ORIGINAL ARTICLE

BACTERIAL ISOLATES AND THEIR SENSITIVITY PATTERNS IN PATIENTS WITH DIABETIC FOOT ULCERS

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ABSTRACT

Objective: To determine the frequency of common microorganisms (bacterial isolates) and their sensitivity and resistance patterns in patients with diabetic foot ulcers

Materials and Methods: This descriptive cross-sectional study was conducted in the Department of Medicine, Khyber Teaching Hospital Peshawar from June 2020 to June 2021. Two hundred and thirty-eight diabetic patients with foot ulcers were included in the study. Deep wound swabs were collected and sent to the microbiology laboratory for culture and sensitivity. The sensitivity patterns of different organisms were identified.

Results: One hundred and thirty-four out of 238 (56.3%) patients were male, and the remaining 104 (43.7%) were female. The mean age of the patients in our study was 57.12±9.58 (32-80) years. Cultures were positive in 216 out of 238 (91%). Gram-negative organisms were 175 (81%), while 41 (19%) were gram-positive bacteria. The common bacteria were Escherichia coli 99 (45.8%), Pseudomonas aeruginosa 49 (22.7%), Staphylococcus aureus including methicillin-resistant staphylococcus aureus (MRSA) 36 (16.6%). The most effective antibiotics against gram-negative organisms were Meropenem (Sensitivity 97%), Amikacin (96%), Piperacillin-Tazobactam (95%), and Cefoperazone-Sulbactam (94%). The most effective antibiotics against Gram-Positive organisms were Vancomycin (Sensitivity 100%), Teicoplanin (100%), and Linezolid (95%). Antibiotics with higher rates of resistance included Doxycycline (89%), Clarithromycin (80%), and Co-Trimoxazole (78.7%).

Conclusion: Diabetic foot ulcers are frequently infected with a variety of organisms. Escherichia coli, Pseudomonas, and staphylococcus aureus are the primary (predominant) organisms. Meropenem, Amikacin, Cefoperazone-Sulbactam, and Piperacillin-Tazobactam Are The most effective antibiotics against Gram-Negative Bacteria, while Vancomycin, Teicoplanin, and Linezolid are effective against gram-positive bacteria.

Keywords: Diabetes Mellitus, Foot Ulcer, Pus Culture, Bacteria, Antibiotic Sensitivity and Resistance

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INTRODUCTION

A diabetic foot ulcer is a major cause of disability, morbidity, and mortality for the diabetic population. It is associated with substantial economic loss and poor quality of life. About 7- 15% of people with diabetes develop a foot ulcer at some stage, and 1% end up with amputation. [1]

Contributing factors in diabetic foot ulcers include peripheral neuropathy, peripheral vascular disease, and increased susceptibility to infection. In DM, wound healing is impaired, leading to gangrene of the foot. [2] About 50 %of diabetic foot ulcers are affected by infection with resultant increased morbidity, limb amputations, and mortality. Common isolates from diabetic foot ulcers include Staphylococcus Aureus, E. coli, Pseudomonas, and Staphylococcus epidermidis. Though the isolates greatly vary from area to area. [3]

The emergence of bacterial resistance to single or multiple antibiotics is another issue leading to poor outcomes in diabetic foot ulcers. In a recent Indian study, it was found that 55-66% of isolates were resistant to commonly used antibiotics. There is also an increased tendency of multiple drug-resistant strains of bacteria, which poses a significant challenge in effectively treating diabetic foot ulcers. [4]

As the bacterial pathogens and their antibiotic sensitivities vary from place to place, this study aimed to determine the frequency of common bacterial isolates from diabetic foot ulcers and their sensitivity patterns to commonly used antibiotics in our local setup. This will help us formulate an empirical antibiotic regimen for diabetic foot ulcers.

MATERIALS AND METHODS

This descriptive cross-sectional study was con-

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ducted in the Out-patients department and wards of medical and surgical units of Khyber Teaching Hospital Peshawar from January 2019 to September 2020. A total of 238 patients were included using an online calculator for sample size calculation.

The research proposal was approved by the institutional ethical and review committee. Informed consent was taken from all the patients in the study. Patients older than 30 years with grade 1 to 5-foot ulcers with fasting blood glucose ≥ 126 mg/dl or random blood glucose ≥ 200 mg or HbA1c > 6.5 were included in the study. Non-diabetic patients with foot ulcers were excluded from the study. The patients were enrolled by non-probability convenient sampling.

After thoroughly cleansing the affected foot with normal saline, pus was collected through a special swab for culture. The samples were taken using a firm circular motion with the swab. One swab was used for gram staining and the other for culture. These swabs were sent to the hospital laboratory for gram staining and culture using a standard microbiological protocol. The samples were directly examined for gram staining while the inoculated plates were incubated at 37^{o}C overnight and the plates were examined for growth the next day. Once the initial growth was achieved, the microorganisms were tested for sensitivities and resistance to different antibiotics using the Kirby Bauer disk diffusion method on Mueller Hinton agar plates in the hospital microbiology laboratory. Standard operating procedures (SOPs) were used for specific purposes for all laboratory procedures. Quality control stains were used to confirm the results of antibiotics, and media. To assess the quality of the general laboratory procedure, the Quality of reagents, antibiotic disk, and media used were according to international guidelines.

Different tested antibiotics included Cefoperazone-Sulbactam, Piperacillin-Tazobactam, Meropenem, Amikacin, Aztreonam, Tigecycline, Doxycycline, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Co-Amoxiclav, Chloramphenicol, Co-Trimoxazole, Vancomycin, Teicoplanin, Linezolid, Flucloxacillin, Clarithromycin, Ciprofloxacin, Levofloxacin, and Colistin sulfate.

Data was collected through a specially designed proforma and analyzed with the SPSS version 25. Microorganisms found in cultures and their sensitivities and resistance to different antibiotics were recorded. Mean and standard deviations were calculated for numerical variables like age and ulcer duration. Frequencies were calculated for nominal variables like the growth of microorganisms and the sensitivity pattern of each antibiotic. Data was presented in pie charts, graphs, and tables.

RESULTS

The mean age of patients was 57±9.55 (32-80) years. The majority of patients were in the age range of 40-70 years. (89%). Out of 238, 134 (56.3%) were male, and 104 (44%) were female. 11 (4.6%) patients had grade 1 ulcers. 123 (51.7%) patients had grade 2, 98 (41%) patients had grade 3, and only 6 (2.5%) patients had grade 4 ulcers. Most patients presented in the second to fourth week after the onset of the ulcer. Among 216 positive cultures, 175 (81%) isolates were gram-positive, and 41 (19%) were gram-negative. Pus culture was positive in 215(91%) patients and negative in the remaining 23 (9%). The common bacterial pathogens are shown in table 1.

The effective antibiotics (high sensitivity and least resistance) included Cefoperazone-Sulbactam, Piperacillin-Tazobactam, Meropenem, and Amikacin. Doxycycline was the least effective antibiotic, with 89% resistance. (Figure 1). The sensitivity of MRSA to vancomycin and Teicoplanin was 100 % (N=21) and 95 % (N=20) for linezolid (See figure 4).

DISCUSSION

Diabetic foot ulcers occur in middle age people who are otherwise active. The mean age of patients in our study was 57±9.55 years. Many of the patients were in age groups that ranged from 40 to 70 years. These find-

Table 1: Common bacterial pathogens in diabetic foot ulcers

Bacteria	n=216	Percentage
E.coli	99	45.8
Pseudomonas aeruginosa	49	22.7
Proteus mirabilis	18	8.3
MRSA	21	9.7
Methicillin sensitive staphylococcus aureus	15	6.9
Acinetobacter	4	1.9
Klebsiella pneumoniae	1	.5
Proteus vulgaris	4	1.9
Strep faecalis	5	2.3

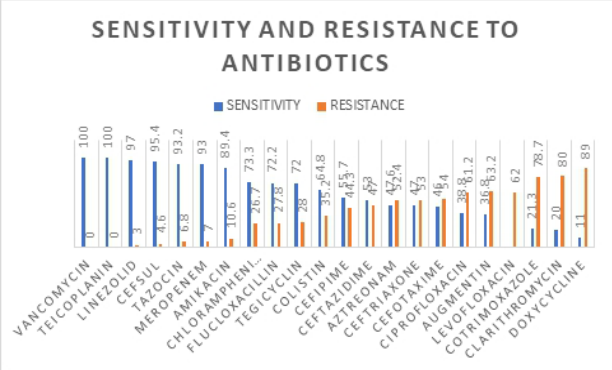

Fig 1: Sensitivity and resistance pattern of different antibiotics

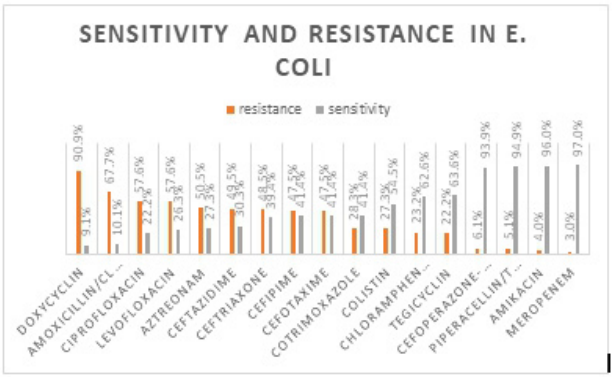

Fig 2: Sensitivity and resistance pattern of E.coli

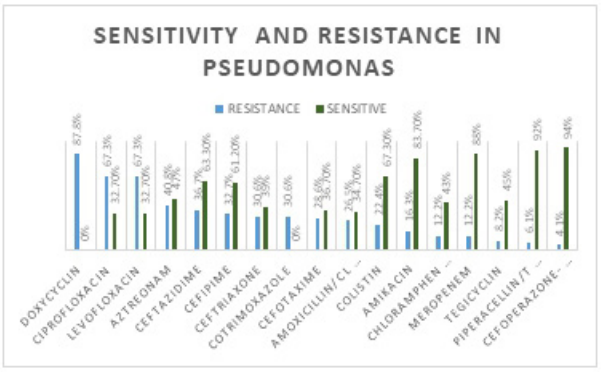

Fig 2: Sensitivity and resistance pattern of Pseudomonas aeruginosa

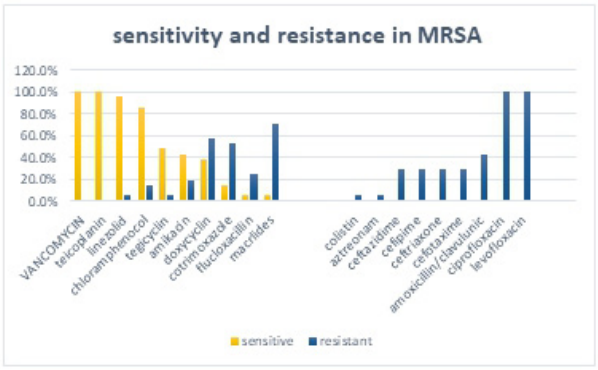

Fig 4: Sensitivity and resistance pattern of MRSA

ings are comparable to studies done worldwide. Since type 2 diabetes mellitus is usually a disease of adults and patients in this age group usually have other complications of diabetes as well, this may be a possible explanation for the higher frequency of diabetic foot ulcers in this age group. [5,6]

In our study, diabetic foot ulcers were more common in males than females. This is comparable to many local and international studies. [7,8] However, some studies have shown a higher frequency of diabetic foot ulcers in females. [9]

According to Wagner's classification, most of our patients had grade 2-3 foot ulcers. [10] This is because most patients ignore grade 1 ulcers or have poor knowledge about the early recognition of diabetic foot wounds. While patients with gangrene are usually referred to orthopedic surgeons for possible amputations. Similar results were found by Latif S et al. [11,12]

Many patients with diabetic foot ulcers have peripheral neuropathy. Initially, the patients may be unaware of the presence of the ulcer, or they underestimate the consequences of an initial ulcer. Therefore, more than 70% of our patients presented from the second to fifth week after the onset of the ulcer. [13,14]

The common isolates of the cultures from diabetic foot ulcers vary by location and setup. Most bacterial isolates in our study were gram-negative (81%) and gram-positive only 19%. The most familiar organisms in diabetic foot ulcers included staphylococcus aureus, Escherichia coli, and pseudomonas aeruginosa. According to Miyan Z et al., the most typical organism in descending order included staphylococcus aureus (20.67%), E. coli (15.72%), Klebsiella pneumonia (13.54%), Pseudomonas (13.54%) and Proteus mirabilis (12.81%). [15] Similar results were also shown in another local study by Amjad SS et al. [16]. According to that study, Staphylococcus aureus was identified in 46% of cases, E. coli in 28%, Pseudomonas in 6% and Klebsiella in 3.5%. According to the study of Saseedharan S et al. in India, the common isolates were Staphylococcus aureus (26.9%), Pseudomonas (20.9%), Enterococcus faecalis (12.7%), Escherichia coli 12%. [8] In our study, the predominant organism was Escherichia coli, followed by Pseudomonas aeruginosa and Staphylococcus aureus. These findings are supported by a similar study done in Iran by Hadadi A et al. [17,18,19]

In our study, E.coli isolates were more sensitive to Meropenem, Amikacin, Piperacillin-Tazobactam, And Cefoperazone-Sulbactam. These results are similar to the studies by Jain SK et al. [20] and Banashankari G et al. [21,] and Ozer B et al. [22]

E.coli showed high resistance to commonly used antibiotics like quinolones, Cephalosporins and Co-Amoxiclav. These results were supported by the study of Alvi et al.[23]Highest resistance was noted to doxycycline (91%). Due to this fact, Doxycycline is not recommended for gram-negative bacteria. [24]

Pseudomonas is an invasive bacterium and usually affects immunocompromised hosts. It is one of the most familiar bacteria responsible for a diabetic foot infection. In our study, Pseudomonas aeruginosa was the second most frequent organism isolated from pus culture. Very few antibiotics are effective against this organism. [25]

In our study, Pseudomonas isolates were more sensitive to Cefoperazone-Sulbactam, Piperacillin-Tazo-

bactam, Meropenem, and Amikacin. Higher sensitivity to these antibiotics was also noted in another local study by Ullah I et al. and Noor S et al. [14,26] In contrast to other studies, our study showed high rates of resistance to quinolones, ceftazidime, doxycycline, and aztreonam. [27,28] The increased resistance to these antibiotics is probably because of the unnecessary use of antibiotics and the emergence of resistant strains of pseudomonas species in diabetic foot ulcers. [29]

Methicillin-resistant Staphylococcus aureus is one of the leading causes of infection in diabetic foot ulcers. In our study, it was noted that 9.7% (n=21) of all the microorganisms and 58.3 % of all staphylococcus aureus cases. This rate is comparable to an international study by Akhi MT et al. and Dadashi M et al.[30,31] According to Nasiri M et al., in Iran, the prevalence of MRSA is 45%.

All isolates were sensitive to Vancomycin and Teicoplanin, while 95% (n=20) isolates were sensitive to Linezolid. According to Pai V et. al., all isolates were susceptible to vancomycin in India. Still, there was high resistance to quinolones, macrolides, aminoglycosides, and chloramphenicol.[32] Although there is widespread emergence of vancomycin resistance in methicillin-resistant staphylococcus aureus,[33] in our study, all isolates were susceptible to vancomycin. One important finding in our study was the high sensitivity of MRSA to the old antibiotic chloramphenicol. This fact has been mentioned in other well-validated studies. [34]

The isolates of Methicillin-sensitive staphylococcus aureus were 100% sensitive to vancomycin. They had higher sensitivity to antibiotics like chloramphenicol, co-amoxiclav, flucloxacillin, and ceftriaxone. Still, their sensitivity to other antibiotics like cefepime, linezolid, quinolones, tetracyclines, macrolides and co-trimoxazole was low. These antibiotics were associated with increased rates of resistance, as shown in a study done in Sri Lanka by Jayatilleke K et al.[35]

Proteus species frequently infect wounds. Proteus mirabilis infection was highly susceptible to cefoperazone-sulbactam, piperacillin-tazobactam, amikacin, quinolones and cefalosporins. At the same time, these isolates were resistant to penicillins, doxycycline, chloramphenicol, co-trimoxazole, and tigecycline.

An African study byMordi RM showed high sensitivity to ciprofloxacin and aminoglycosides but low sensitivity to tetracyclines, erythromycin, chloramphenicol, and penicillins. [36] Similar results were found in another study by Trojan R et al. in Indian Punjab.

In both these studies, proteus showed high sensitivity to ciprofloxacin and other quinolones, cephalosporins, meropenem, amikacins, and cefoperazone-sulbactam. [37]

Acinetobacter is a group of opportunistic microorganisms that usually affects hospitalized people. It is notorious for its widespread resistance to commonly used antibiotics. In our study, its isolates were sensitive only to cefoperazone-sulbactam and colistin. These findings are similar to another study in Iran by Ghaismian R et al., who conducted the study in ICU patients. [38] Similar results were also found in India by Islahi S et al. [39]

Most of the enterococcus faecalis isolates were sensitive to co-amoxiclav, linezolid, quinolones, carbapenems and piperacillin-tazobactam. But they were resistant to ceftriaxone, cefotaxime, doxycycline and co-trimoxazole. These results are similar to the results of a study by Anvarinejad et al. [40]

The majority of our patients lost to follow-ups. Therefore, we were unaware of the ultimate fate of their ulcers. In addition, many patients were already on antibiotics, which could change the culture and sensitivity results.

CONCLUSION

The majority of microorganisms in our set are gram-negative rod and staphylococcus aureus. The most effective antibiotics against gram-negative bacteria are cefoperazone-sulbactam, piperacillin-tazobactam, meropenem, and amikacin. In contrast, the most effective antibiotics against gram-positive organisms are vancomycin, linezolid, teicoplanin and chloramphenicol. Many patients use inappropriate antibiotics before they seek medical advice. This practice has increased the emergence of antibiotic-resistant strains of bacteria. Therefore, the combination of appropriate antibiotics should be started in diabetic foot ulcers before the results of culture reports are available. Appropriate empirical antibiotics can be recommended where pus culture facilities are not available.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Abbas G:	Conceiving the idea, data collection
Khan HA:	Literature search, writing and Statistical analysis
Iqbal S:	Data collection and review
Nabi A:	Literature search and review

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.