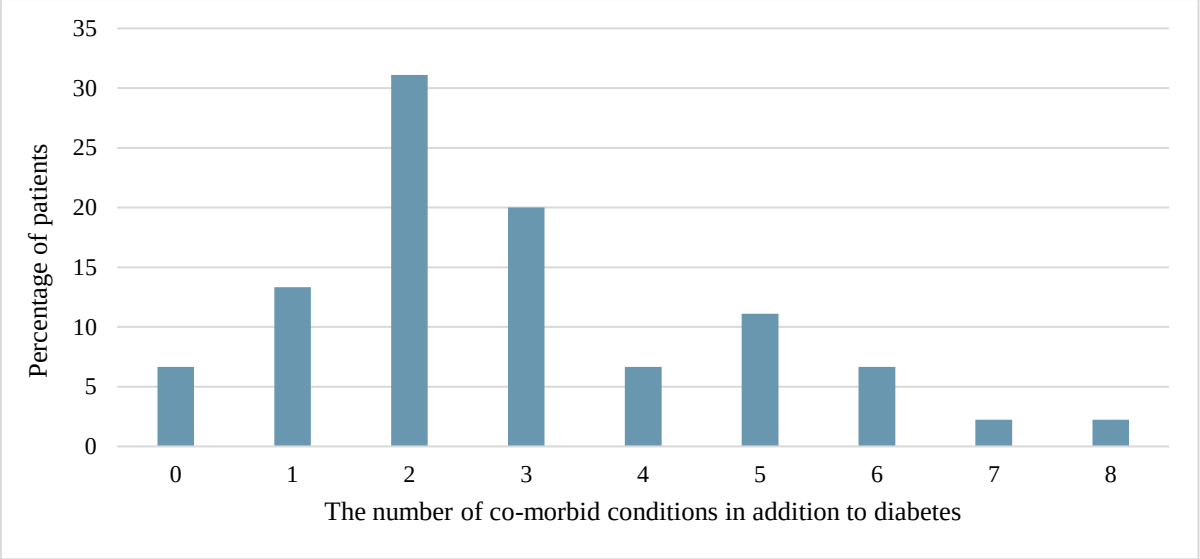


Figure 4.5: The percentage of patients presenting with co-morbidities in addition to diabetes.

The average number of co-morbidities in addition to diabetes mellitus seen in this patient cohort was 3.9 ≈ 4. This corresponds with the findings that South African populations have been experiencing a rise in concurrent chronic diseases (Petersen *et al.*, 2019). Figure 4.6 examines the relationship between ulcer severity and the average number of co-morbidities.

Figure 4.6: The relationship between the average number of co-morbidities seen in patients presenting with various grades of ulcer severity.

Patients who exhibited either Wagner grade 1 or 4 ulcers presented with the highest average of chronic co-morbid conditions (Figure 4.6). The largest proportion of ulcers were classified as grade 1 and grade 2 in severity and these patents presented with an average number of 4.1 and 3.8 co-morbidities, respectively. It has been shown that a high number of co-morbid conditions

had increased the likelihood of amputation and thus ulcer severity (Markowitz *et al.,* 2006). Another study found that the presence of co-morbid conditions increases the risk of DFU reoccurrence (Khalifa, 2018). No relationship was observed in this study between ulcer severity and number of co-morbid conditions and the average number of co-morbid conditions when compared to ulcer severity does not significantly change between Wagner grades. While the number of co-morbid conditions does not affect the ulcer severity in this study, the nature of the co-morbidities does affect ulcer healing. For example, the presence of insufficient renal function and hypertension accelerate ulcer development and prolong healing time (Gazzaruso *et al.,* 2021). It is therefore important to ensure that the patients' entire healthcare team is aware of their history in order to regularly screen for new conditions while monitor existing conditions. If this is carried out in an effective manner in conjunction with improved patient education, the potential to improve patient outcomes is greater.

4.3.3.2. Medication use

Multi-morbidity is a common phenomenon in diabetic patients and as such, most patients will be found to be taking more than one medication on a daily basis (Dobrică *et al.,* 2019). Table 4.5 provides a complete list of the medications noted to be used by this study's patient cohort as well as the frequency in which they were used.

Table 4.5: A complete list of medications used by the patient cohort and the frequency in which they were used.

Medication Name	Dosage	Frequency used
Anti-diabetic medication		
Actraphane*	Patient dependent	19
Protaphane *	Patient dependent	4
Actrapid/ Humulin *	Patient dependent	3
Unspecified insulin	Patient dependent	2
Metformin*	850 mg	27
Metformin*	500 mg	4
Gliclazide	60 mg	2
Glimepiride*	1-6 mg	4
Glibenclamide*	5 mg	1
Lipid lowering agents		
Simvastatin*	10 - 40 mg	18
Atorvastatin**	10 - 40 mg	7
Anti-hypertensive agents and cardiovascular treatment		

Medication Name	Dosage	Frequency used
Enalapril*	10-25 mg	18
Perindopril	4 mg	2
Lisinopril	12.5 mg	1
Bisoprolol	10 mg	1
Atenolol*	25-100 mg	4
Propranolol**	20 mg	1
Carvedilol*	3.125 - 20 mg	7
Furosemide*	40 -80 mg	11
Spironolactone*	25 mg	3
Hydrochlorothiazide*	10-25 mg	15
Amlodipine*	5-10 mg	18
Nifedipine*	30 mg	1
Losartan**	50 mg	2
Aspirin*	25-150 mg	10
Trimetazidine	35 mg	1
Clopidogrel**	75 mg	1
Enoxaparin**	20-110 mg	9
Isosorbide mononitrate*	10 mg	1
Neuropathic and analgesic agents		
Pregabalin	75 mg	1
Amitriptyline*	10-50 mg	11
Pyridoxine*	25-50 mg	8
Nicotinamide*	10-100 mg	4
Tramadol*	50-100 mg	11
Paracetamol*	1 g	15
Drugs for GORD and peptic ulcers		
Cimetidine	200 mg	1
Omeprazole**	30 mg	2
Lansoprazole*	30 mg	4
Pantoprazole	40 mg	1
Antigout preparations		
Allopurinol*	300 mg	1
Colchicine**	0.5 mg	1
Myasthenia gravis management		
Pyridostigmine**	60 mg	1
Anti-asthmatics		
Glycopyrronium bromide	44 mcg	1
Salmeterol/ fluticasone*	25/50 mcg	1
Salbutamol*	100 mcg	2
Antihistamines		
Chlorphenamine*	4 mg	1
Cetirizine*	10 mg	1

Medication Name	Dosage	Frequency used
Thyroid therapy		
Levothyroxine*	50-100 mcg	3
Antiepileptics		
Carbamazepine*	200 mg	1
Topiramate**	50 mg	1
Anxiolytics, antidepressants and hypnotics		
Alprazolam***	0.25 mg	1
Escitalopram	10 mg	1
Citalopram*	20 mg	1
Zolpidem	10 mg	2
Antiemetics		
Metoclopramide*	10 mg	4
Other		
Tamsulosin**	0.4 mg	1
Betamethasone/ salicylic ointment	0.05%/3%	1
Vitamin K*	5 mg	2
Polystyrene sulfonate	15 mg	1
Bevacizumab***	100 mg	1

Key: * - indicated in the South African Essential Medicines list for primary care, ** - indicated in the South African Essential Medicines list for secondary care, *** - indicated in the South African Essential Medicines list for tertiary care

The most commonly used medication was found to be metformin 850 mg taken either twice or three times daily. In total, 68.9% of patients were found to be taking metformin. This is an expected result as the patient cohort is a diabetic population and metformin is the first line treatment in management of this condition according to the STG (The National Department of Health, South Africa, 2020). Insulin was found to be used by 62.2% of patients, with the most frequently used form being actraphane, a biphasic human insulin. Insulin is used to escalate treatment in those patients whose blood glucose is not controlled on metformin and sulphonylureas. The high rates of insulin use in this patient cohort could suggest that patients' blood glucose is generally poorly controlled. This is important because poorly controlled blood glucose exacerbates the development of DFUs and contributes to their chronic nature (Kee *et al.*, 2019).

Of the medications that are not anti-diabetic in nature, the most frequently used include simvastatin (for hyperlipidaemia treatment), enalapril and amlodipine (both used in the treatment of hypertension), with 40% of patients found to be using them. It has already been established that hypertension and dyslipidaemia were the most commonly seen concurrent

conditions in this study population and thus the high prevalence of lipid lowering agents and anti-hypertensive medications was expected. Thereafter, hydrochlorothiazide (a diuretic used in the treatment of hypertension) and paracetamol (an analgesic and antipyretic) use were most frequently seen (33.3%). According to the STG, the first line treatment for diabetic neuropathy includes amitriptyline and paracetamol (The National Department of Health, South Africa, 2020). Adherence to this guideline can be observed with both amitriptyline (24.4%) and paracetamol (33.3%) accounting for the most frequently used neuropathic and analgesic agents.

The treatment of type 2 diabetes is approached in a stepwise manner according to the STG and is summarised in Figure 4.7 (The National Department of Health, South Africa, 2020). What is important to note is that lifestyle modifications, such as dietary changes and increased exercise, are indicated at every stage of treatment. It is important to ensure that patients are adequately educated on appropriate lifestyle modifications in order to allow for the best possible patient outcomes.

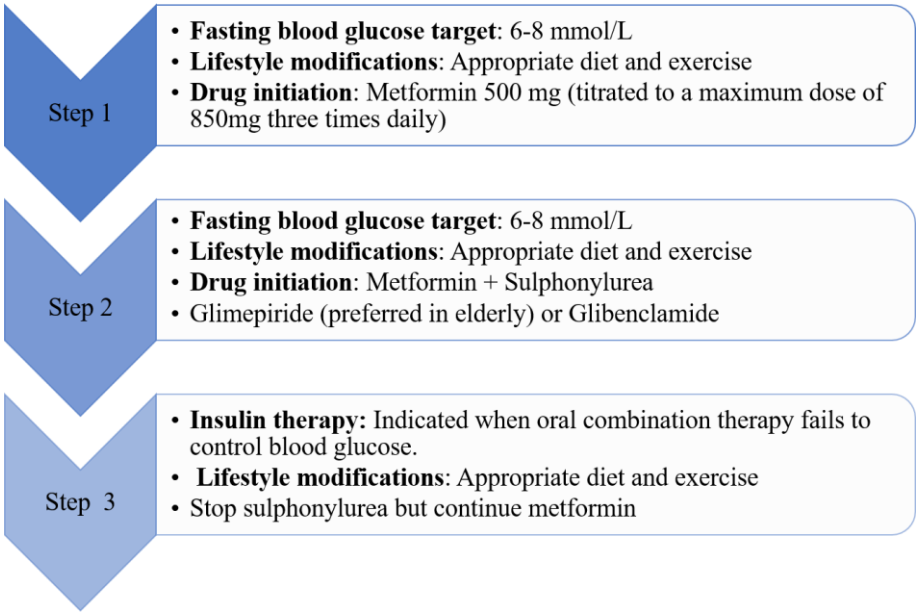


Figure 4.7: The stepwise approach taken to treat type 2 diabetes. Adapted from the STG (The National Department of Health, South Africa, 2020).

Table 4.6 indicates the various combinations of anti-diabetic medication seen in this study's patent cohort and the frequency in which they were seen.

Table 4.6: The frequency of anti-diabetic medications and combinations as observed in the sample population.

Drug or drug combination	Number of patients (%)
Metformin	12 (26.7)
Insulin	12 (26.7)
Metformin + insulin	14 (31.1)
Metformin + glimepiride	4 (8.9)
Metformin + glibenclamide	1 (2.2)
Metformin + gliclazide	2 (4.4)

It can be noted that 26.7% of patients were being treated according to step one of STG, while, 11.1% of patients were being treated according to step two. Glimepiride, the sulphonylurea preferred in the elderly was used in combination with metformin in 8.9% of the patient cohort, while the remaining 2.2% were prescribed glibenclamide in addition to metformin (The National Department of Health, South Africa). Gliclazide, a sulphonylurea not indicated for use in the STG was seen to be used in combination with metformin in two patients. The largest proportion of patients (31.1%) were seen to be initiated on step three of the STG stepwise treatment plan, the combination of metformin and insulin. This is of possible concern as it suggests that the largest proportion of DFU patients were not controlled in earlier phases of treatment. This finding could suggest that patients are not adequately educated in the importance of implementing and adhering to lifestyle modifications. The frequency of this treatment regimen may also occur due to the fact that most patients will ultimately end up on insulin as the duration of diabetes increases in order to maintain glycaemic control (Hemmingsen *et al*., 2012; Ellis *et al.,* 2018). In order to achieve or maintain glycaemic control, insulin use may be initiated in the type 2 diabetes patient, generally in addition to metformin as seen in the STG (Hemmingsen *et al*., 2012; The National Department of Health, South Africa, 2020). In this study, insulin use alone was seen in 26.7% of patients where only one patient was noted to have type 1 diabetes. This suggests that the STG is not followed in its entirety because the final stage of treatment (step three in Figure 4.7) states that metformin therapy should remain in addition to insulin therapy. This prescribing pattern is an indication of poor practice because it has shown that metformin in combination with insulin is more effective in controlling hyperglycaemia than monotherapy with insulin (Paczkowska *et al.,* 2021).

4.3.3.3. Incidence of polypharmacy in diabetic patient management

Polypharmacy is most commonly defined as the use of five or more medications in a day (Masnoon *et al.*, 2017; Dobrică *et al.*, 2019). Patients presenting with type 2 diabetes, in particular, older patients are at risk for polypharmacy due to the nature of treating both macrovascular and microvascular complications of diabetes (Dobrică *et al.*, 2019). It has been found that diabetic patients are twice as likely to experience a drug-drug interaction when compared to patients without diabetes due to the presence of multiple co-morbid conditions in the diabetic populations and it is therefore important to monitor patients' prescriptions (Dobrică *et al.*, 2019). Table 4.7 summarises the total number of medications taken per patient, per day.

Table 4.7: The number of different medications taken daily by the patient cohort.

Number of medications	Frequency of patients (%)
1-4	16 (35.6)
5-10	24 (53.3)
>10	5 (11.1)

In this study's sample population, 36.6% of patients were found to be prescribed between one and four medications. Within this group, 15.5% of patients were prescribed two medications, making it the most frequently used number of medications. Thereafter, 11.1% of patients were found to be prescribed either seven or nine chronic medications. The highest number of medications used by a single patient was 14 and the lowest was one. The majority of the sample population (55.5%) were noted to be prescribed more than five medications.

A review looking at the prevalence of polypharmacy in older diabetic patients found that 64% of patients were prescribed more than four medications a day (Remelli *et al.*, 2022). This review also highlighted that polypharmacy may reduce optimal glycaemic control as well as increase the risk of hospitalisation (Remelli *et al.*, 2022). A more apparent result of polypharmacy would be an increase in the frequency of drug-related interactions (Díez *et al.*, 2022). Table 4.8 provides information on the number of medication interactions observed per patient. The interactions were classified based on information from the South African Medicines Formulary (SAMF) as well as EM Guidance. The EM Guidance interaction checker is based of the most recent international manufacturer information. The interactions were classified as follows: minor interactions, which are minimally clinically significant and the risk or an adverse event

is low; moderate interactions, which are moderately clinically relevant and use of these drugs should only occur when the benefit outweighs the risk; and major interactions, which are highly clinically relevant and the administration of the drugs should be avoided (Soherwardi *et al.,* 2012; Hecker *et al.,* 2022). A detailed list on the possible medication interactions included in this analysis and their potential effects can be found in Appendix I.

Table 4.8: The number of potential interactions found per patient and classified as either minor, moderate or severe. Interaction information was taken from the South African Medicines Formulary as well as the EM Guidance interaction checker (available at: https://emguidance.com/interactions).

Patient code	Wagner grade	Number of medications	Number of minor interactions	Number of moderate interactions	Number of severe interactions	Total number of interactions
A001	1	7	1	5	1	7
A002	2	2	0	0	0	0
A003	2	10	1	3	1	5
A003*	1					
A004	1	2	0	0	0	0
A005	2	9	1	2	1	4
A006	2	2	0	0	0	0
A007	4	6	0	1	1	2
A007*	1					
A008	2	2	0	0	0	0
A009	3	6	0	1	1	2
A009*	1					
A010	3	12	0	8	1	9
A011	2	8	0	6	3	9
A012	2	4	0	1	1	2
A012*	1					
A013	2	5	0	1	1	2
A014	3	1	0	0	0	0
A015	2	4	0	0	0	0
A016	3	9	0	7	6	13
A017	4	4	1	1	0	2
A017*	2					
A018	3	1	0	0	0	0
A018*	1					
A019	4	2	0	0	0	0
A020	2	14	7	5	9	21
A021	1	2	0	0	0	0
A022	1	5	1	1	0	2

Patient code	Wagner grade	Number of medications	Number of minor interactions	Number of moderate interactions	Number of severe interactions	Total number of interactions
A023	1	3	0	0	0	0
A024	1	7	0	3	1	4
A025	1	3	0	0	0	0
A026	1	7	0	0	1	1
A027	4	14	2	12	7	21
A028	2	7	0	2	0	2
A029	4	8	1	6	2	9
A030	4	5	0	0	0	0
A031	3	4	0	0	0	0
A032	4	6	0	1	1	2
A033	5	7	0	3	1	4
A034	3	9	1	3	3	7
A035	2	8	1	4	6	11
A036	2	3	0	0	0	0
A037	3	11	0	5	6	11
A038	1	10	1	7	2	10
A039	3	8	0	0	1	1
A040	1	12	1	12	5	18
A041	2	9	0	4	6	10
A042	2	9	0	5	1	6
A043	1	2	0	0	0	0
A044	1	10	0	7	6	13
A045	2	5	0	0	0	0
Total	**-**	**284**	**19**	**116**	**75**	**210**

* - Patients presenting with a second ulcer

A total of 210 potential drug-drug interactions were observed in the patient cohort. The most frequently seen type of interaction in this study was moderate drug interactions (55.2%). The mean number of drug interactions per patient was 4.7. Only 35.6% of participants in this study did not present with any potential drug-drug interactions while 60% and 57.8% of patients presented with moderate or severe interactions. The largest number of potential drug interactions seen per patient was 21. This number of interactions was seen in one patient and the same patient was noted to be on the largest number of medications (n=14). A study undertaken on outpatients in Jordan found that the average number of medications prescribed per patient was 6.6 and the average number of potential drug interactions per patient was established at 4.2 (Nusair *et al.*, 2020). Similar to the current study, the study undertaken in Jordan found the most frequently seen interaction in terms of severity was moderate (Nusair *et*

al., 2020). Another study evaluated the impact of polypharmacy among diabetic patients and found that 8.7% of patients had potentially serious drug interactions of clinical relevance while 23.4% of patients were found to have prescriptions of potentially inappropriate medications (Al-Musawe *et al.,* 2021). The average number of medications used in patients presenting with both grade one and two DFUs was six. The highest average number of medications used (n=7) was seen in those with DFUs of Wagner grade 3 severity.

Figure 4.8 demonstrates some of the most commonly seen potential drug-drug interactions in the patient cohort. The potential consequences of these interactions include fatal hyperkalaemia, cardiac arrhythmias, nephrotoxicity and rhabdomyolysis. When looking at the most common potential drug-drug interactions observed between enalapril and amlodipine, evidence-based medicine has shown that the benefit of using both agents in combination outweighs the risk and that the predicted interactions often does not translate into clinically relevant events (Rienzo *et al.,* 2009).

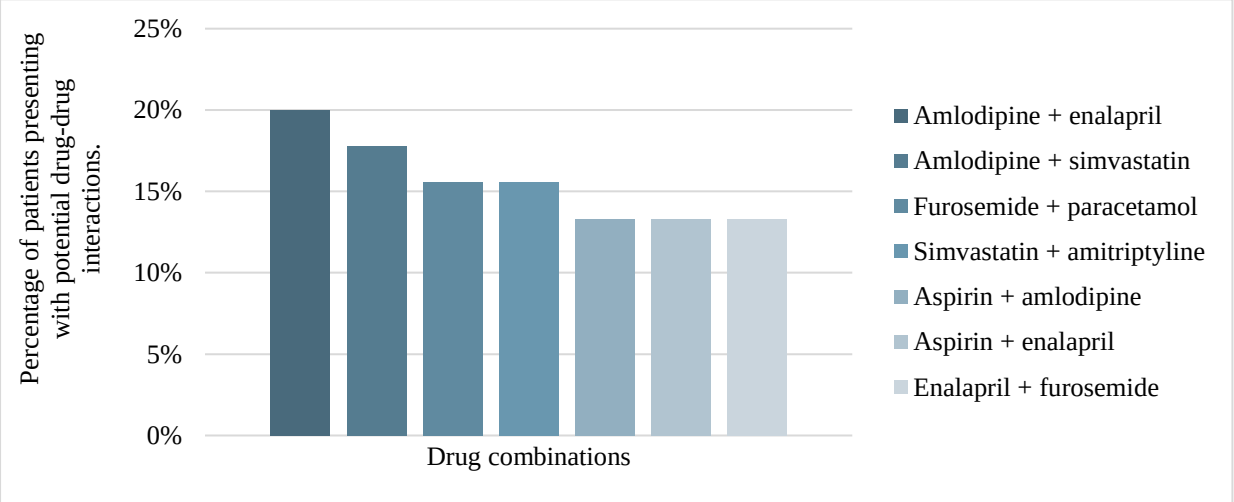


Figure 4.8: The most commonly observed drug-drug interactions.

Step 5 in treating hypertension according to the STG, states that amlodipine, hydrochlorothiazide and enalapril are used in combination in order to reduce blood pressure (The National Department of Health, South Africa, 2020). Similarly, although and interaction exists when simvastatin is used together with amlodipine, a pharmacodynamic response is not commonly observed (Nishio *et al.,* 2005). It is therefore important to include evidence-based practice observations when considering a patient's medication as not all potential interactions warrant intervention. What is important to look for when reviewing a patients medication list

is the duplication of medicines resulting in unnecessary additional medicines that could ultimately negatively affect the patient.

It has been found that the presence of both polypharmacy and subsequent potential drug interactions may alter glycaemic targets and result in a deterioration of kidney functioning (Al-Musawe *et al.,* 2021). This not only poorly effects the overall health of the patient but impaired renal function is also associated with delayed ulcer healing, amputation and mortality (Gazzaruso *et al.,* 2021). It is therefore important to effectively manage patients on multiple medications. This can be accomplished by reviewing the patient's full medication history every time a new drug is considered, improving and increasing clinical pharmacy services, improving communication between healthcare practitioners and finally by implementing effective patient history reporting and record keeping (Nusair *et al.,* 2020). In order to demonstrate the need for effective patient management, a case study is provided.

4.3.4. The holistic clinical analysis of patient DFU management: Patient A020 - A case study

In order to understand the impact of treating patients as a healthcare team and the impact of not carrying out care to the best of one's capabilities, it is important to look at the patient in its entirety. Patient A020 presented to the CMJAH Podiatry unit on the 12th of January 2022 for treatment of a DFU (Figure 4.9).

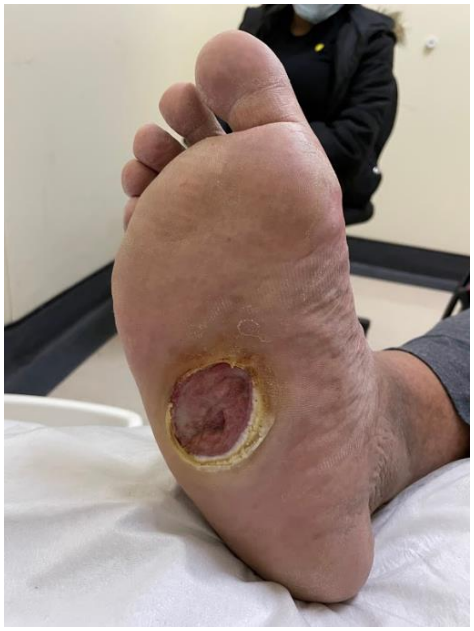


Figure 4.9: Patient A020's diabetic foot wound.

The patient is a 48-year-old, type 2 diabetic female with no history of previous amputation. When the patient was asked about the duration of the DFU it was explained that she had presented with the ulcer one year and three months prior to the 12th of January 2022. The DFU had still not resolved in January 2022 (Figure 4.9). Concurrent chronic conditions for this patient included hypertension, dyslipidaemia, congestive heart failure and asthma. Table 4.9 provides a summary of the patient's medication use and preventative measure use.

Table 4.9: A summary of patient A020's medication and preventative measure use.

Medications	**Indication**
Insulin	Type 2 diabetes mellitus
Atorvastatin	Dyslipidaemia
Simvastatin	Dyslipidaemia
Lisinopril	Hypertension/ heart failure
Atenolol	Hypertension/ heart failure
Propranolol	Hypertension / heart failure
Hydrochlorothiazide	Hypertension/ heart failure
Amlodipine	Hypertension/ heart failure
Amitriptyline	Depression/ neuropathy
Carbamazepine	Epilepsy/ Neuropathic pain
Topiramate	Epilepsy
Alprazolam	Depression
Citalopram	Depression
Zolpidem	Insomnia
Preventative measure use	
Removable walker used when in public Crutches or wheelchair used when at home	

From Table 4.9., it can be seen that the patient was on 14 different chronic medications. Table 4.10 provides a summary of the potentially severe drug-drug interactions as well as their potential consequences faced by this patient.

Table 4.10: Summary of the potential drug-drug interactions patient A020 is at risk of developing.

Drug A	**Drug B**	**Interaction severity**	**Potential effect of interaction**
Atorvastatin	Simvastatin	Minor	Myopathy, rhabdomyolysis, hepatotoxicity
Atorvastatin	Carbamazepine	Minor	Hepatotoxicity due to increased serum levels of carbamazepine

Drug A	Drug B	Interaction severity	Potential effect of interaction
Atorvastatin	Citalopram	Minor	QT interval prolongation, side effects of citalopram increased.
Atorvastatin	Amitriptyline	Minor	QT interval prolongation risk due to increased serum levels of amitriptyline
Simvastatin	Citalopram	Minor	QT interval prolongation, side effects of citalopram increased.
Amitriptyline	Carbamazepine	Minor	Increased anticholinergic effect, hyponatraemia, seizure (especially in elderly)
Simvastatin	Carbamazepine	Moderate	Hepatotoxicity
Amlodipine	Simvastatin	Moderate	Myopathy or rhabdomyolysis
Hydrochlorothiazide	Citalopram	Moderate	Hyponatraemia, QT interval prolongation, Torsade de pointes risk, hypokalaemia, cardiac arrhythmias
Hydrochlorothiazide	Amitriptyline	Moderate	Hyponatraemia, QT interval prolongation, Torsade de pointes risk, nephrotoxicity, cardiac arrhythmias, increased hypotensive effect
Alprazolam	Zolpidem	Moderate	Complex sleep behaviours, CNS depression
Lisinopril	Hydrochlorothiazide	Moderate	Renal failure, increased hypotensive effect
Lisinopril	Atenolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect
Lisinopril	Propranolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect
Lisinopril	Amlodipine	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect
Propranolol	Atenolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, PR interval prolongation, additive bradycardia, increased hypotensive effect
Atenolol	Amlodipine	Severe	Cardiac failure, fatal hyperkalaemia in at risk patients, additive bradycardia, cardiac arrhythmias, increased hypotensive effect

Drug A	Drug B	Interaction severity	Potential effect of interaction
Propranolol	Amlodipine	Severe	Cardiac failure, fatal hyperkalaemia in at risk patients, additive bradycardia, cardiac arrhythmias, increased hypotensive effect
Amitriptyline	Citalopram	Severe	Hyponatraemia, QT interval prolongation, serotonin toxicity, Torsade de pointes risk

From the list of medications, it can be seen that there are various duplications in treatment, where medications have the same mechanism of action and treatment outcome. For example, atorvastatin and simvastatin are both lipid-lowering agents and atenolol and propranolol are both beta-blockers. By eliminating one beta-blocker and one lipid-lowering agent, the number of potential drug-drug interactions is reduced by seven. This point highlights both the importance of reviewing a patient's medication list and the role of the pharmacist in patient healthcare. In the case of patient A020, it should be of concern that the majority or severe interactions result in fatal hyperkalaemia and cardiac arrhythmias and that the additive effect of these medications may put the patient at risk for development of either event. Given the patients history of cardiac concerns, this is a serious matter for review. The patient's medication list should be reviewed at every visit to ensure that unnecessary medications resulting in polypharmacy are not taken by the patient. Consideration should also be taken that in the case of many potential mild and moderate interactions, evidence-based medicine has shown that the likelihood of the interactions occurring is minimal and that the benefit of using the combination of medicines outweighs the risks.

When looking at the preventative measure use by patient A020, it is promising to see that one of the gold standard methods of pressure off-loading, the removable walker, is being used. However, the patient stated that she only makes use of the removable walker in public. Ideally the patient should use the removable walker at all times in order to improve healing time and prevent ulcer reoccurrence. The patient was aware of this fact but complained of the cumbersome nature of wearing the removable walker in the home space. This suggests that the patient has been well-informed about preventative measure use and foot care but patient adherence is a problem. A systematic review evaluating methods to overcome patient non-

adherence concluded that community pharmacist-led interventions have shown to improve medication adherence (Milosavljevic *et al.*, 2018). These interventions included an educational component which once again highlights the importance of continued patient education (Milosavljevic *et al.*, 2018).

When reviewing the treatment history of the DFU itself, no evidence of antimicrobial use was observed. The patient was placed onto a brief course of clindamycin for bronchitis in the period of the DFU. According to the swab sample taken from this patient, the DFU was found to be colonised by *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus aureus. E. faecalis* and *S. aureus* have been shown (Chapter 3) to be among the most common causative pathogens in DFU infection within this study cohort. By not treating the wound in an effective manner, the wound is less likely to heal and further antimicrobial resistance may develop. Based on the finding of this study, gentamicin would be the most effective antimicrobial for this patient, followed by ciprofloxacin.

4.3.5. A holistic treatment plan for addressing diabetic foot ulcers

The prevention of DFUs should be the main focus of the healthcare team treating a diabetic patient and as such forms step one of the treatment plans. Recommendations based on evidence-based guidelines developed by the International Working Group on the Diabetic Foot (IWGDF) (Bus *et al.*, 2020) are as follows;

Step one: Diabetic patients without a DFU

- Annual screening should occur for patients at low risk for developing a DFU. This screening should include the observation of signs and symptoms of peripheral artery disease, peripheral neuropathy, foot deformity as well as renal disease.
- Encouragement and improved patient education should be implemented. This should include encouraging the patient not to walk barefoot, the importance of examining one's feet as well as the need to care for one's feet through the use of emollients, regular nail cutting and ensuring that the feet are kept clean. Infographics may aid in this.
- Screening for other chronic conditions that may occur as a result of diabetes and that may affect the pathophysiology and development of a DFU should occur at each visit.
- Ensure that a thorough patient history is recorded and kept in the appropriate manner in that records are easily accessible and their whereabouts known. This record keeping

should include medication use, preventative measure use, the diagnosis and corresponding treatment plans of concurrent medical conditions. The treatment of previous DFUs should also be well documented to assist in the development of future treatment plans.

Step two: Diabetic patients at higher risk for developing a DFU

- Advise the patient to inspect the temperature of the foot on a daily basis. Should the temperature increase above what is normally observed, the patient should be advised to consult with a healthcare professional as the increase in temperature may indicate inflammation.
- Encourage these patients to wear footwear that reduces planter pressure and fits correctly in order to prevent the development of a DFU.
- In the case of patients with a history of previous DFU or those who present with calluses, consider the use or orthotic pressure off-loading devices to prevent further ulcer development.
- The most ideal preventative measure to be employed is a removable walker. The patient should be educated in its fitting and as to when to wear it.

Step three: The antimicrobial treatment of established and infected DFUs

Using the results from the antimicrobial susceptibility testing, an antibiogram was developed in order to suggest the best approach to treating DFUS using antimicrobials and is found in table 4.11.

Table 4.11: Antibiogram for micro-organisms isolated from DFUs.

Pathogen	**Percentage prevalence**	**Percentage of micro-organism susceptible to the antimicrobial (%)**						**Recommended for treatment**
		AMC	**CN**	**DA**	**CIP**	**VAN**	**FLU**	
Proteus mirabilis	19.33	39.5	94.2	3.5	69.7	1.1	0	Gentamicin
Staphylococcus aureus	13.71	55.7	100	72.1	93.4	0	0	Gentamicin
Pseudomonas aeruginosa	12.58	0	76.8	0	82.1	1.7	0	Ciprofloxacin

Pathogen	Percentage prevalence	Percentage of micro-organism susceptible to the antimicrobial (%)						Recommended for treatment
		AMC	CN	DA	CIP	VAN	FLU	
Enterococcus faecalis	12.36	27.3	65.5	29	30.9	10.9	0	Gentamicin
Escherichia coli	12.13	0	88.9	0	48.1	0	0	Gentamicin
Corynebacterium species	4.72	9.5	14.3	0	9.5	23.8	0	Vancomycin
Staphylococcus epidermidis	3.82	11.7	100	70.6	82.4	0	0	Gentamicin
Enterobacter cloacae	2.92	0	92.3	0	61.5	0	0	Gentamicin
Streptococcus anginosus	2.92	53.8	69.2	53.8	53.8	38.5	0	Gentamicin
Staphylococcus lugdunensis	2.47	0	100	90.9	90.9	9.1	0	Gentamicin
Bacillus species	2.02	22.2	100	11.1	55.6	33.3	0	Gentamicin
Corynebacterium striatum	2.02	22.2	22.2	33.3	0	66.7	0	Vancomycin
Staphylococcus intermedius	1.57	57.1	71.4	85.7	85.7	42.9	0	Clindamycin/ ciprofloxacin
Klebsiella oxytoca	1.35	0	100	0	66.7	0	0	Gentamicin
Citrobacter koseri	1.35	16.7	100	0	50	0	0	Gentamicin
Acinetobacter baumannii	0.67	33.3	66.7	0	100	0	0	Ciprofloxacin
Coagulase negative *Staphylococcus* species	0.67	66.7	100	100	0	0	0	Gentamicin/ clindamycin
Staphylococcus haemolyticus	0.67	66.7	100	100	33.3	0	0	Gentamicin/ clindamycin
Oligella urethralis	0.67	33.3	66.7	33.3	66.7	33.3	0	Gentamicin/ ciprofloxacin
Group B *Streptococcus* species	0.67	66.7	0	100	0	0	0	Clindamycin

Pathogen	Percentage prevalence	Percentage of micro-organism susceptible to the antimicrobial (%)						Recommended for treatment
		AMC	CN	DA	CIP	VAN	FLU	
Gentamicin-methicillin resistant *Staphylococcus aureus*	0.67	0	0	0	0	0	0	None
Shwanella putrifacians	0.45	0	100	50	100	0	0	Gentamicin/ ciprofloxacin
Staphylococcus cohnii	0.22	100	100	100	100	0	0	Amoxicillin/ clavulanic acid/ gentamicin/ clindamycin/ ciprofloxacin

Key: AMC: Amoxicillin/ clavulanic acid, **CN:** Gentamicin, **DA:** Clindamycin, **CIP:** Ciprofloxacin, **VAN:** Vancomycin, **FLU:** Fluconazole

From the antibiogram it is clear that the best performing antimicrobial against pathogens isolated from DFUs was gentamicin followed by ciprofloxacin. Gentamicin was the best performing antimicrobial in 15 of the 23 pathogens found in DFUs. Most notably, gentamicin was effective against four of the five most commonly isolated pathogens from this study cohort. Ciprofloxacin was found to be the most effective antimicrobial against six of the causative pathogens, including *Pseudomonas aeruginosa,* which accounts for 12.58% of all pathogens isolated. Amoxicillin/ clavulanic acid, the current recommended and most commonly used antimicrobial was only seen to be the most effective antimicrobial against *Staphylococcus cohnii,* which is only responsible of 0.22% of pathogens isolated.

4.4. Summary

- The most commonly used form of pressure off-loading was through the use of crutches.
- With the exceptions of those patients classified as Wagner stage 2, the average number of preventative measure use is less than one in all stages of ulcer severity.
- A total of 80% of patients presented with two concurrent chronic conditions in addition to diabetes.

- Patients who presented with Wagner grade 4 ulcers presented with the highest average number of chronic co-morbid conditions.
- In total, 47.9% of treatment protocols used in this study cohort complied with the treatment protocols set out in the STG and SAASP guidelines.
- Only two patients had samples collected for microbiological testing and culture identification.
- A total of 55.5% of patients were prescribed more than five medications to treat on average three co-morbid conditions.
- A total of 210 potential drug interactions were observed in the study population.

Chapter 5: Conclusions

5.1. Overview of the study

The study subject of antimicrobial resistance patterns of diabetic foot ulcers (DFUs) in selected Gauteng public hospitals has been examined and reported on. In addition to this, the use of preventative measures, the role of concurrent medical conditions and previous treatment plans for DFUs has been analysed in detail. The role of antimicrobial resistance in polymicrobial DFU infections complicates the treatment of these wounds. Furthermore, a lack of antibiotic alternatives in the Standard Treatment Guidelines (STG) and Essential Drugs List contributes both towards the ineffective treatment of the wound as well as the worsening of antimicrobial resistance among causative pathogens.

The overall results of this study are highlighted in this chapter in alignment to the initial objectives of the study. The strengths and limitations of the study are also considered.

5.2. The achievement of study objectives

The overall aim of this study was to provide information on local epidemiological data through the identification of the most common causative pathogens isolated in DFU infections amongst South African patients, as well as the antimicrobial susceptibility patterns thereof. Chapter one provided a comprehensive review of the literature and provided insight into global trends pertaining to DFUs from their pathophysiology, prevention, classification and susceptibility patterns. Further to this, the objectives were addressed as follows;

To obtain wound samples from DFU patients in order to identify micro-organisms implicated in these infections.

Sample swabs from 51 DFUs were collected and successfully cultured. A total of 445 isolates were isolated and characterised according to their macromorphological characteristics. It was found that 44 samples (86.3%) resulted in polymicrobial growth and seven samples (13.7%) in monomicrobial growth. The average number of micro-organisms isolated per lesion was three.

To determine the antimicrobial resistance patterns of pathogens obtained from DFU patient samples, by performing antibiotic susceptibility testing using zone of inhibition studies.

Antimicrobial susceptibility testing was carried out on all 445 isolates by means of the antimicrobial disk diffusion assay. The results were recorded and together with the macromorphological characteristics were used to group isolates of similar susceptibility patterns and characteristics. A total of 54 groups was created and a single representative isolated from each group was identified. The most frequently isolated micro-organisms were *Proteus mirabilis* (19.33%), *Staphylococcus aureus* (13.71%), *Pseudomonas aeruginosa* (12.58%), *Enterococcus faecalis* (12.36%) and *Escherichia coli* (12.13%).

In this study, gentamicin proved to be the most effective antimicrobial agent against 91.2% of isolated micro-organisms. Following gentamicin, ciprofloxacin showed susceptible results against 76.8% of the pathogens isolated. Amoxicillin/ clavulanic acid, the first line drug in the STG was effective against 20.4% of micro-organisms.

To determine, by means of a patient interview and thorough review of patient records, the use of preventative measures, the likelihood of past infection and to determine past treatment practices in the management of diabetic foot ulcers.

A total of 45 patients were found to meet the inclusion criteria and consented to participate in this study. From the information obtained from the retrospective review tool, it was established that 48.9% of patients did not make use of any preventative measures. In those patients that did use preventative measure, crutches were most commonly seen intervention (26.7%).

An analysis of past and current treatment practices revealed that 47.9% of the treatment protocols complied to the STG and SAASP guidelines; 14.6% were partially compliant, 33.3% were not compliant and the remaining 4.2% of the protocols could not be assessed for compliancy due to a lack of record keeping. It was evident that a number of missed opportunities in effective patient treatment occurred due to a lack of susceptibility data, patient specific culture assays and a deficit of antibiotic alternatives available in the STG.

In this study, the most frequently observed concurrent chronic conditions were hypertension (84.4%), dyslipidaemia (31.1%) and peripheral neuropathy (26.7%). The average number of co-morbidities in addition to diabetes for this patient population was four.

The medication history of each participant was recorded. The most commonly used medications included metformin (68.9%), insulin (62.2%), hydrochlorothiazide (33.3%), paracetamol (33.3%) and amitriptyline (33.3%). These results correspond to the treatment options for most commonly seen co-morbidities in this study, namely diabetes, hypertension and peripheral neuropathy. In this patient cohort, 31.1% of patients were initiated on step three of the STG stepwise treatment plan, with the combination of metformin and insulin. This suggests that the largest proportion of DFU patients were not controlled in earlier phases of treatment, indicating a potential lack of education in the importance of implementing and adhering to lifestyle modifications. In addition to this finding, insulin use alone was seen in 26.7% of patients, suggesting that the STG is not followed in its entirety because the final stage of treatment calls for the combination of metformin and insulin. This prescribing pattern is an indication of poor prescribing practices.

Finally, polypharmacy was found to be prevalent among this study's patient cohort with 55.5% of patients prescribed more than five medications. A total of 218 potential drug-drug interactions were noted in this study, however, it is estimated that only 1% of potential drug-drug interactions result in a clinically relevant adverse event (Jazbar *et al.*, 2018). Thus, evidence-based practice observations should be considered when reviewing a medication list. Duplication of medicines resulting in unnecessary additional treatment that could ultimately negatively affect the patient should be avoided.

To suggest additional recommendations to the existing local treatment guidelines for diabetic foot ulcers in the public hospitals based on the findings of the above-mentioned objectives.

To prevent the need for antimicrobial use, improve patient outcomes and reduce the burden of DFUs on the healthcare system, the prevention of DFUs should be the main focus of a healthcare team treating a diabetic patient. Using evidence-based guidelines developed by the International Working Group on the Diabetic Foot (IWGDF) (Bus *et al.*, 2020), important guidelines in identifying at risk patients were detailed in step one of the holistic treatment plan

provided for in Chapter 4, Section 4.3.5. Step two of the treatment plan provides guidelines on treating patients at high risk for developing a DFU. These guidelines focus on preventative measure use and patient education. Step three of the treatment plan focuses on the antimicrobial treatment of infected DFUs. An antibiogram is provided and the recommended antimicrobial per pathogen is stated. Overall, the most effective antimicrobials were found to be gentamicin and ciprofloxacin.

5.3. Study limitations

In 2021, a fire at the Charlotte Maxeke Johannesburg Academic Hospital resulted in the relocation of the podiatry unit. As a result, many potential subjects for this study were referred to hospitals not included in this study. Subsequently, an amendment and approval to the ethics application allowed Helen Joseph Hospital to be included in the study. As a result of the fire, some patient files became unaccounted for and as such, new files with no treatment history were provided. This study incorporated information obtained from the files of patients who participated in the study. A common limitation in using patient files for retrospective review is the lack of a full patient history due to ineffective record keeping. On many occasions, files were lost, incomplete or the patient was new and had no file history. This is a limiting factor in that information pertaining previous treatment practices of DFUs, including possible culture identification and susceptibility results. Furthermore, poor record keeping hinders the identification of at-risk patients.

A total of 445 micro-organisms were identified and susceptibility testing was performed on all of these isolates. Isolates were then grouped according to their macromorphological features and susceptibility results. A total of 54 groups were created and a representative isolate from each group was identified. While the utmost care was taken to group isolates as accurately as possible using two different features (macromorphological characteristics as well susceptibility patterns), there remains the possibility that some isolates may have been grouped incorrectly. Due to funding constraints, the identifications of all 445 isolates were not possible. Furthermore, no anaerobes were isolated from the wound samples within this study. This may have occurred due to the fact that anaerobes simply were not present in the wounds or due to the fact that obtaining a deep enough sample was not possible in this study.

5.4. Contribution to the current knowledge of DFUs

This study is the first of its kind known to be undertaken in the South African public healthcare sector. The results obtained from this study contribute to new knowledge regarding local susceptibility patterns of DFUs as well as existing knowledge with regards to causative pathogens worldwide. The main contributions from the findings of this study include:

1. *Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis* and *Escherichia coli* are the most frequently isolated micro-organisms in DFUs from this study sample.
2. Amoxicillin/ clavulanic acid is not an effective empirical treatment for DFUs as suggested in the STG.
3. Gentamicin is an effective antimicrobial agent against the most commonly isolated micro-organisms in this study.
4. Preventative measures are not widely used by patients.
5. Policy developments using patient factors and antimicrobial susceptibility results were defined.
6. The development of a check-list provided to the podiatry units based on the findings of this study to improve the holistic management of DFU (Appendix J).

This study provides insight into commonly seen pathogens causing infection in DFUs, it also highlights their susceptibility patterns. Amoxicillin/ clavulanic acid is currently the most commonly used and the recommended antibiotic in local guidelines. The findings of this research suggest the amoxicillin/ clavulanic acid shows poor susceptibility against the most commonly isolated pathogens and as such in not an effective treatment recommendation. Gentamicin and ciprofloxacin show more promising susceptibility results. The results obtained in this study provide the basis for future research required to establish an optimal treatment plan that effectively targets the pathogens causing DFU infection while at the same time minimising the contribution to antimicrobial resistance in order to improve patient outcomes and reduce the burden of DFUs.

5.5. Future recommendations

This study tested antimicrobials suggested by South African treatment guidelines. Future studies could use a broader range of antimicrobials as used internationally such as cephalosporins and carbapenems. Future studies could consider a larger sample size in multiple South African hospitals in different provinces in order to establish a comprehensive knowledge on common causative pathogens and their susceptibility patterns within varied provinces.

Following the establishment of local susceptibility data, an implementation study should be undertaken in order to establish the uptake of research findings and the use of the subsequent antibiogram. A cost analysis study of the overall economic impact of DFUs should be undertaken in order produce a realistic and cost-effective treatment plan.

It is also recommended that the role of biofilms in DFUs is the South African context be investigated. A biofilm is an organised structure of microbial communities in which cells or attached to a substratum and to each other (Wasfi *et al.,* 2020). Micro-organisms isolated from DFUs have the potential to co-aggregate in a symbiotic manner to form a polymicrobial biofilm which results in chronic infection and delayed healing time, as such their role should be further investigated in the South African context (Pouget *et al.,* 2020).

Most importantly, it is recommended that the STG and Essential Drug List pertaining to the treatment of DFUs be re-addressed in order to improve patient outcomes and reduce the burden of increasing antimicrobial resistance.

5.5. Final conclusion

The results observed in this study have contributed towards new knowledge and a better understanding of the role of the most commonly isolated pathogens from DFUs as well as their susceptibility patterns. The findings and subsequent recommendations serve to reduce the burden DFUs as well as address the growing problem of antimicrobial resistance. The susceptibility patterns of micro-organisms will continue to change and as such, regular surveillance is required in order to ensure that empirical therapy remains both effective and

appropriate so as not to cause increased resistance among micro-organisms implicated in DFU infection.

In conclusion, this study emphasises the need for all members of a healthcare team, including podiatrists, clinicians, microbiologists and pharmacists to work together in order to identify at-risk patients, prevent possible DFUs and effectively treat existing DFUs in a manner that does not contribute to antimicrobial resistance and provides the best possible outcome for the patient. Furthermore, this study highlights the urgent need for updated DFU treatment protocols in the South African public healthcare sector and as such, forms the basis of further research in this area.

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Appendix A

A1: 3rd Annual APSSA Conference on Pharmaceutical Sciences abstract

Diabetic foot ulcers: Is it time to readdress the standard treatment guidelines?

Maxine Grose[1], Stephanie Leigh de Rapper[1], Sandy van Vuuren[1]
[1] Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa
E-mail-address: 1615311@students.wits.ac.za

Purpose: Diabetic foot syndrome is defined as the presence of a diabetic foot ulcer (DFU) associated with neuropathy, peripheral artery disease and infection. While the use of antimicrobials in the treatment of DFUs remains a mainstay, choice of antimicrobial remains problematic due to the presence of polymicrobial infections. The aim of this study was to determine antimicrobial susceptibility patterns and treatment practices of DFUs isolated from patients visiting selected Gauteng public healthcare settings, in order to suggest a clinically effective treatment protocol.

Methods: Microbial sample swabs of 50 patients with DFUs were taken and a structured questionnaire was administered to the patients. A review of each patient's file was completed to assess treatment practices. Each sample swab was spread onto blood agar plates and thereafter, individual pathogens were isolated. The antimicrobial susceptibility patterns of all isolated pathogens were determined using zone of inhibition measurements. Pathogens were grouped according to macromorphological characteristics as well as susceptibility patterns and were then identified.

Results: Based on data obtained from the structured questionnaire, the use of preventative measure among patients is poor with 48.9% of patients reporting that they did not make use of any preventative measures. More than 150 pathogens were isolated and susceptibility testing was done. The most effective antimicrobial was found to be ciprofloxacin followed by gentamicin. Amoxicillin/ clavulanic acid, the first line treatment according to standard treatment guidelines was found to be ineffective in many of the isolated pathogens. The total number of different pathogens identified was 17. The most commonly isolated pathogens were *Proteus mirabilis*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Conclusion: The data obtained through patient questionnaires emphasise the need to encourage patient education and preventive measure use in the management of DFUs. Globally, causative pathogens and corresponding susceptibility patterns differ from country to country and the same is demonstrated in this study. These findings demonstrate the need to reassess the standard treatment guidelines and base treatment plans on local epidemiological data. This study provides valuable data on common causative pathogens in DFU infections as well as the resistance patterns of these pathogens and forms the basis for further research in this field.

A2: Faculty of Health Sciences Research Day.

15 September 2022, University of the Witwatersrand, Johannesburg.

Susceptibility Patterns and Treatment Practices of Diabetic Foot Ulcers in Selected South African Public Hospitals

Maxine Grose, Stephanie Leigh-de Rapper, Sandy van Vuuren

Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.

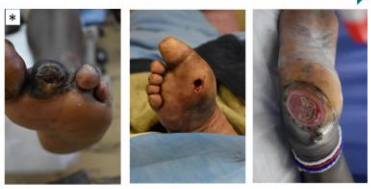
Introduction

Diabetic foot ulcers (DFUs) are one of the most common complications of diabetic foot syndrome and occur in approximately 15-25% of all diabetic patients[1,2]. Inappropriate antibiotic prescribing is hypothesized to be prevalent is South Africa. The treatment of DFUs is based on guidelines set out in the Standard Treatment Guidelines (STG) and the Essential Drugs List (EDL) and a lack of resources prevent healthcare workers from obtaining cultures before empiric treatment is prescribed [3]. By identifying antimicrobial resistance patterns specific to South Africa, more accurate and concise guidelines can be developed thereby reducing the development of further antimicrobial resistance, improving healing times and reducing the risk of re-occurrence and amputation. **Therefore, this study aims to determine the antimicrobial resistance patterns of diabetic foot ulcers in selected Gauteng public healthcare settings, in order to suggest an effective treatment protocol for improved infection management practices.**

Sample and Patient Data Collection

The Levine method was used to swab 50 diabetic foot ulcers from 45 patients [4, 5].

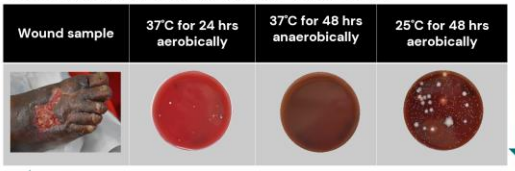
Following the collection of the wound sample, a structured questionnaire was administered to the patient and their file history was recorded.



* - All images presented on this poster were taken by the primary author, with consent from the patient

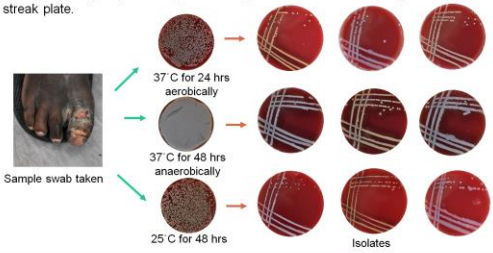
Sample Processing

Once a sample was collected, it was suspended in saline and then placed onto Mueller Hinton agar plates supplemented with 5% sheep's blood.



Sample Isolation

Following sample processing, micro-organisms were isolated by means of a streak plate.



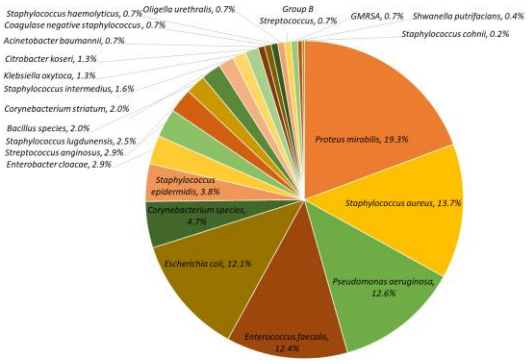
Antimicrobial Testing and Identification

Antimicrobial susceptibility testing was conducted using zone of inhibition measurements. The antibiotics used included amoxicillin/clavulanic acid (3 mcg), gentamicin (10 mcg) and clindamycin (2 mcg) which are included in the STG as well as ciprofloxacin (5 mcg), which is recommended by the South African Antibiotic Stewardship Program (SAASP). Vancomycin (5 mcg) was included in the cohort of antimicrobials to account for methicillin resistant *Staphylococcus aureus* presence and fluconazole to account for yeasts. Isolates were grouped together based on their morphological culture appearance and susceptibility patterns. A single representative isolate from each group was then sent to the National Health Laboratory Services Infection Control and Microbiology Laboratory, University of Witwatersrand for identification.

A sample of the antimicrobial susceptibility plates showing varying susceptibility patterns

Micro-organism Identification

A total of 445 micro-organisms were isolated and divided into 54 groups. The most common pathogen observed was *Proteus mirabilis* followed by *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis* and *Escherichia coli*.



Antimicrobial Susceptibility

The most effective antimicrobial was shown to be gentamicin followed by ciprofloxacin. Amoxicillin/ clavulanic acid was only effective against *S. aureus*.

Pathogen	Percentage prevalence (%)	Percentage of isolated micro-organism susceptible to the antimicrobial (%)						Best performing antimicrobial against the causative pathogen
		AMC	CN	DA	CIP	VAN	FLU	
Proteus mirabilis	19.33	39.5	94.2	3.5	69.7	1.1	0	Gentamicin (CN)
Staphylococcus aureus	13.71	55.7	100	72.1	93.4	0	0	Gentamicin (CN)
Pseudomonas aeruginosa	12.58	0	76.8	0	82.1	1.7	0	Ciprofloxacin (CIP)
Enterococcus faecalis	12.36	27.3	65.5	29.0	30.9	10.9	0	Gentamicin (CN)
Escherichia coli	12.13	0	88.9	0	48.1	0	0	Gentamicin (CN)

*AMC – Amoxicillin/ clavulanic acid; CN – Gentamicin; DA – Clindamycin; CIP – Ciprofloxacin; VAN – Vancomycin; FLU – Fluconazole; Zone of inhibition measurements were interpreted using reference 5

Susceptible | Intermediate | Resistant

Recommendations and Conclusion

The most effective antimicrobial was found to be gentamicin followed by ciprofloxacin. Amoxicillin/ clavulanic acid, the first line treatment according to the STG and used widely in DFU treatment practices, was found to be ineffective against many of the isolated pathogens. Clindamycin, the antimicrobial recommended in the case of penicillin allergy in the STG, was also found to be largely ineffective. This demonstrated the need to reassess the standard treatment guidelines and base treatment plans on updated local epidemiological data. This study provides valuable susceptibility data on common causative pathogens in DFU infections as well as the resistance patterns of these pathogens and forms the basis for further research in this field.

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Appendix B

B1: Human Research Ethics Committee approval certificate.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Miss M Grose

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210431

NAME: (Principal Investigator)	Miss M Grose
DEPARTMENT:	School of Therapeutic Sciences Department of Pharmacy and Pharmacology Medical School University
PROJECT TITLE:	*Antimicrobial susceptibility patterns in diabetic foot ulcers in selected Gauteng public healthcare settings*
DATE CONSIDERED:	2021/04/30
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor S van Vuuren & Ms S Leigh de Rapper
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/08/23

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **April** and therefore reports and re-certification will be due in the month of **April** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2021/05/01
Date

B2: Approval of ethics amendment.

Following the fire are Charlotte Maxeke Johannesburg Academic Hospital.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

2022/03/18

Ms M Grose
School of Therapeutic Sciences
Department of Pharmacy and Pharmacology
Medical School
University

Sent by e-mail to: 1615311@students.wits.ac.za

Dear Ms Grose

Re: **Protocol Ref No:** M210431
Protocol Title: *Antimicrobial susceptibility patterns in diabetic foot ulcers in selected Gauteng public healthcare settings*
Principal Investigators: Ms M Grose

I refer to your letter of 2022/03/08.

I confirm that we have noted and approve of your proposal to move your study site to Helen Joseph Hospital.

Thank you for keeping us informed.

Yours Sincerely

I. Burns

.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

CB Penny

.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

Appendix C

C1: Patient information leaflet.

PARTICIPANT INFORMATION SHEET

Research Project entitled: Antimicrobial susceptibility patterns in diabetic foot ulcer in a South African tertiary care hospital

Dear patient,

Introduction

I, Maxine Grose, am conducting research on the antimicrobial resistance patterns of diabetic foot ulcers in patients presenting to the Charlotte Maxeke Academic Hospital. This study will be done with the aim to guide future protocol development and treatment guidelines.

Invitation to participate

Participation in the study is entirely voluntary. I would like to take this opportunity to invite you to take part in this research study.

What is involved in the study?

Patient Record Review

Your patient records will be reviewed to determine the likelihood of previous foot ulcers. The review will collect information on date of previous ulcers, the treatment offered and the identified cause of the foot ulcer. Any information gathered will be kept confidential.

Examination

A wound culture is a test used to isolate, check antibiotic susceptibility and to identify the micro-organisms responsible for infection. I will be responsible for obtaining a sample of your wound using a swab.

Risks

The risks associated with the wound sample collection are minimal as the sample collection procedure is min invasive. The risks associated include mild pain upon sample collection.

Benefits

Your will be afforded the opportunity to receive the correct treatment for the diabetic foot ulcer by reducing the risk of reoccurrence as well as the risk of antibiotic resistance in future.

Participation is voluntary

You have the right to refuse participation or withdraw at any time during the study. This will not incur any penalty or loss to you.

Confidentiality: When partaking in the study, no identifiable data will be collected by the research group.

Who has reviewed and given ethical approval for this study?

This study protocol has been submitted to the University of the Witwatersrand, Human Research Ethics Committee.

If you have any further queries, or would like to report any problems, please do not hesitate to contact any of the research investigators. Contact details provided below:

Researcher: Maxine Grose
Tel: 071 179 0136
E-mail: 1615311@students.wits.ac.za
Researcher: Stephanie Leigh de Rapper
Tel: 011 717 2268
E-mail: Stephanie.derapper@wits.ac.za
Researcher: Prof. Sandy van Vuuren
Tel: 011 717 2157
E-mail: sandy.vanvuuren@wits.ac.za.

Should you require any information regarding your rights as a participant or if you have any complaints regarding this research study, you may contact the Chairperson of the Human Ethics Committee at the University of the Witwatersrand:

Prof. CB Penny: 011 717 2301

Thank you for taking time to consider participation.

Appendix D

D1: Patient informed consent form.

Appendix C: Informed Consent

PARTICIPANT INFORMED CONSENT FORM

Research Project entitled: Antimicrobial susceptibility patterns in diabetic foot ulcer in a South African tertiary care hospital

I hereby confirm that I have accurately read and understood the information sheet provided by the researcher, and I understand that the following will be done:

1. I will allow the sampling of my wound and the specimen will be collected for culture sensitivity.
2. The wound sample will be cultured and the bacteria causing the infection will be identified.

- I confirm that that I was given an opportunity to ask questions about the study, and all the questions asked have been answered to my satisfaction.
- I confirm that the I have not been coerced into giving consent, and the consent has been given freely and voluntarily, and that I may withdraw participation at any time during the study.
- I hereby agree that I understand the requirements of this project and am willing to participate and provide feedback for research purposes.

Sign: _____ Date: _____

Appendix E

E1: Charlotte Maxeke Johannesburg Academic Hospital site approval.

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)
OFFICE OF THE SENIOR CLINICAL MANAGER

Enquiries: *Ms. TT Mahlangu*
Email: Thandi.Mahlangu4@gauteng.gov.za
Tel: *011 488 3365*
Ref: *1/7/2*
Date: 19 September 2021

GP_202108_026

To: Ms. Grose

RE: FINAL APPROVAL OF STUDY

TITLE: ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN DIABETIC FOOT ULCERS IN SELECTED GAUTENG PUBLIC HEALTHCARE SETTINGS

Permission is granted for you to conduct the above-mentioned study as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not in any way incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall always be observed.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or Sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported/Not Supported
Signed by:Jayshina Punwasi
Signed at:2021-09-19 23:04:56 +02:00
Reason:Witnessing Jayshina Punwasi

Dr J. Punwasi
Clinical Director

Approved/Not Approved
Signed by:Gladys Magugudi Bogoshi
Signed at:2021-09-20 18:44:19 +02:00
Reason:Witnessing Gladys Magugudi Bo

Ms. G Bogoshi
Chief Executive Officer

E2: Helen Joseph Hospital site approval.

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. R. Ncha
Chief Executive Officer
Tel :(011) 489-0306/1087
Fax :(011) 726-5425
E mail: Relebohile Ncha@gauteng.gov.za
Date: 21 October 2021

Dear Ms. Grose

STUDY: Antimicrobial susceptibility patterns in diabetic foot ulcers in selected Gauteng public healthcare.

RESEARCHERS: Ms. Grose

GP_202108_026

The above the study was discussed at the Research Committee meeting. We recommend that permission be granted for Helen Joseph Hospital to be used as a site for the above research,
The researcher is expected to the following:

- Upon completion of the study, copy thereof should be submitted to Helen Joseph Hospital.
- It is the researcher's duty to collect the data from the relevant department after the Research Committee approved the study.

Please liaise with the HOD and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the feedback of your study on completion of the research .
Thank you

Dr. M.D Mukansi
Helen Joseph Hospital
Research Chairperson

Approved

Dr. R. Ncha
Helen Joseph Hospital
CHIEF EXECUTIVE OFFICER
DATE: 25/10/21

Appendix F

F1: Retrospective review tool use for patient data collection.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG
FACULTY OF HEALTH SCIENCES
WITS PHARMACY

PARTICIPANT DATA COLLECTION TOOL

Patient number (as per confidentiality key) _____

Patient demographics

Gender: Male ☐ Female ☐

Is the patient diabetic? Yes ☐ No ☐

If yes, please state if T1DM or T2DM:

_____

Patient history

Does the patient suffer other medical conditions? Yes ☐ No ☐

If yes, please provide details:

_____

Is the patient currently taking any medications? Yes ☐ No ☐

If yes, please provide details:

_____

Has the patient used systemic antibiotics within the last 2 weeks?

Yes ☐ No ☐

Examination

Does the patient present with the following symptoms?

Skin discolouration	Yes ☐	No ☐
Exudate	Yes ☐	No ☐
Bruising	Yes ☐	No ☐
Tenderness/ pain	Yes ☐	No ☐
Ulceration	Yes ☐	No ☐
Odour	Yes ☐	No ☐

Previous preventative methods

Has the patient been prescribed protective footwear? Yes ☐ No ☐

If yes, please provide details:

_____

Has the patient use pressure offloading methods? Yes ☐ No ☐

If yes, please provide details:

_____

PARTICIPANT DATA COLLECTION TOOL continued…

Patient number (as per confidentiality key) _____

Patient diagnosis: _____

Patient record review

Date of infection: _____

Wound sample cultured Yes ☐ No ☐

If yes, what was the micro-organism identified?

_____

Antibiotic prescribed Yes ☐ No ☐

Treatment prescribed:

_____

Date of infection: _____

Wound sample cultured Yes ☐ No ☐

If yes, what was the micro-organism identified?

_____

Antibiotic prescribed Yes ☐ No ☐

Treatment prescribed:

_____

Appendix G

G1: Zone of inhibition readings for all isolates.

Table G1: Zone of inhibition readings for all isolates.

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A001	37˚C/24 hours	1	2.7	7.9	0	13.1	0	0
		2	2.9	11.2	0	12.9	0	0
	37˚C/48 hours anaerobically	1	1.1	5.3	0	14.6	0	0
		2	4.2	8.1	0	13.3	0	0
	25˚C/48 hours	1	0	11	0	13.1	0	0
		2	0	9.5	0	11.4	0	0
		3	0	10.9	0	12.4	0	0
A002	37˚C/24 hours	1	10.1	13.4	8.5	0	0	5.7
		2	7.1	10.2	0	13.5	0	0
		3	9.9	14.2	16.4	0	0	7
		4	11.3	14.8	13.4	0	0	6.4
		5	11.3	12.4	13.2	0	0	5.3
		6	9	13.4	16	13.9	0	4.8
		7	0	12.1	9.7	12.4	0	5.8
	37˚C/48 hours anaerobically	1	8.1	14.9	4.2	0	0	8.7
		2	13.2	12	11.2	0	0	6.3
		3	10.4	13.1	14.1	0	0	5
		4	8.4	7	6	0	0	4.8

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		5	0	0	0	14.1	0	0
		6	6.1	11	4.2	0	0	5.9
	25˚C/48 hours	1	7.4	10.9	10.4	0	0	8.1
		2	9.1	14.6	7.4	0	0	5.2
		3	5.9	9.3	0	11.8	0	0
		4	9.9	14	11.4	0	0	6
A003	37˚C/24 hours	1	0	8.4	0	11.5	0	0
		2	0	8.9	0	15.9	0	8.3
		3	0	0	0	0	0	6.6
		4	7.1	0	0	8.2	0	6
		5	0	8	0	13.3	0	0
	37˚C/48 hours anaerobically	1	12.2	7.1	0	17.9	0	0
		2	4.3	8.2	0	21	0	0
		3	9	7.3	0	12.4	0	0
		4	0	9	0	19.2	0	0
		5	0	7.2	0	20.1	0	0
		6	0	9.1	0	17.3	0	0
	25˚C/48 hours	1	0	11.6	0	17.9	0	0
		2	0	0	0	0	0	7.7
		3	0	7.2	0	16.7	0	0
		4	0	7.9	0	16.9	0	0
		5	0	0	0	0	0	9.8
		6	0	12	0	17.4	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A003*	37˚C/24 hours	1	0	0	0	0	0	9.1
		2	0	8.9	0	12.6	0	0
		3	0	8.8	0	13	0	0
	37˚C/48 hours anaerobically	1	0	0	0	0	0	9
		2	6.1	8.9	10.2	12	0	5.2
		3	7.2	9.9	11.1	13.4	0	6.1
		4	0	9.1	0	19.8	0	0
		5	0	7.8	0	21.1	0	0
		6	0	7.2	0	20	0	0
		7	0	9.2	0	18.2	0	0
	25˚C/48 hours	1	0	0	0	0	0	7.9
		2	4.6	10	10.8	10.1	0	5.9
		3	0	0	0	0	0	8
		4	0	8	0	15.9	0	0
		5	0	7.9	0	19.7	0	0
		6	0	0	0	0	0	8
		7	0	7.7	0	17.9	0	0
		8	0	7.4	0	14.3	0	0
		9	0	0	0	0	0	8.2
A004	37˚C/24 hours	1	7.9	14.5	15	14.6	0	6.1
		2	8.2	14	7.4	8.7	0	6.4
	37˚C/48 hours anaerobically	1	5.1	11.1	12.1	13.9	0	4.4
		2	8.9	4.8	6.1	7.9	0	5.3
		3	7.2	10.5	11.4	12.9	0	5.1

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A005	25˚C/48 hours	1	6.7	14.6	13.3	15.8	0	6.7
		2	7.4	5.1	6.6	8.2	0	6.1
		3	7.6	10.2	7.5	11.6	0	6.6
		4	7.4	6.1	6.6	9.1	0	6.6
	37˚C/24 hours	1	0	9.9	0	14.9	0	0
		2	7.1	6.2	0	8.6	0	6.1
		3	0	10.1	0	14	0	0
	37˚C/48 hours anaerobically	1	4.5	8.5	0	10.8	0	0
		2	6.7	6.3	0	7.4	0	6.2
		3	8.9	10.9	11	12.9	0	5.2
	25˚C/48 hours	1	0	8.9	0	17.1	0	0
		2	6.9	5.9	4.4	7.4	0	5.9
		3	5.6	6.8	4.5	8.7	0	6.5
		4	6.7	7.2	4.2	7.9	0	5.7
A006	37˚C/24 hours	1	9.4	12.1	15	16.1	0	8.6
		2	9.7	12.6	14.3	15.6	0	8.8
	37˚C/48 hours anaerobically	1	6.4	8.6	10.1	12.4	4.1	5.9
		2	6.9	8.3	11.8	14.4	4.2	6.5
		3	0	12.7	0	16.4	0	9.3
	25˚C/48 hours	1	6.8	11.2	13.2	14.6	5.2	6.1
		2	7.5	10.8	10.7	14.2	4.6	7.1
		3	7.4	10.3	12.4	14.3	4.7	6.6
A007	37˚C/24 hours	1	0	0	0	0	0	10.1

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		2	4.2	10.2	0	18.6	0	0
		3	5.3	11	0	18.1	0	0
		4	0	0	0	0	0	5.4
		5	0	0	0	0	0	9.8
	37˚C/48 hours anaerobically	1	0	10.5	0	23.4	0	0
		2	8	9.3	0	9.8	0	6.4
		3	0	0	0	0	0	11.4
	25˚C/48 hours	1	8.8	11.9	0	20.7	0	0
		2	7.9	12.2	0	20.8	0	0
		3	8.7	12	0	19.3	0	0
A007*	37˚C/24 hours	1	10.1	12.3	0	22.3	0	0
		2	8.6	13.1	0	21.4	0	0
		3	5.5	15.1	0	23.8	0	0
		4	4.8	12.5	0	23.6	0	0
	37˚C/48 hours anaerobically	1	9.4	9.7	0	18.9	0	0
		2	10.8	8.6	0	19.7	0	0
		3	5	9	0	16.9	0	0
		4	8.6	9.7	0	19.8	0	0
	25˚C/48 hours	1	9	12.9	0	20.9	0	0
		2	8.7	12.2	0	21	0	0
		3	9.2	12.5	0	21.3	0	0
A008	25˚C/48 hours	1	10.4	14.3	8.2	15.6	0	7.4
A009	37˚C/24 hours	1	17	12.2	14	16.5	0	6.9

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		2	21.2	17.4	20.9	24.4	0	12.6
		3	11.3	6	9	11.6	0	8
		4	9.9	16.5	15.4	5.7	0	7.8
	37˚C/48 hours anaerobically	1	16.4	8.7	11.1	13.9	0	5.3
		2	10.3	8.5	0	20.6	0	0
		3	11.1	8.8	0	13.2	0	0
	25˚C/48 hours	1	10.5	17.1	6.1	24.8	0	0
		2	20	17.3	18.9	23.1	0	12.5
		3	17.5	12.3	14.3	16.4	0	6.7
		4	20.6	18.2	16.7	23.4	0	12.7
		5	18.6	12.2	14.8	17.3	0	8
A009*	37˚C/24 hours	1	9.8	13.2	0	18.9	0	0
		2	9.1	12.2	0	21.9	0	0
		3	10.1	11.7	0	20	0	0
		4	9.9	12.4	0	20.5	0	0
	37˚C/48 hours anaerobically	1	7.1	10.3	0	20.5	0	0
		2	0	10.1	0	15.3	0	0
		3	10.1	9.5	0	20.7	0	0
		4	10.8	10.1	0	17.9	0	0
	25˚C/48 hours	1	10.6	10.9	0	20.7	0	0
		2	9.6	12	0	20.6	0	0
		3	9.8	12.5	0	19.8	0	0
		4	10.4	12.3	0	19.8	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		5	8.4	10	0	17.1	0	0
A010	37˚C/24 hours	1	10.9	11.8	6.8	15.2	0	4.6
		2	8.4	15.7	0	0	0	0
		3	9.2	10.7	12.2	10.8	0	7.4
	37˚C/48 hours anaerobically	1	17.4	9.3	13.9	10.6	0	7
		2	16.7	9.1	13.7	10.3	4.6	6.6
		3	9.1	7.4	0	9.3	0	0
		4	16	8.5	11.7	11.5	0	6.7
	25˚C/48 hours	1	17.4	11.2	12.8	10.2	0	7.3
		2	10.5	11.1	5.9	14	0	0
		3	9.9	7.6	10.1	9.8	0	8.6
		4	16.4	9.9	12.2	9.8	0	6.9
A011	37˚C/24 hours	1	0	8.5	0	14.4	0	0
		2	0	6.8	0	13.1	0	0
	37˚C/48 hours anaerobically	1	0	6.2	0	11.6	0	0
		2	0	6	0	9.5	0	0
		3	0	6.1	0	12.2	0	0
	25˚C/48 hours	1	0	7.7	0	13.5	0	0
		2	0	9.6	0	14.6	0	0
A012	37˚C/24 hours	1	0	9.9	0	13	0	0
		2	0	11.7	8.7	15.1	0	0
		3	6.9	10.9	13.6	13.9	4.4	6.6
		4	8.8	8.1	0	10.8	0	7

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	37˚C/48 hours anaerobically	1	5	8.2	0	13.1	0	0
		2	0	8.4	0	13.2	0	0
	25˚C/48 hours	1	6.6	13	0	13.5	0	0
		2	7.9	13.5	4.1	5.4	0	8
		3	6.3	9.8	0	16.6	0	0
		4	6.5	11	13.4	14.6	0	6.9
		5	0	12.6	0	13.2	0	0
A012*	37˚C/24 hours	1	6.3	10.8	12.3	12.3	0	5.3
		2	0	7.9	0	11.4	0	0
		3	0	11.7	8	16	0	0
	37˚C/48 hours anaerobically	1	0	6.3	0	10.9	0	0
		2	0	6.2	0	12.5	0	0
	25˚C/48 hours	1	0	7.8	0	15.2	0	0
		2	0	6.1	0	13.1	0	0
		3	11	9.6	14.2	5.4	0	8.2
A013	37˚C/24 hours	1	5.6	10.9	0	16.9	0	0
		2	8	11.1	0	17.9	0	0
		3	8.5	11.5	0	17.1	0	0
	37˚C/48 hours anaerobically	1	9.4	8	0	17.7	0	0
		2	6.5	8.6	0	13.3	0	0
		3	7.8	8.1	0	17.4	0	0
		4	5.7	8.6	0	13.5	0	0
	25˚C/48 hours	1	10.4	12.8	0	18.5	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		2	5.2	10.1	0	13.3	0	0
		3	8.5	10.3	0	16.9	0	0
A014	37˚C/24 hours	1	0	8.6	0	14.4	0	0
		2	5.1	10.9	13.8	12.6	0	6.1
		3	0	10.5	12.8	12.9	0	7
	37˚C/48 hours anaerobically	1	0	8.5	0	15.2	0	0
		2	0	10	0	11.9	0	0
	25˚C/48 hours	1	0	9.8	0	14.5	0	0
		2	0	10.4	13.5	12.5	0	6.8
		3	0	9.4	12.5	11.7	0	6.6
		4	0	10.1	12.4	11.8	0	6.4
A015	37˚C/24 hours	1	0	8.1	0	14.2	0	0
		2	5.9	10.6	0	14.4	0	0
	37˚C/48 hours anaerobically	1	0	10	7.7	13.3	0	6.7
		2	6.7	9.5	0	10.3	0	0
	25˚C/48 hours	1	0	7.8	0	12.9	0	0
		2	7.5	9.9	5.1	14.2	0	0
		3	7.1	8.6	0	13.1	0	0
A016	37˚C/24 hours	1	9.7	15.1	9	0	0	9.9
		2	12.9	13.2	13	13.4	0	10.1
		3	5.7	9.5	12.3	10.8	0	6.2
		4	6.8	10	12	11.9	0	6.9
	37˚C/48 hours anaerobically	N/A						

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A017	25˚C/48 hours	1	6.9	9.5	0	14.2	0	0
		2	7.3	10.6	14.4	11.5	0	6.8
		3	10.6	15.5	9	0	0	8.4
		4	0	11.4	7.5	12.1	0	6.5
	37˚C/24 hours	1	8.6	9.6	0	14.9	0	0
		2	8.8	9.6	14.1	7	0	6.5
		3	8.2	9.9	0	13.3	0	0
	37˚C/48 hours anaerobically	1	4.9	8.1	0	11.7	0	0
		2	6.8	8.1	0	16.2	0	0
		3	5.5	7.9	0	11.3	0	0
	25˚C/48 hours	1	6.6	8.4	0	12.9	0	0
		2	8.3	10.1	0	15.2	0	0
		3	8.5	9.5	0	14.7	0	0
A017*	37˚C/24 hours	1	8.2	9.7	11.3	15	0	4.7
		2	8.9	11.7	12.4	13.9	0	4.9
	37˚C/48 hours anaerobically	1	7.1	10.5	11.3	13.2	0	6
		2	7	12	11.9	11.1	0	6.3
	25˚C/48 hours	1	0	9.9	10	14.4	0	7
		2	8.7	9.2	0	14.9	0	0
		3	9.1	10	0	14.8	0	0
A018	37˚C/24 hours	1	0	9.9	8.5	12.4	0	5.5
		2	5.7	9.2	11.2	12.5	0	6.1
		3	5.5	9	9.8	10.2	0	5

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A018*	37˚C/48 hours anaerobically	1	8.1	7.1	0	7.3	0	6
		2	6.6	7.8	9.7	11.2	0	5.3
	25˚C/48 hours	1	6.5	9.9	11.2	10.8	0	6.5
		2	7.6	8.9	8.8	10.2	0	6.6
	37˚C/24 hours	1	6.2	9.5	11.5	12.7	0	5.9
		2	0	10.1	0	5.7	0	0
	37˚C/48 hours anaerobically	1	0	9.1	8.1	12.4	0	5.4
		2	5.2	8.2	9.5	11.5	0	5.4
		3	0	8.4	0	4.2	0	0
	25˚C/48 hours	1	5.4	9.5	0	11.9	0	0
		2	5.6	8.7	10	12.5	0	6.3
		3	0	9	0	6.1	0	0
A019	37˚C/24 hours	1	9.7	10.9	0	0	0	8
		2	9.5	8.6	0	0	0	8.3
	37˚C/48 hours anaerobically	1	9.4	11.6	0	0	0	7.5
		2	9.1	8.8	0	0	0	8.1
	25˚C/48 hours	1	7.9	11.5	0	0	0	8.7
		2	7.4	9.2	0	16.8	0	0
		3	6.9	10	0	15.9	0	0
A020	37˚C/24 hours	1	6.3	9.9	12.2	14	0	6.7
		2	11.8	5.5	10	8.9	0	7.5
		3	5.8	9.8	10.9	0	0	9.7
		1	4.3	9.2	10.6	14.1	0	5.1

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	37˚C/48 hours anaerobically	2	7.2	9.2	10.7	11.3	0	6
		3	5.7	7.8	11.7	13.1	0	5.8
	25˚C/48 hours	1	0	7.9	8.7	0	0	8
		2	11.6	5.6	10.1	9.1	0	7.2
A021	37˚C/24 hours	1	13.6	9.8	11.2	13.7	0	5.9
		2	7.2	11.6	12.5	15.7	0	6.4
		3	11.8	9.2	11.9	8.3	0	8.4
	37˚C/48 hours anaerobically	1	0	7.4	0	10.1	0	0
		2	6.1	8	0	8.1	0	5.7
		3	0	6	0	9.7	0	0
	25˚C/48 hours	1	14.4	10.1	9.9	13.4	0	5.5
		2	0	7.3	0	13.8	0	0
		3	13.4	12.2	14.3	13.2	0	7.1
		4	12.6	9.6	10.5	11.7	0	5.3
A022	37˚C/24 hours	1	0	7.1	0	15.9	0	0
		2	8.2	10.2	0	17.8	0	0
		3	8.4	9.5	0	9.8	0	6.2
	37˚C/48 hours anaerobically	1	9.4	0	0	9.3	0	6.1
		2	0	7.3	0	15.7	0	0
		3	7.2	0	0	8.8	0	0
	25˚C/48 hours	1	0	7.5	0	15.4	0	0
		2	0	0	7.3	0	0	8.2
		3	9	11.6	0	18.8	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		4	9.6	10.9	0	18.5	0	0
A023	37˚C/24 hours	1	0	10	0	13.9	0	0
		2	13.1	9.8	10.9	12.8	0	5.8
		3	7.5	8.8	0	12.2	0	0
	37˚C/48 hours anaerobically	1	7.7	6.4	0	8.6	0	6.9
		2	6.8	7.5	0	11.6	0	0
	25˚C/48 hours	1	6.2	8.4	0	10	0	0
		2	14.2	9.4	10.9	11.6	0	6
		3	0	9.7	0	14.5	0	0
		4	0	9.4	0	12.9	0	0
A024	37˚C/24 hours	1	14.3	11.7	12	12.2	0	6.2
		2	13.2	11.6	5.4	16.6	0	9.9
	37˚C/48 hours anaerobically	1	9	10.2	10.6	9.3	0	6.2
		2	9.5	0	9.4	8.4	0	7.8
		3	8.2	11.6	9.3	9.8	0	5.4
	25˚C/48 hours	1	14.6	12.3	12	11.2	0	5.9
		2	14.3	11.4	10	13.2	0	6.1
A025	37˚C/24 hours	1	0	9.9	0	10.9	0	0
		2	7.1	9.9	11.2	10.7	0	6
		3	7.4	10.3	0	8.5	0	0
	37˚C/48 hours anaerobically	1	0	9.6	0	10.1	0	0
		2	0	7.5	0	9.3	0	0
		3	0	8.1	0	9.1	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	25˚C/48 hours	1	7.2	11.8	0	10	0	0
		2	6.4	10.2	0	7.8	0	0
		3	0	9.2	0	12.9	0	0
A026	37˚C/24 hours	1	10.7	11.6	0	13.6	0	7.9
		2	7.4	12.9	12.6	13.7	0	6.2
		3	8.9	12.9	12.2	15.2	0	6.9
		4	14.9	10.9	11.9	13.8	0	7.6
	37˚C/48 hours anaerobically	1	6.7	11.1	10.6	11.2	0	6.8
		2	15.2	9.1	10.2	14.3	0	7.2
		3	15	9.4	11.6	15.1	0	6.7
		4	8.4	7.5	0	9.7	0	6.1
	25˚C/48 hours	1	9.2	9.8	9	14.1	0	8.5
		2	6.9	15.9	13.9	11.3	0	7.6
		3	9.3	16.2	15	15.1	0	8.6
		4	15.3	10.8	11.6	15	0	7.4
		5	6.9	11.8	9.2	14.4	0	8.6
A027	37˚C/24 hours	1	11.2	0	10.3	9.9	0	7.8
	37˚C/48 hours anaerobically	1	7.9	7	0	10.3	0	7
		2	13.4	9.2	10	11.7	0	6.9
	25˚C/48 hours	1	12.9	9.9	10.3	13	0	6.2
		2	11.7	0	9.6	9.7	0	7.4
A028	37˚C/24 hours	1	7.7	12	11.6	14.2	0	6.4
		1	15.2	8.2	10.4	11.3	0	6.1

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	37˚C/48 hours anaerobically	2	13.4	8.7	10.5	11.3	0	6.5
	25˚C/48 hours	1	8	13.1	13.7	14.9	0	7.4
A029	37˚C/24 hours	1	0	10.5	0	6.8	0	0
		2	0	10.2	0	6.3	0	0
		3	8.1	4.7	0	8.2	0	7.5
	37˚C/48 hours anaerobically	1	0	8.8	0	4.4	0	0
		2	0	7.4	0	0	0	0
		3	0	8.3	0	4.5	0	0
	25˚C/48 hours	1	0	10.4	0	7.5	0	0
		2	0	0	8.5	0	0	9
A030	37˚C/24 hours	1	0	10.4	0	15	0	0
		2	0	10.3	0	17	0	0
		3	0	8.4	0	14.7	0	0
	37˚C/48 hours anaerobically	1	0	6.2	0	12.8	0	0
		2	0	6.4	0	12.9	0	0
	25˚C/48 hours	1	0	8	0	13.5	0	0
		2	0	10.3	0	17.1	0	0
		3	0	8.1	0	13.7	0	0
A031	37˚C/24 hours	1	6.1	9.2	0	15.4	0	0
		2	5.7	10.2	11.6	11.1	0	5.3
		3	13.4	15	9.8	6.7	0	10.8
	37˚C/48 hours anaerobically	1	6.8	7.2	0	15.6	0	0
		2	5.8	6.9	0	15.3	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A032	25˚C/48 hours	1	5.3	7.3	0	17.1	0	0
		2	6.1	11.2	13.5	12.3	0	6.5
		3	9.7	0	11.2	10.5	0	8.2
	37˚C/24 hours	1	0	9.2	0	14.1	0	0
		2	0	9.2	0	14.4	0	0
		3	6.7	7.2	6.1	9	0	6.9
	37˚C/48 hours anaerobically	1	12.3	11.6	0	20.7	0	0
		2	8.3	8.6	0	18.2	0	0
	25˚C/48 hours	1	7	10.6	0	11.1	0	0
		2	6.7	9.9	0	10.8	0	0
		3	6.8	7.7	0	14.2	0	0
		4	0	7.5	0	14.3	0	0
A033	37˚C/24 hours	1	0	0	0	0	0	7.9
		2	0	0	0	0	0	0
	37˚C/48 hours anaerobically	1	0	6.4	0	13	0	0
		2	0	0	0	0	0	8.2
	25˚C/48 hours	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	8.2
A034	37˚C/24 hours	1	0	8.1	0	12.2	0	0
		2	0	10.1	0	13.2	0	0
		3	0	10.2	0	11.9	0	0
		1	0	8.3	0	14.7	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	37˚C/48 hours anaerobically	2	0	8.2	0	13.3	0	0
	25˚C/48 hours	1	0	8	0	13.8	0	0
		2	0	8.8	0	14.5	0	0
		3	0	9.4	0	12.4	0	0
A035	37˚C/24 hours	1	0	9.7	0	16.5	0	0
		2	0	10.6	0	17.4	0	0
		3	0	12.5	0	18	0	0
	37˚C/48 hours anaerobically	1	0	0	0	8.4	0	0
		2	13.8	14.6	0	0	0	8.7
	25˚C/48 hours	1	0	11.1	0	19.4	0	0
		2	0	11.2	0	19.7	0	0
		3	0	12.3	0	19.3	0	0
A036	37˚C/24 hours	2	9.4	11.9	0	19.9	0	0
		3	9.9	10.9	0	18.7	0	0
	37˚C/48 hours anaerobically	1	9	7.7	0	17	0	0
		2	0	8.8	0	13.2	0	0
	25˚C/48 hours	1	0	9.8	0	11.1	0	0
		2	10.3	12.4	0	21.4	0	0
		3	0	0	0	11.1	0	0
A037	37˚C/24 hours	1	0	9.6	0	14.8	0	0
		2	0	8.5	0	15.8	0	0
	37˚C/48 hours anaerobically	1	0	9.2	0	15.6	0	0
		2	0	9.3	0	14.6	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
	25˚C/48 hours	1	0	8.1	0	14.5	0	0
		2	0	8.4	0	14.5	0	0
		3	0	8.2	0	16.2	0	0
A038	37˚C/24 hours	1	12.7	9.2	11.7	14.6	0	6.8
		2	10.2	11.1	11.3	13	0	6.5
		3	11.1	9	9.4	12.5	0	5.9
	37˚C/48 hours anaerobically	1	14	8.5	9.7	11.9	0	5.8
		2	13.2	8	9.9	12.4	0	6
		3	13.8	8.4	9	12.5	0	6.5
	25˚C/48 hours	1	9.2	8.8	10	13.2	0	5.8
		2	12.9	9.9	10.5	13.7	0	5.9
		3	9.3	10.6	11.1	11.5	0	5.3
A039	37˚C/24 hours	1	9.3	12.6	0	18.7	0	0
	37˚C/48 hours anaerobically	2	8.9	9.3	0	12.5	0	0
		3	8	8.7	0	12.7	0	0
	25˚C/48 hours	1	0	10.4	0	12.7	0	0
		2	9.9	12.4	0	20.7	0	0
A040	37˚C/24 hours	1	13.4	9.7	10.6	11.1	0	5.4
		2	0	10.5	0	12.9	0	0
		3	9.2	5.2	10	10.4	0	7.3
		4	13.4	9.8	10.3	11.3	0	5.9
	37˚C/48 hours anaerobically	1	0	9	0	13.8	0	0
		2	12.9	9.9	9.8	10.7	0	6.5

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
		3	8.8	12.1	7.4	8	0	6.1
	25˚C/48 hours	1	0	9.7	0	12.9	0	0
		2	13.2	9.4	9.9	12.6	0	5.6
		3	10.3	5.2	10.1	8.6	0	6.8
A041	37˚C/24 hours	1	0	0	0	0	0	6.9
		2	0	0	0	0	0	8.3
	37˚C/48 hours anaerobically	1	18	11.4	11.3	14.6	0	5.6
		2	0	0	0	0	0	7.8
	25˚C/48 hours	1	0	0	0	0	0	8.7
		2	8.9	12	10.8	11.3	0	5.7
A042	37˚C/24 hours	1	7.7	10	0	12.4	0	0
		2	7.8	6.9	0	8.5	0	6.1
		3	11.8	14.1	0	20.2	0	0
	37˚C/48 hours anaerobically	1	6.9	6.3	0	10.3	0	0
		2	10.8	8.4	0	14.7	0	0
		3	9.5	7.7	0	19.2	0	0
	25˚C/48 hours	1	7.4	9.4	0	11.3	0	0
		2	7.4	6.5	0	7.8	0	6.6
		3	8	11.6	0	20	0	0
A043	37˚C/24 hours	1	0	8.9	0	13.7	0	0
		2	11.5	17.1	0	20.4	0	10
	37˚C/48 hours anaerobically	1	0	8.4	0	10.6	0	0
		2	0	9.5	0	12.5	0	0

Sample	Incubation Condition	Isolate Number	Zone of Inhibition (radius from the centre of the disk to the edge of the ZOI in mm)					
			Amoxicillin/ Clavulanic Acid	Gentamicin	Clindamycin	Ciprofloxacin	Fluconazole	Vancomycin
A044	25˚C/48 hours	1	11.3	14	0	12	0	6.1
		2	15	10.8	0	11.1	0	5.5
	37˚C/24 hours	1	8.2	11	11.8	16.2	0	6
		2	6.9	12	12.3	14	0	6.1
	37˚C/48 hours anaerobically	1	8.9	10.2	9.4	13.2	0	6.9
		2	8.7	9.6	10.3	12.9	0	7.3
	25˚C/48 hours	1	9.1	13.6	15.1	17.7	0	7
		2	8.8	12.7	11.5	14.4	0	6.4
A045	37˚C/24 hours	1	0	0	6.9	0	0	8.6
		2	15.8	5.2	14.7	8	0	9.6
	37˚C/48 hours anaerobically	1	0	0	6.2	0	0	9.5
		2	0	0	6.2	0	0	10.1
		3	0	0	5.4	0	0	7.2
	25˚C/48 hours	1	0	0	9.3	0	0	12
		2	18.3	6.3	17.9	10.9	0	13

* - Patients presenting with a second DFU; ZOI – Zone of inhibition

Appendix H

H1: The full file review for all patients including patient specific notes.

Table H1: Full file review for all patients including patient specific notes.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A001	21/04/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	Patient notes: Still treating the same ulcer, no antibiotic use further antibiotic use recorded.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A002	07/03/2021	No	No	Mupirocin (topical)	Ulcer still not healed	No alignment to local or international guidelines
A003	12/03/2021	No	No	No	Ulcer still not healed	No alignment to local or international guidelines
	20/08/2021	No	No	Systemic antibiotics (not specified) then Amoxicillin/ clavulanic acid (1000 mg)	Patient hospitalised for HF and renal failure, antibiotics given to address foot ulcer, no improvement	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A004	20/08/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	Ulcer still not healed	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A005	06/08/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	Ulcer still not healed	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A006	*	*	*	*	File missing	*
A007	01/04/2019	No	No	No	Amputation of the right limb below the knee	No evidence of empiric antibiotic treatment noted in the file.
	02/09/2019	No	No	Cloxacillin	DFU reoccurrence	No alignment to local or international guidelines
	30/07/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg)	Ulcer still not healed	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A008	23/08/2021	No	No	Amoxicillin/ clavulanic acid 1,2 g IV followed by Amoxicillin/ clavulanic acid orally (1000 mg)	Ulcer still not healed; however wound bed is improving	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A009	21/06/2021	No	No	No	Ulcers not healed	No evidence of empiric antibiotic treatment noted in the file.
A010	17/02/2020	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD	Initial DFU treatment	Initial treatment aligned to international guidelines however, lack of resistance data

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
	11/08/2020	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 7 days followed by Amoxicillin/ clavulanic acid orally (1000 mg) 3 days	DFU still not healed, evidence of bone protruding	to constitute antibiotic alternatives.
A011	13/07/2021	No	No	Amoxicillin/ clavulanic acid 1,2 g IV TDS and clindamycin 600 mg IV * hourly	Right amputation below the knee as a result of recurrent chronic DFU. Current ulceration on right stump due to pressure of dressing	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
A012	03/06/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD; Metronidazole (400 mg) BD	Recurrent ulceration.	Metronidazole not included in STG/ SAASP. Metronidazole used in the treatment of necrotic and gangrenous wounds internationally.
A013	13/09/2021	No	No	No	Patient treated at different facility and cannot recall antibiotic prescribed. "C" - used long term over the period of 1 month	Insufficient information reported in the file.
A014	*	*	*	*	File missing	*
A015	09/2021	No	No	No	File not present, information based on patient description	Insufficient information reported in the file.
A016	*	*	*	*	File missing	*

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A017	07/12/2021	No	No	Amoxicillin/ clavulanic acid (1000 mg) BD	Ulceration on the toes still present, signs of gangrene.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A018	16/11/2021	No	No	Amoxicillin/ clavulanic acid (250 mg) TDS	Wound not healed, evidence of bone protruding. Patient reports improvement over significant time frame	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
A019	**	**	**	**	New patient, no file history	**
A020	20/12/2021	No	No	Clarithromycin 500 mg BD	Patient was treated for bronchitis Patient had ulcer for 1 year and 3 months and still not healed - no antibiotic use for DFU seen in file history Patient seems to remember something about a resistant *Staphylococcal* infection.	No alignment to local or international guidelines
A021	2021 - 2022	No	No	No	Patient has presented with multiple ulcers that have healed without the use of antibiotics. Treatment includes cleaning and debriding and dressing with povidone iodine.	No evidence of empiric antibiotic treatment noted in the file.
A022	25/12/2021 - 31/12/2021	Unknown	Unknown	Yes - Iv antibiotics	Patient presented without file but recalls nosocomial infection that required IV antibiotics	None

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A023	***	***	***	***	New file created on account of fire	**
A024	***	***	***	***	New file created on account of fire	**
A025	7/02/2022 - 11/02/2022	No	No	Amoxicillin/ clavulanic acid 500 mg D	Patient complained of sore throat in addition to DFU	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
	29/12/2020	No	No	Clindamycin IVI 600mg 8 hrly; Amoxicillin/ clavulanic acid IVI 1,2 g 8 hrly for 10 days	Left leg sepsis originating from chronic DFU, resulted in amputation below the left knee	Clindamycin is used alone in the case of penicillin allergy or severe ulceration in the SA guidelines. Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A026	**	**	**	**	New patient, no file history	**
A027	03/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly and then azithromycin 500mg PO 8 daily	Patient admitted for surgical debridement of the right foot. Patient has had this procedure three times in the past and has lost a toe on the same foot due to recurrent DFU.	Azithromycin not indicated for use in the STG or SAASP guidelines or internationally.
A028	22/02/2022	No	No	Clotrimazole 20 mg BD, Fluconazole 200 mg Weekly	Patient was treated for onychomycosis	Treatment was not initiated for the purpose of treating the DFU, however, the treatment may have affected healing of the ulcer itself.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
	24/03/2022	No	No	Amoxicillin/ clavulanic acid 1 g BD 7/7 and Mupirocin	Patient was admitted for surgical debridement of DFU, wound healing slowly	Mupirocin not indicated in local or international guidelines
A029	08/03/2022	Yes	E. Coli	Piperacillin/ Tazobactam (4.5g) IVI 6 hrly Ertapenem 1 g IVI Daily (7 days)	Patient admitted for treatment of infected amputation site as a result of diabetic foot complications. Patient scheduled for amputation of the remaining toe.	Treatment aligns to international guidelines for GPC, GNR as well as obligate anaerobes
A030	07/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly and then amoxicillin/clavulanic acid 600 mg IV 8 hrly	Patient has been wearing only socks due to pressure of shoes. Strong odour from wound, patient scheduled for amputation of 1st toe to avoid systemic involvement as a result of the DFU.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A031	07/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IVI 8 hrly	Previous amputation at different hospital of the 1st and 2nd toes of the right foot. New ulceration at site causing infection.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A032	07/03/2022	No	No	No	Patient admitted for amputation of left foot due to gangrene of the forefoot. Patient also had right leg amputated in 2021 but at a different hospital (no file history).	No evidence in the file that the patient is on any antibiotics.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A033	07/03/2022	Yes	*Streptococcus agalactiae, Peptostreptococcus anaerobis*	Piperacillin/ Tazobactam (4.5 g) IVI 6 hrly	Patient presented with poorly controlled diabetes according to file notes, patient not compliant to medications. Patient admitted due to septic DFU. Pus culture taken	Treatment aligns to international guidelines for GPC, GNR as well as obligate anaerobes
	13/03/2022	Yes	Gram (+) diplococci and Gram (+) cocci in chains	As above	Blood culture	Treatment aligns to international guidelines for GPC, GNR as well as obligate anaerobes
A034	09/03/2022	No	No	Amoxicillin/clavulanic acid (1000 mg) PO TDS	Patient newly diagnosed with diabetes, presented with septic ulcer. Patient admitted for surgical debridement of the wound.	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
A035	03/03/2022	No	No	No	Patient not admitted on account of DFU, patient admitted due to heart and renal failure. Patient had foot amputated in 2017, no indication of medical records that date back to this time.	No evidence in the file that the patient is on any antibiotics.
A036	18/03/2022	No	No	Amoxicillin/ clavulanic acid (1000 mg) PO BD	Patient seen at casualty for DFU, prescribed Augmentin and referred to podiatry. New patient thus no previous file history	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A037	13/03/2022	No	No	Amoxicillin/ clavulanic acid (1000 mg) PO BD	Patient admitted for second toe amputation as a result of chronic diabetic foot wound (unrelated to right heel sampled)	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
A038	26/02/2021	No	No	Amoxicillin/ clavulanic acid 1,2 g IVI TDS	Patient admitted for amputation of the second toe as a result of infected DFU	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
	12/03/2022	No	No	Ceftriaxone 250 mg IMI STAT, azithromycin 1g PO D, amoxicillin/ clavulanic acid (1000 mg) PO BD, metronidazole 1 g PO STAT	Patient not admitted on account of DFU, patient admitted due to fluid overload, renal dysfunction and enlarged testicle.	Patient not treated for DFU alone, however, treatment aligns to international guidelines
A039	09/02/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly	Patient admitted for debridement of the initial wound	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
	21/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly	Patient admitted for further debridement and amputation of the large toe as the ulcer had become infected again after initial debridement.	Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A040	22/03/2022	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly Then Amoxicillin/ clavulanic acid 1g PO BD Mupirocin	Patient admitted for heart issues. Mupirocin given as a topical treatment and patient was referred to a podiatrist.	Mupirocin not indicated in local or international guidelines. Initial treatment aligned to international guidelines, however, lack of resistance data to constitute antibiotic alternatives.
A041	01/04/2022	No	No	Clindamycin IVI 600mg BD; Amoxicillin/ clavulanic acid IVI 600mg 8 hrly	Patient admitted for cellulitis of lower left limb due to chronic ulceration.	Clindamycin is used alone in the case of penicillin allergy or severe ulceration in the SA guidelines. Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A042	16/07/2016	No	No	No	Patient admitted for amputation of the right leg above the knee and shortly thereafter, amputation of the left big toe	No evidence in the file that the patient was on any antibiotics.
	10/06/2022	No	No	No	Recurrent ulceration.	No evidence in the file that the patient is on any antibiotics.
A043	10/06/2022	No	No	No	No evidence of previous infection or antibiotic use noted in file. Only evidence of eye clinic appointments.	-

Patient code	Date of current and previous	Culture Collected	Identified Micro-organism	Antibiotic Prescribed	Patient notes	Treatment regimen comment
A044	23/08/2021	No	No	Amoxicillin/ clavulanic acid 1.2 g IV 8 hrly Then Amoxicillin/ clavulanic acid 1g PO BD	Toe amputation	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
	17/06/2022	No	No	Amoxicillin/ clavulanic acid 1,2 g IVI TDS	Patient admitted for new ulcer as sampled. Patient was seen by podiatry for consult.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.
A045	21/06/2022	No	No	Amoxicillin/ clavulanic acid 1g PO TDS	Patient admitted for first time DFU caused by an untreated corn.	Initial treatment aligned to international guidelines however, lack of resistance data to constitute antibiotic alternatives.

Key: * - patient file unaccounted for; **- new patient, no file history; *** - new file created and previous file lost due to CMJAH fire thus no file history; **IV** – intravenously; **BD –** twice daily; **TDS** – three times daily; **IMI** – intramuscular injection; **STAT** – immediately

Appendix I

I1: Full table of the identified interactions as well as the potential consequences thereof.

Table I1: The identified potential drug-drug interactions and their possible consequences Interaction information was taken from the South African Medicines Formulary as well as the EM Guidance interaction checker (available at: https://emguidance.com/interactions).

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Amlodipine	Enalapril	Severe	Fatal hyperkalaemia, cardiac arrhythmias	9
Amlodipine	Simvastatin	Moderate	Myopathy or rhabdomyolysis	8
Furosemide	Paracetamol	Moderate	Nephrotoxicity	7
Simvastatin	Amitriptyline	Minor	QT interval prolongation risk due to increased serum levels of amitriptyline	7
Aspirin	Amlodipine	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	6
Enalapril	Aspirin	Severe	Fatal hyperkalaemia in at risk patients, acute renal failure, cardiac arrhythmias	6
Enalapril	Furosemide	Moderate	Hyperkalaemia, renal failure, increased hypotensive effect	6
Insulin	Carvedilol	Moderate	Hypoglycaemia without tachycardia	5
Insulin	Aspirin	Moderate	Hypoglycaemia	5
Furosemide	Aspirin	Moderate	Ototoxicity, nephrotoxicity, decreased diuretic effect	5
Aspirin	Paracetamol	Moderate	Nephrotoxicity	5

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Atorvastatin	Amitriptyline	Minor	QT interval prolongation risk due to increased serum levels of amitriptyline	4
Amitriptyline	Tramadol	Severe	QT interval prolongation, respiratory depression, seizures, serotonin toxicity, sedation	4
Amitriptyline	Paracetamol	Moderate	QT interval prolongation, nephrotoxicity	4
Enalapril	Carvedilol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	4
Hydrochlorothiazide	Paracetamol	Moderate	Nephrotoxicity	4
Amitriptyline	Aspirin	Moderate	Nephrotoxicity	4
Enalapril	Enoxaparin	Severe	Hyperkalaemia, cardiac arrhythmias	4
Carvedilol	Enoxaparin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	3
Metformin	Carvedilol	Moderate	Hyperglycaemia, Hypertensive effect and compensatory bradycardia with masked hypoglycaemia.	3
Hydrochlorothiazide	Amitriptyline	Moderate	Hyponatraemia, QT interval prolongation, Torsade de pointes risk, nephrotoxicity, cardiac arrhythmias, increased hypotensive effect	3

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Furosemide	Hydrochlorothiazide	Moderate	Hypokalaemia, hyponatraemia, nephrotoxicity, increased hypotensive effect	3
Atenolol	Amlodipine	Severe	Cardiac failure, fatal hyperkalaemia in at risk patients, additive bradycardia, cardiac arrhythmias, increased hypotensive effect	3
Hydrochlorothiazide	Enalapril	Moderate	Renal failure	2
Aspirin	Lansoprazole	Moderate	Nephrotoxicity	2
Carvedilol	Aspirin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	2
Carvedilol	Amlodipine	Severe	Cardiac failure, fatal hyperkalaemia in at risk patients, additive bradycardia, cardiac arrhythmias, increased hypotensive effect	2
Furosemide	Spironolactone	Minor	Hyperkalaemia, hyponatraemia, increased hypotensive effect	2
Aspirin	Enoxaparin	Severe	Hyperkalaemia, bleeding risk, cardiac arrhythmias	2
Furosemide	Lansoprazole	Moderate	Hypomagnesemia, nephrotoxicity	2
Furosemide	Salbutamol	Moderate	Hypokalaemia, QT interval prolongation, Torsade de pointes risk	2
Hydrochlorothiazide	Aspirin	Moderate	Nephrotoxicity	2

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Hydrochlorothiazide	Losartan	Moderate	Nephrotoxicity, increased hypotensive effect	2
Hydrochlorothiazide	Lansoprazole	Moderate	Hypomagnesemia, nephrotoxicity	2
Hydrochlorothiazide	Metoclopramide	Moderate	QT interval prolongation, Torsade de pointes risk, hypokalaemia	2
Furosemide	Amitriptyline	Moderate	Hyponatraemia, QT interval prolongation, Torsade de pointes risk, increased hypotensive effect	2
Amitriptyline	Omeprazole	Moderate	QT interval prolongation, nephrotoxicity	2
Amitriptyline	Carbamazepine	Minor	Increased anticholinergic effect, hyponatraemia, seizure (especially in elderly)	2
Enalapril	Atenolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	2
Glimepiride	Carvedilol	Moderate	Hyperglycaemia, Hypertensive effect and compensatory bradycardia with masked hypoglycaemia.	1
Glimepiride	Aspirin	Moderate	Enhanced hypoglycaemic effect	1
Glibenclamide	Carvedilol	Moderate	Hyperglycaemia, Hypertensive effect and compensatory bradycardia with masked hypoglycaemia.	1

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Atorvastatin	Simvastatin	Minor	Myopathy, rhabdomyolysis, hepatotoxicity	1
Atorvastatin	Carbamazepine	Minor	Hepatotoxicity due to increased serum levels of carbamazepine	1
Atorvastatin	Citalopram	Minor	QT interval prolongation, side effects of citalopram increased.	1
Atorvastatin	Allopurinol	Moderate	Hepatotoxicity	1
Simvastatin	Carbamazepine	Moderate	Hepatotoxicity	1
Simvastatin	Citalopram	Minor	QT interval prolongation, side effects of citalopram increased.	1
Enalapril	Tamsulosin	Moderate	Increased hypotensive effect	1
Enalapril	Allopurinol	Moderate	Anaphylaxis, leukopenia, myocardial infarction, can render infection unresponsive to antibiotic therapy	1
Enalapril	Colchicine	Minor	Can render infection unresponsive to antibiotic therapy	1
Enalapril	Spironolactone	Severe	Fatal hyperkalaemia in at risk patients, increased hypotensive effect	1
Perindopril	Nifedipine	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Perindopril	Bisoprolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Perindopril	Amitriptyline	Moderate	Nephrotoxicity, increased hypotensive effect	1
Perindopril	Aspirin	Severe	Fatal hyperkalaemia in at risk patients, nephrotoxicity, cardiac arrhythmias	1
Lisinopril	Atenolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Lisinopril	Propranolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Lisinopril	Hydrochlorothiazide	Moderate	Renal failure, increased hypotensive effect	1
Lisinopril	Amlodipine	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Bisoprolol	Aspirin	Severe	Hearing loss, fatal hyperkalaemia in at risk patients, cardiac arrhythmias	1

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Propranolol	Atenolol	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, PR interval prolongation, additive bradycardia, increased hypotensive effect	1
Atenolol	Enoxaparin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	1
Atenolol	Spironolactone	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Atenolol	Aspirin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	1
Propranolol	Amlodipine	Severe	Cardiac failure, fatal hyperkalaemia in at risk patients, additive bradycardia, cardiac arrhythmias, increased hypotensive effect	1
Carvedilol	Spironolactone	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Furosemide	Losartan	Moderate	Hyperkalaemia, renal failure, increased hypotensive effect	1
Furosemide	Salmeterol	Moderate	Hypokalaemia, QT interval prolongation, Torsade de pointes risk	1
Furosemide	Metoclopramide	Moderate	QT interval prolongation, Torsade de pointes risk, hypokalaemia	1

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Furosemide	Allopurinol	Moderate	Nephrotoxicity, antagonistic effect on allopurinol	1
Furosemide	Colchicine	Moderate	Nephrotoxicity	1
Furosemide	Clopidogrel	Moderate	Nephrotoxicity	1
Furosemide	Polystyrene sulfonate	Moderate	Severe hypokalaemia	1
Spironolactone	Losartan	Severe	Fatal hyperkalaemia in at risk patients, nephrotoxicity, cardiac arrhythmias, increased hypotensive effect	1
Spironolactone	Enoxaparin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	1
Spironolactone	Aspirin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, decreased diuretic effect, sodium retention	1
Spironolactone	Amlodipine	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect	1
Hydrochlorothiazide	Citalopram	Moderate	Hyponatraemia, QT interval prolongation, Torsade de pointes risk, hypokalaemia, cardiac arrhythmias	1
Amitriptyline	Citalopram	Severe	Hyponatraemia, QT interval prolongation, serotonin toxicity, Torsade de pointes risk	1
Amitriptyline	Metoclopramide	Moderate	QT interval prolongation, Torsade de pointes risk	1
Amitriptyline	Allopurinol	Moderate	Nephrotoxicity	1
Amitriptyline	Colchicine	Moderate	QT interval prolongation, nephrotoxicity	1

Medication 1	Medication 2	Interaction classification	Description	Number of patients
Amitriptyline	Escitalopram	Moderate	Hyponatraemia, QT interval prolongation, serotonin toxicity, Torsade de pointes risk	1
Amitriptyline	Lansoprazole	Moderate	QT interval prolongation, nephrotoxicity	1
Amitriptyline	Clopidogrel	Moderate	Nephrotoxicity	1
Amitriptyline	Isosorbide mononitrate	Severe	Fatal hypotension in at risk patients	1
Aspirin	Omeprazole	Moderate	Nephrotoxicity	1
Aspirin	Pantoprazole	Moderate	Nephrotoxicity	1
Aspirin	Clopidogrel	Moderate	Acute haemorrhage, nephrotoxicity, upper GIT bleeding	1
Amlodipine	Losartan	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias, increased hypotensive effect, oedema	1
Amlodipine	Enoxaparin	Severe	Fatal hyperkalaemia in at risk patients, cardiac arrhythmias	3
Omeprazole	Paracetamol	Moderate	Nephrotoxicity	1
Lansoprazole	Paracetamol	Moderate	Nephrotoxicity	1
Pantoprazole	Paracetamol	Moderate	Nephrotoxicity	1
Glycopyrronium bromide	Chlorphenamine	Moderate	Additive anticholinergic and antimuscarinic effect	1
Alprazolam	Zolpidem	Moderate	Complex sleep behaviours, CNS depression	1

Appendix J

J1: Suggested patient management recommendations provided to the podiatry units based on the results of this study.

PATIENT MANAGEMENT RECOMMENDATIONS – A TICK LIST

Section 1: Patient History

- ☐ The patient has presented with a full file history (Yes/ No).
- ☐ If no, establish the health history of the patient in as much detail as the patient will provide.
- ☐ Take the full medication history of the patient.
- ☐ Use the patients history to determine their risk for diabetic foot ulcer development.
- ☐ Record all appointments and examinations in order to refer back at any time.
- ☐ Record patient prevnetative measure use and how often they use the measure.

Section 2: Screening

- ☐ Screen patients at low-risk for diabetic foot ulcer development anually.
- ☐ Observe for signs and symptoms off:
    - Peripheral artery disease
    - Peripheral neuropathy
    - Foot deformity
- ☐ Refer the patient for additional screening with other healthcare practitioners in order to address chronic conditions that exacerbate the development of diabetic foot ulcers (Eg: renal disease, hypertension and cardiovascular disease).

Section 3: Patient education

- Educate the patient at every appointment:
  - Encourage the patient not to walk barefoot.
  - Examine ones feet regualry.
  - Correct nail cutting technique.
  - Good hygiene practices and the use of emollients.
  - Encourage at risk patients to check their foot temperature as an early sign of inflammation.

Section 4: Footwear and preventative measure use

- Encourage these patients to wear footwear that reduces planter pressure and fits correctly.
- In the case of patients with a history of previous DFU or those who present with calluses, consider the use or orthotic pressure off-loading devices to prevent further ulcer development.
- The most ideal preventative measure to be employed is a removable walker. The patient should be educated in its fitting and as to when to wear it.
- Emphasise the importance of using the preventative measures consistently.

Section 5: Pharmacological interventions

- In the case of a diabetic foot ulcer that has become infected or one that is not improving, refer the patient for antimicrobial treatment;
  - Advise the healthcare practiontoner on the established local epidemiolocal patterns (provided).
  - Encourgae the healthcare prctioner to carry out microbiological testing where possible.

Appendix K

K1: Turnitin Plagiarism Report

Maxine Turner (nee Grose) Thesis

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