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Bacteriological Profile of Diabetic Foot Infections in a Teaching hospital in Saudi Arabia

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The microbiological characteristics of diabetic foot infections (DFIs) have not been extensively studied in Saudi Arabia (SA). In addition, there are no data on the frequency of multidrug-resistant organisms (MDROs) causing DFIs in SA. This study investigated and evaluated the bacteriology of DFIs and their resistance patterns to antibiotics that will be helpful to manage DFIs appropriately. The microbiological records of infected DF wounds for patients treated at an University Hospital in Riyadh from December 2009 until December 2011 were retrospectively reviewed. A total of two hundred and sixty-eight positive samples from patients with DFIs were reviewed during the study period. A total of 308 isolates were detected. A pure growth of single bacteria from culture was detected in 231 specimens (86.2%). However, polymicrobial growths were detected only in 37 samples (13.8%). Gram-negative bacilli were more prevalent (65.6%) than Gram-positive cocci (34.4%). However, the most frequently isolated pathogens were *S.aureus* (21.4%), followed by *Pseudomonas* spp. (13.3%) and *E.coli* (9.7%). Thirty-three percent of *S.aureus* were methicillin-resistant *Staphylococcus aureus* (MRSA) and 19.4% of *Enterobacteriaceae* species were extended-spectrum beta-lactamase producers. *S. aureus* and *P. aeruginosa* were the most common causes of DFIs. Approximately 36% of patients with DFIs were infected by MDROs.

Key words: bacteria, antibiotic sensitivity, multi-drug resistant organisms, ESBL, MRSA.VRE.

The prevalence of diabetes in some Arab countries ranks among the top 10 prevalence's worldwide.[1]A recent report showed an increase in the prevalence of diabetes mellitus of up to 30% in Saudi Arabia(SA).[2] The role of diabetes mellitus as a major risk factor for the development of foot infections is well established. The prevalence of diabetic foot (DF) problems varies among different countries in the world. In the Arab world, including SA, several factors make the prevalence of DF higher than in the West (e.g., weather and footwear).[1, 3]Studies have suggested that 2.5% of diabetic patients develop DF each year, and 15% of these patients develop DF at some point during their life.[4] In SA, DF was prevalent in 13.5% of the diabetic patients referred to the nephrology clinic.[5] DF is the most frequent cause of hospitalization for patients with diabetes, representing up to 25% of all diabetic hospital admissions.[6]Also, it is the most common cause of non-traumatic lower limb amputation[7]and precedes 85% of such cases.[4]The rate of mortality is higher in patients with DF and is approximately twice the rate of diabetic patients without DF.[7] Therefore, systemic antibiotic treatment must be administered as early as possible for DF infections (DFIs). Initially DFIs were treated empirically based on the clinical knowledge of the treating physician and on the prevalence of the microbial pattern in the locality and the hospital.

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The patterns of microbes infecting DF wounds have been studied widely.[8-23] However, the results have been varied and have often been contradictory, suggesting that area-specific studies should be conducted to assess the problem of DFI and institute effective treatments.

Diabetic patients with foot ulcers have several factors that may be associated with a high risk of multidrug-resistant organisms (MDROs) carriage and infections. Several studies have found that the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in diabetic wounds ranges from 16–56 % of isolated *S. aureus*.[8,12,15-18,20,21,23-26] MDROs were positive in 35.2% of patients with DFIs.[27] Extended-spectrum beta-lactamase (ESBL) production was noted in 44.7 % of gram-negative bacilli[25] and 56% of *E. coli* isolated from DFIs.[12] Infection with MDROs may increase hospital stay durations, costs, and additional morbidity and mortality.[27]

Knowledge of the bacteriological profile of DFIs in our community will guide health professionals to manage foot ulcers.[1,3] However, few reports have focused on that problem in the Arab countries. To the best of our knowledge, no studies that focus on patterns of DFIs have been reported from our region, except for one older study conducted in 2000.[8] Although increasing antimicrobial resistance is a pertinent problem in SA,[28-31] there are no data related to the frequency of MDRO infections, including ESBL-producing bacteria and MRSA among DFIs in this region.

The aim of this study is to investigate the bacterial spectrum responsible for DFIs, analyze their antibiotic susceptibility patterns, and determine the frequency of MDROs, including ESBL-producing bacteria and MRSA isolated from DFIs. Our results will be fruitful for taking appropriate measures to manage these infections.

METHODS

This study is a retrospective study reviewing the laboratory records of all positive results from DF samples from all patients presented with infected diabetic foot wounds seen as out or inpatients during the study period at King Khalid University Hospital (KKUH). This hospital provides primary and secondary care services for patients from the northern Riyadh area. It also provides tertiary care services to all on referral bases.

The microbiological records of infected DF wounds were reviewed during the period of December 2009 to December 2011. The information regarding the age, sex, diagnosis, nature of the specimen, examination required, and diagnosis, as well as type of bacteria isolated present and antibiotic susceptibility were obtained from department of Microbiology laboratory information system (LIS). Patients with foot infections due to any other causes such as non diabetics - post traumatic, arterial disorder alone, venous disorder alone, non diabetic peripheral neuropathy and secondary to implant infection were excluded. Only clinically infected diabetic wounds and only non-duplicate isolates were included in the study.

All specimens were Gram-stained , and the bacteria were isolated by inoculation of specimens on a set of selective and non-selective media such as blood agar MacConkey agar and 5% BA supplemented with vitamin K1 (1 g/ml), haemin (5 g/ml) and gentamicin (75 g/ml) (GBA) in addition to Robertson's cooked meat medium (RCM). All of the inoculated plates were incubated under the appropriate atmospheric conditions for 24-48 h. Bacteria were identified to the species level using an automated system (MicroScan Walkaway, Siemens) or by using API . The antimicrobial susceptibility testing was performed using an automated system (MicroScan Walkaway, Siemens) and confirmed by the disk diffusion method or E-Test. All procedures were performed according to the manufacturer's directions. Organisms were identified, tested and antimicrobial susceptibility testing results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[32]MDROs were defined as methicillin resistant *S. aureus* (MRSA), vancomycin resistant Enterococci (VRE) and, bacteria producing extended spectrum beta-lactamase (ESBL) and, *P. aeruginosa* resistant to both ceftazidime and imipenem, and Acinobacter baumannii resistant to imipenem. In additions to GNB resistant to three or more classes of antimicrobials.

Detection of extended spectrum Beta-lactamase

The integrated Lab- Pro version 1.12 that includes the Alert expert system uses growth in the presence of cefpodoxime (4 g/ml) and ceftazidime (1

g/ml), i.e., at concentrations recommended by the CLSI for ESBL screening,[32] as primary indicators for possible ESBL production. MICs obtained for ceftriaxone, cefotaxime, and aztreonam are interpreted according to CLSI breakpoints,[32] and results may also trigger rules which alert users to possible ESBL production. These results were considered a positive ESBL screening result. Screening with this system is limited to *E. coli, K. pneumoniae, K. oxytoca*, and *Proteus* i.e., those species that are primarily dealt with in the CLSI guideline. Other *Enterobacteriaceae* isolates which commonly harbor AmpC enzymes but additionally may produce ESBLs, such as *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp may also produce a positive screening result.

A positive ESBL screening result was confirmed using three ESBL E test strips (AB Biodisk, Solna Sweden) containing ceftazidime, cefotaxime and cefepime with and without clavulanate, according to the manufacturer's recommendations. ESBL production was determined by a 3 twofold concentration decrease in any minimal inhibitory concentration (MIC) of cefotaxime, ceftazidime, or cefepime combined with clavulanate versus its MIC when tested alone.[32]

This study was approved by Institutional Review Board (IRB) committee of KKUH and the College of Medicine.

Statistical analysis

Statistical analysis was performed by the Chi-square test using SPSS 19.0 statistical software (SPSS Inc. Wacker Drive, Chicago, IL USA) and a *p* value of either equal to or less than 0.05 was considered statistically significant. The percentage and the mean values were applied to determine the significance, and used where applicable.

RESULTS

A total of 268 positive samples collected from patients with DFIs were reviewed in this study. Males were predominant and significantly higher than in females (M=72.4%, F=27.6% and

Table 1. The distribution of etiological agents isolated from diabetic foot infections

	Number of Microbial Etiology				Frequency%		Proportion%	
	Mono*	Poly*	Total	MRO	Total	MRO	Total	MRO
Gram negative bacteria(GNB)								
Eschericia coli	24	6	30	16	11.2	6	9.7	5.2
Klebsiella	16	13	29	8	10.8	3	9.4	2.6
Proteus	9	1	10	2	3.7	0.7	3.3	0.6
Morganella	9	3	12	4	4.5	1.5	3.9	1.3
Enterobacter	17	8	25	7	9.3	2.6	8.1	2.3
Citrobacter	9	5	14	4	5.2	1.5	4.6	1.3
Serratia	12	2	14	1	5.2	0.4	4.6	0.3
Pseudomonas	30	10	40	9	14.9	3.4	12.9	2.9
Acinetobacter	16	6	22	19	8.2	7	7.1	6.2
Other gram negative	6	0	6	2	2.2	0.7	1.9	0.6
Total of GNB	148	54	202	72	75.4	26.8	65.6	23.3
Gram positive bacteria (GPB)								
Staphylococcus aureus	55	11	66	22	24.6	8.2	21.4	7.1
Streptococcus spp	9	8	17	0	6.3	0	5.5	0
• Strep group A	2	1	3	0	1.1	0	0.9	0
• Strep group B	5	5	10	0	3.7	0	3.3	0
• Strep group F	1	2	3	0	1.1	0	0.9	0
• Strepto mitis/oralis	1	0	1	0	0.4	0	0.3	0
Enterococcus Spp	19	4	23	2	8.5	0.8	7.5	0.7
• *E .faecalis*	16	4	20	0	7.4	0	6.5	0
• *E. faecium*	3	0	3	2	1.1	0.7	1	0.7
Total OF GPB	83	23	106	24	39.5	9	34.4	7.8

*= Microbial Etiology

Frequency = % of the organism to the total number of patients (268). Proportion = % of isolate to the total number of isolated organisms(308)

P<0.001). The mean age of cases was 59.6 years.

The 268 specimens yielding pathogens included 199 wound swabs, 49 tissue (including 2 bone samples) and 20 pus specimens .A total of 308 isolates were detected from 268 those different specimens, with an average of 1.2 bacteria per sample. 231 specimens (86.2%) have pure growth of single isolate in culture. However, polymicrobial growths were detected only in 37 samples (13.8%). Among these 231 specimens grew only single isolate, *S. aureus* was the most predominant isolate being recovered from 55 (23.8%) patients followed by *Pseudomonas* species from 30 (13%)and *Ecoli* from 24 (10.4%). However, among 37 specimens which grew mixed culture of two or more isolates, *Klebsiella* species were the most commonly isolated pathogen and detected in 13 (35%) patients, followed by *S. aureus* in 11(29.7%) and *Pseudomonas* species in 10 (27%). The details of the organisms isolated from different DFIs and specimens have been tabulated in Table-1 and 2.

Gram-negative bacteria (GNB) were isolated more frequently and statistically significant than gram positive bacteria (GPB) among different DF specimens (swabs P<0.0001, and tissue P=0.0046) with exception of pus samples, where GPB were more predominant than GNB, but were not statistically significant (65% vs. 45%, P=0.6217).*S. aureus* was the most predominant isolate being recovered from half of the pus samples. The distributions of isolated bacteria among different specimens were shown in Table-2.

Among the 268 DF specimens, 96 (35.8%) isolates were MDROs. Among GNB, the rate of

Table 2. Comparative frequency of bacteria by specimen collection type

	Total number268*	Swab199*	Tissue49*	Pus20*
Number of Specimens growing				
Pure culture =One bacterium	231(86.2%)	169(84.9%)	44(89.8%)	18(90%)
Mixed culture	37(13.8%)	30 (15.1%)	5(10.2%)	2(10%)
Two bacteria	34	27	5	2
Three bacteria	3	3	0	0
Total of isolated bacteria	308	199	54	22
Gram negative bacteria**	202 (75.4%)	155(77.9%)	38(77.6%)	9(45%)
Eschericia coli	30 (11.2 %)	24 (12.1%)	4 (8.2 %)	2 (10 %)
Klebsiella spp	29 (10.8 %)	26 (13.1%)	1 (2%)	2 (10 %)
Proteus spp	10 (3.7 %)	5 (2.5%)	4 (8.2 %)	1 (5%)
Morganella morganii	12 (4.5 %)	7 (3.5 %)	5 (10.2%)	0
Enterobacter spp	25 (9.3 %)	16 (8 %)	9 (18.4%)	0
Citrobacter spp	14 (5.2 %)	8 (4 %)	3 (6.1%)	3 (15%)
Serratia marcescens	14 (5.2 %)	8 (4 %)	5 (10.2 %)	1 (5%)
Pseudomonas spp	40 (14.9%)	38 (19 %)	2 (4.1%)	0
Acinetobacter baumannii	22 (8.2 %)	19 (9.6 %)	3 (6.1 %)	0
Other gram negative	6 (2.2 %)	4 (2 %)	2 (4.1%)	0
Gram positive bacteria	106 (39.6%)	77 (38.7%)	16 (32.7%)	13 (65%)
Staphylococcus aureus (SA)	66 (24.6%)	46 (23.1%)	10 (20.4%)	10 (50%)
• Methicillin Susceptible SA	44 (16.4%)	29(14.5%)	8 (16.3%)	7 (35%)
• Methicillin Resistant SA	22 (8.2%)	17(8.5%)	2 (4.1%)	3 (15%)
Streptococcus spp	17 (6.3%)	14 (7%)	1(2%)	2(10%)
• group B Streptococci	10 (3.7%)	8 (4%)	1 (2%)	1 (5%)
• Other streptococcus spp	7 (2.6%)	5 (2.5%)	0	1 (5%)
• Strep group A	3 (1.1%)	2 (1%)	0	1 (5%)
• Strep group F	3 (1.1%)	3 (1.5%)	0	0
• Strepto mitis/oralis	1 (0.4%)	1 (0.5%)	0	0
Enterococcus Spp	23 (8.6%)	17 (8.5%)	5 (10.2%)	1 (5%)
• *E .faecalis*	20 (7.5%)	15(7.5%)	4 (8.2%)	1 (5%)
• *E. faecium*	3 (1.1%)	2(1%)	1 (2%)	0

* number of specimen ** number of isolated bacteria

resistance was found to be 26.8% of patients and 23.3% of isolates, Gram negative multi-drug resistant bacteria to more than 3 antibiotics classes were 66 isolates including 2 isolates were *Stenotrophomonas maltophilia* . Among 26 of ESBL producing enterobacteria , 6 were only ESBL producers. Gram positive multi-drug resistant bacteria were isolated in 24 cases (9%), comprising 22 (8.2%) cases of MRSA and 2 (0.8%) cases ofVRE (Table1).

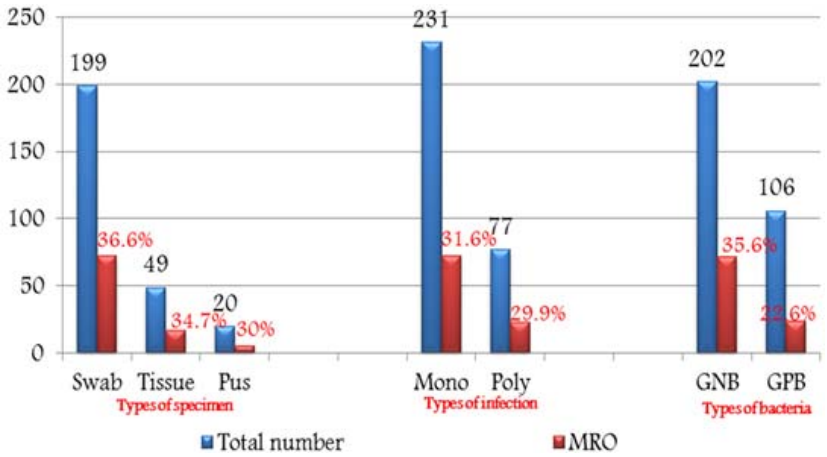
As shown in Figure 1, there was no statistically difference in MDRO isolation rate among different types of specimens, infections or bacteria.

The antimicrobial susceptibility pattern of the GPB was shown in Table-3.Thirty-three percent of *S.aureus* were MRSA. The rate of resistance of MRSA to ciprofloxacin, clindamycin, cotrimoxazole and tetracycline were 31.8%, 31.8%, 36.4%, and 45.4% respectively.

Table 3. Antimicrobial Resistance pattern of Gram Positive Bacteria isolated from DFIs

Gram positive bacteria	*S. aureus* N=66		*Streptococcus* sppN=17		*Enterococcus* SppN=23	
Antibiotic	MSSA 44(66.7%)	MRSA 22(33.3%)	SGB* 10(58.8%)	Others 7 (41.2%)	*E.faecalis* 20(87%)	*E.faecium* 3(13%)
PG Penicillin	-	-	0	0		
AMC Amoxicllin –clavulanic acid	0	R	-	-		
CRD Cefazolin	0	R	0	2 (28.5%)		
CRO Ceftriaxone	0	R	-	-		
SXT Co-trimoxazole	0	8 (36.4%)	-	-		
GM Gentamicin	0	4 (18.1%)	-	-		
CLN Clindamycin	5 (11.4%)	7 (31.8%)	0	0		
ERY Eryhromycin	9 (20.5%)	8 (36.4%)	2 (20%)	0		
RD Rifampin	0	3 (13.6%)	-	-		
TET tetracycline	1 (2.3%)	10 (45.4%)	9 (90%)	1 (14.3%)		
Amp ampicilln	-	-	0	0	0	3 (100%)
CIP ciprofloxacin	1 (2.3%)	7 (31.8%)	-	-	11 (55%)	3 (100%)
VA vancomycin	0	0	0	0	0	2 (66.7%)

SGB= Strep group B. (%of resistant bacteria)



(MRO Swab vs MRO Tissue = P=0.8939).(MRO Swab vs MRO Pus= P=0.9056). (MRO Tissue vs MRO Pus = P=0.7692)
(MRO Monomicrobial vs MRO Polymicrobial= P=0.9410)
(MRO Gram negative vs MRO Gram positive= P=0.3539)

Fig. 1. Microbiological data of two groups of bacteria according the antimicrobial resistance pattern

Table 4. Antimicrobial Resistance pattern of Gram negative bacteria isolated from DFIs

Antibiotic	*E.coli* 30	*Klebsiella* Spp29	Proteus Spp10	Morganella Spp12	Enterobacter Spp25	Citrobacter Spp14	Serratia 14	Total 134	Acineto-bacter22	Pseud. Spp40	Total 62
Ampicillin	24 (80%)	R	8 (80%)	R	R	R	R	126 (94%)			
Amoxicllin –clavulanic acid	18 (60%)	11 (37.9%)	2 (20%)	R	R	R	R	96 (71.6%)			
Cephalothin	16 (53%)	13 (44.8%)	4 (40%)	R	R	R	R	98 (73.1%)			
Cefuroxime	16 (53%)	11 (37.9%)	4 (40%)	11 (91.7%)	19 (76%)	8 (57%)	13 (92.9%)	82 (61.2%)			
Ceftriaxone	16 (53%)	8 (27.6%)	4 (40%)	5 (41.6%)	15 (60%)	7 (50%)	1 (7.1%)	56 (41.8%)			
Cefotaxime	16 (53%)	8 (27.6%)	4 (40%)	5 (41.6%)	15 (60%)	7 (50%)	1 (7.1%)	56 (41.8%)			
Ceftazidime	-	-	-	-	-	-	-		19 (86.4%)	10 (25%)	29 (46.8%)
cefepime	16 (53%)	7 (24.1%)	1 (10%)	2 (16%)	9 (36%)	5 (35.7%)	0	40 (29.9%)	19 (86.4%)	10 (25%)	29 (46.8%)
Pipercillin-tazobactam	4 (13.3%)	4 (13.7%)	0	0	5 (20%)	4 (28.6%)	1 (7.1%)	18 (13.4%)	19 (86.4%)	7 (17.5%)	26 (41.9%)
ESBL	16 (53.3%)	8 (27.6%)	2 (20%)	-	-	-	-	-	-	-	
Imipenem	0	0	0	1 (8.3%)	1 (4%)	1 (7.1%)	0	3 (2.2%)	19 (86.4%)	7 (17.5%)	26 (41.9%)
Merpenem	0	0	0	0	1 (4%)	1 (7.1%)	0	2 (1.5%)	19 (86.4%)	6 (15%)	25 (40.3%)
Gentamicin	9 (30%)	9 (31%)	2 (20%)	4 (33.3%)	6 (24%)	5 (35.7%)	1 (7.1%)	36 (26.9%)	11 (50%)	10 (25%)	21 (33.9%)
MRO	13* (43.3%)	6* (20.7%)	1* (10%)	4 (33.3%)	7 (28%)	4 (28.6%)	1 (7.1%)	36 (26.9%)	19 (86.4%)	9 (22.5%)	28 (45.2%)

*=Resistant to 3 classes of antimicrobial agents , including ESBL+ R to other 2 classes of antimicrobial agents
(%of resistant bacteria)

All *streptococcus* species were susceptible to penicillin, ampicillin, and clindamycin. The rate of resistance of streptococci to cefazolin was 28% and 20% of *Streptococcus agalactiae*(Streptococcus Group B = SGB) were found to be resistant to erythromycin.Among 23 *Enterococci* species isolated only two strains of the *E. faecalis* (8.7%)were vancomycin resistance(VRE),but both were susceptible to linezolid .

The Antimicrobial susceptibility test results for GNB were presented in Table 4.Among the GNB, The rate of resistance to carbapenem was 22.5% of *Pseudomonas* species and 86.4% of the *Acinetobacter* spp isolates. All these resistant isolate were susceptible to colistin.

Twenty-six isolates (19.4%) of *Enterobacteriaceae* species were ESBL producers. The rate of ESBLs among *E. coli and Klebsiella* species *was* 53.3% and 27.6% respectively. All of the ESBL producing isolates were susceptible to imipenem. In general, imipenem followed by amikacin were the most active agents against *Enterobacteriaceae* isolates with the susceptibility rate of 97 % and 90 respectively. Around 42%, 40%,45%, and 59% of the *Enterobactericeae* isolates were resistant to the 3rd , 4th generation cephalosporins ,ciprofloxacin, and Cotrimoxazole respectively. While amoxicillin-clavulanate, doxycycline, and cephalexin were the least active of the drugs tested.

DISCUSSION

In this study, a comprehensive evaluation of the microbiological profiles and antimicrobial susceptibility of infected feet in diabetic patients was performed. The prevalence of DFIs was found to be significantly higher in males (72.4%) than in females (27.6%). Similar findings were obtained in other studies. [10,12-18,21,23]

A total of 308 organisms were isolated from 268 samples, with a rate of 1.2 isolates per sample. DFIs are typically polymicrobial in nature, and this characteristic has been well documented in the literature.[9,12,17,26] However we observed predominantly mono-microbial infections in this study; this finding is consistent with several recent studies.[13-16,21,23] In one study, 50% of the samples grew a single isolate, and mixed bacterial growth was observed in the remaining 50 % of the samples. [19] Severe and moderate DFIs are typically found to be polymicrobial in nature,[10,12,17,25,26] whereas mild DFIs are primarily monomicrobial.[12,15,23]

In general , GNB were more prevalent (65.6%) than GPB (34.4%),and this difference was statistically significant. However, the most frequently isolated pathogens were *S. aureus* (21.4%), followed by *Pseudomonas* spp. (13.3%) and *E.coli* (9.7%).These organism were also predominant among the mixed growth samples, in a similar order. However, *S. aureus, Klebsiella* spp. and *Pseudomonas* spp. were predominant among the monobacterial isolates. Although GNB were the most prevalent pathogens, the predominance of *S. aureus* isolates was consistent with results of a previous retrospective study that was conducted in Jeddah[8] and more recent studies that were performed in Kuwait[18] and other countries. [15,23,25] The main causative isolated pathogen was consistent with the existing literature.[9-10,16-18,20,24,27] In contrast, many other studies reported a predominance of Gram-negative aerobes.[12-15,19,21,25-27] An approximately equal distribution of aerobic Gram-positive and Gram-negative pathogens was reported among DFIs in Turkey, during both the last 20 years and the most recent 5 years.[22] These discrepancies could be relatedto the differences in the types , severity of the infections and the methods used to collect the specimens included in the studies. In addition, differences in causative organisms could occur over time and across geographical regions.

The role of anaerobes is particularly unclear, as in recent studies[14,16,19,21,23] anaerobes were not isolated or discussed because anaerobic cultures were not routinely performed. In addition, many institutions lack an anaerobic culture setup. Among studies detected anaerobes, the isolation rate was variable and ranged from 0–15 % of cases.[8,11-13,17,18,20,24]. Among 31 studies of DFIs from different regions of Turkey, only 12 (40%) reported anaerobes, and the isolation rate of anaerobes was 5.9% (range, 0–13.4%)[24]. Several factors may explain the low isolation rate of anaerobes, including improper sampling, unnecessary delays in the transportation of samples to the microbiology laboratory, and the media and culture methods applied in laboratory, as well as previous treatment of patients with multiple antibiotics and patients

with relatively mild infections.[11,20,24] However, a higher isolation rate (31.4%) was observed among 54 patients for whom a pus sample was aspirated and anaerobic cultures were performed.[26] In addition, an isolation rate of 49% was observed among patients with moderate-to-severe DFIs when the specimens were obtained properly and processed optimally.[10]

Of major concern is the increasing incidence of MDROs, particularly MRSA and ESBL-producing bacteria. The present study confirms that MDRO infection is common in patients with DFIs. In this study, MDROs constituted up to 35.8% of the cases. This finding is consistent with the report of Kandemir et al. in Turkey.[27] However, this rate was lower in our population than in studies conducted in India. [12,19,25,26] On the other hand, this rate was higher than the rate reported in France in 2004.[24]When the MDRO growth rate was compared, no significant differences were observed among the different types of specimens, infections, or bacteria. However, the potential risk factors for DFIs caused by MDROs were investigated and analyzed in several studies.[24-27] The most important and common causes were the occurrence and duration of previous antibiotic therapy, a high frequency of hospitalizations for the same diabetic foot ulcer (DFU), the depth of the DFU, the presence of osteomyelitis and neuropathy, and poor glycemic control.

MRSA emerged as an important pathogen in DFIs, comprising between 16 % and 56% of *S. aureus* isolates.[12,15-18,20,21,23-26]. None of those MRSA isolates were resistant to vancomycin. In our study, the isolation rate of MRSA was nearly 33.3% of *S. aureus* isolates, which was similar to the rate (30%) reported in a previous study performed in SA.[8] Only two (8.7%) of the isolated *Enterococcus* strains showed resistance to vancomycin. This VRE isolation rate was consistent with a published study that was conducted in Iran[17] but much lower than the rate found in India, where 50% of the isolated *Enterococcus* strains were resistant to vancomycin.[16] In contrast, other studies reported that all aerobic Gram-positive strains, including *Enterococcus* strains, were fully susceptible to vancomycin.[8,10,18,19,25]

In this study, ESBL producers accounted for approximately 13% of GNB and 19% of Enterobacteriaceae. This finding was consistent with a study carried out in Italy[20] but lower than the rates reported in studies conducted in India.[12, 19, 23, 25, 26] On the other hand, this finding was higher than the rate reported in France in 2004.[24]*E. coli* was the highest ESBL producer in this study, with 53.3% of isolates being ESBL producers. This finding was consistent with a study from India that was published in 2011 and reported that 56% of *E. coli* isolates were ESBL producers.[12] In 2013, another study from India showed that 80% of the *Proteus mirabilis* isolates were ESBL producers.[19]

The prevalence of ESBL-producing *E. coli* and *Klebsiella* spp. was higher in our population of patients with DFIs than in other studies that were conducted previously in SA[8] and recently in Kuwait.[18] Thus, this study emphasizes the importance of routine screening for ESBL-producing Enterobacteriaceae in clinical laboratories.

Enterobacteriaceae typically exhibited resistance to beta-lactams, with up to 41.8% resistance to 3rd generation cephalosporins. This finding was higher than that reported in an old study from SA[8] and in more recent studies from Kuwait,[18] Italy[20] and the USA.[10] This high resistance rate may be due to the higher ESBL producer rate in our study and the greater number of AmpC-producing species, such as *Enterobacter* and *Serratia*. All members of the Enterobacteriaceae, including MDROs, were found to be susceptible to carbapenems, except *Citrobacter* and *Enterobacter* spp. This result correlated with the findings of a recent study that was performed in India.[19] A recent study conducted in Turkey[21] demonstrated that all *E. coli* and *Klebsiella* spp. isolates were susceptible to imipenem. However, 76-94% of other members of the Enterobacteriaceae were found to be susceptible to carbapenem.

In the current study, the rate of carbapenem resistance was 14.4% among GNB, with 2.2% of Enterobacteriaceae, 17.5% of *Pseudomonas* and 86.4% of *Acinetobacter* species isolates being resistant to imipenem. These results were compatible with the findings of other studies that were conducted in different countries and indicated that the rate of imipenem resistance among *P. aeruginosa* strains and other GNB is rising in DFIs.

In 2013, Turhan et al. from Turkey[25] reported that the prevalence of carbapenem sensitivity was 49.4% among *Pseudomonas* isolates and that 25% of *Acinetobacter* spp. isolates had imipenem resistance. In 2011, Zubair et al. from India reported a carbapenem resistance rate of 18.4% (*P. aeruginosa* (52.2%), *E. coli* (36.6%), *K. pneumoniae* (11.1%) and surprisingly, *Acinetobacter* sp. (0%).[26] In contrast, 10% of *P. aeruginosa* isolates were resistant to meropenem in a report from Iran in 2012.[17] Imipenem was active against only 74% of strains.[20] Shanmugam et al. from India[19] reported in their study, which was published in 2013, that over 31% of GNB were carbapenemase producers, including *Pseudomonas, Acinetobacter* spp. and members of the Enterobacteriaceae, based on the modified Hodge Test, but all of those isolates were susceptible (100%) to colistin.

Our study has several limitations. The most important limitation is that these results were based on retrospective data,. Comprehensive empirical antimicrobial coverage is a key factor for successful DFI management; hence, larger, prospective and controlled studies from several different regions of SA that use appropriate sampling and culture procedures are recommended.

CONCLUSION

In conclusion, our findings have important clinical implications, particularly with respect to the causes of DFIs and the selection of empirical treatment for DFIs. *S. aureus* and *P. aeruginosa* were the most frequently isolated pathogens .The prevalence of MDROs is alarmingly high (35.8%) among DFIs. Detailed knowledge about susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance. Empirical antimicrobial therapy for DFIs should cover both MRSA and *Pseudomonas*.

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