ORIGINAL ARTICLE

AEROBIC BACTERIAL PROFILE IN DIFFERENT GRAGES OF DIABETIC FOOT ULCERS OF SUDANEASE PATIENTS: A CROSS SECTIONAL STUDY

| Saada Nour [1*] | Maowia Mukhtar [2] | Mohamed ElMakk [3] | Elshibli Mohamed [4], Walyeldin Elnour [5] | Mahdi Shamad [6] | Nuha Ibrahim [7] | Mohamed Fadl [8] | and | Safia Ahmed [9] |

[1.] University of Bahri | College of Medicine | Department of Microbiology | Khartoum | Sudan |
[2.] University of Khartoum | Institute of Endemic Diseases | Department of infectious Diseases | Khartoum | Sudan |
[3.] University of Khartoum | Faculty of Medicine | Department of Surgary | Khartoum | Sudan |
[4.] Alsafa Academy Khartoum | Sudan |
[5.] University of Bahri | College of Medicine | Department of Pediatric | Khartoum | Sudan |
[6.] University of Bahri | College of Medicine | Department of Dermatology | Khartoum | Sudan |
[7.] National Public Health Laboratory| National Tuberculosis Reference Laboratory | Khartoum | Sudan |
[8.] University of Khartoum | Faculty of Medicine | Khartoum Sudan |
[9.] National Public Health Laboratory | Bacteriology Reference Laboratory | Khartoum | Sudan |

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ABSTRACT

Background Diabetic foot ulcer infection causes great morbidity and mortality among diabetic patients and it is a major cause of lower extremity amputation worldwide. **Objectives:** This study aimed to determine the aerobic bacterial profile in diabetic foot infection DFI in different Wagner's grades to speculate the relation between the bacterial organisms and the depth of the wounds and to find out the correlation between the peripheral neuropathy and different ulcer grades in diabetic patients. **Methods**: This study was conducted in the period from December 2017 - March 2018 in a Diabetes Center in Sudan. A total of 152 diabetic patients who admitted with different grades of foot ulcers were randomly enrolled in the study. The patients were grouped using Wagner's classification. Tissue biopsies and deep swabs were collected from the ulcers for aerobic cultures. The cultured isolates were identified using phenotypic and biochemical properties. The sensation of patients was examined clinically. **Results**: The mean age of the patients was 54.31 (SD ± 12.1) years, males to females' ratio was 8: 1. The mean duration of diabetes was 14 (SD ± 8) years. The ulcers varied in duration from 1 day to 10 years. Of 152 samples 181 aerobic bacteria were isolated. Cultures yielded 1-3 isolate per culture. The maximum number of bacteria was isolated from grade 3 ulcers followed by long standing ulcers 50.8% and 28% respectively. Polymicrobial infection was higher in long standing ulcers (30.4%). The isolates were mostly Gram-negative bacteria. The most frequent pathogens were *proteus* spp. (35.3%), Methicillin Resistant *Staphylococcus aureus* MRSA 14.4% and Coliform 12.2%, respectively. The most common isolates in grade 3 ulcers were *P. Mirablis, Staphylococcus* and Coliform, while in long standing ulcers they were *P. Mirablis, S. aureus* MRSA and Coagulase negative *staphylococcus*. The most common associated bacteria in polymicrobial infection were *P. Mirablis* with *P. Aerginosa, S. aureos* MRSA and Coliform respectively, respectively. **Conclusion**: Gram-negative bacteria were more prevalent and the most frequent pathogen was Proteus spp. The most common polymicrobial infections were due to *P. mirablis* with; *P. aerginosa, S. MRSA* and Coliform respectively. Major isolates in low grades 1 and 2 are Gram-positive and in high grades 3, 4 are Gram-negative bacteria. No significant relation between Wagner grades and neuropathy was detected.

Keywords: *diabetic foot ulcer, aerobic bacteria, Wagner's classification.*

1. INTRODUCTION

Diabetic foot infection (DFI) is a serious complication of diabetes and a major cause of lower extremity amputation worldwide [1]. Approximately one-fourth of diabetic patients develop an ulcer during their lifetime, and about half of these ulcers become infected [2, 3]. Diabetic foot problems result in high economic cost and a large national economic burden [4]. Bacterial pathogens isolate from diabetic foot ulcer (DFU) vary with the grades and severity of infection. Early infections are generally monomicrobial, whereas advanced infections are mainly polymicrobial while low grades are generally infected with gram-positive organisms [4]. The identity of the bacteria infecting diabetic foot differs among patients and hospitals [5]. The International Diabetes Federation has anticipated that the number of persons with diabetes will increase from 240 million in 2007 to 380 million in 2025 [6]. This increment needs more research programs.

Impaired diabetic wound healing constitutes a major health problem in patients with diabetes which is estimated to occur in 15% of diabetic patients and often requires prolonged hospitalization for its management. Treatment of DFU is complicated and healing may take several months or sometimes years [7]. Chronic diabetic wounds need 3-6 months for dressing and follow up and sometime exceed 6 months J.D.C. [8]. Amputees need restoration and physical therapy to return them to their normal social life [9]. The costs of chronic ulcer care represent a major portion of the health care budget and continue to grow at exponential rates, and this is an important issue in developing countries

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[10]. The aim of this study was to determine the aerobic bacterial profile in DFI in different Wagner's grades to speculate the relation between the bacterial organisms and the depth of the wounds and to find out correlation between the peripheral neuropathy and different ulcer grades in diabetic patients. A few studies identified the bacterial isolates in Sudan but to our knowledge this report on bacterial isolates from different grades of ulcers considering the depth of the ulcers was not done in Sudan.

2. MATERIALS AND METHODS

2. 1. Study area and setting

The study was conducted in Jaber Abu Eliz diabetes Center (J.D.C) in the capital Khartoum. The center is the largest diabetes clinic specialized in treatment and care of diabetic foot and it receives patients referred from different region of the country. J.D.C is a multidisciplinary specialized Diabetic Centre in the Sudan. It caters for 70, 000 registered diabetic patients, utilized by people from Khartoum state, its surrounding areas and from other states.

2. 2. Study design and period

This study is cross-sectional which was conducted from December 2017 to March 2018.

2. 3. Sample size and sampling technique

One hundred fifty two diabetes patients with foot ulcers were enrolled in the study. The patients were attending the outpatient clinic in the Surgery Unit at J. D. C. They were enrolled using systemic random sampling.

2. 4. Base line data

The demographic data including age, sex, duration of diabetes, duration of the ulcer, and duration of the sensation loss was collected by face to face interview using a predesigned questionnaire. All the questionnaires were checked for accuracy and completeness. The questionnaire was prepared in English version and translated to Arabic which is the language of the study participants.

2. 5. Patients

The investigations were designed to identify aerobic bacteria in each ulcer grade.The patients were grouped according to Wagner diabetic foot ulcer classification into 5 groups [grade 1 , grade 2, grade 3, grade 4 and long standing ulcers (maturation stage)]. The number of patients in each group was as following: 8 patients were grade 1, 19 patients were grade 2, 70 patients were grade 3, 12 patients were grade 4, and 43 patients were long standing ulcer (maturation phase). Specimens used in this study were obtained from every patient group. The ulcer duration was determined verbally based on the patient response. While the sensation of patients was examined clinically, osteomyelitis was diagnosed by probe to bone test and bone biopsy for microbiological investigation. Gender, ulcer grades and sensation loss of the participants are shown in Table 1.

2. 6. Collection of microbiological samples

The ulcers were cleaned vigorously with saline and extensively debrided first to avoid the isolation of colonizing flora. Specimens were collected by 2 methods, tissue biopsy (soft tissue and bone) from the central region of the ulcer bed using a 6-mm disposable sterile punch biopsy (Stiefel Laboratories, Ltd., Sligo, Ireland) and placed immediately into a sterile vial containing 2 ml of sterile normal saline. Some samples were collected by deep swab technique from patients with new wound (grade 1) and long-standing wound (maturation phase). All specimens were taken from patients on dressing table. The specimens were transferred within 1 hour to Bacteriology Department at the National Public Health Laboratory, where optimal microbiological culture techniques were used.

2. 7. Bacterial isolation and identification

The specimens were inoculated on blood agar and MacConkey agar plates for the isolation of aerobic bacteria. Inoculated blood agar was put into candle jar with carbon dioxide which was needed to enhance bacterial growth [11], and it was incubated for 18-24 h at 35 °C -37°C. Inoculated mac agar was incubated for 18-24 h at 35 °C - 37°C. Gram stain was done from bacterial colonies. Selected isolates from Mac and blood agar were sub-cultured into nutrient agar and incubated to refresh the sample. Additionally, mannitol salt agar was inoculated and incubated at 37°C for 24 hours. The isolates were identified based on colony morphology, colour change, gram-staining results, Urea and indole motility (SIM), and biochemical reactions for catalase, oxidase, coagulase, and other biochemical tests [12, 13, 14]. In this study, anaerobic bacteria were not investigated due to the limited laboratory facilities.

2. 8. Data quality control

Aseptic technique was used throughout sampling and handling procedures by using sterile materials, flaming and icebox. For remarkable studies of microorganism, pure culture was used. Solutions and equipment containing water were autoclaved at 121°C for 15 to 20 minutes. The sterility of the media was detected by incubating 5% of the batch at 37°C for 18-24h.

2. 9. Statistical methods

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21 with significance level at 0.05 and CLs 95%, descriptive statistics (mean ±SD) obtained for quantitative variables, while qualitative variables described using frequency and percent difference between frequencies in (table 3) tested by goodness of fit test using chi-square test or Fisher Exact test when needed. The relationships between qualitative variables tested by independence test using chi-square or Fisher Exact test when needed, to obtain the p-value.

2. 10. Ethics approval and consent to participate

The ethical approval for this study was obtained from the Ethics Committee of Research Department at the Ministry of Health, Khartoum State, Sudan on 17/8/2017. A written consent was obtained from all participants before their enrolment after explaining the aim of the study. The youngest patient was nineteen years old, so there was no need to obtain informed consent from parent or guardian.

3. RESULTS

A total of 152 diabetic patients were enrolled in this study out of which 135 patients (88.8%) were male and 17 patients (11.2%) were females. The mean age of the patients in the study group was 54.31 with SD ± 12.1years. The gender, ulcer grades and sensation loss of the participants are shown in Table 1. The mean duration of diabetes was 14 SD ± 8 years. The highest numer of patients 66 (43.7%) were in diabetic duration 12-24 years and 125 (82.2%) were in the age of 40-65 years. The ulcers varied in duration from 1 day to 10 years. The frequencies of age, diabetic duration, ulcer duration and sensation loss duration are shown in Table 2. Concerning patients' protective sensations, 82 patients (53.6%) lost sensation and the sensation loss duration ranged from 7 days to 24 years among all patients. The percent of patients with peripheral neuropathy in each ulcer grade is plotted in Figure 1.

Table 1: The gender, ulcer grades and sensation loss of the participants

		Count	Column N %
Sex	Females	17	11.2%
	Males	135	88.8%
Ulcer Grade	Grade 1	8	5.3%
	Grade 2	19	12.5%
	Grade 3	70	46%
	Grade 4	12	7.9%
	Long standing ulcer	43	28.3%
Sensation Loss	Yes	82	53.9%
	No loss	70	46.1%
Sample	Swab	45	37.16%
	Tissue	103	62.84%

Table 2: Frequencies of age groups, diabetes duration, ulcer duration and sensation loss duration among participants.

Groups	Frequencies	Count	Percentage
Groups of Age	15 to 40 years	12	7.9%
	40 to 65	125	82.2%
	65 to 90	15	9.9%
Groups of D. Duration	Less than 12 months	10	6.6%
	1 to 12	59	38.8%
	12 to 24	66	43.4%
	24 to 36	17	11.2%
Groups of U-Duration	Less than 12 months	135	88.8%
	12 to 36	14	9.2%
	More than 36	3	2.0%
Groups of Loss of Sensation	Less than 12 months	30	36.6%
	12 to 60	29	35.4%
	60 to 120	15	18.3%
	More than 120	8	9.7%

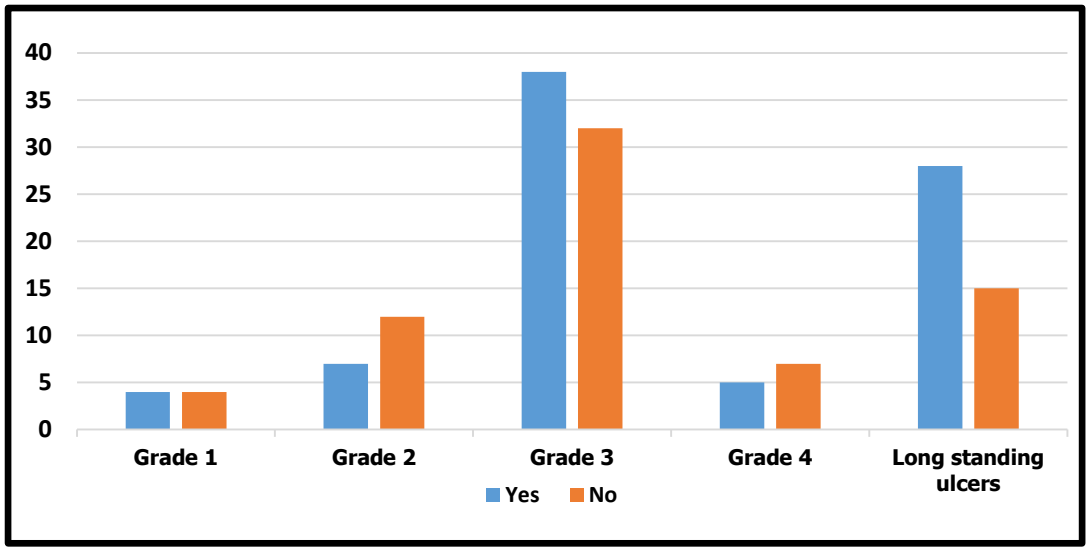

Figure 1: Ulcer grades and associated peripheral neuropathy. The p-value of test of independence=.365

3. 1. Microbiological findings

Bacterial growth was detected in 138 specimens (90.8%) and no growth was seen in 14 specimens (9.2%). Cultures yielded a total of 181 aerobic isolates from different ulcer grades (Figure 2), with a range of 1-3 organisms per sample. The average numbers of isolates was 1.2 per case. In the present work, two types of bacterial infections were detected, monomicrobial and poly microbial infections, 63.82% and 26.97% respectively (Figure 3). Most of poly microbial infections were from long standing and grade 3 ulcers, 30.4% and 27%, respectively. Frequencies of bacteria isolated from the ulcers of the participants are tabulated in Table 3. The number of Gram- negative rods was 120 (66.3 %), higher than Gram-positive 61 (33.7 %), out of 61 Gram- positive bacteria 55 were cocci and 6 were coccobacilli.

Table 3: Frequency of bacteria isolated from the ulcers of the participants

		Count	N %	P-value
Gram	G (-ve) rods	120	66.3%	.000
	G (+ve) Cocobacilli	6	3.3%	
	G (+ve)Coci	55	30.4%	
Isolates	Acinobacter	6	3.1%	.000
	Coliforms	22	11.3%	
	E. coli	8	4.1%	
	K. oxytoca	3	1.5%	
	K. pneumonia.	5	2.6%	
	P. mirables	48	24.7%	
	P. vulgaris	16	8.2%	
	P.aeruginosa	19	9.8%	
	S.aureas	8	4.1%	
	S.aureas (MRSA)	26	13.4%	
	S.aureas (VRSA)	4	2.1%	
	Saph. Coagulase –ve	16	8.2%	

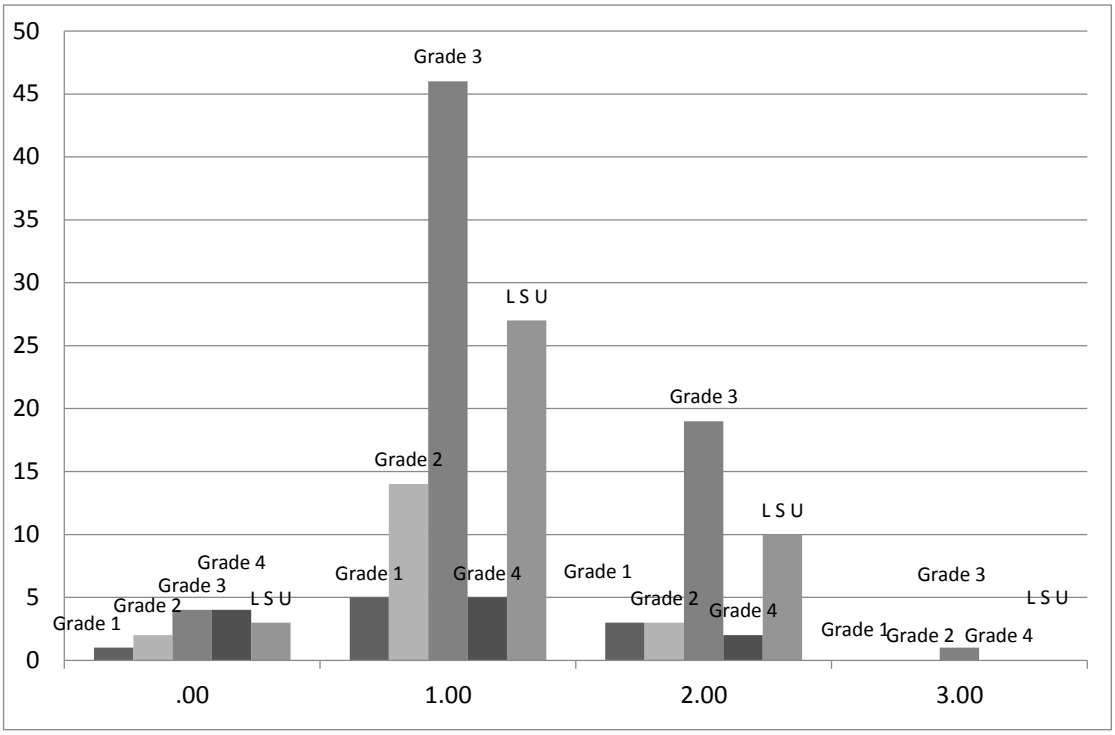

Figure 2: The relation between wound grade and number of bacterial species isolated.

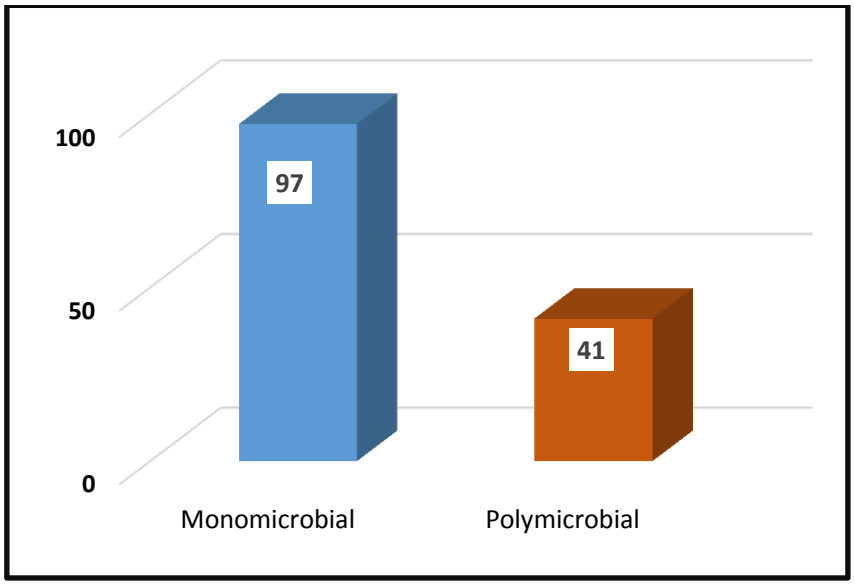

Figure 3: Types of microbial infection of diabetic wounds.

The percentages of Gram negative to Gram-positive bacteria in each ulcer grades are shown in Table 4. The most frequent pathogens of all isolates were *Proteus* 35.3%, [*P. mirablis* 26.5%+ *P. vulgaris* 8.8%] followed by *S. aureus* MRSA 14.4% and Coliform 12.2% respectively. The maximum number of bacteria was isolated from grade 3 ulcers followed by long standing ulcers 50.8% and 28%, respectively.

Table 4: Wagner classification and associated aerobic bacteria and peripheral neuropathy.

Wound grade	No of patients	No of patients with Pripheral neuropathy	% of patients with Pripheral neuropathy	Typical pathogenes
Zero				Normal skin flora
One	8	4	(50%)	The percent of Gram-negative to Gram positive 2:1 the most common speicies were *E.coli* and *staph.coagulase –ve.*
Two	19	7	(36.8%)	The percent of Gram-negative to Gram positive 1:1 the most common speicies were *Staphylococcus* and different spp. of Gram negative.

Three	70	38	(54.3%)	The percent of Gram-negative to Gram positive 5:1 the most common speicies were *P. Mirablis, Staphylococcus* and Coliform.
Four	12	5	(41.6%)	No Gram-positive bacteria isolated in this grade, the most common Gram negative were *P. aeruginosa* and *P. Mirablis.*
Long standing ulcers (maturatio n phase)	43	28	65.1%	The percent of Gram-negative to Gram positive 4:3 the most common speicies were *Proteus, s. sureus* MRSA, *Co agulase negative Staph.* And *P. aeruginosa.*

The most common isolates in grade 3 ulcers were *P. Mirablis, Staphylococcus* and Coliform where in long standing ulcers they were *P. Mirablis, S. aureus* MRSA and Coagulase negative *staphylococcus*. The most common associated bacteria in polymicrobial infection were *P. Mirablis* with *P. Aerginosa, S. aureos* MRSA and Coliform, respectively.

4. DISCUSSION

Foot infections in diabetes patients are a complex problem and a common cause of morbidity, ultimately leading to severe complications like gangrene and amputation [15]. Effective management of the infection requires isolation and identification of the bacteria [16]. Diabetes and diabetic foot infections are on the rise in Sudan with little data available to guide doctors to achieve effective cure.

This study aimed to isolate and identify aerobic bacterial pathogens associated with diabetic foot infections in different grades of wounds. As reported in other studies males were more represented in this study with the males to females' ratio reaching 8:1. High prevalence of diabetic foot infection among males has been reported by others studies [1, 3, 4] and this could be attributed to more outdoor activities among males compared to females. In contrast, a study done in J. D. C. in 2012 reported that males to females' ratio was 3:3.3 [17]. In our study, we found that the ulcers varied in duration from 1 day to 10 years which was a long duration range in comparison to a previous study done in the same center [17]. Two patterns of bacterial infections were detected in the present work; monomicrobial infection 63.82% and polymicrobial infections 26.97% with aerobic Gram-negative, especially *proteus* spp as the most common causative agents. The average numbers of isolates were 1.2 per case which is similar to a study by Eithar and Omer (2015) in the same Centre [18]. In addition, another study in Sudan reported a similar number of isolates per case 1.39 [19]. Polymicrobial nature of DFIs has been reported in several studies conducted both in Sudan and abroad [20]. A study in India found that the majority of DFIs were polymicrobial with aerobic Gram-positive cocci, especially staphylococci as the most common causative agents [21]. This disagreement with our result could be due to epidemiological factors. In a study in Manipal- India, of the 108 specimens from the diabetic foot lesions, culture showed polymicrobial growth in 44.4% which was equal to monomicrobial growth in 44.4% [22]. These discrepancies suggest differences in diabetic foot infections, with severe infections usually having polymicrobial isolates and mild infections usually having monomicrobial isolates [23, 24]. To our research group, these discrepancies may be attributed to differences in the hygiene, hospital practice and usage of antibiotics. However, according to Citron et al., (2007) these discrepancies could be due to differences in the causative organisms occurring over time, geographical variations, or the type and severity of infection included in the studies [23]. Charles et al., (2001) reported variation of the bacterial pathogens encountered with the Wagner grades, early infections are generally monomicrobial, whereas advanced infections tend to be polymicrobial [4]. Low grades are generally infected with gram-positive and these findings were highly consistent with the present work, in which Gram-positive bacteria found in grades 1, 2 and decreased in grade 3 where the ratio of Gram-negative to Gram-positive was 5:1 then it disappeared towards grade 4 and reappeared in long standing ulcers which were in maturation phase. Widatalla et al., (2012) found that the most common pathogens were *Staphylococcus aureus* (33.3%), *Pseudomonas aeruginosa* (32.2%), and *Escherichia coli* (22.2%) [25]. A study in Sudan (J. D. C) [18] also found that the most common isolated organism was *S. aureus* (46%). Those two studies [18, 25] are consistent with most international reports [26, 27, 28, 29] where *S. aureus* was found as the most predominant and this contradicts with our findings. A recent study in Sudan [30] identified proteus spp. (*mirabils* and *vulgaris*) as the most frequent bacteria in diabetic wounds (37.5%) and this agrees with our findings. In the present research, microbiological investigation revealed that the percentage of Gram-negative organisms 66.3 % higher than Gram-positive which was 33.7 %. In addition, a study conducted in North India found that Gram-negative aerobes were most frequent 63.8% higher than Gram-positive aerobes 36.1% [31] which match the results of the current study. However, similar results were also obtained by two Indian studies [3, 4]. Benwan et al., (2012) stated that Gram-negatives were more prevalent, but predominant organisms isolated were members of the Enterobacteriaceae [32].

In the present study the percentage of diabetic patients with peripheral neuropathy was in range of 36.8% - 65.1% with average of 51%. This is in line with estimation of Bagchi and Sreejayan (2012) which was 45% - 60% of all

ulcerations in diabetic patients were mainly due to neuropathy. Diabetic peripheral neuropathy (DPN) is the most frequent neurological complication of diabetes mellitus (DM) [33]. It is recognized that peripheral neuropathy along with foot deformities and acute or repetitive trauma are the triad of factors that contribute ultimately to diabetic foot ulcers [34]. In the current study, no significant association between different grades of wounds (Wagner grades) and neuropathy was detected. This suggests that there was no impact of peripheral neuropathy on DFU healing although the association between peripheral neurpathy and non-healing diabetic ulcers was reported from developing countries [35, 36, 37, 38].

5. CONCLUSION

Gram-negative bacteria were more prevalent, and the most frequent pathogens were Proteus spp. The most common polymicrobial infection were *P. mirablis* with; *P. aerginosa, S. aureus* MRSA and *Coliform,* respectively. Polymicrobial infection was higher in long standing ulcers (30.4%). Low grades 1 and 2 are infected with Gram-positive and high grades 3, 4 are infected with Gram-negative. No significant relation between Wagner grades and neuropathy was detected suggesting no impact of peripheral neuropathy on DFU healing. Epidemiological studies are needed to clarify the reason beyond the variations in the pathogens encountered from diabetic foot infection in different studies. Therefore, based on the findings of this study early diagnosis, proper microbiological cultures are essential for effective management of diabetic wound infection.

Conflicts of interest: The authors state that they have no competing interests.

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