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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY IN DIABETIC FOOT ULCER PAITIENTS

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Abstract

Diabetic foot ulcers (DFUs) affect 5-10% of diabetic patients and often arise from neuropathy, vascular insufficiency, and impaired immunity, complicating wound healing. Recurrence risk factors include gender, smoking, diabetes duration, prior ulceration, peripheral artery disease, and painful diabetic neuropathy. DFUs are frequently colonized by polymicrobial infections, with increasing antimicrobial resistance complicating treatment. Hospital-based studies show higher prevalence rates of DFUs compared to population-based studies, with a notable prevalence in male patients and those with type 2 diabetes mellitus. This study aimed to determine the frequency of bacterial infections and evaluate antimicrobial susceptibility patterns in DFUs at a tertiary care hospital in Rawalpindi. A cross-sectional study over six months September 2024-Feburary 2025 involved collecting tissue samples (n=225) from DFU patients. Bacterial cultures were analyzed using standard microbiological techniques, and antibiotic susceptibility was tested via the Kirby-Bauer method. Among the 225 samples, 170 were from males, with a mean age of 56.4 years. The microbial profile revealed a polymicrobial composition, predominantly Pseudomonas aeruginosa (24.9%) and E. coli (21.5%) as the most common gram-negative pathogens, while Staphylococcus aureus (21.5%) was the most prevalent gram-positive pathogen. Antibiotic susceptibility testing indicated Pseudomonas aeruginosa had high sensitivity to Tobramycin (60.9%) and Ciprofloxacin (60%), but resistance to Cefixime (64.7%). E. coli showed high responsiveness to piperacillin-tazobactam (91.8%) and resistance to tetracycline (83%). Staphylococcus aureus exhibited sensitivity to Tobramycin (70.6%) but resistance to tetracycline (58.8%). The findings highlight the diverse microbial environment and antimicrobial resistance in DFUs, emphasizing the need for susceptibility testing to guide effective therapy and tailored management strategies for improved treatment outcomes.

KEYWORDS:

Bacterial Infections , Antimicrobial Resistance, Ciprofloxacin Sensitivity, Tobramycin Susceptibility, Polymicrobial Colonization, Pseudomonas aeruginosa, Escherichia coli (E. coli), Staphylococcus aureus

Introduction

Diabetes is a disorder related to lifestyle and is characterized by chronic hyperglycemia and poses serious complications in many cases (1). The most common complication in diabetes mellitus is Diabetic peripheral neuropathy (DPN). Pathogenesis of DPN is the impaired metabolism and bioenergetics failure to support the long axons of neurons (2). It can affect both somatic and autonomic nervous systems(3). Diabetic foot ulcer (DFU) is a complicated and multifactorial clinical problem that affects about 6.3% of diabetes mellitus patients. According to the international working group on diabetic foot (IWGDF) defined DFU as symptoms (current or previous) of diabetes such as skin chapping, infections, ulcerations and destruction of skin tissues combined with neuropathy and peripheral artery disease (PAD) that defoliates skin (epidermis and dermis) of the foot and exposes the underneath sterile tissues which ultimately leads to formation of thick lesions (4). About 18.6 million individuals are affected by diabetes worldwide and 80% of these leads to lower extremity amputations. Approximatly 50-60% ulcers become infected with pathogens. About 20% of the severe infections lead to amputation of the lower extremities. Previous 5 year mortality rate for diabetic foot ulcer is 30% (5). Infections may be of gram-negative origin, gram-positive origin or fungal. Among gram-positive organisms, *staphylococcus aureus, staphylococcus epidermidis, Enterococcus faecalis* and *streptococcus hemolyticus* are reported to cause diabetic foot ulcer (DFU) infections. *Escherichia coli, pseudomonas aeruginosa, klebsiella pneumoneae, proteus mirabilis* and *acetobacter baumannii* are some of the gram-positive species reported to cause DFU infections. Some fungal infections are also reported to cause DFU infections (6). Infections caused by strains of antibiotic resistance are more hectic to deal with. Antibiotic resistance becomes a challenge in itself when it comes to treating diabetic foot ulcers. *Multi-drug resistance (MDR), Extended spectrum β-lactamase* producing bacterial resistance and *methicillin resistant staphylococcus aureus (MRSA)* are the most common encountered and hard to deal with resistance patterns (7, 8). Presence of these resistance strains complicates the disease even more by restricting the treatment choices to deal with infections. This leads to further complications such as ischemia and neuropathy (9). Ischemia accompanied by neuropathy usually leading to necrosis is common at the site of infection and may require extreme measures such as debridement or even sometimes amputation if debridement doesn't work (10).

The objective of this study is to isolate and identify different strains of bacteria from patients suffering from diabetic foot ulcers.

Methodology

Study design

A cross sectional study was performed at Tertiary Care Hospital of Rawalpindi and Islamabad for the duration of six months period in tertiary care hospital in order to harness the bacterial infection and antimicrobial susceptibility testing from diabetic foot ulcer patients.

Sampling Technique

Total (n=225) samples from patients with diagnosed diabetic foot ulcer were taken following non probability purposive sampling. And the size of sample calculated analyzed by openEpi calculator software.

Selection criteria

The following criteria was followed.

Inclusion criteria:

- Patients with DFU without taking the account of age and gender
- Diabetic patients having level of HbA1c greater than 7.5%

Exclusion criteria:

- Patients not undergoing any antibiotic therapy within past 2 weeks prior to enrollment
- Patients having foot ulcers due to non diabetic causes e.g trauma and venous ulcers

Sample Collection

Tissue sample from patients of diabetic foot ulcers were collected after area around the ulcer was cleaned with disinfectant. Using sterile biopsy needles sample were taken from the base of the ulcer ensuring to avoid any contamination from surface and put them in sterile transport medium and sent immediately for microbiological analysis to lab.

Bacteriological identification

Culture media used:

After collection of samples, they were inoculated on blood and macConkey agar media and plates were incubated at 37 C for 24 hours for possible growth of aerobic bacteria. After incubation period, the growth of bacteria identified by morphological characteristics, hemolysis, pigmentation and odour.

Gram staining and Microscopy:

The cultured bacterial growth further accessed by smear on glass slide for gram stain. This technique helps to differentiate bacteria using colony either its gram positive and negative. Following standard staining protocol, gram staining was done.

Biochemical tests:

Different standard biochemical test inlcluding (catalase, coagulase, oxidase) were performed for further specie level identification.

Antibiotic susceptibility testing

Antibiotics susceptibility testing was performed for the isolated bacteria on Mueller hinton agar media with antibiotics following the technique of Kirby bauer disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines 2020 (Karlsson, 2020).

Bacterial suspension was prepared by picking colony form overnight culture and dissolving in normal sterile saline (0.85%) in autoclaved test tube and vortexed and turbidity matched by adjusted to 0.5 Mcfarland standard. After it using sterile swab, bacterial suspension was inoculated evenly on the plate, left for few minutes, and antibiotic disks were applied.

For both gram positive and gram negative pathogens susceptibility profiles, following antibiotic disks were used including Tobramycin, Ciprofloxacin, Cefexime, Piperacillin-tazobactam, and tetracycline.

Plates were incubated for 24 hours at 37 C temperature, following incubation zones of inhibition were determined by measuring. And results were reported based on CLSI standards. All procedure were done under aseptic conditions and regularly monitored.

Statistical analysis

Result interpretation and data analysis was done through SPSS software version 21, utilizing descriptive statistics.

Results

This study has been conducted to determine the prevalence and analysis of bacteriological profiles as well as the antimicrobial susceptibility among patients with diabetic foot ulcers in Tertiary Care Hospitals of Rawalpindi and Islamabad.Atotalof226sampleshavebeenexamined,includingbothgenders. In the study group, 56 are females (n=56) and 170 are males (n=170). The pie chart illustrates the gender distribution of the study population indicating the predominance of male patients over female patients. Patientsofalmostallagegroupsareincludedinthestudy,approximately20to80years.

Thishistogramillustratesthatthemajorityofcasesarebetween40to70yearsold.The most commonly observed is in patients of 56 years old. The standard deviation is 10.961, indicating some variation among the ages of affected patients.

Outof226samples,(9)gram-negativeorganismsand(3)gram-positiveorganismsare identified, among which gram-negative species are isolated more frequently. *Pseudomonas aeruginosa* is at 24.9%, whereas Staphylococcus aureus is at 21.5%, which shows a prevalence of diabetic foot ulcers. Outofthetotal12 organisms,only3arecategorizedasgram-positiveorganisms,with a percentage of 21.5% for *Staphylococcus aureus*, 1.7% for *Streptococcus pyogenes*, and 0.4% for *Streptococcus agalactiae*

Among these organisms, gram-negative organisms are found to be more prevalent including*Pseudomonasaeruginosa*at24.9%,*Escherichiacoli*at21.5%,*Acinetobacter baumanii* at 14.3%, *Klebsiella spp & M.morganii* at 4.6%, *Proteus species* at 3%, *Enterobacter spp* at 1.7%, *Citrobacter spp* at 1.3%, and *Burkholderia cepacia* representing 0.4% of total samples.

Acinetobacter baumanii is a gram-negative bacterium that shows high resistance to Erythromycinat83.3%,Ciprofloxacinat55.6%,andTobramycinat35.7%.Incontrast, itshowslessresistancetoGentamicinat21.4%whereGentamicinprovedtobehighly effective against *Acinetobacter baumannii* at 78.6%, the efficacy percentages for Tobramycin, Ciprofloxacin, Erythromycin at 64.3%, 44.4% and 16.7% respectively.

*Escherichiacoli*isarod-shaped,withacylinderinthecenter,gram-negativebacterium that shows high sensitivity to Pip-tazobactam (Tazocin) at 91.8%, and Cefepime at 68.9%, Ceftriaxone at 62.0% whereas it shows very high resistance to Tetracycline at 17%Tetracycline83.0%,Ceftriaxone38%,Cefepime31.1%,andPip-tazobactam
(Tazocin)

Klebsiella pneumoniae is a gram-negative, rod-shaped bacterium that shows high sensitivity to both Augmentin and Pip-tazobactam (Tazocin) at 77.8%, Cefepime at 44.4%, and Ampicillin at 22.2%. In contrast, it shows high resistance to Ampicillin at 77.8%, Cefepime at 55.6%, Augmentin, and Pip-tazobactam (Tazocin) at 22.2%.

*Staphylococcusaureus*isagram-positivebacteriumthatissmallandroundinshape.It is the most prevalent gram-positive bacteria and it shows great sensitivity to Tobramycin at 70.6%, Augmentin at 68.9%, Levofloxacin at 62.2%, and Tetracycline at 41.2%. Whereas it shows resistance to Tetracycline at 58.8%, Levofloxacin at 37.8%Augmentin at 31.1%, and Tobramycin at 29.4%.

Streptococcus pyogenes is a gram-positive bacterium, which is non-motileand round- shaped. It shows high sensitivity to Ceftriaxone at 75%, Vancomycin at 66.7%, Ciprofloxacin at 50%, and Erythromycin at 25%. In contrast, it shows high resistance for Erythromycin at 75%, Ciprofloxacin at 50%, Vancomycin at 33.3%, and Ceftriaxone at 25.0%.

Proteus is a rod-shaped gram-negative bacterium. It shows a swarming capability and shows a high sensitivity for Cefepime at 80%, Ciprofloxacin at 70%, Azithromycin at 50%, and Augmentin at 37.5%. In contrast, it shows high resistance to Augmentin at 50%, Ciprofloxacin at 30%, Azithromycin, and Cefepime at 10%.

Pseudomonas aeruginosa is a gram-negative, motile bacterium that shows high sensitivity to Tobramycin at 60.9%, Ciprofloxacin at 60%, Augmentin at 59.2%, and Cefiximeat35.3%.Ontheotherhand,itshowshighresistanceforCefiximeat64.7%, Augmentin at 40.8%, Ciprofloxacin at 40%, and Tobramycin at 39.1%.

Table1:Frequencyofdifferentparameterofstudy.

Parameter	Frequency		Percentage	
Gender	Female	**56**	Female	**24.8**
	Male	**170**	Male	**75.2**

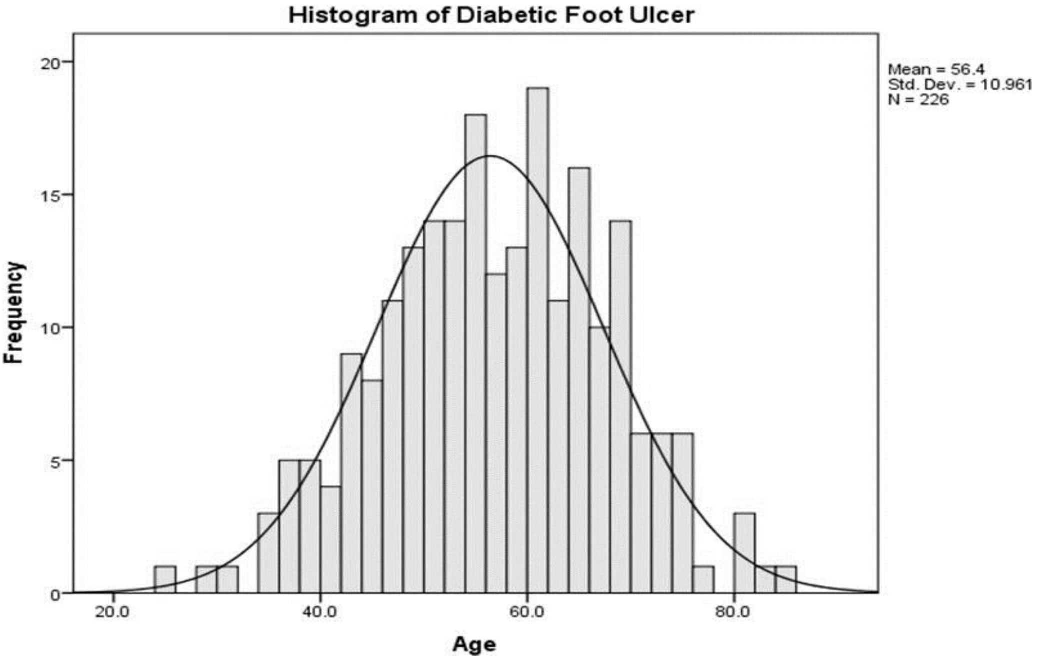

Figure1:HistogramofageCategoriesofdiabeticfootulcerpatients

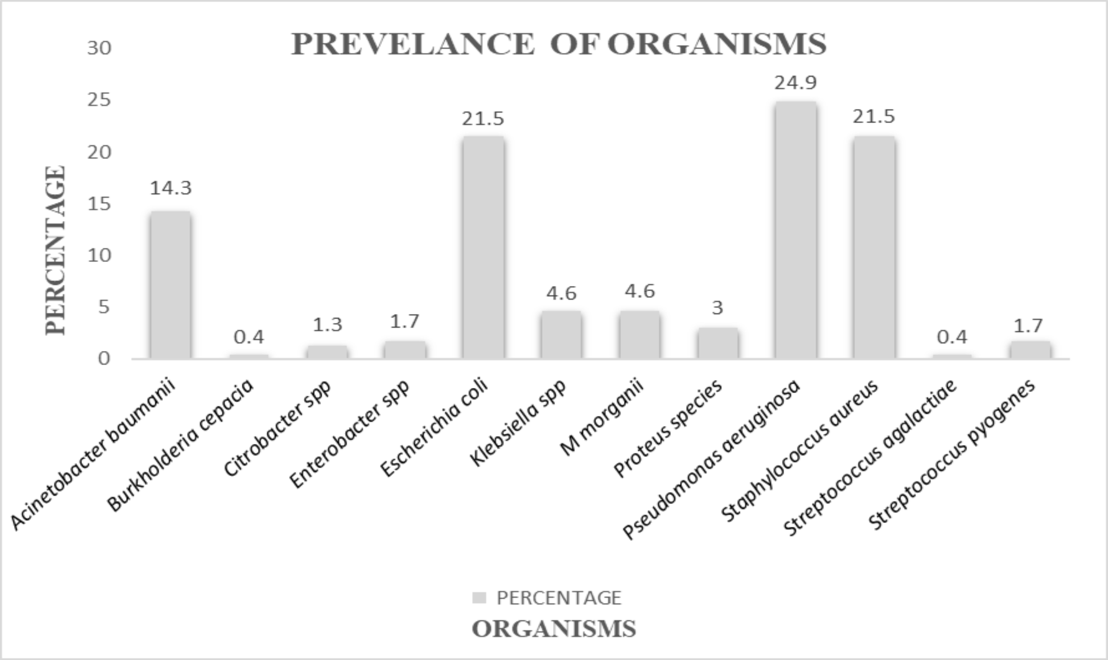

Figure2:Prevalenceofmicroorganismsisolatedfromdiabeticfootulcerpatients.

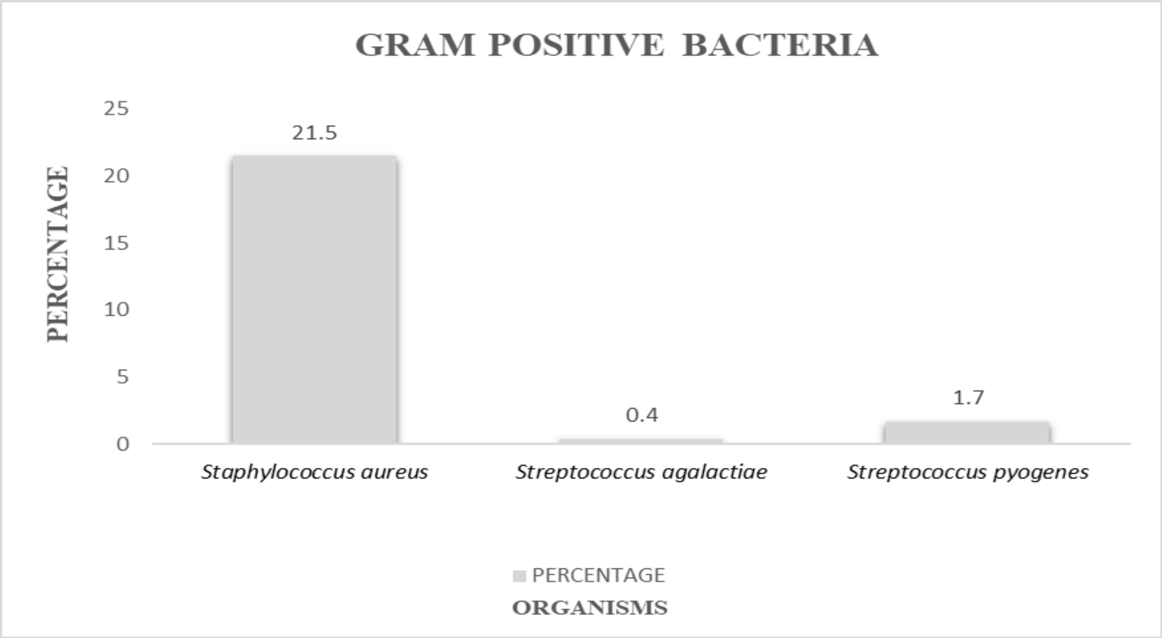

Figure3:Prevalenceofgram-positiveorganisms.

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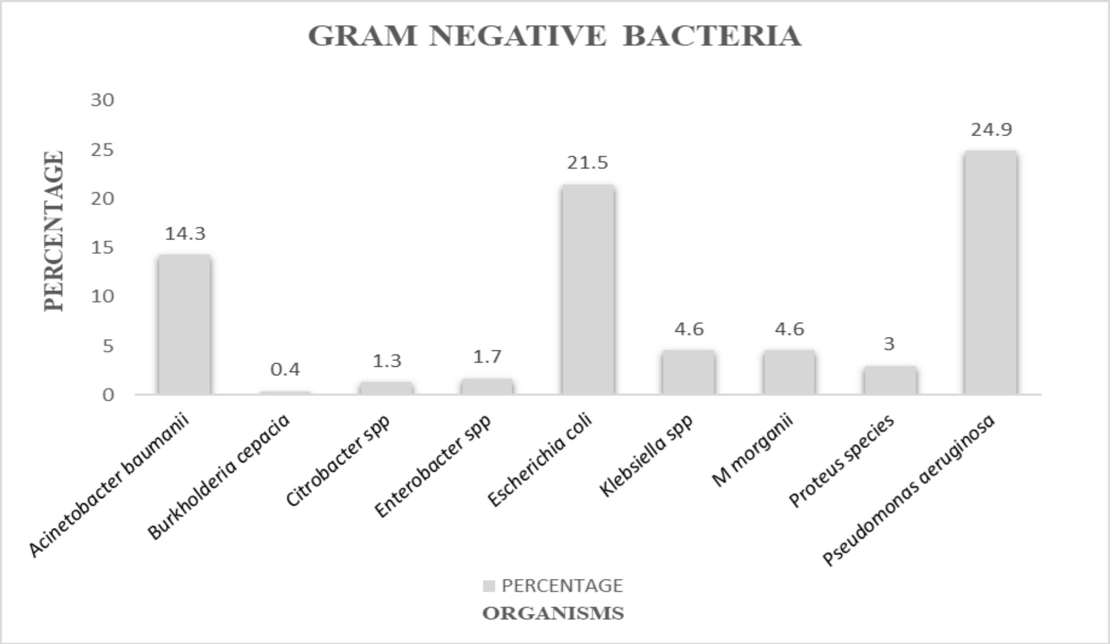

Figure4:Prevalenceofgram-negativeorganisms.

at 8.2%.

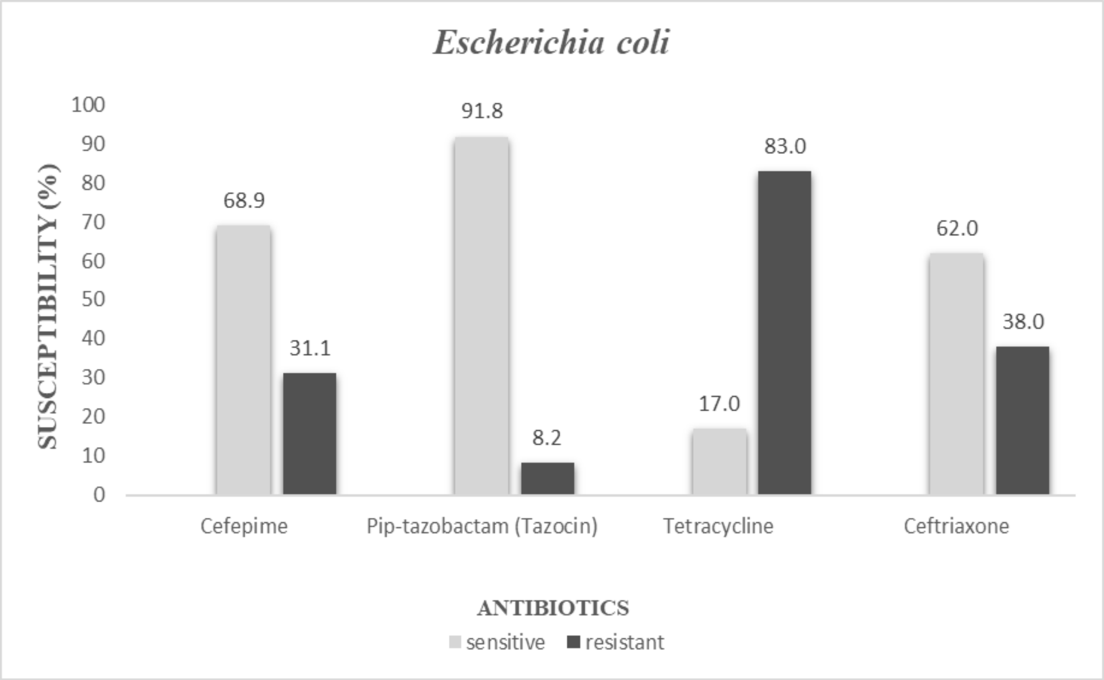

Figure5:Antibioticsusceptibilityprofileof*Escherichiacoli.*

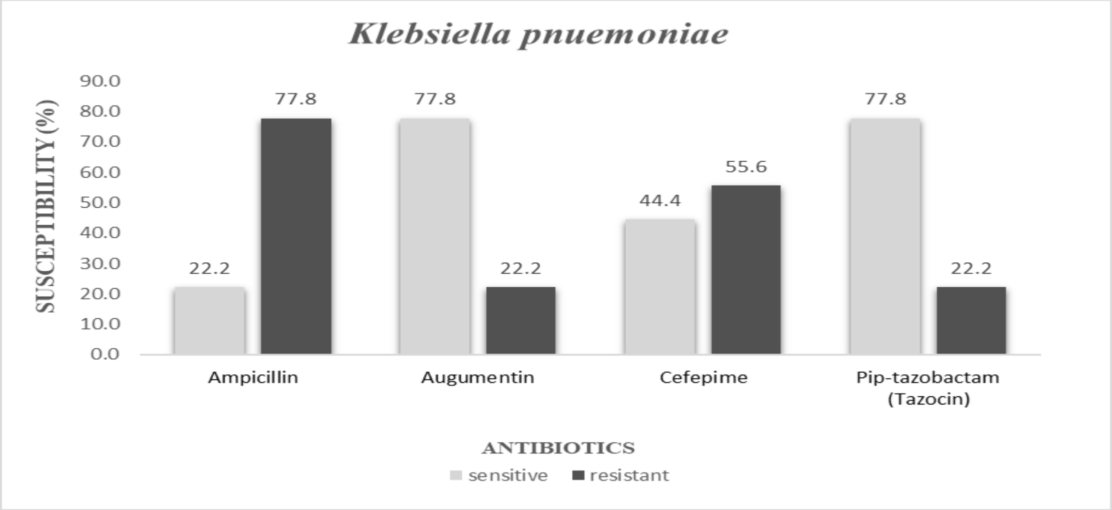

Figure6:Antibioticsusceptibilityprofileof*Klebsiellapnuemoniae.*

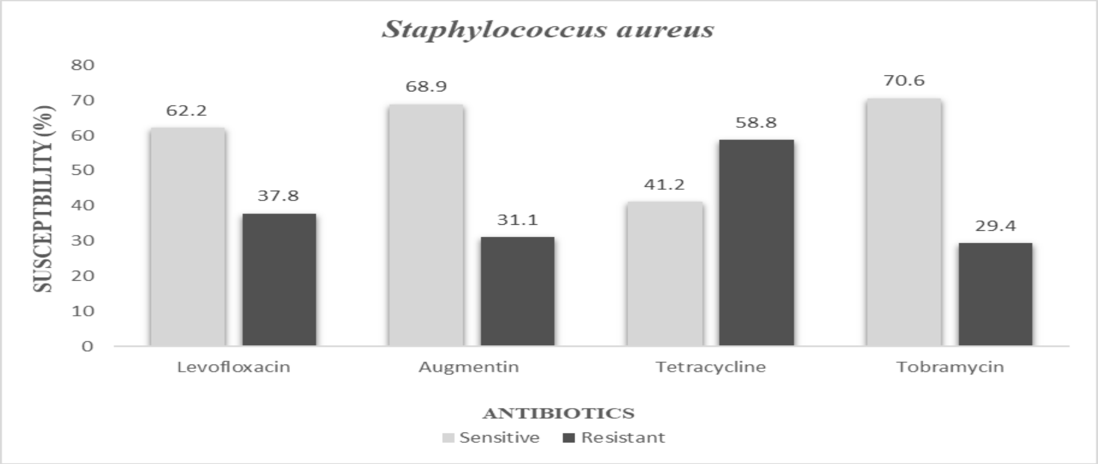

Figure7:Antibioticsusceptibilityprofileof*Staphylococcusaureus.*

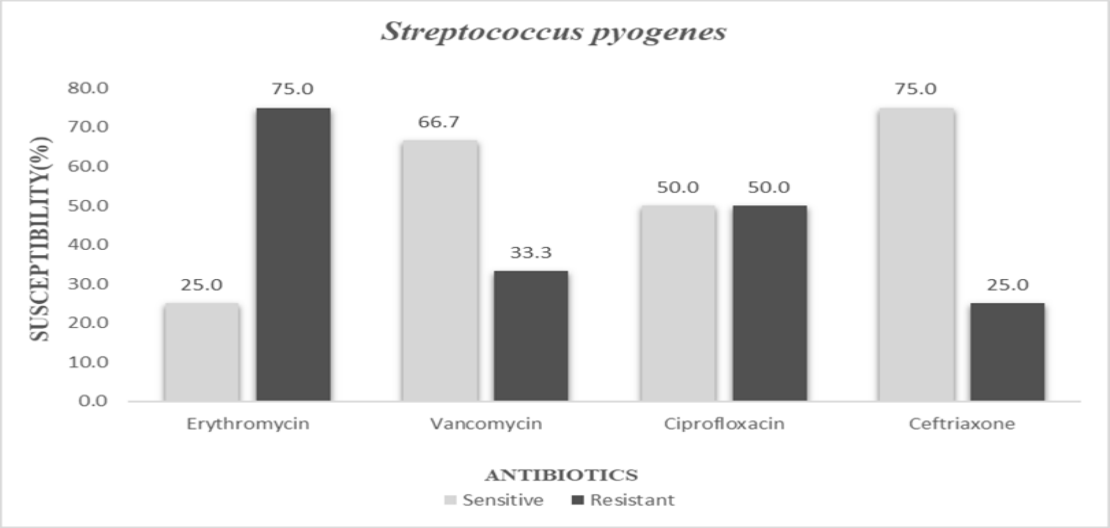

Figure8:Antibioticsusceptibilityprofileof*Streptococcuspyogenes*.

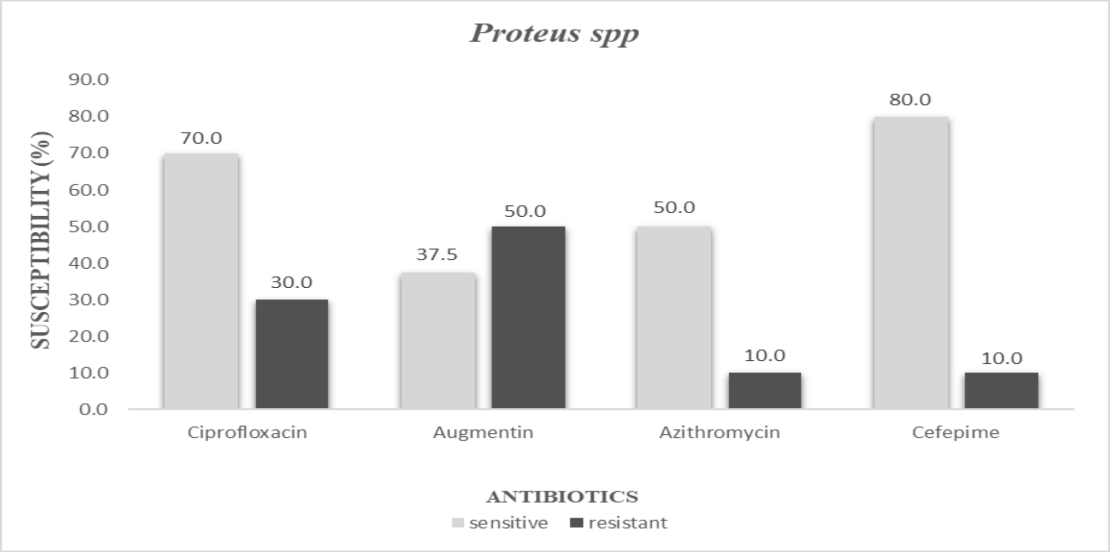

Figure9:Antibioticsusceptibilityprofileof*Proteus spp*

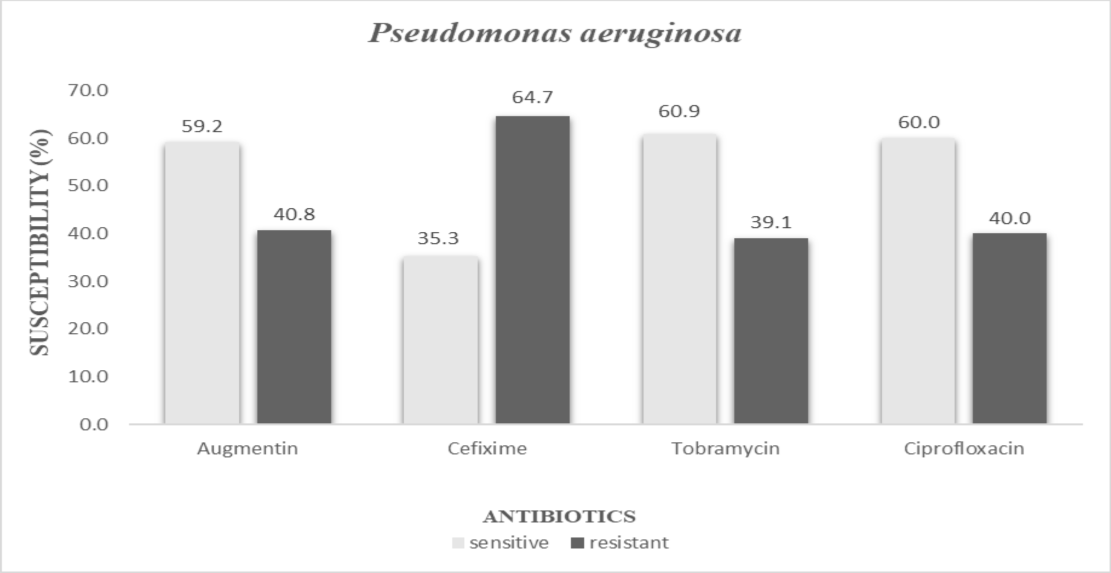

Figure10:Antibioticsusceptibilityprofileof*Pseudomonasaeruginosa*

Discussion

Diabetic foot ulcers (DFUs) are a significant complication of diabetes, often affecting deeper tissues. Poorly controlled diabetes and inadequate management contribute to DFU progression [1]. Key factors include diminished sensation, restricted blood flow, and contributing factors like foot structure and poorly fitting shoes [2].

While treatable, DFUs remain a leading cause of hospitalization among diabetic patients. This study aimed to analyze the prevalence of DFUs, identify specific bacteria, and assess antibiotic susceptibility patterns [3]. We collected 226 DFU samples from tertiary care hospitals in Rawalpindi and Islamabad, finding a higher prevalence in males (N=170, 75%) compared to females (N=56, 25%). This may be due to biological, behavioral, and social factors, as men often face higher mechanical stress and are less likely to seek medical attention [4].

The highest prevalence of DFUs was in the 54-56 years age group (56.4%), consistent with previous studies [3][5]. The risk of DFUs increases with age due to prolonged diabetes duration and associated vascular complications [6].

In our study, Pseudomonas aeruginosa was the most common isolate (24.9%), followed by Escherichia coli (21.5%) and Acinetobacter baumannii (14.3%). These findings differ from Akwah et al., who identified Proteus mirabilis as the most prevalent organism [3]. Among Gram-positive bacteria, Staphylococcus aureus had a prevalence of 21.5%, aligning with previous studies [3][5].

Our study revealed significant sensitivity of Staphylococcus aureus to tobramycin (70.6%), contrasting with Goh et al., who found 100% sensitivity to vancomycin [7]. Pseudomonas aeruginosa showed sensitivity to tobramycin (60.9%) but resistance to cefixime (64.7%) [7].

Klebsiella pneumonia demonstrated high sensitivity to augmentin and piperacillin/tazobactam (77.8%), differing from other studies [8]. Acinetobacter baumannii displayed 78.6% sensitivity to gentamicin [9].

Conclusion

The findings of this study showed that, both Gram negative and Gram positive organisms manifest at different levels of antimicrobial resistance to various drugs. Due to the inadvisable use of antibiotics has led to a surge in antibiotic resistance among all soft tissue infections in Pakistani population, to a great extent. To control this pathogen-specific profiling is crucial for developing effective, targeted antimicrobial treatment plans which are refined through continuous monitoring.

Limitations

he study's 225 diabetic foot ulcer-positive samples may not fully represent the broader population, limiting the generalizability of the findings. Additionally, the lack of detailed patient histories restricts our ability to capture the diversity of risk factors, such as smoking, obesity, and poor glycemic control. This absence of data on risk factors and ulcer grading hinders our capacity to identify patterns and establish correlations between these factors and the severity, occurrence, and recurrence of ulcers.

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