Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i16.3358

IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILE OF MICROORGANISMS ISOLATED FROM DIABETIC FOOT ULCERS

Neha Goel[1], Hina Singh[2], Sagar Kashyap[3*]

[1]Associate Professor, Department of Microbiology, GS Medical College & Hospital
[2]Associate Professor, Department of General Medicine, GS Medical College & Hospital
[3]*Assistant Professor, Department of Microbiology, GS Medical College & Hospital

***Corresponding Author: -**Sagar Kashyap
*Assistant Professor, Department of Microbiology, GS Medical College & Hospital,
Email: drsagarkashyap88@gmail.com

Abstract

Introduction: Diabetes increases the susceptibility of wounds to a variety of infections, including bacteria. It is a major factor in lower limb amputations and can progress from a soft tissue infection to a bone infection. For a good prognosis, it's crucial to make an early diagnosis and choose a course of therapy based on the identification of the pathogens and their antimicrobial susceptibility patterns. As a result, the goal of this study was to identify the bacteria that cause foot ulcers and assess their profile of antibiotic resistance.

Materials and Methods: Using sterile swabs, samples of diabetic foot ulcers were aseptically taken. Culture, Gram staining, and biochemical tests were used to identify the isolates. The Kirby-Bauer disc diffusion method was used to assess the antibiotic susceptibility of the isolated bacteria.

Results: Out of 150 patients with diabetes, 67 patients had diabetic foot ulcer. 37.3% of them were female and 62.7% of them were male. Of these, 55.2% were Gram negative and 41.7% were Gram positive isolates. The most predominant bacteria among the isolated microorganisms were *Staphylococcus aureus* (31.3%), followed by *Pseudomonas species* (17.9%). Most of the Gram positive bacteria were resistant to dicloxacillin and Cefuroxime. The majority of Gram negative bacteria were resistant to Cefepime followed by tetracycline, cefotaxim, and cefuroxime.

Conclusion: Numerous multidrug resistant bacteria and a wide range of pathogens can infect diabetic foot ulcers. The outcome demonstrated a general rise in bacterial resistance to antimicrobial drugs and highlights the significance of microbiological analysis and antimicrobial susceptibility testing prior to beginning antibiotic treatment for infections in diabetic foot ulcers.

Keywords: Antimicrobial agents; diabetic mellitus; diabetic foot ulcer; Gram-positive bacteria; gram-negative bacteria.

1. Introduction:

Diabetes mellitus affects around 422 million people worldwide and is accountable for an estimated two million deaths per year[1]. Diabetic foot ulcer is a serious and debilitating complication of untreated diabetes that typically appears as ulceration on the plantar portion of the foot[2]. It will appear in about 25% of diabetic individuals throughout the course of their lives, and more than 50% of these ulcers will become infected[3]. DFU is brought on by repeated trauma as a result of a confluence of conditions including immune impairment, peripheral vascular disease, and loss of protective sense[4]. Ulcers are

most likely to occur in the weight bearing areas such as plantar metatarsal head, heel, tips of hammer toes and other major areas[5]. The lack of protective sensation in the feet predisposes diabetes patients to developing trauma and ulcers. This sensory impairment is brought on by the over-expression of sorbitol dehydrogenase and aldose reductase, which leads to an increase in fructose and sorbitol production. These glucose byproducts build up and cause osmotic stress, which lowers myoinositol production and nerve conduction in nerve cells[6]. Moreover, diabetes can cause sensory neuropathy as well as neural autonomic dysfunction, which impair sweat production and make the foot more prone to dryness, skin cracking and fissures[7]. Muscle atrophy and anatomical flaws in the foot can also result from motor neuron dysfunction. This raises the risk of ulceration by causing focally raised pressures at distinct plantar foot zones[8]. In addition, significant changes to the extracellular matrix (ECM) and inflammation both contribute significantly to the persistence of the non-healing DFU[9].
Moreover, a large number of microorganisms would colonize and multiply in the ulcer, accentuating tissue and causing infection[10]. The protein and carbohydrate components found in diabetic foot ulcer can act as nidus for infection[11]. In the early acute stage, diabetic foot infection is typically monomicrobial and brought on by Gram positive cocci; in the chronic stage, it is typically polymicrobial and brought on by a combination of Gram negative aerobes, anaerobes, and fungi[12]. Bacteria that show drug resistance to three or more widely used antibiotics that are typically sensitive are referred to as multi-drug resistant pathogens[13]. It is critical to concentrate on evaluating the risk factors of multi-drug resistant bacterial infections in order to find more effective treatment options[14]. Continuous updates of the microorganisms responsible for infection and their resistance pattern remain a keystone in the management process, since infection with resistance strains is increasing and poses additional morbidity and mortality[15]. The treatment of diabetic foot infection requires the selection of the appropriate antimicrobial. In an effort to determine the best antimicrobial treatment options for patients, the goal of this study is to identify the bacteria that cause foot ulcers and assess their profile of antibiotic resistance.

2.Material and Methods

2.1 Study design and participant subjects

This study was conducted in the department of Microbiology at the G.S. Medical College& Hospital. The current study included 300 participants from G.S medical college. Out of the 300 participants chosen, 150 had type 2 diabetes, while the remaining 150 acted as the control group. The American Diabetes Association (ADA, 2022)'s criteria were used to determine the diagnosis of T2DM in each subject[16]. After receiving proper approval from the institutional ethical committee, the study got underway. Prior to the event, each participant got counseling about diabetes, including its causes, symptoms, complications, etc. Before registering, all subjects were made aware of the study's goals. Before giving their written agreement, every subject was fully informed of the advantages and disadvantages of the study.

2.1.1 Inclusion and exclusion criteria employed for selection of subjects

Inclusion Criteria

This study included all adult diabetic patients with ulcers who visited the clinic during the study period, provided informed consent, and whose ulcers were higher than or equivalent to the Wagner first degree grading scale.

Exclusion criteria

Subjects with additional disease accompanied by diabetic foot ulcer were not admitted to the Department of Diabetic foot and those subjects presently using any other drug which interferes with the interpretation of trial results were excluded.

2.2 Collection of samples

Using two sterile swabs that had been dipped in sterile glucose broth, samples were collected from the deepest area of the ulcer. The swab was used to take the samples while rotating it around like a film. Gram staining was done on one swab, while culture was done on the other. Socio-demographic and other clinical data were gathered using semi-structured questionnaires.

2.3 Pathogen identification

The ulcerated secretions were taken on the day of admission using ulcer swabs and cultured within 1 h after collection. Directly from the sample, a Gram smear was evaluated. Three different agar plates—blood agar, MacConkey agar, and chocolate agar —were used to inoculate the samples. After being incubated at 37°C for the entire night, the inoculation plates were examined the next day for growth. According to the Wagner Diabetic Foot Ulcer Classification System, ulcers were categorized in this study.

2.4 Antibiotic susceptibility test

The Kirby Bauer disc diffusion technique was used to assess the antibiotic susceptibility of the isolated bacteria on Mueller Hinton Agar in accordance with the Clinical Laboratory Standard Institute (CLSL) guidelines 2020[17]. To make the inoculums for each isolate, colonies from the purified culture were overnight emulsified in test tubes with sterile saline (0.85%) and turbidity adjusted to standard 0, 5 McFarland. A sterile swab was used to evenly distribute the bacterial suspension over the MHA plate. After waiting three minutes, the antibiotic discs were added. Amikacin, Cefotaxim, Cefuroxime, Imipenem, Ciprofloxacin, Tetracycline, Gentamicin, Cefepime, Chloramphenicol, and Meropenem were among the antibiotic discs used for Gram-negative bacteria. Ampicillin, Cefotaxim, Cefuroxime, Dicloxacillin, Levofloxacin, Tetracycline, Gentamicin, Vancomycin, Erythromycin, and Penicillin were the antibiotics used for Gram-positive bacteria. Using a Vernier calliper, the diameter of the zones of inhibition was measured after the plates were incubated for 16–18 hours at 35°C. The data were then interpreted in accordance with CLSL standards.

For each and every laboratory procedure, standard operating procedures were employed. To confirm the outcome of antibiotics, media, and to evaluate the quality of the overall laboratory procedure, quality control strains of *Escherichia coli* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *K. pneumonia* (ATCC 1705) were used.

3. Result

Patient data: 67 of the 150 diabetic individuals had diabetic foot ulcers. The mean age of diabetes patients was 49.7 years, with a mean fasting blood glucose level of 183.7 mg/dl and a mean postprandial blood glucose level of 260.6 mg/dl. Of these, 42 (62.7%) were male and 25 (37.3%) were female. 9.4% was the mean HbA1c level. Among the patients who were included, the proportion of Wagner grade III patients was noticeably higher.

Table 1: Baseline characteristics of subjects recruited to the study

Parameters	Variables	Normal (n=150)	Diabetic (n=150)
Mean age(years)	30-70	48.5	49.7
Sex (No.)	M/F	105/45	100/50
DFU	M/F	-	42/25
BMI (Kg/m^2)18-30		24.5±0.8	25.1±0.7
Duration of diabetes	≤ 1	-	32
(Years)	1-10	-	88
	11-20	-	28
	21-30	-	02
Systolic blood pressure (mmHg)		127.9±3.2	130.5±3.3
Diastolic blood pressure (mmHg)		78.4±1.4	79.3±1.0
Wagner's classification system	Grade I	-	10
Grade II	-	21	
Grade III	-	27	

Grade IV	-	09
Grade V	-	-

BMI: Body Mass Index; DFU: Diabetic foot ulcer

3.1 Identification of microorganisms

32 of the 67 samples were monobacterial in origin, while 35 of the samples displayed polymicrobial growth. Of these 28 isolates, 41.7% of them were Gram positive and 37 (55.2%) were Gram negative. *Staphylococcus aureus* (31.3%) was the most prevalent bacteria among the isolated bacteria, followed by *Pseudomonas species* (17.9%) and *Escherichia coli* (14.9%). *Acinetobacter species*, *Klebsiella pneumonia*, *Enterococcus species*, and *Proteus vulgaris* were among the other isolates. One of the two isolated fungi was recognized as a *Candida species*, and the other as *Aspergillus niger*. No significant difference was found among the isolates. Out of 67 diabetes subjects with diabetic foot ulcer, 27 subjects were classified to have Wagner grade III diabetic foot ulcer followed by Grade II.

Table 2: Profile of microorganism isolated from Diabetic foot ulcer

Isolated Microorganism	Frequency (N=67)	Percentage (%)
Staphylococcus aureus	21	31.3
Pseudomonas species	12	17.9
Escherichia coli	10	14.9
Streptococcus species	05	7.4
Acinetobacter species	06	8.9
Klebsiella pneumoniae	07	10.4
Enterococcus species	02	2.9
Proteus species	02	2.9
Candida species	01	1.4
Aspergillusniger	01	1.4

3.2 Antibiotic resistant profile of Gram positive isolates

Using various antibiotic combinations, 28 Gram positive bacteria were tested for antibiotic susceptibility. The majority of Gram-positive bacteria were resistant to dicloxacillin, cefuroxime, cefotaxim, gentamicin, and tetracycline. Most *Staphylococcus aureus* and *Enterococcus species* isolates were found to have high levels of resistance and to be resistant to dicloxacillin, cefotaxim, gentamicin, and tetracycline. Gram positive bacteria have significantly greater levels of dicloxacillin resistance.

Table 3: The Antimicrobial susceptibility pattern of Gram positive bacteria

Antimicrobial Agents	***Staphylococcus aureus* (N=21)**		***Streptococcus species* (N=5)**		***Enterococcus species*(N=2)**	
	S (%)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Ampicillin	07 (33.3)	14 (66.6)	2 (40)	3 (60)	1 (50)	1 (50)
Cefotaxim	05 (23.8)	16 (76.1)	3 (60)	2 (40)	0 (00)	2 (100)
Cefuroxime	04 (19.0)	17 (80.9)	2 (40)	3 (60)	2 (100)	0 (00)
Dicloxacillin	04 (19.0)	17 (80.9)	2 (40)	3 (60)	0 (00)	2 (100)
Levofloxacin	14 (66.6)	07 (33.3)	2 (40)	3 (60)	2 (100)	0 (00)
Tetracycline	07 (33.3)	14 (66.6)	2 (40)	3 (60)	0 (00)	2 (100)
Gentamicin	06 (28.5)	15 (71.4)	2 (40)	3 (60)	0 (00)	2 (100)
Vancomycin	16 (76.1)	05 (23.8)	3 (60)	2 (40)	1 (50)	1 (50)
Erythromycin	09 (42.8)	12 (57.1)	4 (80)	1 (20)	0 (100)	2 (100)
Penicillin	08 (38.0)	13 (61.9)	3 (60)	2 (40)	0 (100)	2 (100)

3.3 Antibiotic resistant profile of Gram negative isolates

The antibiotic susceptibility of 37 distinct Gram negative bacteria was tested using various antibiotic combinations. Tetracycline, Cefotaxim, and Cefuroxime were resistant against Gram-negative bacteria after Cefepime. Tetracycline and Cefotaxim resistance was found in *Escherichia coli* isolates. Tetracycline, cefotaxim, and cefepime were not effective against *Pseudomonas* *spp.*Imipenem and Meropenem were not resistant to any *Proteus species.* The majority of antibacterial medications, including tetracycline, cefepime, chloramphenicol, cefotaxim, and meropenem, were resistant against *Acinetobacter spp.* and *Klebsiella spp.*

Table 4: The Antimicrobial susceptibility pattern of Gram negative bacteria

Antimicrobial Agents	***Pseudomonas spp.*** **(N=12)**		***Escherichia coli*** **(N=10)**		***Acinetobacter spp.*** **(N=06)**		***Klebsiella pneumonia*** **(N=07)**		***Proteus species*** **(N=02)**	
	S (%)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
Amikacin	07 (58.3)	05 (41.6)	08 (80)	02 (20)	2 (33.3)	4 (66.6)	02 (28.5)	5 (71.4)	1(50.0)	1(50.0)
Cefotaxim	04 (33.3)	08 (66.6)	03 (30)	07 (70)	1 (16.6)	5 (83.3)	02 (28.5)	5 (71.4)	2 (100)	0 (00)
Cefuroxime	09 (75)	03 (25.0)	09 (90)	01 (10)	2 (33.3)	4 (66.6)	01 (14.2)	6 (85.7)	1 (50.0)	1(50.0)
Imipenem	07 (58.3)	05 (41.6)	08 (80)	02 (20)	3 (50.0)	3 (50.0)	03 (42.8)	4 (57.1)	2 (100)	0 (00)
Ciprofloxacin	06 (50)	06 (50.0)	06 (60)	04 (40)	2 (33.3)	4 (66.6)	04 (57.1)	3 (42.8)	1 (50.0)	1(50.0)
Tetracycline	01 (8.3)	11 (91.6)	01 (10)	09 (90)	1 (16.6)	5 (83.3)	00 (00)	7 (100)	00 (00)	2(100)
Gentamicin	11 (91.6)	01 (8.30)	08 (80)	02 (20)	4 (66.6)	2 (33.3)	01 (14.2)	6 (85.7)	1 (50.0)	1(50.0)
Cefepime	00 (00)	12 (100)	00 (00)	10 (10)	0 (00)	6 (100)	00 (00)	7 (100)	00 (00)	2(100)
Chloramphenicol	10 (83.3)	02 (16.6)	08 (80)	02 (20)	3 (50.0)	3 (50.0)	00 (00)	**7** (100)	1 (50.0)	1(50.0)
Meropenem	08 (66.6)	04 (33.3)	07 (70)	03 (30)	1 (16.6)	5 (83.3)	05 (71.4)	2 (28.5)	2 (100)	0 (00)

4. **Discussion**

According to Wang et al. (2022) a diabetic foot ulcer is a serious condition that affects more than just the superficial subcutaneous tissue[18]. Diabetic foot ulcers arise as a result of poorly managed or uncontrolled diabetes[18]. Diabetes-related foot ulcers may become infected and cause gangrene, osteomyelitis, or even amputation if left untreated[19]. The methods utilized to treat this infection include surgery and antibiotic therapy[20]. This analysis was carried out to determine the predominant pathogenic bacterial infections linked to diabetic foot ulcers and their patterns of antimicrobial sensitivity to widely prescribed antibiotics at the study sites. The majority of diabetic foot ulcer patients in the current study (62.7%) were male; these results are in line with those of previous studies[21,22]. This could be explained by the greater active participation of males in outdoor sports, which exposes them to accidents and ulcer formation.

According to the Wagner diabetic foot ulcer categorization system, ulcers were categorized in this study[23]. According to research done in Egypt, where grade III was identified in 50% of participants and grade II in 25%, the most prevalent was grade III (40.2%), followed by grade II (31.3%)[12]. In contrast to these results, an Indian study revealed that grade II (69.2%) outperformed grade III (5.1%). The recent study shows that a variety of organisms can infect diabetes individuals, but Gram negative bacteria are the most persistent and hazardous pathogens that cause systemic symptoms. Gram negative bacteria (55.2%) made up a higher percentage than Gram positive bacteria (41.7%). In contrast to these results, several researches have found that Gram positive bacteria, as opposed to Gram negative bacteria, are more commonly identified from diabetic foot ulcers[12,24].

This result is consistent with a previous study in which Gram negative bacteria were recovered in 88.5% of cases compared to 7% of cases for Gram positive bacteria. In a study from Egypt, there were 56% of the samples were Gram negative and 27.7% were Gram positive samples; in another study from northeast India, there were 79% Gram positive samples and 21% Gram negative samples[25,26].

S. aureus, *E. coli* and *P. aeruginosa* were determined to be the main isolates in our investigation. This has a strong connection to several indigenous researches from the southern region of the nation where *S. aureus* was the main pathogen[27]. According to Alkhudhairy and Al-Shammari (2020), *P. aeruginosa* is also a cause of the significant tissue damage that diabetes individuals experience[28]. *P. mirabilis* and Gram negative bacteria are additional microorganisms[29]. The most frequently isolated organisms were *Staphylococcus species*, specifically *Staphylococcus aureus* and *Staphylococcus* *epidermidis*, followed by *Enterococcus species*. This raises a serious health issue in subjects suffering from diabetes foot ulcers and diabetes[26]. Xie et al. stated that 59.8% of their samples exhibited polymicrobial infections, the current investigation found that 52.2% of samples revealed polymicrobialinfection[30].According to Akhi*et al.* (2015), monobacterial infections typically cause mild infections, whereas polymicrobial infections typically cause severe infections[25]. These findings are in line with the findings of the current study, which found that the majority of patients with polymicrobial infections were identified as having Wagner III grade diabetic foot ulcers. Furthermore, the present study found that Gram positive and Gram negative species were identified with mild to severe infections in diabetes patients who did not receive the antibacterial medication treatment. When selecting an antibiotic for early treatment, the majority of practitioners will prescribe an antibiotic based on their expertise and observations[31]. Prior to treatment, a proper understanding of antibiotic resistance will aid in the effective management of the illness. The findings of the current study will offer recommendations to doctors regarding possible antibiotics to be used in the treatment of diabetic foot ulcers. The Gram positive bacteria in this investigation were shown to be more likely to be resistant to dicloxacillin followed by Cefuroxime, Cefotaxim, Gentamicin, and Tetracycline. The results of this investigation corroborated those of Sanchez-Sanchez et al., who discovered that dicloxacillin resistance was most prevalent among Gram-positive bacteria[32]. The Gram-negative bacteria that were recovered in the current study were more likely to be resistant to cefepime than to tetracycline, cefotaxim, or cefuroxime. A higher proportion of the Gram-negative bacteria in Sanchez-Sanchez et al.'s 2017 study were ampicillin, cefotaxime, and ceftriaxone resistant[32]. Levofloxacin, netilmicin, and amikacin are the three antibiotics that have been shown to be the most effective in prior studies[32,33]. Despite the small study sample size, the findings indicated that altering empirical techniques to stop the spread of antibiotic resistance was necessary. The present investigation confirmed that DFU infection is caused by both Gram positive and Gram negative bacteria. Due to their antimicrobial resistance profile, these bacteria can pose difficulties for patient management and increase complications like osteomyelitis and potentially necessitate amputation of the limbs.

5. Conclusion

Numerous multidrug resistant bacteria and a wide range of pathogens can infect diabetic foot ulcers. The most common isolate in the investigation was *Staphylococcus aureus*, which was followed by other Gram-negative bacteria. The present study found a high prevalence of resistance to commonly used antibiotics, highlighting the need for vigilance while treating diseases with antibiotics. Dicloxacillin resistance was higher among the isolates in the current investigation, followed by cefuroxime, cefotaxim, gentamicin, and tetracycline. The results highlighted the importance of microbiological analysis and antimicrobial susceptibility testing before starting antibiotic treatment for infections in diabetic foot ulcers and showed a general increase in bacterial resistance to antimicrobial medications.

6. References

1. GBD 2019 Diabetes in the Americas Collaborators. Burden of diabetes and hyperglycaemia in adults in the Americas, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Diabetes Endocrinol.*2022; 10: 655-667.
2. Kale DS, Karande GS, Datkhile KD. Diabetic foot ulcer in India: Aetiological trends and bacterial diversity. *Indian J EndocrMetab*. 2023; 27:107-14.
3. Yazdanpanah L, Shahbazian H, Nazari I, Arti HR, Ahmadi F, Mohammadianinejad SE, Cheraghian B, Hesam S. Incidence and Risk Factors of Diabetic Foot Ulcer: A Population-Based Diabetic Foot Cohort (ADFC Study)-Two-Year Follow-Up Study. *Int J Endocrinol.* 2018 Mar 15; 2018:7631659.
4. Tuttolomondo A, Maida C, Pinto A. Diabetic foot syndrome as a possible cardiovascular marker in diabetic patients. *J Diabetes Res.* 2015; 2015:268390.

5. Sabapathy SR, Periasamy M. Healing ulcers and preventing their recurrences in the diabetic foot. *Indian J Plast Surg*. 2016; 49(3):302-313.
6. Ramirez-Acuña JM, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, Flores-Morales V, Martinez-Fierro ML. Diabetic Foot Ulcers: Current Advances in Antimicrobial Therapies and Emerging Treatments. *Antibiotics (Basel).* 2019; 8.
7. Boyko EJ, Ahroni JH, Stensel V, Forsberg RC, Davignon DR, Smith DG. A prospective study of risk factors for diabetic foot ulcer. The Seattle Diabetic Foot Study. *Diabetes Care.* 1999; 22: 1036-1042.
8. Tesfaye S. Recent advances in the management of diabetic distal symmetrical polyneuropathy. *J Diabetes Investig.* 2011; **2**:33-42.
9. Skopljak A, Sukalo A, Batic-Mujanovic O, Muftic M, Tiric-Campara M, Zunic L. Assessment of diabetic polyneuropathy and plantar pressure in patients with diabetes mellitus in prevention of diabetic foot. *Med Arch.* 2014; 68: 389-393.
10. Akkus G, Sert M. Diabetic foot ulcers. A devastating complication of diabetes mellitus continues non-stop in spite of new medical treatment modalities. *World J Diabetes.* 2022 Dec 15;13(12):1106-1121.
11. SadeghpourHeravi F, Zakrzewski M, Vickery K, G Armstrong D, Hu H. Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. *J Clin Med.* 2019 Nov 10; 8(11):1935.
12. Ismail AA, Meheissen MA, Elaaty TAA, Abd-Allatif NE, Kassab HS. Microbial profile, antimicrobial resistance, and molecular characterization of diabetic foot infections in a university hospital. *Germs.* 2021 Mar 15; 11(1):39-51.
13. Nikaido H. Multidrug resistance in bacteria. *Annu Rev Biochem.* 2009; 78:119-46.
14. Yan X, Song JF, Zhang L, Li X. Analysis of risk factors for multidrug-resistant organisms in diabetic foot infection. *BMC EndocrDisord.* 2022 Feb 21; 22(1):46.
15. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health.* 2015; 109(7):309-18.
16. American Diabetes Association Professional Practice Committee; 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes—2022*. *Diabetes Care*. 2022, 45: S17–S38.
17. Bauer AW, Kirby WMM, Sherris JC, and Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. 1966; 36:493-496.
18. Wang X, Yuan CX, Xu B, Yu Z. Diabetic foot ulcers: Classification, risk factors and management. *World J Diabetes.* 2022 Dec 15; 13(12):1049-1065.
19. Armstrong DG, Lipsky BA. Diabetic foot infections: stepwise medical and surgical management. *Int Wound J.* 2004 Jun; 1(2):123-32.
20. Atlaw A, Kebede HB, Abdela AA, Woldeamanuel Y. Bacterial isolates from diabetic foot ulcers and their antimicrobial resistance profile from selected hospitals in Addis Ababa. *Ethiopia. Front Endocrinol (Lausanne).* 2022; 13:987487.
21. Murshed M. Bacteriological profile of diabetic foot infection and its effect on limb salvation. *J Surg Sci.*2020; 24(1):21–5.
22. Shah P, Eswarawaka M, Anne D, Shah P, Srivastava N. Bacteriological profile of diabetic foot. IntSurg J. 2021; 8(2):704–9.
23. Shah P, Inturi R, Anne D, Jadhav D, Viswambharan V, Khadilkar R, Dnyanmote A, Shahi S. Wagner's Classification as a Tool for Treating Diabetic Foot Ulcers: Our Observations at a Suburban Teaching Hospital. *Cureus.* 2022 Jan 22; 14(1):e21501.
24. Dwedar R, Ismail D, Abdulbaky A. Diabetic foot infection: Microbiological causes with special reference to their antibiotic resistance pattern. *Egyptian J Med Microbiol*. 2015; 24:95–102.
25. Akhi MT, Ghotaslou R, Asgharzadeh M, Varshochi M, Pirzadeh T, Memar MY, ZahediBialvaei A, SeifiYarijanSofla H and Alizadeh N: Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran. *GMS Hyg Infect Control*. 2015; 10: Doc02.

26. Anvarinejad M, Pouladfar G, Japoni A, Bolandparvaz S, Satiary Z, Abbasi P and Mardaneh J: Isolation and antibiotic susceptibility of the microorganisms isolated from diabetic foot infections in Nemazee hospital, Southern Iran. *J Pathog.* 2015; 328796.
27. Nageen, A. The most prevalent organism in diabetic foot ulcers and its drug sensitivity and resistance to different standard antibiotics. *J. Coll. Physicians Surg. Pak.* 2016; 26 (4), 293–296.
28. Alkhudhairy, M.K., Al-Shammari, M.M. Prevalence of metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. *New Microbes New Infect.* 2020; 35: 100661.
29. Hassan MA, Tamer TM, Rageh AA, Abou-Zeid AM, Abd El-Zaher EH, Kenawy ER. Insight into multidrug-resistant microorganisms from microbial infected diabetic foot ulcers. *Diabetes Metab. Syndr.* 2019; 13 (2), 1261–1270.
30. Xie X, Bao Y, Ni L, Liu D, Niu S, Lin H, Li H, Duan C, Yan L, Huang S and Luo Z. Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, southern China: Focus on the differences among different Wagner's grades, IDSA/IWGDF grades, and ulcer types. *Int J Endocrinol*. 2017: 8694903.
31. Leibovici L, Shraga I, Andreassen S. How do you choose antibiotic treatment? *BMJ.* 1999 Jun 12; 318(7198):1614-6.
32. Sanchez-Sanchez M, Cruz-Pulido WL, Bladinieres-Camara E, Alcala-Duran R, Rivera-Sanchez G and Bocanegra-Garcia V: Bacterial prevalence and antibiotic resistance in clinical isolates of diabetic foot ulcers in the northeast of Tamaulipas, Mexico. *Int J Low Extrem Wounds.* 2017; 16: 129-134.
33. Sugandhi P and Prasanth DA: Microbiological profile of bacterial pathogens from diabetic foot infections in tertiary care hospitals, Salem. *Diabetes MetabSyndr* 8: 129-132, 2014.