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RESEARCH ARTICLE

PATHOGENIC BACTERIAL ISOLATES FROM DIABETIC FOOT INFECTIONS PATIENTS AND THEIR SUSCEPTIBILITY TO ANTIBIOTICS IN SELECTED PUBLIC HOSPITALS, SANA'A, YEMEN

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Abstract: Diabetic foot infections remain a major public health problem and cause socioeconomic burdens to affected people. Clinically infected foot ulcers require treatment guided by appropriate cultures and antimicrobial susceptibility testing. This study aimed to assess the bacterial profile and antimicrobial susceptibility pattern of isolates from diabetic foot infections in selected public hospitals, Sana'a, Yemen. A cross-sectional study was conducted from June 2021 to July 2022 at Al-Gomhori teaching hospital, Kuwait teaching hospital, and Al-Thawrah public hospital in Sana'a, the capital city of Yemen. The study included 135 adult patients with infected diabetic foot ulcers. Convenient sampling was employed. Wound aspirates from the foot ulcers were collected aseptically and inoculated into Blood, MacConkey, Chocolate and Mannitol salt Agar. The antimicrobial susceptibility patterns were conducted by disk diffusion method. The data was analyzed with SPSS v.20 for windows. The results revealed that; One hundred ninety bacterial isolates were identified among 135 patients. Among them, 62.96% had mono-bacterial infection while 37.04% had mixed bacterial infections. Gram negative aerobic bacterial infections were more accounting cases 63.7% than, Gram positive aerobic bacteria 36.3%. The most commonly isolated bacteria were *S. aureus* 26.3%, followed by *Klebsiella spp* 22.1% and *Proteus spp* 11.1%. In general, 73.68% of the isolates developed multidrug resistance to at least one drug in three different classes of antibiotics. Meropinem and amikacin appeared to be the best antibiotics for therapy against Gram negative and cefoxitin and vancomycin against Gram positive organisms.

KEYWORDS: Diabetic Mellitus, diabetic foot ulcer, bacterial isolates antimicrobial susceptibility test

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INTRODUCTION:

In the past 20 years the prevalence of diabetes among the world's adult population has raised by more than threefold, growing to over 463 million adults worldwide. During this time global prevalence of diabetes mellitus has dramatically increased from 4.6% to 9.3%.[1] As the prevalence of diabetes mellitus increased, complications associated with the condition also have increased dramatically in recent decades.[2] Diabetic foot ulcer (DFU) infection is classified as mild, moderate or severe according to the extent and severity of the clinical signs, and whether systemic symptoms are present.[3] The diagnosis of infection in a DFU is made largely on a clinical basis. However, if infection is suspected, the DFU ought to be sampled for microbiological analysis.[4-5] Foot infection is one of the most common bacterial infections in clinical practice of diabetes. Many research stated at the bacteriology of diabetic foot infections (DFIs) over the last 25 years, however the results have variations and have often been contradictory. The difference could partly have been due to the variations in the causative organisms, over time, geography, or the type and the severity of the infection, as were reported in the studies.[6] Diabetic foot infections are predominantly poly microbial.[7]

A combination of Gram positive and Gram-negative aerobes (e.g., *Escherichia coli*, *Proteus species*, and *Klebsiella species*) with anaerobes is likely to be found at the site of infection.[8] For patients who haven't been treated with antibiotics within the past thirty days and have a mild DFI, infections are often mono microbial. The most common causative organisms are aerobic Gram-positive bacteria present on the skin surface such as *β Hemolytic Streptococci* or *Staphylococcus aureus*. Methicillin-resistant *Staphylococcus aureus* is present in 10% to 32% of diabetic infections and is associated with a higher rate of treatment failure in patients with diabetic foot infection.[9] In contrast, infections are typically poly microbial in patients with diabetes who have used antibiotics within the past thirty days and in people with deep, limb threatening infections or persistent non healing wounds. Anaerobic bacteria are generally part of poly microbial infections in wounds with malodorous discharge, limb ischemia, or gangrene.[6]

Bacteriological assessment of diabetic foot ulcer infection is essential to identify those agents that are involved in the development of the foot lesions. Knowledge of the bacteriology of diabetic foot infections is significant in guiding antibiotic selection and appropriate definitive therapy that will help health care professionals to manage diabetic patients and prevent from subsequent amputation.[10] Antibiotic susceptibility test is also a requirement for the management of infections which can help to make better therapeutic choices. Hence, this study was aimed to determine the organisms associated with diabetic foot infection (DFI) and their antibiotic sensitivity pattern in selected public hospitals in Sana'a, Yemen.

MATERIALS AND METHODS:

Study Area

The study was a cross-sectional, conducted from June 2021 to July 2022 in patients with only diabetic foot infections attending selected public hospitals (Al-Gomhori teaching hospital, Kuwait teaching hospital, and Al-Thawrah public hospital) in Sana'a, the capital city of Yemen. It was conducted in the medical, surgical, orthopedic wards and also at the diabetes outpatient clinics. Sample size for the present study was 135 informed and consented diabetic adult patients infected with diabetic foot ulcer investigated for bacterial, based on a previous study conducted on diabetes mellitus among hospitalized patients: prevalence, symptoms and complications in three main hospitals of Mukalla City, Yemen.[11]

Sampling Technique

A consecutive sampling technique was used to enroll the study participants. All patients with diabetic foot infection, above 18 years, not on antibiotic treatment within 14 days and agree to participate in the study and

give informed consent were included. Permission to carry out the study was sought from the medical directors of the hospitals, and the consultants in charge of the diabetes outpatient clinic or the wards at all the three hospitals. Primarily physicians in the diabetic clinic and the wards were communicated for their collaboration in the sampling of the wound aspirates. And a data collection activity was performed with the assistant researchers and a laboratory technologist helped in the laboratory bench work activities. The researchers promised to keep the participant's information confidential. Isolation, identification and the antimicrobial sensitivity patterns were done in Microbiology Laboratory, Department of Laboratory Medicine, Faculty of Medical Sciences, Al-Nokhbah International University, Sana'a. All data were kept under lock and key, with accessibility limited to the researchers only.

Cultivation and Identification

The specimen was collected by Levine technique on sterile cotton swab by rotating with sufficient pressure.[12] Culture, gram staining and biochemical tests were used. Swabs collected from patients were streaked on a Blood agar (5% sheep blood), Chocolate agar and MaCconkey agar (Oxoid) and Mannitol salt agar (MSA) by sterile inoculating loop. The MacConkey agar plate and MSA were incubated aerobically while chocolate and blood agar were incubated in microaerophilic atmosphere (5-10% CO_2) candle jar. Biochemical tests were performed on colonies from pure cultures for identification of the isolates. Gram negative rods were identified by performing a series of biochemical tests using Triple sugar iron, Indole test, Simmons citrate agar, Urea, Malonate and motility. Gram positive cocci were identified based on their gram reaction, catalase and coagulase test results.[13]

Antimicrobial Susceptibility Testing

Kirby-Bauer disk diffusion technique was used according to criteria set by Clinical and Laboratory Standard Institute (CLSI) 2018.[14] The inoculum was prepared from pure culture by picking parts (3-5) of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of the inoculation suspension was determined by comparison with the turbidity standard in a 0.5 barium sulfate solution from McFarland. Spreading of the test organisms evenly on the surface of Mueller-Hinton agar (Oxoid) and exposing with the antibiotic impregnated paper disks into the agar medium was performed, and then incubated aerobically at 37°C for 16–18 hours. Diameters of zone of inhibition around the discs were measured to the nearest millimeter using a clipper and classified as sensitive, intermediate, and resistance according to the standardized table supplied by CLSI 2018. The routine antibiotics that were frequently used in the study area were considered and all the disks that were used for the test are from Oxoid. For Gram positive bacteria; clindamycin (2µg), cefoxitin (30µg), penicillin (10µg), trimethoprim sulphamethoxazole (1.25/23.75µg), gentamycin (10µg), tobromycin (10µg), erythromycin (15µg), ciprofloxacin (5µg), ampicillin (10µg), vancomycin (30µg), doxycycline (30µg) were employed and for Gram negative bacteria, tobromycin (10µg), amoxicilin-clavulanate (20/10µg), amikacin (30µg), gentamycin (10µg), ampicilin (10µg), piperacillin-tazobactam (100/10µg), cefotaxim (30µg), cefepime (30µg), ceftriaxone (30µg), cefuroxime (30µg), chloramphenicol (30µg), ceftazidime (30µg), ciprofloxacin (5µg), impenem/meropinem (10µg), trimethoprim sulfamethoxazole (1.25/23.75µg) were tested.

Data Analysis

The data was entered and double checked before analysis. Then the data was exported to SPSS version 20 for analysis.

Ethical Consideration

The study was conducted after getting ethical clearance from the research and ethics review committee of the Department of Laboratory Medicine, Faculty of Medical Sciences, Al-Nokhbah

International University. An official permission was also obtained from Al-Gomhori teaching hospital, Kuwait teaching hospital, and Al-Thawrah public hospital. Written consent was obtained from each study subjects before collection of swab samples and other relevant clinical information. Study participants did get appropriate treatments based on the findings from the culture and AST. Information obtained at any course of the study was kept in confidential.

RESULTS

Among 135 patients recruited in this study, 85 (62.96%) had mono-bacterial, while 50 (37.04%) had mixed bacterial infections. Gram negative aerobic bacterial infections were more at 121 (63.7%), than Gram positive aerobic bacteria 69 (36.3%). The most commonly isolated microorganism was *S. aureus* 26.3%, followed by *Klebsiella spp* 22.1%, *Proteus spp* 11.1%, *E. coli* 10.5% and *Acinetobacter* 10.5%, Coagulase Negative *Staphylococcus* (CONS) 6.3%, *Enterobacter clocae* 3.2%, *E. faecalis* 2.6%, *P. aeruginosa* 2.6%, *P. retgeri* 2.6%, *M. Morgani* 1.1% and *Viridian streptococci* 1.1%. The proportion of each bacterial isolate to the total isolates is presented in **Table 1**.

Table 1: Magnitude of bacterial isolates

Bacteria Isolates	Freq.	(%)
Gram Positive Bacteria	**69**	**36.3**
Staphylococcus aureus	50	26.3
Coagulase negative staphylococcus (CONS)	12	6.3
Enterococcus spp	5	2.6
Viridian streptococci	2	1.1
Gram Negative Bacteria	121	63.7
Proteus spp.	21	11.1
Pseudomonas aeruginosa	5	2.6
Klebsiella spp.	42	22.1
Escherichia coli	20	10.5
Enterobacter clocae	6	3.2
Acinetobacter spp.	20	10.5
Providencia retigeri	5	2.6
Morganella morgani	2	1.1
Total	**190**	**100**

Table 2: Antimicrobial susceptibility pattern of Gram-positive bacterial isolates from DFIs

Isolates		Antimicrobial agents in number (%)											
		FOX	P	E	CN	VAN	SXT	AMP	GEN	CHL	CIP	DOX	TORB
S. Aureus	S	31 (62)	2 (4)	23 (46)	30 (60)	NA	27 (54)	NA	25 (50)	26 (46)	28 (50)	20 (40)	25 (50)
	I	0 (0)	0 (0)	2 (4)	1 (2)	NA	1 (2)	NA	1 (2)	0 (0)	3 (4)	0 (0)	2 (4)
	R	19 (38)	48 (96)	25 (50)	19 (38)	NA	22 (44)	NA	24 (48)	24 (54)	19 (46)	30 (60)	23 (46)
CoNS	S	5 (41.7)	3 (25)	5 (41.7)	5 (41.7)	NA	4 (33.3)	NA	5 (41.7)	4 (33.3)	5 (41.7)	4 (33.3)	5 (41.7)
	I	0 (0)	0 (0)	0 (0)	0 (0)	NA	0 (0)	NA	0 (0)	0(0)	0 (0)	0 (0)	0 (0)
	R	7 (58.3)	9 (75)	7 (58.3)	7(58.3)	NA	8 (66.7)	NA	7 (58.3)	8 (66.7)	7 (58.3)	8 (66.7)	7 (58.3)
Enterococcus spp.	S	NA	NA	NA	NA	0 (0)	NA	3(60)	NA	NA	NA	NA	NA
	I	NA	NA	NA	NA	4 (80)	NA	0(0)	NA	NA	NA	NA	NA
	R	NA	NA	NA	NA	1 (20)	NA	2(40)	NA	NA	NA	NA	NA
Viridian streptococci spp.	S	NA	1 (50)	1 (50)	1 (50)	NA	NA	1(50)	NA	NA	NA	NA	NA
	I	NA	0 (0)	0 (0)	0 (0)	NA	NA	0(0)	NA	NA	NA	NA	NA
	R	NA	1 (50)	1 (50)	1 (50)	NA	NA	1(50)	NA	NA	NA	NA	NA

Key: FOX=Cefoxitin, P=Penicillin, E=Erythromycin, CN=Clindamycin, VAN=Vancomycin, SXT=Cotrimoxazole, AMP=Ampicillin, GEN=Gentamycin, CHL=Chloramphenicol, CIP=Ciprofloxacillin, DOX=Doxycycline, TORB=Torbamycin, S=Sensitive, I=Intermediate, R=Resistance, n=number.

Antimicrobial susceptibility patterns of Gram-positive bacterial isolates is shown below in **Table 2**; the predominant S. *aureus* isolate among the Gram positive isolates showed resistance for penicillin 96%, doxycycline 60%, chloramphenicol 54%, and erytromycin 50%. Most of the CON'S isolates showed resistance to peniciline 75%, chloramphenicol 66.7%, cotrimoxazole 66.7%, doxycycline 66.7%. 58.3% resistance was seen to cefoxitin, clindamycin, erytromycin, ciprofloxacin and torbamycin. *Enterococcus spp* exhibited resistance against ampicillin 40% and vancomycin 20%. On the other hand, *Viridian Streptococci* 50%, sensitivity level was seen on penicillin, erythromycin, clindamycin and ampicillin.

Antimicrobial susceptibility patterns of Gram-negative bacterial isolates is shown below in **Table 3**, among Gram negative isolates, all of the isolates showed highest sensitivity against amikacin 85-100% and meropenem 72.2-100% except for *Acinetobacter* which showed only 65% & 40% sensitivity for both antibiotics consecutively. All Gram-negative isolates showed high resistance for ampiciline 100%. *Morganella Morgani* was highly resistant 100% for augmentine. Resistance to second and third generation cephalosporins (cefotaxime, cefuroxime, ceftriaxone and ceftazidime) was observed for *Klebsiella spp*

87.5-100%, *Enterobacter Spp.* 83.3%. *Acinetobacter Spp* showed high level of resistance for most antibiotics like ceftazidime and tazobactam-peperazine 100%, 90% for cotrimoxazole and cefepime.

Table 3: Antimicrobial susceptibility pattern of Gram-negative bacterial isolates from DFIs

Isolates (n)		Antimicrobial agents in number (%)														
		SXT	AMP	AMK	GEN	CHL	CIP	CRO	CTX	CAZ	AUG	CFP	MEM	TZP	CFX	TORB
Proteus mirabilis	S	2 (28.6)	0	6 (85.7)	5 (71.4)	5 (71.4)	5 (71.4)	4 (57.1)	4 (57.1)	4 (57.1)	2 (28.6)	5 (71.4)	7 (100)	5 (71.4)	4 (57.1)	5 (71.4)
	I	0	0	0	0	1 (14.3)	0	0	0	0	0	1 (14.3)	0	0	0	0
	R	5 (71.4)	7 (100)	1 (14.3)	2 (28.6)	1 (14.3)	2 (28.6)	3 (42.9)	3 (42.9)	3 (42.9)	5 (71.4)	1 (14.3)	0	2 (28.6)	3 (42.9)	2 (28.6)
Proteus vulgaris	S	9 (60)	0	15 (100)	13 (86.7)	7 (46.7)	13 (86.7)	10 (66.7)	10 (66.7)	10 (66.7)	6 (40)	12 (80)	15 (100)	12 (80)	10 (66.7)	13 (86.7)
	I	0	0	0	0	1 (6.6)	0	1 (6.6)	0	0	1 (6.6)	0	0	1 (6.6)	0	0
	R	6 (40)	15 (100)	0	2 (13.3)	7 (46.7)	2 (13.3)	4 (26.7)	5 (33.3)	5 (33.3)	8 (53.3)	3 (20)	0	2 (13.3)	5 (33.3)	2 (13.3)
Klebsiella pneumoniae	S	8 (44.4)	0	18 (100)	15 (83.3)	11 (61.1)	14 (77.8)	0	0	0	0	10 (55.6)	13 (72.2)	3 (16.7)	0	16 (88.9)
	I	0	0	0	0	0	0	0	0	0	0	0	0	2 (11.1)	0	0
	R	10 (55.6)	18 (100)	0	3 (16.7)	7 (38.9)	4 (22.2)	18 (100)	18 (100)	18 (100)	18 (100)	8 (44.4)	5 (27.8)	13 (72.2)	18 (100)	2 (11.1)
Klebsiella oxytoca	S	4 (26.7)	0	13 (86.7)	8 (53.3)	9 (60)	8 (53.3)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	7 (46.7)	14 (93.3)	5 (33.3)	4 (26.7)	8 (53.3)
	I	0	0	0	0	0	0	0	0	0	0	2 (13.3)	0	3 (20)	0	0
	R	11 (73.3)	15 (100)	2 (13.3)	7 (46.7)	6 (40)	7 (46.7)	13 (86.7)	13 (86.7)	13 (86.7)	13 (86.7)	6 (40)	1 (6.7)	7 (46.7)	11 (73.3)	7 (46.7)
Klebsiella ozenae	S	2 (25)	0	7 (87.5)	6 (75)	6 (75)	5 (62.5)	1 (12.5)	1 (12.5)	1 (12.5)	0	5 (62.5)	7 (87.5)	2 (25)	1 (12.5)	7 (87.5)
	I	0	0	0	0	0	0	0	0	0	0	1 (12.5)	0	2 (25)	0	0
	R	6 (75)	8 (100)	1 (12.5)	2 (25)	2 (25)	3 (37.5)	7 (87.5)	7 (87.5)	7 (87.5)	8 (100)	2 (25)	1 (12.5)	4 (50)	7 (87.5)	1 (12.5)
Escherichia coli	S	4 (20)	0	19 (95)	16 (80)	11 (55)	10 (50)	7 (35)	7 (35)	7 (35)	5 (25)	9 (45)	20 (100)	11 (55)	7 (35)	18 (90)
	I	0	0	1 (5)	0	1 (5)	1 (5)	0	1 (5)	1 (5)	0	1 (5)	0	0	1 (5)	0
	R	16 (80)	20 (100)	0	4 (20)	8 (40)	9 (45)	13 (65)	12 (60)	12 (60)	15 (75)	10 (50)	0	9 (45)	12 (60)	2 (10)
Pseudomonas aeruginosa	S	NA	NA	5 (100)	5 (100)	NA	3 (60)	NA	NA	2 (40)	NA	4 (80)	5 (100)	3 (60)	NA	5 (100)
	I	NA	NA	0	0	NA	0	NA	NA	1 (20)	NA	1 (20)	0	1 (20)	NA	0
	R	NA	NA	0	0	NA	2 (40)	NA	NA	2 (40)	NA	0	0	1 (20)	NA	0
Acinetobacter spp.	S	2 (10)	NA	13 (65)	11 (55)	NA	4 (20)	NA	NA	0	NA	1 (5)	8 (40)	0	NA	10 (50)
	I	0	NA	1(5)	0	NA	1(5)	NA	NA	0	NA	1(5)	0	0	NA	0
	R	18 (90)	NA	6 (30)	9 (45)	NA	15 (75)	NA	NA	20 (100)	NA	18 (90)	12 (60)	20 (100)	NA	10 (50)
Providencia rettgeri	S	2 (40)	0	5 (100)	3 (60)	3 (60)	3 (60)	3 (60)	4 (80)	4 (80)	2 (40)	5 (100)	5 (100)	4 (80)	4 (80)	4 (80)
	I	0	0	0	0	0	0	0	0	0	0	0	0	1 (20)	0	0
	R	3 (60)	5 (100)	0	2 (40)	2 (40)	2 (40)	2 (40)	1 (20)	1 (20)	3 (60)	0	0	0	1 (20)	1 (20)
Morganella morgani	S	1 (50)	0	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	0	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)
	I	0	0	0	0	0	1(50)	0	0	0	0	0	0	0	0	0
	R	1 (50)	2 (100)	0	1 (50)	1 (50)	0	1 (50)	1 (50)	1 (50)	2 (100)	0	0	1 (50)	1 (50)	1 (50)
Enterobacter cloacae	S	6 (100)	0	6 (100)	6 (100)	6 (100)	5 (83.3)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	5 (83.3)	6 (100)	1 (16.7)	1 (16.7)	6 (100)
	I	0	0	0	0	0	0	0	0	0	0	0	0	3(50)	0	0
	R	0	6 (100)	0	0	0	1 (16.7)	5 (83.3)	5 (83.3)	5 (83.3)	5 (83.3)	1 (16.7)	0	2 (33.3)	5 (83.3)	0

Key: FOX=Cefoxitin, P=Penicillin, E=Erythromycin, CN=Clindamycin, VAN=Vancomycin, SXT=Cotrimoxazole, AMP=Ampicillin, GEN=Gentamycin, CHL=Chloramphenicol, CIP=Ciprofloxacillin, DOX=Doxycycline, TORB=Torbamycin, S=Sensitive, I=Intermediate, R=Resistance, n=number.

Table 4 shown, the antibiogram of the isolates in this study, it showed that, 100% multidrug resistance among *Klebsiella pneumoniae*, *Klebsiella ozenae*, *Morganella morgani and Acinitobacter* spp. Gram positive isolates, *S. aureus* 60%, *CoNS* 67%, and *Viridian streptococci* 50% of isolates were resistant to four and more antibiotics. On the other hand, among Gram negative isolates *Pseudomonas aeruginosa* 20%, *E. coli species* 85%, *P. mirabilis* 71.43%, *P. vulgaris* 53.33%, *P.retigeri* 60% showed resistance to three antibiotics. Majority of isolates in *Klebsiella oxytoca* 86.6%, and *Entrobacter cloacae* 83.33%, were resistant to six up to ten antibiotics. In general, 73.68% of the isolates in our study developed multidrug resistance to at least one drug in three different classes of antibiotics (≥3 antibiotics).

Table 4: Antibiogram of bacteria isolated from patients with Diabetic foot infections

Bacterial Isolates (n)	No. (%) of resistance							
	R0	R1	R2	R3	R4	R5	R6-10	MDR
Staphylococcus aureus (n=50)	2 (4)	16 (32)	2 (4)	0	2 (4)	3 (6)	25 (50)	30 (60)
(CONS) (n=12)	2 (17)	2 (17)	0	0	1 (8)	0	7 (58)	8 (67)
Enterococcus spp. (n=5)	3 (60)	1 (20)	1 (20)	0	0	0	0	1 (20)
Viridian streptococci (n=2)	1 (50)	0	0	0	1 (50)	0	0	1 (50)
Klebsiella pneumoniae (n=18)	0	0	0	0	0	0	18 (100)	18 (100)

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Klebsiella oxytoca (n=15)	0	1 (6.7)	1 (6.7)	0	0	0	13 (86.6)	13 (86.6)
Klebsiella ozenae (n=8)	0	0	0	0	1 (12.5)	0	7 (87.5)	8 (100)
Escherichia coli (n=20)	0	2 (10)	1 (5)	2 (10)	2 (10)	0	13 (65)	17 (85)
Proteus vulgaris (n=15)	0	3 (20)	4 (26.67)	3 (20)	0	0	5 (33.33)	8 (53.33)
Proteus mirabilis (n=7)	0	1 (14.29)	1 (14.29)	2 (28.57)	0	0	3 (42.85)	5 (71.43)
Pseudomonas aeruginosa (n=5)	2 (40)	2 (40)	0	1 (20)	0	0	0	1 (20)
Enterobacter cloacae (n=6)	0	1(16.67)	0	0	0	0	5 (83.33)	5 (83.33)
Providencia rettgeri (n=5)	0	1 (20)	1 (20)	1 (20)	0	0	2 (40)	3 (60)
Morganella morgani (n=2)	0	0	0	1 (50)	0	0	1 (50)	2 (100)
Acinetobacter spp. (n=20)	0	0	0	0	0	0	20 (100)	20 (100)
Total	10 (5.3)	30 (15.7)	11 (5.8)	10 (5.3)	7 (3.7)	3 (1.6)	119 (62.6)	140 (73.7)

Key: R0=no resistance to antibiotic, R1=resistance to 1 antibiotic, R2=resistance to 2 antibiotics, R3=resistance to 3 antibiotics, R4=resistance to 4 antibiotics, R5 =resistance to 5 antibiotics, R 6-10=resistance to 6-10 antibiotics.

DISCUSSION

DFIs are a common and serious complication of diabetes which is present in up to 50% of DFUs, and 80% of non-traumatic lower-limb amputations are a consequence of DFU infection.[15-16] The bacterial etiologies and risk factors associated with the diabetic foot infection are not well studied and published is scarce in Yemen. Therefore, the present study was undertaken to identify and characterize bacterial etiologies of diabetic foot infection, to determine their antimicrobial susceptibility pattern.

A total of 190 microorganisms were isolated from 135 patients, with an average of 1.41 microorganisms isolated from each patient. This study has similar proportion with studies conducted in Malaysia, Saudi Arabia, and southern Iran in the rate of 1.47, 1.45 and 1.42, respectively. [17-19] Studies from abroad showed similar bacterial proportion may be due to bacteria isolates were from infected ulcers however another study was conducted generally on DFUs. However, this is quite different to hospital-based study conducted in Ethiopia where cultures yielded an average of 0.77 organisms per case.[20]

Spectrums of bacteria vary widely in diabetic foot infections. In our study, Gram negative bacteria were isolated predominantly 63.7%, while Gram positive cocci accounted for 36.3%. The most commonly isolated microorganism was *S. aureus* 26.3%, followed by *Klebsiella spp* 22.1%, *Proteus spp* 11.1%, *E. coli* 10.5% and *Acinetobacter* 10.5%, *Coagulase Negative Staphylococcus* (CONS) 6.3%, *Enterobacter clocae* 3.2%, *E. faecalis* 2.6%, *P. aeruginosa* 2.6%, *P. retgeri* 2.6%, *M. Morgani* 1.1% and *Viridian streptococci* 1.1%. This is in agreement with the previous study done on diabetic infections.[21] Similar finding has been published elsewhere.[19,22-23] However it is somehow different from the retrospective study conducted on diabetic foot and indicated *Klebsiella species* to be the predominant bacteria.[20] This contradiction reinforces the fact of variability of organisms infecting DFUs across different regions and even within the same settings and at different times as has been demonstrated in studies.[24] The variation with the other study may also be related to difference in the method and the time gap between the studies which can result in the change in the spectrum and the antibiogram of the isolates.

In this study, Gram positive and Gram-negative bacteria showed decreased sensitivity to most of the antimicrobial agents tested; the predominant Gram positive bacteria isolate was *S. aureus.* This was consistent with the reviewed articles on microbiology and antimicrobial therapy by (Kwon KT *et al.,* 2015*)* in Korea.[25] *Staphylococcus aureus* showed the highest sensitivity to cefoxitine 62%, followed by clindamycin 60%. In our study S. *aureus* showed resistance for penicillin 96%, doxycycline 60%, chloramphenicol 54%, and erytromycin 50%. This is an indication of the alarming levels of resistance for different group of antibiotics by *S. aureus* at the hospitals. This is contrary to the findings in Egypt by (Hefni *et al.,* 2013)[24] where, *S. aureus* showed high sensitivity to chloramphenicol, erythromycine and tetracycline but similar to the findings by (Hena *et al.,*2010).[26]

Our study indicated *Staphylococcus* (CoNS) 6.3% as the second most prevalent bacteria followed by *E. faecalis* 2.6% and *Viridian streptococci* 1.1%. This result is close to the study conducted in Egypt where *Staphylococci* (CoNS) 9.7% was the succeder to the most prevalent Gram-positive cocci *Staphylococcus aureus*.[22] Most of the CoNS isolates showed resistance to peniciline 75%, chloramphenicol 66.7%, cotrimoxazole 66.7%, doxycycline 66.7%. 58.3% resistance was seen to cefoxitin, clindamycin, erytromycin, ciprofloxacin and tobramycin. This finding is not agreed comparable with other study from India.[27] This emanated possibly from the differences in the sociodemographic, severity level or grades, health care system and methods used during sample collection. The antimicrobial susceptibility testing also showed that vancomycin 80% was the most effective against *Enterococcus faecalis*. This is in line with previous study done in Iran where most of the isolate were sensitive to vancomycin.[28]

Gram negative aerobes were the leading in our study comprised 63.7% of the total isolates. A Study from Egypt and Turkey also showed similar reports in which Gram-negative bacteria were more isolated at 67% and 61.3% compared with Gram positive bacteria. [24,28] The concordance between the studies could be due to similarities in type of sample and the methods implemented. Our findings showed members of the *Enterobacteriaceae* family were the predominant group among the Gram negative aerobes in line with other study from Brazil.[29] The second, third and fourth major isolates were *Klebsiella spp* 22.1%, *Proteus spp* 11.1% and, *E. coli* 10.5% species. Considerable shares were also possessed by other isolate from the family like, *Enterobacter clocae 3.2%, P.retgeri* 2.6% and *M. Morgani* 1.1%.

With regard to the susceptibility patterns, meropinem and amikacin appeared to be the best antibiotics for therapy against Gram negative organisms. All Gram-negative isolates showed high resistance for ampiciline 100%. *Morganella Morgani* was highly resistant 100% for augmentine. Resistance to second and third-generation cephalosporins (cefotaxime, cefuroxime, ceftriaxone and ceftazidime) was observed for *Klebsiella spp* 87.5-100% and *Enterobacter Spp.* 83.3%. Studies in Kuwait and Egypt also supported the finding most effective treatments for the Gram negative bacteria were amikacin and imepinem.[30,24]

CONCLUSION

The present study concluded that major bacterial isolates were identified in this study with Gram negative bacteria being the predominant. Diabetic foot infections were associated with mono-microbial etiology. With regard to the susceptibility patterns, meropinem and amikacin appeared to be the best antibiotics for therapy against Gram negative organisms. The antimicrobial susceptibility testing also showed that vancomycin and cefoxitin were still the effective antimicrobials against the Gram-positive organisms.

Authors' Contributions

Nabilah Shiaf conceived and designed the research idea and performed proposal writing, data collection and analysis, interpretations of the findings and manuscript writing. ***Al-Samet Ebrahim M*** contributed in the study design, analysis and interpretations of the findings, drafting and writing the manuscript. ***Jameel Nosibah L*** contributed in data collection, laboratory work, analysis and interpretations, and manuscript writing.

Conflict of Interest

The authors declare that, no conflict of interest.

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