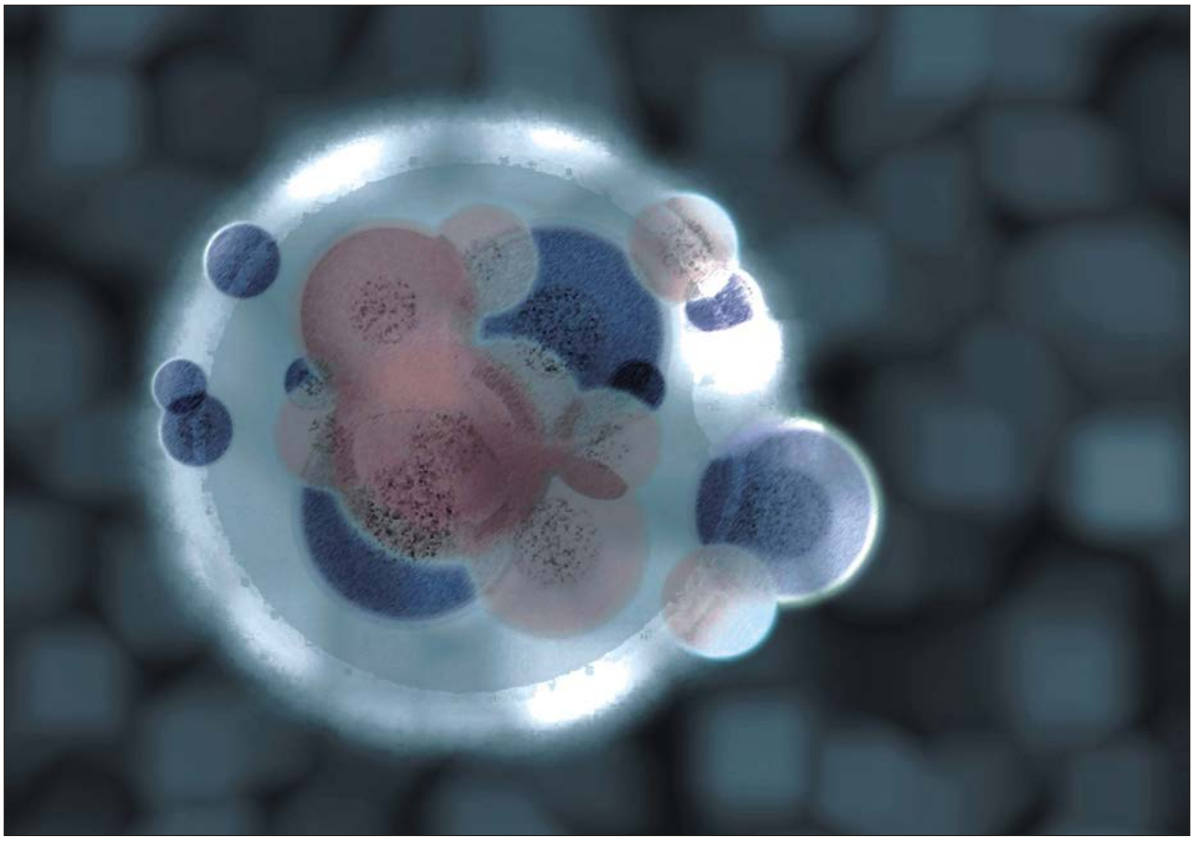

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Research Article
Antibiotic Susceptibility Patterns of Bacteria Recovered from Wounds of Diabetic Patients in Some Northern Kwazulu-Natal Hospitals, South Africa

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Abstract

Background and Objective: Antimicrobial susceptibility testing of bacteria recovered in diabetic wounds remains an important way of monitoring infection and the emergence of antibiotic-resistant bacteria. Early diagnosis of microbial resistance patterns is aimed to help institute the appropriate antibacterial therapy and improve the adverse effects of diabetic wound infection. This study aimed to determine the phenotypic resistance patterns of the bacteria isolated from the wounds of hospitalized diabetic patients. **Materials and Methods:** Antimicrobial disk diffusion assay was performed on 42 bacteria isolates using antibiotics of different classes. Variants were analysed using one-way ANOVA and two-way ANOVA through Graphpad prism version 6. **Results:** Multidrug resistant patterns were noted among different bacterial groups such as Enterococci (83%), Enterobacteriaceae (55%), Non-Enterobacteriaceae (50%), Staphylococci (43%) and Gram-positive rods (33%). The bacteria conferred resistance to penicillin (100%), ampicillin (91%), cefepime (60%), ceftazidime (55%) and gentamicin (52%). Hospital X's bacteria were found to be most resistant to erythromycin (80%) and ciprofloxacin (70%), while hospital Z's bacteria were most resistant to vancomycin (50%) and penicillin (50%), with Hospital Y's bacteria showing the most resistance to Imipenem (45%). *Proteus mirabilis* showed 86% resistance to Imipenem while *Klebsiella* spp. and *Escherichia coli* also exhibited resistance to important antibiotics. **Conclusion:** The noted high levels of antibiotic resistance and multi-drug resistance patterns, observed in the study are of grave concern as it limits treatment options thereby negatively impacting on the health and quality of life of the affected diabetic patients. It is therefore, imperative that these findings be taken into consideration during public health policy making and awareness programmes.

Key words: Antibiotic resistance, diabetic patients' wounds, multi drug resistance, disk diffusion assay, enzyme activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Despite the role played by antibiotics in managing bacterial infections, antibiotic resistance has become a serious international health drawback compromising the safety of the population[1]. Antibiotic resistance occurs when bacteria becomes insensitive to a drug to which it was once susceptible[2,3]. Resistance is the result of bacteria mutation and selection pressure from the irrational use of antibiotics[4]. The World Health Organization (WHO) has reported that, globally, there are very high statistics of antibiotic resistance and they continue to rise[3] and threaten the success of medical intervention at all levels of health care[5]. South Africa has made efforts to tackle antibiotic resistance by introducing several infection prevention and control training programs countrywide, however, these programs have not been implemented in some healthcare facilities[6] especially in deep-rural areas. The South African surveillance data verified that there is increasing resistance in all major infection causing bacteria[1].

Gram-negative bacteria are a major therapeutic challenge causing severe infections in hospital settings[7,8]. A number of critically ill patients particularly with sepsis are treated with antibiotics but clinical outcomes are not improving due to the emergency of multidrug-resistant bacteria that limit the choice for therapy[7,9]. The resistance conferred by bacteria such as Enterobacteriaceae (*Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus*) and Pseudomonas may be due the production of different β-lactamase enzymes that have activity against penicillin, 1st, 2nd, 3rd generation cephalosporin which has magnified antibiotic resistance in hospital settings, mainly in patients with wounds[10,11].

Diabetics with non-healing and slow healing wounds are prone to antibiotic-resistant bacterial infections due to inappropriate use of antibiotics and frequent hospitalization. Diabetics also suffer from peripheral arterial diseases which may lead to poor penetration of antibiotics into the lower limb tissues and promote selection of resistant bacterial strains[12-14]. Some bacterial strains have the ability to spread in clinical settings and cause cross infection which makes health care settings an emergence point of resistant bacterial phenotypes[8]. In some cases, this has resulted in worse outcomes such as death of patients[3].

In addition, bacteria can be intrinsically resistant to antibiotics through inherent structural and functional characteristics which result in absence or reduced access of the drug to its target[2,14]. For example, most diabetic wounds are inhabited by bacteria in a biofilm form which has been reported to limit antibiotic diffusion[2]. These bacteria are favored by invasive procedures or contaminating substrates in wound care that make them proliferate[5]. Bacteria can acquire resistance through horizontal gene transfer carried on plasmids, transposons and integrons by conjugation or mating and mutation[6,4,14]. Horizontal gene transfer has played a major role in transmission of the β-lactam antibiotics that has major contribution to resistance[15]. Diabetic wounds are thus increasingly inhabited by antibiotic-resistant bacteria[16].

Antimicrobial susceptibility testing is essential in providing treatment regimes that will help reduce morbidity and mortality in patients infected by bacteria[17]. Antimicrobial susceptibility testing *in vitro* is also used to characterize multidrug resistant bacteria[18]. The common microbiological method is the Kirby-Bauer disk diffusion method that determines the sensitivity or resistance of pathogenic bacteria to various antimicrobial agents[19]. The data obtained is important for compilation of surveillance reports on antimicrobial resistance which serve as guidance in antimicrobial therapy[17].

In a previous study conducted by Mthembu *et al*.[20] 42 bacteria isolated from the wounds of hospitalized diabetic patients and characterized through 16S rDNA have been reported. In this manuscript the antimicrobial susceptibility profiles of the bacteria isolates were also evaluated so as to assess the effectiveness *in vitro* of the conventional antibiotics that will be used to treat bacterial infections in diabetic patients.

MATERIALS AND METHODS

The details of the materials and methods used in the isolation and characterization of the bacteria have been previously described by Mthembu *et al*.[20]. Briefly wound specimens of 18 (22% male and 78% female) randomly selected hospitalized diabetic patients were collected from three rural-based Northern KwaZulu-Natal hospitals (named X, Y and Z) in South Africa between 2015 and 2016. To collect the wound specimen medical doctors firstly cleaned the wound with sterile saline and sterile cotton pads, after which they then introduced sterile swabs at the base of the wound and subsequently inserted the swab into Amies transport media to maintain the specimen during transportation to the University of Zululand's Biochemistry laboratory.

Antibiotics: All the antibiotic discs were supplied by Polychem (OXOID). Nine antibiotics representing different antibiotic classes were selected and are as shown in Table 1.

Antibiotic susceptibility test: Antibiotic susceptibility testing of the bacteria isolates was done according to the Bauer *et al*.[21] method as recommended by the Clinical Laboratory Standard Institute (CLSI)[22]. The test isolates were grown on

Mueller-Hinton broth overnight for 18-24 h at 37°C after which turbidity of the suspension was adjusted to match 0.5 McFarland's standard. A volume of 100 µL of the diluted suspension was inoculated into Mueller-Hinton agar containing plates and streaked with sterile swabs. Antibiotic disks were tested against different bacterial groups such as *Staphylococci*, Enterococci, Gram-negative bacilli from Enterobacteriaceae, non-Enterobacteriaceae Gram-negative bacilli and Gram-positive rods. The antibiotic disks were evenly placed on plates aseptically and incubated for 18-24 h overnight at 37°C. The choices and interpretive standards of antibiotics were based on the CLSI[23] guideline. The zones of inhibition of the bacteria were measured and classified into three groups: resistant, intermediate and susceptible according to the CLSI[23] guideline. Multidrug-resistant patterns were assessed whereby, bacteria identities resistant to two or more classes of antibiotics were recorded as multi-drug resistant[18].

Table 1: Antibiotics used in the study and their respective classes

Antibiotic class	Antibiotics
β-lactams	Penicillin (10 units)
	Ampicillin (10 µg)
	Ceftazidime (30 µg)
	Cefepime (30 µg)
	Imipenem (10 µg)
Glycopeptides	Vancomycin (30 µg)
Aminoglycosides	Gentamicin (10 µg)
Fluoroquinolones	Ciprofloxacin (5 µg)
Macrolides	Erythromycin (15 µg)

Statistical analysis: Variants were analyzed using one-way ANOVA and two-way ANOVA through Graphpad prism version 6, whereby graphs and figures were constructed at a 95% confidence level unless stated otherwise.

RESULTS

Antibiotic susceptibility test: The 5 groups of bacteria showed varying susceptibility patterns as shown in Table 2. Multidrug resistance patterns were noted among the different groups, Enterococci (83%), Enterobacteriaceae (55%), Non-Enterobacteriaceae (50%), Staphylococci (50%) and the Gram-positive rods (33%). Table 3 shows the percentage resistance observed for each bacteria group against an individual antibiotic. Resistance percentages of 100% were noted among the Staphylococci and Enterococci group against the antibiotic penicillin, while non-Enterobacteriaceae and the Gram-positive rods exhibited 100% resistance against ampicillin. The highest resistance was observed against β-lactams in all groups. Table 4 shows the overall susceptibility patterns of each individual antibiotic against all the bacteria across the different groups. It is of interest that a 100 and 91% resistance against penicillin and ampicillin was noted, respectively. Antibiotic resistance was further evaluated in each of the hospitals under study to obtain the percentage resistance of the bacteria recovered against each individual antibiotic and the results are presented in Fig. 1. Bacteria from

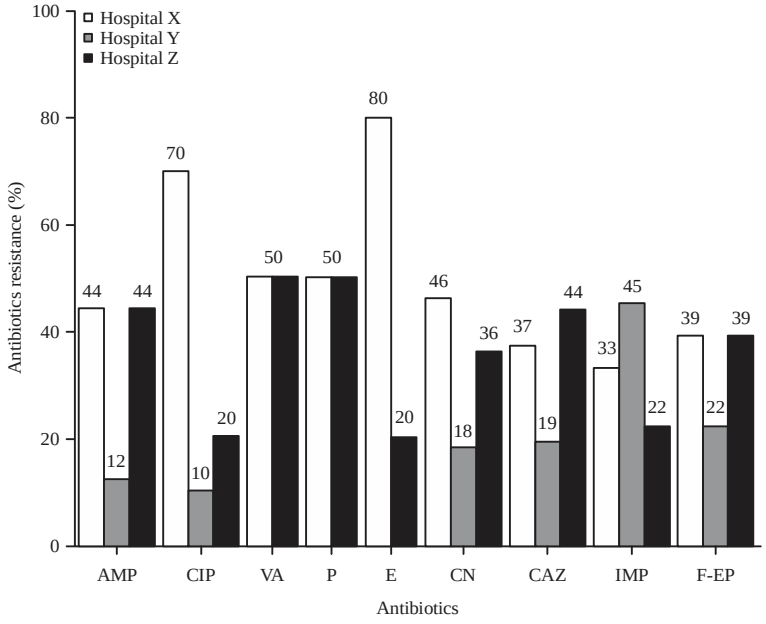

Fig. 1: Antibiotic resistance percentages (for each antibiotic) against bacteria from the different hospitals. AMP: Ampicillin, CIP: Ciprofloxacin, CAZ: Ceftazidime, VA: Vancomycin, P: Penicillin, IMP: Imipenem, CN: Gentamicin, E: Erythromycin, F-EP: Cefepime

Table 2: Antibiotic sensitivity profiles of the bacteria

		Antibiotic sensitivity profiling								
Codes	Identities	AMP	CIP	CAZ	VA	P	IMP	CN	E	F-EP
Staphylococci group										
S1	*Staphylococcus aureus*	-	S	-	I	R	-	S	S	-
S2	*Staphylococcus sciuri*	-	I	-	I	R	-	R	I	-
A2	*Staphylococcus aureus*	-	S	-	R	R	-	S	I	-
S4	*Staphylococcus aureus*	-	R	-	I	R	-	R	I	-
S6	*Staphylococcus aureus*	-	S	-	I	R	-	S	S	-
N/S 4	*Staphylococcus aureus*	-	S	-	I	R	-	S	S	-
S5	*Staphylococcus epidermidis*	-	R	-	I	R	-	R	R	-
Enterococci group										
A3	*Corynebacterium striatum*	R	R	-	R	R	-	S	R	-
A4	*Enterococcus faecalis*	R	R	-	R	R	-	R	R	-
St1	*Enterococcus faecalis*	S	R	-	I	R	-	R	I	-
ST2	*Enterococcus faecalis*	R	R	-	I	R	-	R	R	-
P1	*Enterococcus faecalis*	R	I	-	R	R	-	R	R	-
E1	*Desemziaincerta*	R	S	-	S	R	-	I	I	-
Enterobacteriaceae group										
P2	*Proteus mirabilis*	R	S	R	-	-	S	S	-	R
P3	*Proteus mirabilis*	R	S	R	-	-	R	R	-	R
P4	*Proteus mirabilis*	R	I	S	-	-	R	R	-	R
S3	*Proteus mirabilis*	I	S	R	-	-	R	S		R
SS1	*Proteus mirabilis*	R	S	R	-	-	R	S	-	R
SS2	*Proteus mirabilis*	I	S	R	-	-	R	R	-	R
E3	*Proteus mirabilis*	R	S	R	-	-	R	R	-	R
PP1	*Citrobacterkoseri*	R	S	S	-	-	S	S	-	I
PP2	*Klebsiellaoxytoca*	R	S	S	-	-	I	S	-	S
A5	*Klebsiellaoxytoca*	R	I	R	-	-	R	R	-	R
A1	*Klebsiellapneumoniae*	R	S	R	-	-	I	R	-	R
NEE3	*Klebsiellapneumoniae*	R	I	R	-	-	S	R	-	R
NE2	*Klebsiellapneumoniae*	R	S	S	-	-	S	S	-	S
NEE4	*Klebsiellapneumoniae*	R	S	S	-	-	S	S	-	S
B1	*Klebsiellapneumoniae*	R	S	I	-	-	S	S	-	S
NE1	*Morganellamorganii*	R	R	S	-	-	S	R	-	S
NE4	*Escherichia coli*	R	R	R	-	-	I	R	-	R
NEE6	*Escherichia coli*	R	R	I	-	-	S	R	-	R
NEE2	*Enterobacterxiangfangensis*	R	S	S	-	-	S	S	-	S
NEE5	*Enterobacterxiangfangensis*	R	I	R	-	-	I	R	-	R
Non-Enterobacteriaceae bacilli group										
NS 1	*Aeromonashydrophila*	R	S	S	-	-	S	S	-	S
NS 2	*Aeromonashydrophila*	R	S	S	-	-	S	S	-	S
NS 3	*Rhizobium radiobacter*	R	S	S	-	-	S	S	-	S
NP3	*Pseudomonas aeruginosa*	R	S	S	-	-	R	R	-	S
E2	*Janthinobacterium* sp.	R	R	R	-	-	R	R	-	R
B2	*Acinetobacterbaumannii*	R	I	R	-	-	S	R	-	R
Gram-positive rod group										
NE3	*Bacillus* sp.	R	S	R	-	-	S	S	-	R
NP1	Bacillus sp.	R	S	R	-	-	S	R	-	R
NP2	Bacillus sp.	R	I	R	-	-	I	I	-	R

-: Means not tested, S: Susceptible, I: Intermediate, R: Resistant, AMP: Ampicillin, CIP: Ciprofloxacin, CAZ: Ceftazidime, VA: Vancomycin, P: Penicillin, IMP: Imipenem, CN: Gentamicin, E: Erythromycin, F-EP: Cefepime

Hospital X showed the highest resistance patterns against erythromycin (80%) and ciprofloxacin (70%), while bacteria from hospital Z showed the highest resistance at 50% against vancomycin and penicillin. Hospital Y bacteria was most resistant to imipenem (45%). The data shown to be significantly different ($p<0.0001$).

DISCUSSION

This study revealed very high levels of antibiotic resistance of up to a 100% among the different bacterial species isolated from the wounds of diabetic patients, this was noted mainly within the β-lactam class of antibiotics (Table 3)

of which, some are among the first line of treatment given in the event of infection. This therefore limits the treatment options, further compromising the health and quality of life of the patients as diabetic wounds are generally slow healing in nature. This also corresponds to Zubair *et al.*[12] and Shahi *et al.*[13] findings which allude that the burden of antibiotic-resistant bacteria has become common in diabetic patients with wounds. Multidrug resistance patterns were also noted in the study with the highest percentage being against the *Enterococci* group (Table 2). The *Enterococci* spp. have been reported to be resistant to multiple antibiotics in clinical settings, with various resistant mechanisms being reported against all antimicrobial agents in clinical practice[24]. This indicates the pressure imposed on diabetics who are contaminated by multidrug resistant bacteria and become immuno-compromised[1]. The other clinical challenge of antibiotic resistance is the decline in efficacy of antibiotic treatment, with the dry pipeline of developing new antibiotic agents which threatens the provision of prompt treatment[4].

Table 3: Resistance of different bacteria groups against antibiotics

Bacteria Group	Antibiotic	Resistance (%)
Staphylococci	Ciprofloxacin	33
	Vancomycin	17
	Penicillin	100
	Gentamicin	50
	Erythromycin	17
Enterococci	Ampicillin	83
	Ciprofloxacin	67
	Vancomycin	50
	Penicillin	100
	Gentamicin	67
	Erythromycin	67
Enterobacteriaceae	Ampicillin	95
	Ciprofloxacin	15
	Ceftazidime	55
	Imipenem	35
	Gentamicin	55
	Cefepime	65
Non-Enterobacteriaceae	Ampicillin	100
	Ciprofloxacin	17
	Ceftazidime	33
	Imipenem	33
	Gentamicin	50
	Cefepime	33
Gram-positive rods	Ampicillin	100
	Ciprofloxacin	0
	Ceftazidime	100
	Imipenem	0
	Gentamicin	67
	Cefepime	100

The bacteria were mostly resistant to β-lactam (penicillin, ampicillin, cefepime and ceftazidime) and aminoglycoside (gentamicin) (Table 3, 4). The observed resistance may be exhibited by bacterial cell walls (β-lactam) and ribosomal proteins (gentamicin). β-lactams are bactericidal and inhibit the cell wall synthesis of different organisms[25]. Penicillin is most active against Gram-positives while ampicillin is an excellent broad-spectrum antibiotic[26]. Cephalosporins and carbapenems are active against Gram-negatives[27] however, in this study most bacteria were resistant to these antibiotics. Bacteria can protect themselves against β-lactams through alteration in penicillin binding proteins (PBPs) that reduce the affinity of β-lactams to the sites of action and the production of β-lactamases that are able to hydrolyze the β-lactam ring[28]. The frequent use of β-lactams as the first line of treatment has played a major role in the development of antibiotic resistant bacteria[29]. As a result, infections caused by β-lactam resistant organisms have increased over the years[27]. To make matters worse, penicillin is still being prescribed without considering its effects on resistance[30].

The declining effectiveness of antibiotics is believed to be also driven by mismanagement of antibiotics[31] in hospitals where most infection causing bacteria are isolated[1]. In the study, hospital X was implicated in resistance against ciprofloxacin, a broad spectrum bactericidal antibiotic that is a last line of defense in antibiotic treatment[26,32] and erythromycin, which is considered bacteriostatic and targets gram-positives [26,33]. Resistance to quinolones can be a severe clinical problem due to their importance in health[34]. Therefore, the use of ciprofloxacin needs to be well monitored. Bacteria

Table 4: Overall antibiotic susceptibility patterns

Mode of Action	Antibiotic class	Antibiotics	Resistance (%)	Intermediate (%)	Susceptibility (%)
Inhibition of peptidoglycan synthesis	B-Lactam	Ampicillin (n = 35)	91	6	3
		Penicillin (n = 12)	100	0	0
		Ceftazidime (n = 29)	55	7	38
		Cefepime (n = 30)	60	3	37
		Imipenem (n = 30)	33	17	50
Inhibition of the DNA gyrase and topoisomerase IV activity	Fluoroquinolones	Ciprofloxacin (n = 42)	24	19	57
Disruption of the peptidoglycan cross-linkage	Glycopeptides	Vancomycin (n = 12)	33	58	9
Inhibition of the protein synthesis at the 30S ribosomal unit	Aminoglycosides	Gentamicin (n = 42)	52	5	43
Inhibition of the protein synthesis at the 50S ribosomal unit	Macrolides	Erythromycin (n = 12)	42	42	16

n: number of organisms tested, Mode of action (CLSI[25])

resistant to ciprofloxacin can mediate resistance through mutations that alter the drug targets or reduce drug accumulation and develop plasmids that protect bacterial cells from the lethal effect of quinolones[32,34] while erythromycin and vancomycin can be resisted through methylation of bacterial ribosomes altered cell-wall precursors, respectively[33], which all compromise the available treatment and increase the burden of antibiotic resistance.

Enterobacteriaceae showed varying susceptibility patterns, with 55% resistance to antibiotics. It has been reported to be resistant to a number of antibiotics through the production of β-lactamase[10,27]. All *Proteus mirabilis* (*P. mirabilis*) (predominant) isolates were shown to be susceptible to ciprofloxacin while 86% was resistant to Imipenem. Imipenem resistance has been reported to be natural in *P. mirabilis*[35]. Imipenem is normally the last line of treatment in extended-spectrum β-lactamase producing organisms[10,35]. Some multidrug resistant strains of *P. mirabilis* produce these enzymes[9,29]. Among the *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* species, multidrug resistant patterns were observed against β-lactams, ciprofloxacin and gentamicin in which resistance has been previously reported by Iredell *et al.*[36]. The ability of *Klebsiella* species to rapidly spread in the hospital environment contributes to their resistance which has been closely associated with the production of extended-spectrum β-lactamases (ESBL), mostly mediated by plasmids[37]. In 2011 *K. pneumoniae* was documented with the most extreme cases of multidrug resistant bacterial infection in South Africa[1]. This emphasizes the importance of routine susceptibility testing before treatment is prescribed[14].

The resistance observed on skin commensals, *Staphylococcus epidermidis* and *Corynebacterium striatum*, is normally experienced in patients receiving treatment through antibiotics, especially the broad-spectrum[38] such as ciprofloxacin and gentamicin. *Enterococcus faecalis*, also regarded as normal skin flora[39], showed multidrug resistant patterns with one species (*Enterococcus faecalis* (A4)), showing resistance to all test antibiotics. The resistance shown by the normal skin flora poses a threat to other sensitive bacteria and patients[38]. Kiser *et al.*[26], reported that susceptibility testing of bacteria from an anatomic site for which they are found should be avoided because results may encourage the physician to treat normal conditions. The problem is that skin microflora has become resistant to the current treatment[40], as evidenced by their multidrug-resistant patterns recorded in the study. Treatment in immune compromised patients such as diabetics where bacteria are most likely to become pathogenic is now a challenge.

CONCLUSION

These findings show that it is important to evaluate possible resistance patterns of bacteria since some bacterial strains have become resistant to current treatment. The resistance conferred by Gram-negative bacilli and some skin commensals pose a threat to antibiotics used in the treatment of wound infection and may lead to a public health crisis.

SIGNIFICANCE STATEMENT

This study discovers the antibiotic resistance patterns of some bacteria isolated from wounds of diabetic patients in some Northern KwaZulu-Natal hospitals in South Africa, which can provide beneficial information for policy makers in the health care system and for public health awareness programmes. This study will help the researcher to uncover the critical areas of antibiotic resistance in rural based hospitals in South Africa that many researchers were not able to explore. Thus evidence is now available in literature revealing the presence of multidrug resistant bacteria inhabiting the wounds of hospitalized diabetic patients in these hospitals.

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