ORIGINAL ARTICLE

Assessment of Bacterial Isolates from Diabetic Foot Ulcers and Their Antimicrobial Susceptibility Patterns among Diabetic Patients Attending Kafr El Sheikh University Hospital

[1]Ayat S. El-Nahal[*], [2]Moustafa H. Mabrouk, [1]Samar A. Eissa, [1]Sally A. Saleh, [3]Hebatalla A. Ahmed, [1]Sally H. Essawy

[1]Department of Medical Microbiology and Immunology -Faculty of Medicine-Kafrelsheikh University, Egypt
[2]Department of Vascular Surgery, Faculty of Medicine-Kafrelsheikh University, Egypt
[3]Department of Community Medicine and Public Health, Faculty of Medicine, Kafrelsheikh University, Egypt

ABSTRACT

Background: *Diabetic foot ulcers (DFU) are one of the most frequent consequences of diabetes mellitus. Having an ulcer in a diabetic foot significantly affects a person's quality of life due to reduced physical function and reduced mobility.* **Objectives**: *to recognize the bacteria responsible for ulcers in the diabetic foot, to assess their antibiotic sensitivity, and to ascertain if infections in the diabetic foot frequently contain multi-drug-resistant bacteria.* **Methodology**: *Fifty diabetic individuals with diabetic foot ulcers attending the Vascular Surgery Department at Kafr El Sheikh University Hospital participated in this work. Sterile swabs were utilized to take pus samples from infections in the diabetic foot then the samples were immediately transferred and processed in the microbiological laboratory under aseptic conditions.* **Results**: *our study showed that 11 samples out of 30 were polymicrobial while 19 were monomicrobial with Pseudomonas aeruginosa as the most prevalent isolated pathogen followed by Staphylococcus aureus. Our study revealed that 15 (33.3%) isolated bacterial strains were multi-drug resistant.* **Conclusion**: *Early detection of ulcers in diabetic foot and adequate sample collection are crucial for identifying bacteria and determining antibiotic susceptibility patterns before starting antimicrobial therapy. This study guides the selection of empirical antibiotics for infections in diabetic feet.*

INTRODUCTION

Diabetic foot ulcers (DFU) are one of the most frequent consequences of diabetes mellitus. Having a diabetic foot ulcer significantly affects a person's quality of life due to reduced physical function and reduced mobility.[1,2] In addition to obvious physical morbidity and mortality, DFUs also present significant psychological challenges for patients, including emotional distress, depression, and anxiety disorders. [3,4]

Diabetic foot ulcer is the main cause of diabetes-related hospitalization, which is characterized by difficulty in treatment, longer hospitalization, and high medical costs. [5,6]

When DFU develops, the biggest problem is the increased vulnerability to different microorganisms that can cause major consequences including osteomyelitis, gangrene, infection, amputation, or even death with 50% five-year survival rate following an amputation and a 70% five-year survival rate following a DFU, where the five-year mortality and direct healthcare expenses are equivalent to cancer. [7,8]

Infection from diabetic foot ulcers is generally polymicrobial. Bacterial infections are the primary reason for many DFU problems, which can be decreased by early detection of infections and their management.[9] More than one million diabetic patients suffer lower limb loss annually, as a result of failure of therapeutic interventions for treating diabetic foot infections.[10]

This work aims to recognize the bacteria responsible for ulcers in diabetic foot, to assess their antibiotic sensitivity, and to determine if diabetic foot infections frequently contain multi-drug resistant bacteria.

METHODOLOGY

Subjects:

This work was carried out in the Medical Microbiology and Immunology Department, Faculty of Medicine, Kafr El Sheikh University. Fifty diabetic individuals with diabetic foot ulcers attending the Vascular Surgery Department of Kafr El Sheikh University Hospital participated in this work. All patients provided informed permission following an explanation of the work. All patients were subjected to full history taking, and a thorough clinical examination.

The Wagner Classification System for Ulcer in Diabetic Foot was applied to categorize ulcers in diabetic foot. As (Grade 0-Pre-ulcerative; no cellulitis or open lesions, Grades 1–2: Superficial ulcer; Grade 3–Deep ulcer with osteomyelitis, abscess, and joint infection; Grade 4–Localized gangrene of the forefoot and Grade 5–Global gangrene of the whole foot.

Sampling collection and technique:

Sterile swabs were utilized to take pus samples from infections in diabetic feet. The date, time, and patient identification number were written on each sample's label. Afterward, the specimens were immediately transferred to the microbiological laboratory under aseptic conditions.

Isolation and identification of pathogenic bacteria:

Each swab was cultured on blood agar, nutrient agar, and McConkey agar plates by sterile inoculation loop. Petri dishes were incubated at 37°C for 24-48 hours. The preliminary identification of microorganisms was performed through colony morphology, Gram staining, and type of hemolysis on blood agar. Identification of gram-negative and gram-positive bacteria was confirmed by the routine microbiological methods.

Antimicrobial susceptibility test:

Antibiotic susceptibility assays of bacteria isolated from the previous step were conducted employing the disc diffusion method according to CLSI, 2024. [11]

The 0.5 McFarland standard was used to adjust turbidity. On the Muller-Hinton agar surface, the bacterial inoculum was evenly distributed. Selected discs of antibiotic were placed over the agar plates and incubated for 16-18 hours at   35°C. The different classes of standard antibiotics were selected and their sensitivity pattern was studied for the bacterial isolates (Gram-positive and Gram-negative) according to CLSI, 2024.[11] Subsequently, MDR bacteria was detected as lack of sensitivity to at least one antimicrobial agent across three or more categories.[12]

Statistical analysis:

The data were analyzed using the Statistical Package for the Social Sciences" SPSS 22.0 software (IBM Microsoft). Quantitative data normality was tested by Kolmogorov's test.   Qualitative variables were prescribed using numbers and percent, the Chi-square test was chosen for analysis or Fisher's exact test and the Monte Carlo exact test (if more than 20% of the expected cell value is less than 5). Numerical variables were expressed as means and standard deviations or median (IQR), Independent t-test or Mann-Whitney U-test was chosen for comparison between groups. P-value (< 0.05) was adopted as the level of significance.

RESULTS

Our study showed that most diabetic patients suffering from ulcer diabetic foot were males (86.7%). Type 2 diabetes was the most common type (73.3%). Ninety percent (90%) of the patients had peripheral neuropathy and (80%) were hypertensive. The median time length of disease duration was 15.0 years (10.0-20.0). Regarding inflammatory markers, the CRP median value was 90mg/dl. The Wagner Diabetic Foot Ulcer Classification System was used in this work to categorize ulcers. The most common type was grade 3 (43.3%) followed by grade 2 (26.7%) followed by grade 4 (23.3%) and grade 5 (6.7%).The result is shown in table 1.

Table 1: Demographics and Clinical Characteristics of the Study

Clinical Characteristics variables		No %
Age (years)		47.0±13.0
Sex	Female	4(13.3%)
	Male	26(86.7%)
Diabetes type	Type 1	7(23.3%)
	Type 2	23(73.7%)
Haemoglobin A1C (mmol/L) Test		12.0(10.0-15.0)
Duration of diabetes in years		15.0(10.0-20.0)
Hypertension	Yes	24(80.0%)
	No	6(20.0%)
Peripheral Neuropathy (PN)	Yes	27(90.0%)
	No	3(10.0%)
CRP (mg/dl)		90.0(50.0-140.0)
Wagner's classification system	Grade 2	8(26.7%)
	Grade 3	13(43.3%)
	Grade 4	7(23.3%)
	Grade 5	2(6.7%)

-Values are presented as Number (%), Mean±SD, and Median (IQR).

Regarding culture results, 11 out of 30 cultures were polymicrobial while 19 cultures were monomicrobial with only one organism isolated. Higher Haemoglobin A1C and CRP median values were detected in participants with polymicrobial infections. For Wagner's classification grades of the participants in this work and type of culture results, it was estimated that polymicrobial infections were strongly detected in higher grades as all cases of grade 4 and 5 were polymicrobial infections. The result is shown in table 2.

Table 2: Demographics and Clinical Characteristics of the Study Participants According to Culture Results

Clinical Characteristics variables		Culture results		P value
		Polymicrobial (N=11)	Monomicrobial (N=19)	
Age (months)		51.0±6.0	45.0±16.0	0.142
Sex	Female	1(25.0%)	3(75.0%)	1.000
	Male	10(38.5%)	16(61.5%)	
Diabetes type	Type 1	2(28.6%)	5(71.4%)	
	Type 2	9(36.7%)	14(63.3%)	1.000
Haemoglobin A1C (mmol/L) Test		15(10-18)	12(10-15)	0.216
Duration of diabetes in years		15(15-25)	14(10-15)	0.134
Hypertension	Yes	10(41.7%)	14(58.3%)	
	No	1(16.7%)	5(83.3%)	0.372
Peripheral Neuropathy (PN)	Yes	10(37.0%)	17(63.0%)	
	No	1(33.3%)	2(66.7%)	1.000
CRP(mg/dl)		140(90-190)	70(30-100)	0.002*
Wagner's classification system	Grade 2	0(0.0%)	8(100.0%)	
	Grade 3	2(15.4%)	11(84.6%)	0.000*
	Grade 4	7(100.0%)	0(0.0%)	
	Grade 5	2(100.0%)	0(0.0%)	

*Significant.
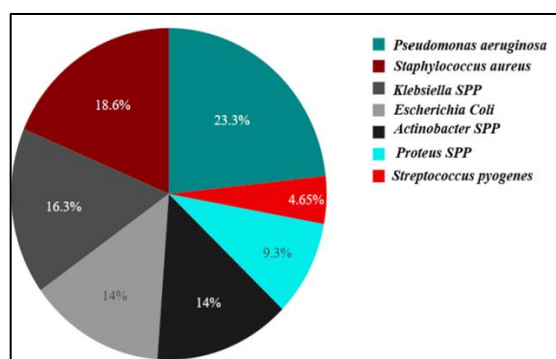Values are presented as Number (%), Mean±SD, and Median (IQR).

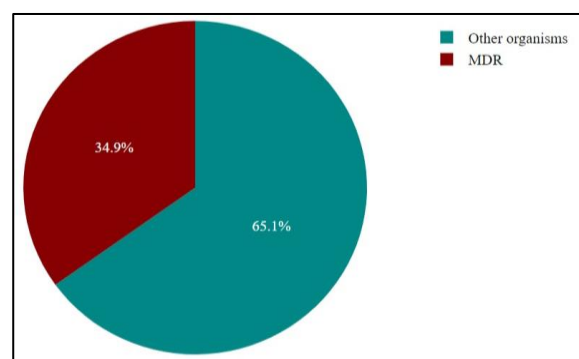**Fig. 1:** Percentage of microorganisms isolated from diabetic foot ulcer

Fig. 2: Percentage of multi-drug-resistant bacteria isolated from diabetic foot ulcer

Regarding culture results, the most prevalent pathogen was Pseudomonas *aeruginosa* following that *Staphylococcus aureus* and *Streptococcus pyogenes* was the least isolated pathogen.

Our study showed that a total of 43 isolated strains, (33.3%) were multi-drug resistant strains.
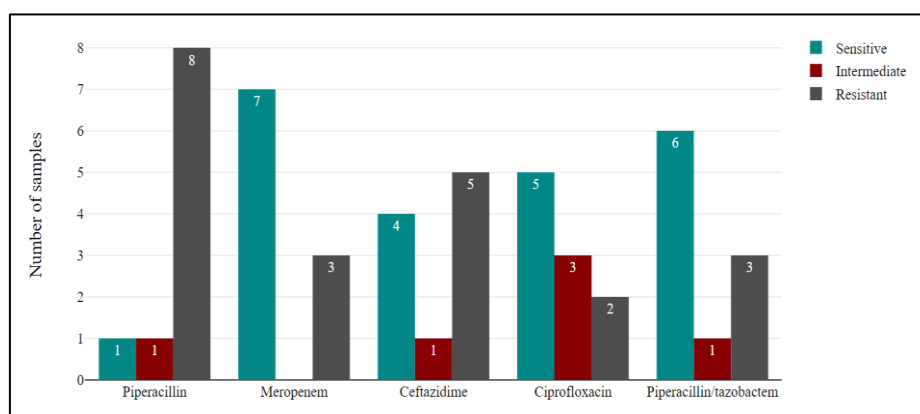

Fig. 3: Susceptibility of *Pseudomonas aeruginosa* to antimicrobial drugs.

Our study showed that *Pseudomonas aeruginosa* had highest sensitivity to meropenem then Piperacillin/tazobactam then ciprofloxacin while least sensitivity was to Piperacillin.
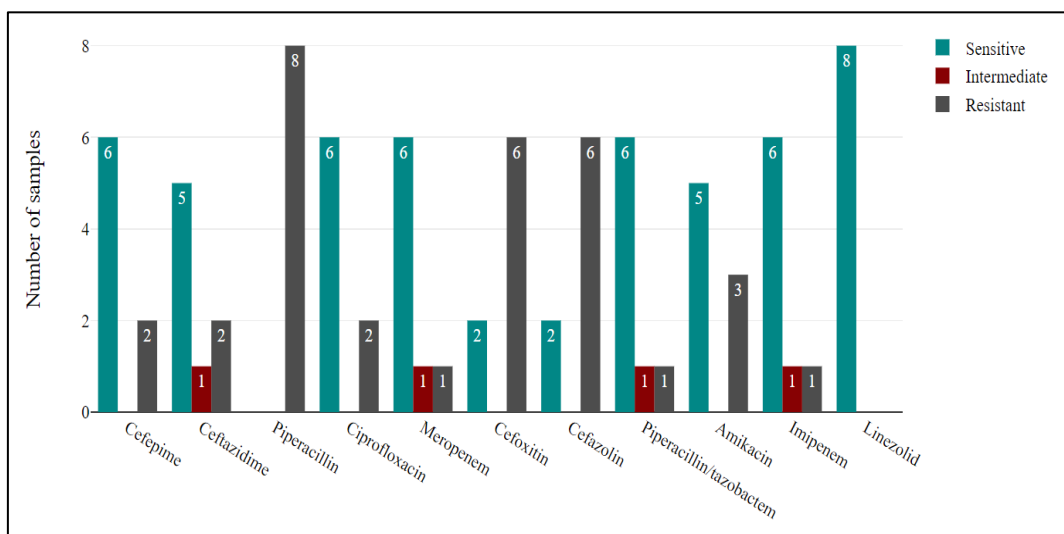

Fig. 4: Susceptibility of *Staphylococcus aureus* to antimicrobial drugs.

Regarding *Staphylococcus aureus*, linezolid had the highest sensitivity while cefoxitin and cefazolin had the least sensitivity among antibiotics.
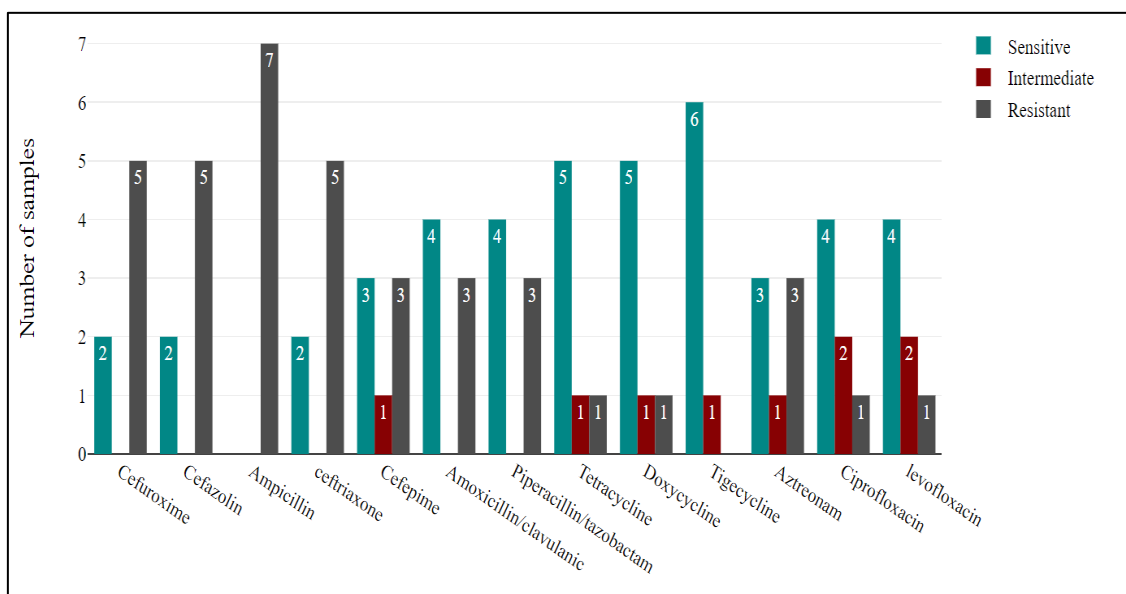

Fig. 5: Susceptibility of *Klebsiella Species* to antimicrobial drugs.

Our study showed that Klebsiella species had the highest sensitivity to Tigecycline then Tetracycline and doxycycline while the lowest sensitivity to Cefazoline, and Ceftriaxone.
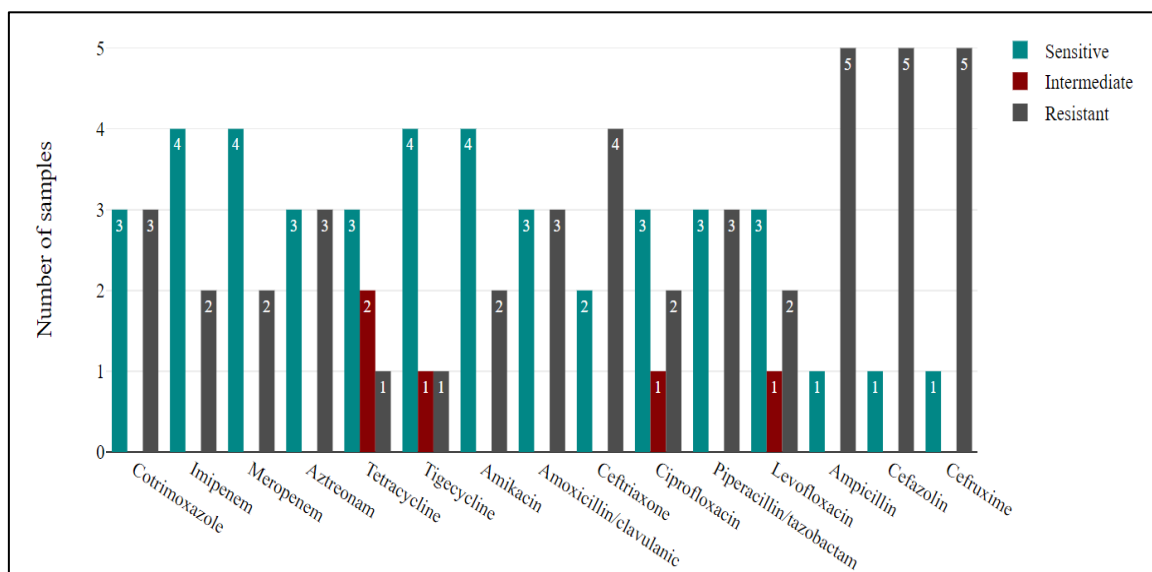
Fig. 6: Susceptibility of *E. coli* to antimicrobial drugs.

Our study revealed that *E.coli* had the highest sensitivity to Tigecycline, Amikacin, and Carbapenems while the least sensitivity to Ampicillin, Cefazoline, and Ceftriaxone.
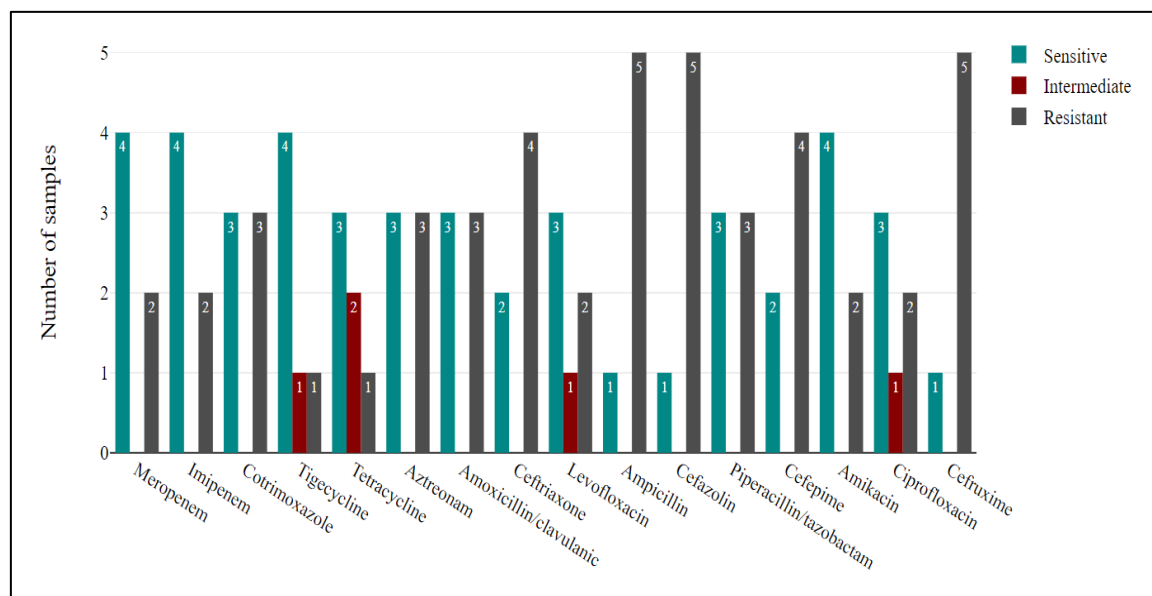

Fig. 7: Susceptibility of *Acinetobacter* species to antimicrobial drugs.

Our work showed that *Acinetobacter* s*pecie*s had the highest sensitivity to Tigecycline, Amikacin, and Carpabenems while the least sensitivity to Ampicillin, cefazoline, and Ceftriaxone.
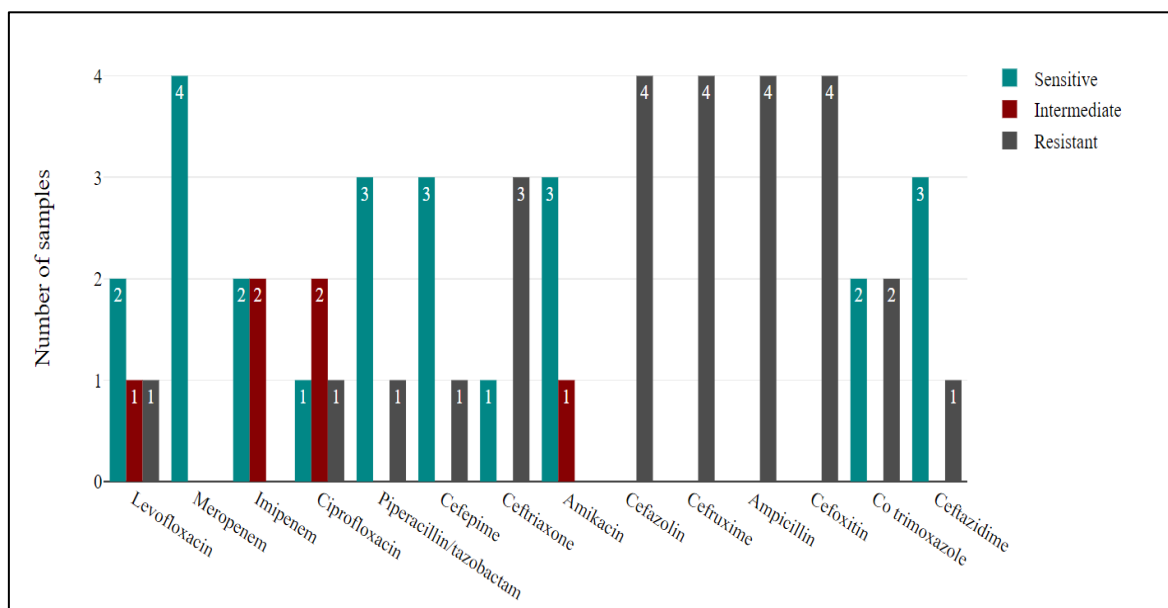
Fig. 8: Susceptibility of *Proteus spp* to antimicrobial drugs.

Our study showed that *proteus species* had the highest sensitivity to Meropenem then Cefepime, Piperacillin/tazobactem ceftazidime, and amikacin while the least sensitivity to Ceftuxime, Cefazoline, Ampicillin, and Cefoxitin.
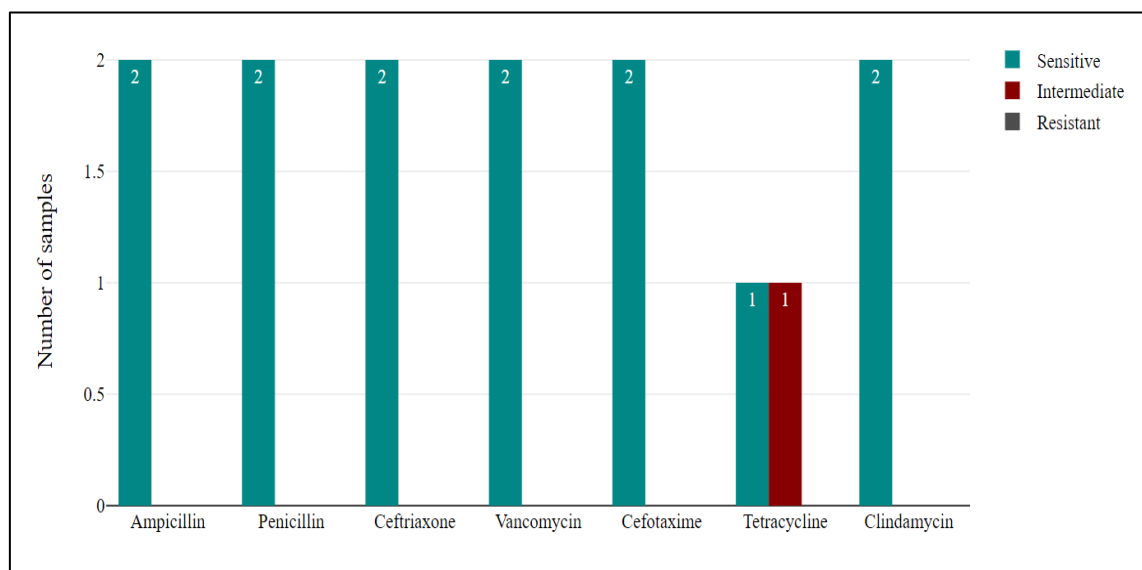

Fig. 9: Susceptibility of *Streptococcus pyogenes* to antimicrobial drugs

Streptococcus pyogenes strains were highly sensitive to Vancomycin, Penicillin, Ampicillin, Ceftriaxone, and Clindamycin**.**

DISCUSSION

The most prevalent side effect of diabetes mellitus is diabetic foot ulcer. If this ulcer is left untreated, the patients will be susceptible to infections and other problems including osteomyelitis, gangrene, and amputation. The two methods utilized to treat this illness are surgery and antibiotics. The purpose of this study was to recognize the bacterial infections linked to DFU and their antimicrobial sensitivity patterns.

This study showed that most of the diabetic individuals suffering from ulcers in the diabetic foot were males (86.7%) and the mean age of the subjects was 47.1 ± 13.0 years. More than two-thirds (73.3%) were Type 2 diabetes. Almost all patients (90%) had peripheral neuropathy and (80%) were hypertensive.

Similarly, a study by Uivaraseanu et al.[13] stated that ulcers in the diabetic foot are more prevalent in males than in females and increased in type 2 diabetic patients than in type 1 diabetic patients. Patients suffering from diabetic foot ulcers were older, extended duration of diabetes, and had more diabetic complications, such as diabetic polyneuropathy diabetic kidney disease, and retinopathy than patients without diabetes.

The Wagner Classification System of Ulcers in Diabetic Foot was used in this work to categorize ulcers. The highest percentage was in grade 3 (43.3%) followed by grade 2 (26.7%) which is in harmony with another study carried out in Egypt[14], where 50% of the patients had grades 3, and 25% had grade 2. Contrary to these results, a study by Jos et a .,[15] suggested that grade 2 (92.9%,) was the predominant type.

Regarding culture results, (36.6%) were polymicrobial while (63.4%) were monomicrobial. Polymicrobial infections were strongly detected in higher grades as all cases of grades 5 and 4 were polymicrobial infections.

In accordance with our work, a study by Atlaw et al.[9] revealed that increasing ulcer severity was correlated with an increase in rate and kind of bacterial isolates.

Our work showed that *Pseudomonas aeruginosa* was the prevalent isolated pathogen (23.3%) followed by *Staphylococcus aureus* (18.6%) and *Streptococcus pyogenes*(4.65%) as the least isolated pathogen. Similarly, research by Goh, et al[16] demonstrated that the predominant pathogen was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*, which is per the results of the work by Kleef et al. [17] who showed that *Pseudomonas aeruginosa* was the common pathogen (33%),

However, research from Ethiopia revealed that the common isolate was S. *aureus*, and also a study by Dawaiwala, I., et al., [18] revealed that the most prevalent Gram-negative& Gram-positive organisms isolated were *Escherichia coli and Staphylococcus aureus* respectively and MDR organisms constituted up to 52 (47.2%)

Our study showed that (33.3%) of isolated strains were multi-drug resistant. Similarly a study by Yan et al.[19] revealed that 42.5% of isolates were identified as MDR. On the contrary study from Ethiopia[4] revealed that multidrug-resistant bacteria represent a high proportion of isolated bacteria (92.9%).

In this work, most of the *Pseudomonas aeruginosa* and *Proteus species* had the highest sensitivity to Meropenem. *Klebsiella* species, *E.coli,* and Acinetobacter had the highest sensitivity to Tigecycline. All *Staphylococcus aureus* strains were susceptible to linezolid.

In line with our results, Liu et al.[20] showed that *Staphylococcus* and *Streptococcus* were resistant to clindamycin and erythromycin but susceptible to Vancomycin, Linezolid, and Tigecycline. Gram-negative bacteria remained highly sensitive to Meropenem, Tigecycline, and Amikacin. Previous studies showed a different pattern of susceptibility[9].

CONCLUSION

Early detection of ulcers of the diabetic foot and adequate sample collection are crucial for identifying bacteria and determining antibiotic sensitivity patterns before starting antimicrobial therapy. This study guides the selection of empirical antibiotics for infections with diabetic foot.

Conflicts of interest:
- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Fund: none

Approval of Ethical Committee:
- Ethical approval was obtained from the Scientific Research Ethical committee at Kafr El-Sheikh University before starting the study (approval number: KFSIRB200-142 and date of final approval : 29_1_2024).
- Informed consent obtained from participants after explaining the purposes of the study.
- No harmful methodology used with participants.
- Human rights were granted. Data was confidential, and a coding system for data was used

REFERENCES

1. Tejaswi L, Angadi N. The Impact of Diabetic Foot Ulcer on Health Related Quality Of Life and Employment among Diabetics Attending Tertiary Care Teaching Hospital, Davangere. Natl. J. Community Med. 2018: 9:794–797.

2. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. New England Journal of Medicine, 2017, 376.24: 2367-2375

3. Crocker RM, Palmer KNB, Marrero DG, Tan T.-W. Patient Perspectives on the Physical, Psycho-Social, and Financial Impacts of Diabetic Foot Ulceration and Amputation. J. Diabetes Complicat. 2021; 35, 107960

4. Polikandrioti M, Vasilopoulos G, Koutelekos I, Panoutsopoulos G, Gerogianni G, Alikari V, Dousis E, Zartaloudi A. Depression in Diabetic Foot Ulcer: Associated Factors and the Impact of Perceived Social Support and Anxiety on Depression. Int. Wound J. 2020; 17: 900–909.

5. Kerr M, Barron E, Chadwick P, et al. The cost of diabetic foot ulcers and amputations to the National Health Service in England. Diabetic Medicine, 2019, 36.8: 995-1002.

6. Tchero H, Kangambega P, Lin L, et al. Cost of diabetic foot in France, Spain, Italy, Germany and United Kingdom: A systematic review. In: Annales d'endocrinologie. Elsevier Masson, 2018. p. 67-74.

7. Hitam S, Asma'hassan S, Maning N. The significant association between polymicrobial diabetic foot infection and its severity and outcomes. The Malaysian Journal of Medical Sciences: MJMS. 2019; 26.1: 107.

8. Armstrong DG, Swerdlow, MA, Armstrong AA, et al. Five year mortality and direct costs of care for people with diabetic foot complications are comparable to cancer. J Foot Ankle Res. 2020; 13: 16.

9. 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. Frontiers in Endocrinology. 2022; 13: 987487.

10. Ndosi M, Wright-Hughes A, Brown S, Backhouse M, Lipsky BA, Bhogal M, Reynolds C, Vowden P, Jude EB, Nixon J, et al. Prognosis of the Infected Diabetic Foot Ulcer: A 12-Month Prospective Observational Study. Diabet. Med. 2018; 35:78–88

11. CLSI M100. Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition. 2024.

12. Rafailidis PI, Kofteridis D. Proposed amendments regarding the definitions of multidrug-resistant and extensively drug-resistant bacteria. Expert Review of Anti-infective Therapy. 2022; 20.2: 139-146.

13. Uivaraseanu B, Bungau S, Tit DM, et al. Clinical, pathological and microbiological evaluation of diabetic foot syndrome. Medicina. 2020; 56.8: 380.

14. 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; 11(1):39–51.

15. José AR, Sara, J, José Luis Lázaro-Martínez. Journal of Clinical Medicine. 2020; 9(9):3009.

16. Goh TC, Bajuri MYC. Nadarajah S, et al. Clinical and bacteriological profile of diabetic foot infections in a tertiary care. Journal of foot and ankle research. 2020; 13: 1-8.

17. Kleef DG, Hussein KR. Isolation of Bacterial Causative agents for Diabetic Foot patients and Antibiotic Susceptibility test against Bacterial Isolates. University of Thi-Qar Journal of Science. 2023; 10.1 (SI).

18. Dawaiwala, I, Awaghade S, Kolhatkar P, Pawar S, Barsode S. Microbiological pattern, antimicrobial resistance and prevalence of MDR/XDR organisms in patients with diabetic foot infection in an Indian tertiary care hospital. The International Journal of Lower Extremity Wounds. 2023; 22.4: 695-703.

19. Yan X, Song JF, Zhang L, et al. Analysis of risk factors for multidrug-resistant organisms in diabetic foot infection. BMC Endocrine Disorders. 2022; 22.1: 46.

20. Liu W, Song L, Sun W, Fang W, Wang C. Distribution of microbes and antimicrobial susceptibility in patients with diabetic foot infections in South China. Frontiers in Endocrinology. 2023; 14: 1113622.