The antimicrobial susceptibility patterns of diabetic foot ulcers in the South African public healthcare sector

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Background. 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 DFU infection remains a mainstay, the choice of antimicrobial remains problematic owing to the presence of multidrug-resistant polymicrobial infections. In the South African public healthcare sector, the treatment of DFUs is based on the Standard Treatment Guidelines (STGs) and the Essential Drug List. These guidelines are developed using evidence-based medicine and are based on global susceptibility patterns rather than local susceptibility data, and may not provide the most appropriate treatment options.
Objectives. To determine the antimicrobial susceptibility patterns of DFUs isolated from patients visiting selected Gauteng provincial public hospitals in order to determine a clinically effective treatment protocol for the management of these infections.
Methods. Sample swabs were taken from 51 DFUs using the Levine method. 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 a representative isolate from each group was then identified.
Results. A total of 51 DFU ulcer swabs from 45 patients were included in the study. From the sample swabs, a total of 445 pathogens were isolated. The most effective antimicrobial was found to be gentamicin, followed by ciprofloxacin. Amoxicillin/clavulanic acid, the first line treatment according to the STGs, was found to be ineffective for many of the isolated pathogens. The most commonly isolated pathogens were *Proteus mirabilis*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*.
Conclusion. These findings demonstrate the urgent need to reassess the STGs 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.

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Diabetes mellitus (DM) is a metabolic disorder that affects approximately 537 million people worldwide.[1] Diabetic foot syndrome, a complication of diabetes mellitus, is the most common cause of hospitalisation and lower limb amputation among patients with diabetes.[2,3] A diabetic foot ulcer (DFU) is a complication of diabetic foot syndrome that occurs in a person with diagnosed DM, and typically presents alongside neuropathy or peripheral artery disease in the lower extremity.[4] These wounds often present as open lesions, with infection.[5,6] South African (SA) data indicate that the prevalence of DFUs is 5.4% - 6.0% among patients with diabetes,[7,8] with more recent studies noting that 28% of patients with diabetes presenting to primary healthcare facilities in SA had developed DFUs.[9] It is estimated that >50% of DFUs become infected.[6,10] Infection of a DFU occurs when a virulent micro-organism invades the host and ultimately results in local tissue damage.[11] The micro-organisms that cause infection in DFUs depend on patient factors such as the degree of immunosuppression and duration of diabetes, as well as pathogen-specific resistance patterns.[12]

Gram-positive bacteria implicated in diabetic foot ulcers and infections may comprise *Streptococcus, Staphylococcus* as well as *Enterococcus* species,[13] with the most common causative pathogen in DFUs being *Staphylococcus aureus*.[6,13,14] Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a commonly reoccurring pathogen in skin and soft-tissue infections, and more so in the feet of patients with diabetes.[6] While it seems that Gram-positive organisms 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 developing countries, or continents with warmer climates such as Asia and Africa, Gram-negative organisms are more prevalent.[6,15,16] Among the Gram-negative bacilli, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus* spp. are the most common causative pathogens.[17]

Antimicrobial resistance compounds the problem of rising diabetic foot infection incidences.[6] International guidelines such as the Wound Healing Society (WHS) guidelines suggest that if infection is suspected in the DFU, cultures should be performed before the administration of antibiotics.[17,18] Empirical treatment should be guided by the severity of the infection and changed to directed therapy as soon as culture results are obtained.[19] However, the use of inappropriate empirical antimicrobials or low concentrations of these agents could result in the development and distribution of resistant pathogens.[20] It is therefore important that antimicrobial resistance patterns specific to geographical location be investigated in order to prescribe the most appropriate treatment regimen in these cases. In the studies carried out on the African continent, including Nigeria, Kenya and Egypt, the causative pathogens found in DFUs differ greatly. In Kenya, *S. aureus* and *Proteus* spp. were the most commonly identified micro-organisms, which were susceptible to imipenem, piperacillin/tazobactam and ciprofloxacin, and were resistant to amoxicillin/clavulanic acid and clindamycin.[21] 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.[22] This was different once again in Egypt, where erythromycin was found to be one of the antimicrobials with the most resistance.[20]

In the SA public healthcare sector, the treatment of DFUs is based on the Standard Treatment Guideline (STGs) and the Essential Drug List (EDL).[23] These guidelines are developed using evidence-based medicine and are based on global susceptibility patterns. However, differing susceptibility patterns 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.[6] According to the STGs, patients treated at a public hospital in SA presenting with DFUs are treated with amoxicillin/clavulanic acid taken orally, while 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 are recommended.[24] The SA Antibiotic Stewardship Programme (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 SA. SA treatment guidelines do not account for nosocomial or methicillin-resistant *S. aureus* infections or recent exposure to antibiotics. Furthermore, there is no consideration for the treatment of chronic DFUs given in the STGs. The limited treatment options suggest a 'one size fits all' approach when it comes to treating DFUs in the SA public healthcare sector. This may result in poor treatment outcomes and higher recurrence rates.

Objective

The aim of this research was to determine the antimicrobial susceptibility patterns of bacteria isolated from the DFUs of patients visiting selected Gauteng Province public healthcare settings, and to suggest a clinically effective treatment protocol.

Methods

Setting

The setting of this study was two tertiary public healthcare facilities in Gauteng Province, SA, in patients visiting the podiatry and wound clinics.

It was established that an average of 100 patients attend these two public healthcare facilities on a yearly basis for diabetic foot care. However, this prevalence had decreased in these settings due to the COVID-19 pandemic at the time of the study. Based on this prevalence rate, a total minimum sample size of 50 DFUs was required for this study to attain a 50% attrition rate at a 95% confidence interval. Participation was on the day of study enrolment, and no follow-up visits were required.

Participants

Patient selection was based on purposive, homogenous sampling (selecting participants who share similar characteristics, traits, or attributes, and employed to reduce variability within the sample), as the goal of this research was to identify only those patients who present with foot ulceration as a consequence of diabetes. All patients (female or male) who were included in the study had to be a diagnosed as a patient with diabetes (type 1 or type 2) presenting with ulceration of the foot. Paediatric patients were excluded from the study, as well as those patients presenting with venous ulcers of the foot. Patients were requested to participate in the study following provision of study information shared by means of a patient information sheet, which included information as to why the study was being conducted, ethical considerations and what was expected from the patient. Upon positive affirmation of consent to participate, the patient was asked to sign an informed consent form.

Record review

A review of past records took place to determine previous treatment plans and subsequent adherence to local guidelines. This was carried out at the time of patient enrolment. The patients' records were reviewed, and information pertaining to the treatment of previous DFUs was recorded, including the date of presentation, whether a culture was taken for analysis and the antimicrobial treatment prescribed.[25]

Wound sample collection

Sample collection was carried out during patient consultation with the study-site podiatrist. In this study, clinical pathogens were collected directly from the wound of each participant using the Levine swabbing method.[26,27] The wound area was swabbed with a nylon-flocked swab soaked in 150 µL of 0.9% sterile saline.[27,28] The nylon-flocked swab was rotated over a 1 cm^2 area with enough pressure to express fluid within the wound for 5 seconds.[27,29] The swab was then placed into a sealed sterile receptacle. This process was repeated a second time, and the second swab was transferred via 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, Microbiology laboratories, Faculty of Health Sciences, University of the Witwatersrand and immediately processed for culture growth.

Culture of sample

To culture the collected patient samples, the swab was aseptically immersed in 1 mL of a 0.9% sterile saline solution for elution of the culture by vortex (Lasec) at ambient temperature for 3 minutes at 3 200 rpm. A suspended sample (0.1 mL) was aseptically applied to the surface of Mueller Hinton agar (Thermo-Fisher) 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 hours to culture aerobic bacteria. A second plate was cultured at 5% CO_2 at 37°C for 48 hours to culture anaerobic bacteria, and the third plate was cultured at 25°C for 48 hours to culture any possible yeasts.[30] This process was carried out in duplicate to ensure that no contamination of the samples had occurred during the spread plate process.

Colony morphology

Microbial colonies were isolated from the spread plates by means of the streak plate technique and incubated in the conditions mentioned previously. Each isolate was described according to: macromorphological characteristics; size as either large (1 - 3 mm), small (<1 mm) or granulated or tiny (pinpoint); pigment; and growth pattern, described as round, irregular, spreading, filamentous or rhizoid. The margins of the isolates were further classified as entire, undulate or filiform. The elevation of the isolates was classified as either flat, raised, convex, umbonate or irregular.[31] This was done to identify isolates that were similar to one another to assist in grouping them for further identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted using zone of inhibition measurements. Culture (0.1 mL) was applied to the surface

of the Mueller Hinton agar supplemented with 5% sheep blood.[32] Commercially prepared antibiotic disks (Oxoid, Thermo-Fisher), each of which were 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 µg), gentamicin (10 µg) and clindamycin (2 µg), which are included in the SA STGs. In addition, ciprofloxacin (5 µg) was included, as is the recommended antimicrobial by SAASP. Vancomycin (5 µg) was included to account for MRSA presence. Although this study focused on bacterial isolates, fluconazole (25 µg) was also included as a control to account for infection that may be of fungal origin. A blank disk with no antimicrobial activity was used as a negative control. Antimicrobial susceptibility testing was carried out in duplicate on all isolates. Zone of inhibition susceptibility measurements were then recorded and interpreted using the Clinical and Laboratory Standards (CLSI) guidelines as resistant, intermediate or susceptible.

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 selected for identification (National Health Laboratory Services Infection Control and Microbiology Laboratory, University of the Witwatersrand). Biochemical examination, which included starch hydrolysis, lipid hydrolysis, iron agar test, catalase test, indole production test and methyl red test, was used 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 API20C AUX system (Biomerieux, France).

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (ref. no. M210431), and the study was registered on the National Health Research Database (re. no. GP_202108_026). Approval of the study was obtained from both facilities.

Results

Antimicrobial susceptibility

From the micro-organisms cultured, it was found that 44 samples (86.3%) resulted in polymicrobial growth, and 7 samples (13.7%) in monomicrobial growth. A total of 445 micro-organisms were isolated from the 51 original wound samples. The average number of micro-organisms isolated per lesion was established at 2.97.

Antimicrobial susceptibility patterns (Fig. 1.) demonstrated that only 20.4% of micro-organisms were susceptible to amoxicillin/clavulanic acid, the first-line drug for SA treatment guidelines.[24] The majority of isolated micro-organisms (91.2%) were susceptible to gentamicin. Only 24% of the pathogens isolated were susceptible to clindamycin. In total, 76.8% of the pathogens were found to be susceptible to ciprofloxacin. A further 10.8% of isolated pathogens demonstrated intermediate susceptibility to vancomycin. 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. 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. Based on the analysis of the different groups, a total of 229 micro-organisms (51.5%) were Gram-negative, while the remaining 216 (48.5%) were Gram-positive. The most frequently isolated micro-organisms were *Proteus mirabilis* (19.3%), *S. aureus* (13.7%), *P. aeruginosa* (12.6%), *E. faecalis* (12.4%) and *E. coli* (12.1%). Less prevalent micro-organisms isolated from DFUs in this study included coagulase-negative *Staphylococci* (7.6%) and *Corynebacterium* species (4.7%). Of the 20.4% of micro-organisms found to be susceptible to amoxicillin/clavulanic acid, *S. aureus* accounted for the majority (13.7%). When looking at the susceptibility patterns found with ciprofloxacin, only *E. faecalis* demonstrated resistance out of the top five most isolated pathogens. *C. striatum* (accounting for 2.02% of causative pathogens) demonstrated complete susceptibility to vancomycin, highlighting the current poor choice of antimicrobial treatment for DFUs in the SA public healthcare sector.

Antibiogram

Table 1 is an antibiogram showing the most effective antimicrobial for each identified pathogen. From this Table it is clear that the most effective antimicrobial in the majority of causative pathogens is gentamicin. Amoxicillin/clavulanic acid, the mainstay in SA treatment guidelines, was only found to be the most effective antimicrobial against *S. cohnii,* which was the least frequently isolated pathogen (0.22%).

Past treatment practices and adherence to local guidelines

Table 2 describes the antimicrobial treatment protocols observed in the study population and compares the treatment practices observed to both local and international guidelines. It was determined that 47.9% complied to those set out in the SA guidelines, 14.6% were partially compliant and 33.3% were not compliant. It was further found that 60.4% of the treatment protocols implemented complied partially to international guidelines.

Discussion

In those patients who develop DFU infections, the cultures are generally polymicrobial in nature.[6,13,33] In this study, 86.3% of the cultures from DFU wound swabs were found to be polymicrobial. The results seen in this study are congruent with those seen in a study carried out in Malaysia, where 85% of patients presented with a polymicrobial infection.[34] In another study undertaken in Kuwait, polymicrobial infection was reported as occurring in 75% of patients.[35] A similar predominance of polymicrobial infection was seen in multiple studies in Pakistan, Oman and India.[33,36,37] Infections that are polymicrobial in nature are more complex to treat and are more likely to develop into chronic infections, making it important to treat them in the most effective manner.[13,38] In addition, within this study, the average number (three) of micro-organisms isolated per lesion was higher than the average reported number in other studies.[33,35,37] This could be attributed to multiple reasons specific to the SA context. Lack of access to basic amenities and general healthcare could be the causative factor. The impact of the COVID-19 pandemic cannot be overlooked, where patient adherence to wound care was drastically reduced because of severe lockdown restrictions.[39] Furthermore, in SA, a shortage of podiatrists and insufficient staff training are barriers to patient care, and contribute negatively towards wound progression.[40,41]

Worldwide, Gram-positive micro-organisms are mostly responsible for infection of DFUs. However, in tropical and developing countries,

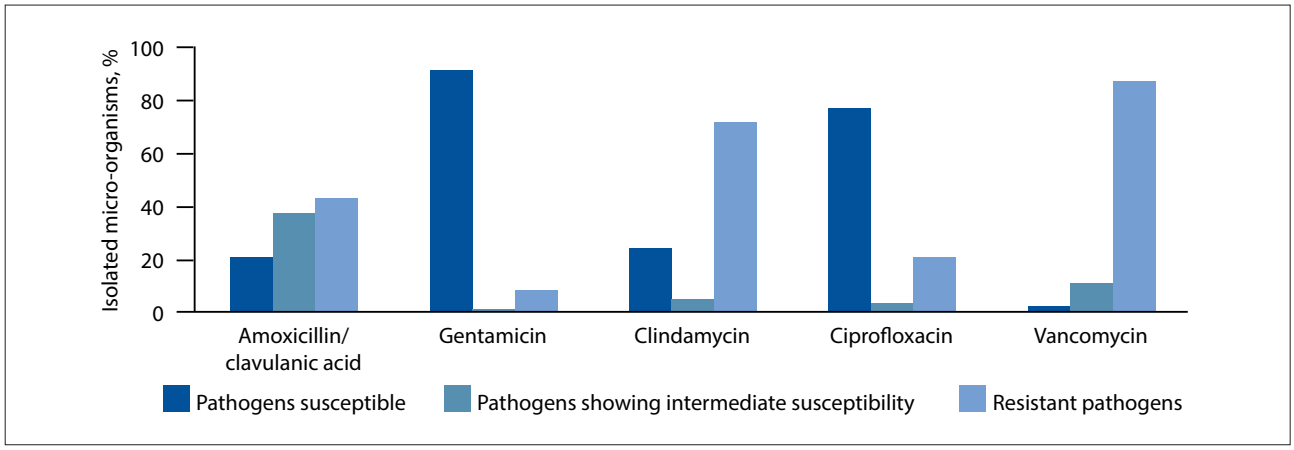

Fig. 1. The antimicrobial susceptibility patterns observed for all isolated pathogens.

Table 1. Antibiogram for all isolated and identified pathogens

Pathogen	Percentage prevalence (*n*=445)	Percentage of micro-organism susceptible to the antimicrobial (*n*=2)						Recommended antibiotic
		AMC	CN	DA	CIP	VAN	FLU	
Proteus mirabilis	19.3	39.5	94.2	3.5	69.7	1.1	0	Gentamicin
Staphylococcus aureus	13.7	55.7	100	72.1	93.4	0	0	Gentamicin
Pseudomonas aeruginosa	12.6	0	76.8	0	82.1	1.7	0	Ciprofloxacin
Enterococcus faecalis	12.4	27.3	65.5	29	30.9	10.9	0	Gentamicin
Escherichia coli	12.1	0	88.9	0	48.1	0	0	Gentamicin
Corynebacterium spp.	4.7	9.5	14.3	0	9.5	23.8	0	Vancomycin
S. epidermidis	3.8	11.7	100	70.6	82.4	0	0	Gentamicin
Enterobacter cloacae	2.9	0	92.3	0	61.5	0	0	Gentamicin
Streptococcus anginosus	2.9	53.8	69.2	53.8	53.8	38.5	0	Gentamicin
S. lugdunensis	2.5	0	100	90.9	90.9	9.1	0	Gentamicin
Bacillus species	2.0	22.2	100	11.1	55.6	33.3	0	Gentamicin
Corynebacterium striatum	2.0	22.2	22.2	33.3	0	66.7	0	Vancomycin
S. intermedius	1.6	57.1	71.4	85.7	85.7	42.9	0	Clindamycin/ciprofloxacin
Klebsiella oxytoca	1.4	0	100	0	66.7	0	0	Gentamicin
Citrobacter koseri	1.4	16.7	100	0	50	0	0	Gentamicin
Acinetobacter baumannii	0.7	33.3	66.7	0	100	0	0	Ciprofloxacin
Coagulase-negative Staphylococcus spp.	0.7	66.7	100	100	0	0	0	Gentamicin/clindamycin
S. haemolyticus	0.7	66.7	100	100	33.3	0	0	Gentamicin/clindamycin
Oligella urethralis	0.7	33.3	66.7	33.3	66.7	33.3	0	Gentamicin/ciprofloxacin
Group B Streptococcus spp.	0.7	66.7	0	100	0	0	0	Clindamycin
Gentamicin-methicillin resistant S. aureus	0.7	0	0	0	0	0	0	None
Shwanella putrifacians	0.5	0	100	50	100	0	0	Gentamicin/ciprofloxacin
S. cohnii	0.2	100	100	100	100	0	0	Amoxicillin/clavulanic acid/ gentamicin/clindamycin/ ciprofloxacin

AMC = amoxicillin/clavulanic acid; CN = gentamicin; DA = clindamycin; CIP = ciprofloxacin; VAN = vancomycin; FLU = fluconazole

Gram-negative micro-organisms are reported to be predominant.[42] In this study, the prevalence of Gram-negative micro-organisms (51.5%) was 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.[43] Similar results to this study were observed in a Cameroonian study 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.[44] 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 in 2019 in Bangladesh.[45] A meta-analysis examining the microbiology of diabetic foot infections found the prevalence of coagulase-negative *Staphylococci* to be 5.8%.[46] 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.[47,48]

The overall antimicrobial susceptibility trends seen in this study show that ciprofloxacin and gentamicin are the two best performing

Table 2. A comparison between observed treatment protocols and both local and international guidelines

Patient code	Date of current and previous treatments	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A001	21 April 2021	No	Amoxicillin/clavulanic acid (1 000 mg)	C	PC
A002	7 March 2021	No	Mupirocin (topical)	NC	NC
A003	12 March 2021	No	No	NC	NC
	20 August 2021	No	Systemic antibiotics (not specified) then amoxicillin/clavulanic acid (1 000 mg)	C	PC
A004	20 August 2021	No	Amoxicillin/clavulanic acid (1 000 mg)	C	PC
A005	6 August 2021	No	Amoxicillin/clavulanic acid (1 000 mg)	C	PC
A006	*	*	*	*	*
A007	1 April 2019	No	No	NC	NC
	2 September 2019	No	Cloxacillin	NC	NC
	30 July 2021	No	Amoxicillin/clavulanic acid (1 000 mg)	C	PC
A008	23 August 2021	No	Amoxicillin/clavulanic acid 1.2 g IV followed by Amoxicillin/clavulanic acid orally (1 000 mg)	C	PC
A009	21 June 2021	No	No	NC	NC
A010	17 February 2020	No	Amoxicillin/clavulanic acid (1000 mg) BD	C	PC
	11 August 2020	No	Amoxicillin/clavulanic acid 1.2 g IV for 7 days followed by amoxicillin/clavulanic acid orally (1 000 mg) daily for 3 days	C	PC
A011	13 July 2021	No	Amoxicillin/clavulanic acid 1.2 g IV TDS and clindamycin (600 mg) IV	C	PC
A012	3 June 2021	No	Amoxicillin/clavulanic acid (1 000 mg) BD; metronidazole (400 mg) BD	PC	PC
A013	13 September 2021	No	No	NC	NC
A014	*	*	*	*	*
A015	September 2021	No	No	NC	NC
A016	*	*	*	*	*
A017	7 December 2021	No	Amoxicillin/clavulanic acid (1 000 mg) BD	C	PC
A018	16 November 2021	No	Amoxicillin/clavulanic acid (250 mg) TDS	C	PC
A019	†	†	†	†	†
A020	20 December 2021	No	Clarithromycin (500 mg) BD	NC	NC
A021	February 2021	No	No	NC	NC
A022	25 - 31 December 2021	Unknown	Yes - IV antibiotics	Unknown	Unknown
A023	‡	‡	‡	‡	‡
A024	‡	‡	‡	‡	‡
A025	7 February 2022	No	Amoxicillin/clavulanic acid (500 mg) daily	C	PC
	29 December 2020	No	Clindamycin IV (600 mg) 8 hourly; amoxicillin/clavulanic acid IV 1.2 g 8 hourly for 10 days	PC	PC
A026	†	†	†	†	†
A027	3 March 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly and then azithromycin (500 mg) orally for 8 days	PC	PC
A028	22 February 2022	No	Clotrimazole (20 mg) BD, Fluconazole (200 mg) weekly (treatment regimen for onychomycosis)	N/A	N/A
	24 March 2022	No	Amoxicillin/clavulanic acid (1 000 mg) BD for 7 days and Mupirocin	PC	PC
A029	8 March 2022	Yes (Escherichia coli)	Piperacillin/tazobactam (4.5 g) IV 6 hourly Ertapenem (1 g) IV daily for 7 days	NC	C
A030	7 March 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly and then amoxicillin/clavulanic acid (600 mg) IV 8 hourly	C	PC
A031	7 March 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly	C	PC
A032	7 March 2022	No	No	NC	NC
A033	13 March 2022	Yes (*Streptococcus agalactiae, Peptostreptococcus anaerobis;* Gram-positive diplococci and Gram-positive cocci in chains)	Piperacillin/tazobactam (4.5 g) IV 6 hourly	NC	C

continued

Table 2. (continued) A comparison between observed treatment protocols and both local and international guidelines

Patient code	Date of current and previous treatments	Identified micro-organism	Antibiotic treatment regimen prescribed	Alignment to the STG or SAASP	Alignment to international guidelines
A034	9 March 2022	No	Amoxicillin/clavulanic acid (1 000 mg) orally TDS	C	PC
A035	3 March 2022	No	No	NC	NC
A036	18 March 2022	No	Amoxicillin/clavulanic acid (1 000 mg) orally BD	C	PC
A037	13 March 2022	No	Amoxicillin/clavulanic acid (1 000 mg) orally BD	C	PC
A038	26 February 2021	No	Amoxicillin/clavulanic acid 1.2 g IV TDS	C	PC
	12 March 2022	No	Ceftriaxone (250 mg) IMI STAT, azithromycin (1 g) orally daily, amoxicillin/clavulanic acid (1 000 mg) orally BD, metronidazole 1 g orally STAT	PC	C
A039	9 February 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly	C	PC
	21 March 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly	C	PC
A040	22 March 2022	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly then amoxicillin/clavulanic acid (1 000 mg) orally BD; mupirocin	PC	PC
A041	1 April 2022	No	Clindamycin IV (600 mg) BD; amoxicillin/clavulanic acid IV (600 mg) 8 hourly	PC	PC
A042	16 July 2016	No	No evidence of antibiotic use recorded in file	NC	NC
	10 June 2022	No	No evidence of antibiotic use recorded in file	NC	NC
A043	10 June 2022	No	No evidence of antibiotic use recorded in file	NC	NC
A044	23 August 2021	No	Amoxicillin/clavulanic acid 1.2 g IV 8 hourly then	C	PC
	17 June 2022	No	amoxicillin/clavulanic acid (1 000 mg) orally BD Amoxicillin/clavulanic acid 1.2 g IVI TDS	C	PC
A045	21 June 2022	No	Amoxicillin/clavulanic acid (1 000 mg) orally TDS	C	PC

*Patient file unaccounted for.
†New patient, no file history.
‡New file created and previous file lost due to Charlotte Maxeke Johannesburg Academic Hospital fire thus no file history.
STGs = Standard Treatment Guidelines; SAASP = South African Antibiotic Stewardship Programme; C = compliant; PC = partially compliant; NC = non-compliant; IV = intravenously; BD = twice daily; TDS = three times daily; IMI = intramuscular injection; STAT = immediately

antimicrobials against most pathogens isolated from the DFUs within this study. What is important to note from this is that the first-line treatment used in the SA public healthcare sector, amoxicillin/clavulanic acid, shows poor susceptibility against most 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.[49] In most Dutch hospitals, the empirical treatment recommended is ciprofloxacin and clindamycin.[50] Another study,[51] undertaken in India, found amikacin and gentamicin to be the most effective antimicrobials in DFU treatment. Similar to the results of this study, a study in Nigeria found that both ciprofloxacin and gentamicin exhibited good susceptibility patterns against DFU pathogens.[52] In contrast to the above-mentioned studies, a study undertaken in Pakistan established that Gram-positive isolates showed 53.7% resistance to ciprofloxacin, while Gram-negative isolates showed 74% resistance to ciprofloxacin.[33] 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.[53] Similarly, a study in Brazil found that a large proportion of *S. aureus* isolates showed resistance to ciprofloxacin (55.5%), and 43.5% of Gram-negative bacteria were resistant to ciprofloxacin.[54] The vastly different antimicrobial susceptibility results established in different regions of the world provide reasons as to why DFUs should be treated according to local epidemiological data and not solely on global trends.

International guidelines recommend that a polymicrobial infection of a DFU with both Gram-negative and Gram-positive micro-organisms should be treated empirically with amoxicillin/clavulanic acid, a second- or third-generation cephalosporin or a fluoroquinolone.[15,55] Both the STGs and the SAASP guidelines recommend amoxicillin/clavulanic acid as the mainstay in empirical antimicrobial treatment for DFUs. Our study shows that is not an appropriate choice for first-line treatment in SA, as it is only effective against 20.4% of the micro-organisms isolated. Furthermore, amoxicillin/clavulanic acid is not effective against *P. mirabilis*, *P. aeruginosa*, *E. faecalis* and *E. coli*, four of the top five most frequently observed pathogens in DFUs in this study. 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.[15,55]

P. aeruginosa was found the third-most isolated pathogen, and was responsible for 12.6% of DFU infection. Both the STGs and SAASP guidelines do not account for the presence of this pathogen in DFUs, and therefore infection with *P. aeruginosa* often results in a wound of chronic nature owing to its enhanced virulence and ability to produce biofilms.[56] International guidelines suggest either amoxicillin/clavulanic acid, penicillin with ceftazidime, penicillin with ciprofloxacin or a group two carbapenem for treating a wound infected by *P. aeruginosa*.

During the data collection phase of this study, a fire occurred at one of the study sites, which affected the podiatry unit and records store. Due to missing files, new patients with no file history, as well as files from existing patients that were lost on account of the fire resulted in seven patients (15.6%) who did not have comprehensive records of treatment protocols for previous or current DFUs. While the majority of patients were treated in a manner that adhered to the STGs 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. It was further found that 60.4% of the treatment protocols implemented complied only partially with international guidelines. A lack of local susceptibility data prevented further compliance with international guidelines owing to 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.[57] In contrast, an Australian study determined that 83% of patients received empirical antibiotic treatment that complied with national guidelines.[58] A study carried out in Poland yielding outcomes similar to our findings found that although there was good adherence to local prescribing guidelines, the guidelines themselves did not cover the most common pathogens responsible for DFU infection.[59] Only two patients had samples collected for microbiological testing and culture identification. The subsequent treatment protocols implemented for these patients were found to be non-compliant with local guidelines, but compliant with 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 a study carried out in India, it was found that 44% of patients had undergone culture and sensitivity testing within 48 hours of being admitted.[57] 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.

Conclusion

The susceptibility patterns observed in this study demonstrate that gentamicin and ciprofloxacin are more effective treatments for DFUs, rather than the recommended standard treatment of amoxicillin/clavulanic acid. 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. Furthermore, this study highlights the urgent need for updated DFU treatment protocols in the SA public healthcare sector, and as such, forms the basis of further research in this area.

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Author contributions. SdR and SvV designed the study. MT conducted the study and collected the data. SdR and SvV are co-supervisors to MT, as the study forms part of a postgraduate degree. TPM provided expert input into sample collection design. All authors contributed towards writing the manuscript and approved the final submission.

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