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Microbial profile and susceptibility patterns of diabetic foot ulcers in patients treated at a tertiary hospital

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ABSTRACT

The increase in the number of patients with diabetes mellitus has been associated with a rise in its complications, including a diabetic foot. The study aimed to determine types of microorganisms present in diabetic foot ulcers in [location deleted...], and to analyse their susceptibility patterns to commonly used antibacterial drugs. In this retrospective cross-sectional study, analysis of specimens from wounds of diabetic patients seen [Affiliated institutions deleted…] was carried out. Stored data for a five-year period starting from 01 July 2017 to 30 June 2022 was used. The study consisted of 97 patients. There were 53 (54.6%) males and 44 (45.4%) females between the ages of 15 and 83 years. From the specimens, 150 bacteria were isolated with Gram positive isolates being 80 (53%) and Gram negative isolates 70 (47%). Staphylococcus aureus was the predominant organism, and displayed an overall resistance of 80% against penicillins and with 19% of its isolates being methicillin resistant. Considering the above, a question remains about which empiric drug to use for resistant Gram positive organisms, as Gram positive isolates form most of the organisms cultured in diabetic foot ulcers in our setting. The results of the study are crucial in guiding clinicians on the best choice of empiric antibiotics in our setting. Until now, such information in literature from Ga-Rankuwa and its vicinity does not exist.

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Introduction

People living with diabetes mellitus in 2019 were estimated to be around 500 million according to data from the International Diabetes Federation (IDF). From this group approximately 19.4 million people between the ages of 20 and 79 years came from Africa. South Africa reported 4.5 million cases of diabetes during that period. Increasing urbanisation has led to changes in lifestyle with easy access to fast foods and a more sedentary way of life, and this has seen both diabetes and obesity increasing in incidence in recent years (Atlas, 2015).

The rise in the incidence of diabetes mellitus has been associated with a proportional increase in its complications. Foot complications in particular account for over half of all hospital admissions among diabetic patients, and this has been linked to significant morbidity, high financial costs and increased risk of amputations and death. Global healthcare expenditure in managing diabetes was estimated at USD 673 billion in 2015 (Atlas, 2015; Mutonga, 2018).

A diabetic foot is characterised by worsening infection, ulceration, necrosis and destruction of deep tissues of the foot [see image 1 & 2] as a result of a complex interplay between immunopathy, (motor, sensory and autonomic) neuropathy as well as varying degrees of peripheral arterial disease in a diabetic patient (Bekele, Fekadu, Bekele, & Dugassa, 2019; Schaper et al., 2020). A diabetic foot ulcer (DFU) is a much common and serious complication of diabetes (Yazdanpanah et al., 2018). The lifetime risk of developing a foot ulcer in diabetic patients is estimated at 15-25 % (Yazdanpanah, et al., 2018). Diabetic foot sepsis (infection) is the presence of two clinical signs or symptoms of infection in or around a diabetic foot ulcer which may include purulence, erythema, pain, swelling, warmth and indurations (Bekele, et al., 2019).

A favourable outcome in managing a diabetic foot depends on being familiar with microbial profile of the infection, appropriate selection of effective antibacterial drugs as well as understanding principles of basic wound care and above all, early medical and surgical intervention before irreversible complications, such as extensive tissue necrosis and gangrene, ensue (Lipsky, 1999). Appropriate selection of antibiotics which should be guided by culture proven effectiveness is therefore paramount. The frequent emergence of antimicrobial resistance makes the use of empirical treatment rather daunting unless a special system of ongoing monitoring of hospital dwelling organisms is operationalized (Lipsky, 1999).

Method

The study aimed to determine microbial profile and susceptibility patterns of diabetic foot ulcers in patients treated at a tertiary hospital in Ga-Rankuwa, Pretoria. In this cross-sectional study, retrospective data was collected for the period 01st July 2017 until 30th June 2022. Analysis was performed on specimens of pus, serous fluid and tissues that were coming from wounds of diabetic patients who were seen at [Affiliated institutions deleted…] in the emergency unit, surgical wards and hospital wound care clinic. All patients with DFU who were seen at [Affiliated institutions deleted…] and whose wounds were swabbed for microbiological studies during the study period were included. This population included both gender and all age groups. Non-diabetic patients with foot ulcers (e.g. critical limb ischaemia) were not included. Patients with missing or inaccurate information in their files were eliminated from the study.

A treating doctor or nurse was responsible for swabbing the wounds after irrigating them with normal saline and wiping them with sterile gauzes. Measures were taken to avoid cross contamination. The swabs were labelled with a unique barcode sticker that corresponded with the one on the request form. The wounds were swabbed up to their base with aseptic swabs, and then swabs were bottled for transportation to [Affiliated institutions deleted…] National Health Laboratory Service (NHLS).

Each patient's demographic information and microbiological results of specimens were transcribed on to a data collection sheet. This data was subsequently exported to Excel spreadsheet for characterisation and analysis. Descriptive statistics was done to demonstrate basic demography of the study population using Microsoft Excel spreadsheet. All statistical procedures were performed on SAS (SAS Institute Inc., Cary, NC, USA), Release 9.4. running under Microsoft Windows on a personal computer. The study was approved by [Affiliated institutions deleted…]. Confidentiality was maintained throughout the study and patients' data was used exclusively for research purposes.

Results and Discussions

A total of 97 patients were included in the study. Fifty-three (54.6%) were males and forty-four (45.4%) females. The study population included patients from the ages 15 to 83 years with the mean age of 56 years. More than 50 percent of patients were between the ages of 35 and 60 years. The patients were seen and swabbed at the emergency unit, surgical wards and hospital wound clinic. However, about 70 percent of patients were captured from the hospital wound clinic. The demographic characteristics have been summarised in Table 1.

Hundred and fifty isolates were identified from specimens of 97 patients in the study. Of these, there were 80 (53%) Gram positive isolates and 70 (47%) Gram negative isolates [see tables 2 & 3]. In eight patients the specimens yielded no growth [see fig. 1]. Approximately 46% of Gram positive isolates were Staphylococcus aureus, followed by Enterococcus faecalis (22%) and Streptococcus species (19%) [see table 2]. Streptococcus species included S. pyogenes (n: 4), S. viridans (n: 4), S. agalactiae (n: 3), S. Group F (n: 3) and S. sanguinis (n: 1). Amongst the Gram negative isolates Pseudomonas aeruginosa was predominant (27%), followed by Proteus species (20%), Enterobacter species (13%), Klebsiella species (11%), Escherichia coli (10%) and Morganella (9%). [see table 3] Proteus species included P. mirabilis (n: 7), P. hauseri (n: 3), P. penneri (n: 3) and P. vulgaris (n: 1). Enterobacter species were E. cloacae (n: 8) and E. asburiae (n: 1). Klebsiella had species K. pneumoniae (n: 5) and K. oxytoca (n: 3). While Morganella species M. morganii (n: 4) and M. sibonii (n: 2) were present. Overall, amongst both Gram negative and positive isolates, Staphylococcus aureus had the greatest percentage occurrence (23%) with Pseudomonas, Enterococcus and Streptococcus species following in descending order. [see fig. 1]

The antimicrobial sensitivity and resistance profile of Gram positive isolates is demonstrated in Table 4. High resistance pattern of approximately 80% of S. aureus against penicillin/ampicillin is observed with moderate sensitivity of 76% for macrolides. Penicillin/ampicillin has a sensitivity of 100 and 80 percent against Enterococcus and Streptococcus species, respectively. Gentamycin was found to be highly sensitive (> 80%) against Gram negative isolates, i.e. Pseudomonas, Morganella, Escherichia coli, and Citrobacter. Escherichia coli were highly resistant to trimethoprim-sulfamethoxazole (> 70%), yet sensitive to gentamycin (86%). Morganella morganii species demonstrate 100% resistance against amoxicillin-clavulanic acid and cephalosporins with good sensitivity to the carbapenems. [see Table 5].

Gram positive bacteria demonstrated overall average sensitivity of 72% and resistance of 27%. While Gram negative organisms were roughly 60% sensitive, 3% intermediately sensitive and 37% resistant against conventional antibiotics. [see fig.2 & 3] Of the 37 Staphylococcus aureus isolates, 7 (19%) were methicillin resistant staphylococcus aureus (MRSA). [see table 6] Multi-drug resistant Gram negative isolates included strains from Morganella morganii (4 isolates; 66%), Klebsiella (3 isolates; 37%), Escherichia coli (2 isolates; 28%), Proteus (2 isolates; 14%), and Pseudomonas (1 isolate; 5%).

Table 1 <Patient Characteristics>

Variables	Mean (yr)	Range (yr)	Frequency (n=97)	Percentage, %
Gender	–	–		
Male			53	54.6
Female			44	45.4
Age (years)	56.6	15 – 83		
< 35			7	7.2
35–60			51	52.6
> 60			39	40.2
Hospital units	–	–		
Wound clinic			67	69.0
Emergency unit			15	15.5
Surgical ward			15	15.5

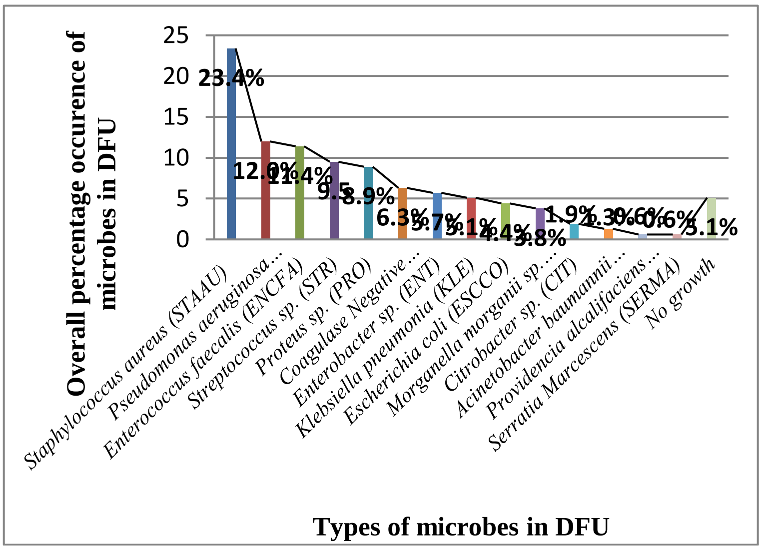

Figure 1. Types of Microbes in DFU

Table 2 <Percentage Distribution of Gram Positive Microbes in DFU>

Bacteria (Gram positive)	Frequency, n=80	Percentage, (%)
Staphylococcus aureus (STAAU)	37	46.2
Enterococcus faecalis (ENCFA)	18	22.6
Streptococcus sp. (STR)	15	18.7
Coagulase Negative Staphylococcus (CNS)	10	12.5

Table 3 <Percentage Distribution of Gram Negative Microbes in DFU>

Bacteria (Gram negative)	Frequency, n=70	Percentage (%)
Pseudomonas aeruginosa (PSEAE)	19	27.1
Proteus sp. (PRO)	14	20
Enterobacter sp. (ENT)	9	12.8
Klebsiella sp. (KLE)	8	11.4
Escherichia coli (ESCCO)	7	10
Morganella morganii sp. (MOGM)	6	8.6
Citrobacter sp. (CIT)	3	4.3
Acinetobacter baumannii complex (ACIBC)	2	2.8
Providencia alcalifaciens (PRVAL)	1	1.4
Serratia Marcescens (SERMA)	1	1.4

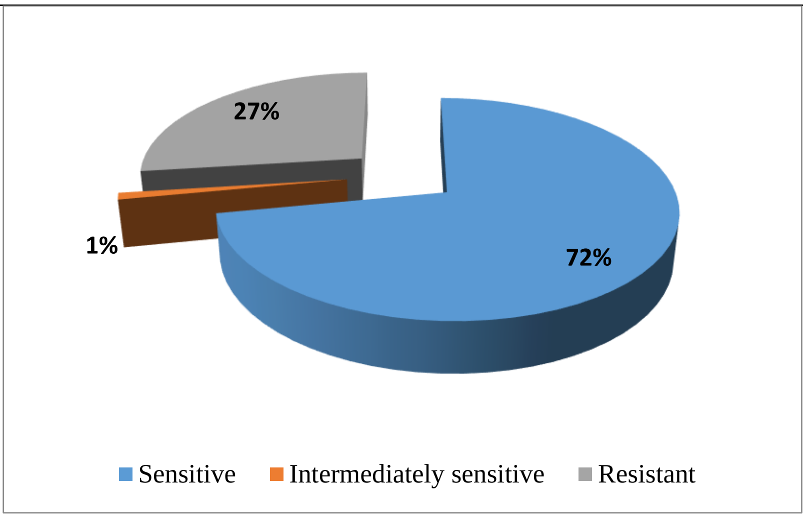

Figure 2 <Average Percentage Sensitivity and Resistance of Gram Positive Organisms from DFU>

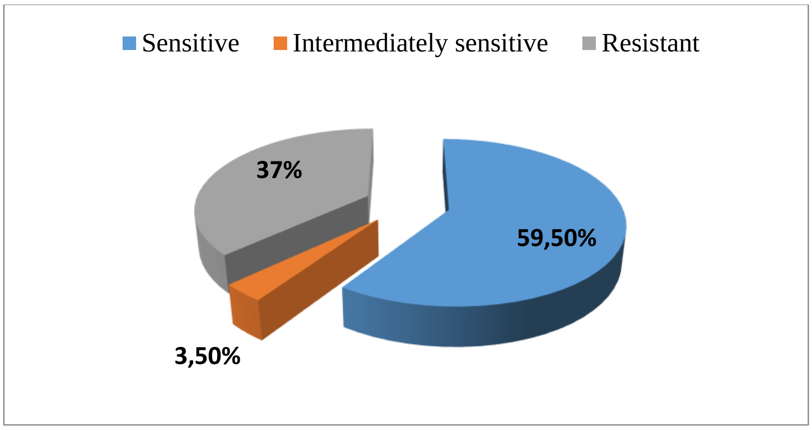

Figure 3 <Average Percentage Sensitivity and Resistance of Gram Negative Organisms from DFU>

Discussion

According to this study, there were more males (54.6%) than females (45.4%) with DFU. This observation is echoed in similar studies done in Nigeria (Bello, Oyekanmi, Kelly, Mebude, & Bello, 2018), Sudan (Nyamu, Otieno, Amayo, & McLigeyo, 2003), Tanzania (Chalya et al., 2011) and Ethiopia (Deribe, Woldemichael, & Nemera, 2014). However, this is in contrast to findings of a few studies in Kenya (Mutonga, 2018). Data from large randomised studies have demonstrated a relatively lower risk of DFU in females, owing to a much lesser risk of severe peripheral neuropathy, better joint flexibility and decreased likelihood of foot pressure ulcers (Dinh & Veves, 2008).

More than half of the patients were within the age group 35-60 years with an average age of 56. This is consistent with findings from other studies. Diabetic foot ulcers often present in patients in their fifth and sixth decades of life (Nyamu, et al., 2003). Most of the specimens (70%) were collected from patients at the wound clinic. These were outpatients who come for regular wound care at the hospital. Considering the high resistance patterns of about 27% and 37% for Gram positive and negative isolates respectively, it is likely that this subset of patients was previously admitted and treated in-hospital on antibiotics. This is further supported by the observation of *Pseudomonas aeruginosa* being the commonest Gram negative isolate in a population that carries 70% of walk in patients from home.

The study reveals a much greater frequency (approximately 46%) of *Staphylococcus aureus* compared with other Gram positive isolates. *S. aureus* was also the predominant isolate (23%) amongst all bacteria in DFU. The dominance of *S. aureus* was reflected in a study from West Africa by O.O. Bello and colleagues (Bello, et al., 2018). They showed that *S. aureus* was the commonest isolate in DFU, followed *Pseudomonas, Staphylococcus epidermidis* and *Proteus mirabilis* (Bello, et al., 2018). On the contrary, a local study from Kwa Zulu Natal demonstrated that the most common bacteria in DFU were Gram negative bacilli, particularly *Enterobacter sp.*, followed by *Staphylococcus* species and *Enterococcus faecalis* (Penduka et al., 2018*).* Gram negative bacteria, *Acinetobacter baumannii, Providencia alcalifaciens* and *Serratia marcescens*, were the least common. The wide variation in microbial diversity in DFU between geographic locations, even within the same country, speak to differences in local antibiotic selection and usage policies, which should encourage clinicians and policymakers to employ measures to safeguard against rising antimicrobial resistance.

S. aureus' resistance pattern with high levels of methicillin resistant strains is a matter that is greatly concerning. With its overall resistance of 80% against penicillin/ampicillin and with 19% of its isolates being methicillin resistant, this trend is particularly worrisome given that S. aureus is in fact the most predominant isolate (23%) in DFU. All S. aureus strains in this study were sensitive to vancomycin. However, the use of vancomycin as an empiric treatment would be inappropriate as this would lead to a rise in its resistance.

On the contrary, the results of O.O. Bello and colleagues showed that in their setting S. aureus was essentially sensitive to penicillin/ampicillin, ceftriaxone, amikacin, trimethoprim-sulfamethoxazole and carbapenems (Lipsky, 1999). A study by Mingxia Wu and colleagues found that the percentage occurrence of MRSA in S. aureus isolates to be around 20%, consistent with the findings of this study (Bello, et al., 2018). Other reports have demonstrated higher numbers of MRSA, up to 78%, like in an Iranian study by Mojtaba Anvarinejad and colleagues (Anvarinejad et al., 2015).

Gentamycin has demonstrated its usefulness as it remained highly sensitive against a wide range of Gram negative bacteria. The shortcomings of this antibacterial drug lie in its toxicity profile which includes kidney failure and hearing loss as well as its availability as an intravenously drug only. Though less frequent in numbers, Morganella morganii strains demonstrated 100% resistance to amoxicillin-clavulanate. Morganella morganii species accounted for 9% of Gram negative bacteria in DFU, and whether this should motivate for withdrawal of the use of amoxicillin-clavulanate in DFU remains an open question. Overall, Gram negative isolates showed greater resistance pattern (37%) against conventional antibiotics compared to Gram positive isolates (27%).

From the study, clinicians are made aware that gentamycin is an effective drug against Gram negative bacteria, and can serve in the 'first line regimen'. While vancomycin is perfectly potent against Gram positive organisms, this drug cannot be used as first line as it will quickly lead to resistant organisms and is one of the few 'last defence' antibiotics available. In light of the perceived observation of rising levels of antibiotic resistance, [Affiliated institutions deleted…] had embarked on an ongoing surveillance and monitoring of hospital dwelling micro-organisms and their resistance prolife. However, the programme was interrupted during the period of heightened Covid-19 scourge. The authors of this manuscript hope to encourage and revive such initiatives so as to improve the lives of the people of Ga-Rankuwa and its surrounding region.

Conclusions

Diabetes mellitus and its feared complication of a diabetic foot pose a major health concern to the affected individual and can negatively impact any nation's public health budget. To isolate, characterise and determine sensitivity patterns of bacteria in diabetic foot ulcers (DFU) is one step towards formulating a solution to this global crisis.

S. aureus is the predominant isolate in DFU and is highly resistant to penicillins. Its exact resistance profile to amoxicillin-clavulanate, in particular, could not be determined. Gentamycin has shown superiority in its effectiveness against Gram negative isolates. This advantage seems to be dampened by its nephrotoxicity and neurotoxicity profile. However, questions remain about which drug to use as first line for the resistant Gram positive isolates, as Gram positive bacteria account for the majority of organisms cultured in DFU in our setting.

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