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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL INFECTION IN DIABETIC FOOT ULCER IN DIFFERENT REGIONS OF KHYBER PAKHTUNKHWA

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Abstract

Background: Diabetic foot infections (DFIs) represent a significant public health challenge, and understanding the pathogens responsible is essential for effective antibiotic therapy. The microorganisms causing these infections, along with their antibiotic resistance patterns, vary by geographic region, necessitating localized empirical treatment. Objective: This study aims to investigate the incidence of antibiotic sensitivity and resistance patterns of bacterial infections in diabetic foot ulcers in Khyber Pakhtunkhwa. Material and method: A cross-sectional study was conducted at the Diabetes Center, Hayatabad Medical Complex, Peshawar, over four months, involving 500 randomly selected diabetic patients with foot ulcers. Samples were collected aseptically using wound swabs and cultured on various media, including Mueller-Hinton agar, MacConkey agar, and CLED. Bacterial identification was carried out using biochemical assays (catalase, coagulase, TSI), Gram staining, and the use of specific media. Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Data were analyzed using SPSS version 25. Result: Out of 500 samples, 200 (40%) showed positive bacterial growth, with the highest sample contribution from Peshawar (35%), followed by Bannu (25%). Female participants accounted for 58%, with the majority (68%) aged between 41 and 60 years. Staphylococcus aureus and Klebsiella pneumoniae were the principal isolates, confirmed through Gram staining and biochemical assays. Antibiotic susceptibility testing showed high sensitivity to Amikacin (91%) and Gentamicin (89%), while resistance rates for Cotrimoxazole and Imipenem exceeded 90%. Conclusion: This study highlights the significant prevalence of Staphylococcus aureus and Klebsiella pneumoniae in diabetic foot ulcers across Khyber Pakhtunkhwa and the high rates of antibiotic resistance. The results underscore the critical need for localized antibiotic management strategies to reduce resistance and improve patient outcomes.

Keywords: Antibiotic susceptibility, Bacterial infection, diabetic foot ulcer.

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Introduction

Diabetes mellitus (DM) is a set of metabolic disorders that is characterized by the presence of continuously high levels of sugar in the blood as a result of impaired insulin work or the lack production [1]. It is divided into four broad categories: type 1, type 2, gestational diabetes and others. Type 1 diabetes or insulin-dependent diabetes is the most common called juvenile diabetes and it is caused by the autoimmune destruction of 5-10% of the population, pancreatic beta-cells [2,3]. DM is one of the few diseases whose incidence has been on the increase in the past several decades all over the world. According to the International Diabetes Federation in 2019, there are 463 million people living with diabetes in the world and

the worldwide prevalence is 9.3 percent [5]. Projections indicate that there will be 578 million and 700 million by the year 2030 and 2045 respectively [6].

The number of people suffering diabetes is excessive in Pakistan [7], with almost 33 million individuals affected by this temperature, which is the third highest in the world [8]. According to previous research, the rates of diabetes progress gradually increased the percentage of this disease among the population [9]. This increase is associated with the rates of diabetic foot ulcers (DFUs) that are experienced by 14.3% of diabetics and being related to critical complications such as amputation in low income country [10,11]. Risk of DFUs is increased with factors related to advanced age, long duration of diabetes, smoking, raised blood pressure and diabetic retinopathy. Largely, there is a vast rural-urban disparity regarding access to healthcare, with a higher prevalence rate of DFUs being reported by the rural population of regions such as north region KP [12].

The pathogenesis of DFUs involves a combination of factors, including compromised skin integrity due to peripheral neuropathy, reduced tissue oxygenation, and impaired immune function [13]. The presence of infection, especially in chronic ulcers, complicates treatment and can lead to life-threatening conditions such as sepsis or gangrene. Effective management of DFUs requires prompt identification of bacterial infections, with the most common pathogens being Gram-positive cocci like *Staphylococcus aureus* and Gram-negative bacteria like *Pseudomonas aeruginosa*. The increasing prevalence of multidrug-resistant bacteria complicates treatment, with a rising need for targeted antibiotic therapy [14,15].

Inadequate access to healthcare facilities, a lack of foot care education, and absence of multidisciplinary care teams, which specialize in the treatment of DFUs, exist in countries that face low incomes, such as Pakistan, and account for the exacerbation of DFUs. Numerous diabetic-related amputations are unavoidable due to poor care of the feet. The development of strong health sector including forestation of diabetic foot care clinics is important in combating rising cases of DFUs. The increasing awareness of antibiotic resistance, especially in Gram-negative bacteria adds to the demands of an overall approach to this problem of DFUs with its complications and consequently antimicrobial stewardship and optimization of treatment regimens.

Materials and Methods

This study was a cross-sectional research that took place at City Hospital, Peshawar to examine bacterial infection on diabetic foot problems. The sample size was 500 within both genders in various parts of Khyber Pakhtunkhwa. The research was carried out during the period of four months after being approved by the research and ethical scientific board City Hospital, Peshawar. The participants were chosen using random sampling and by extension all the individuals with diabetes and foot ulcers were adequately represented.

The number of participants that met particular inclusion criteria being confirmed diabetic patients with foot ulcers and age between 20 to 80 years, was included in the study. The exclusion criteria ruled out patients with diabetes and foot ulcers as well as other infections (HIV, HCV, or HAV) foot ulcers and no diabetes. Further, people outside Khyber Pakhtunkhwa were also not considered [16]. Having finalized the criteria the participants were sampled and wound swabs were taken of diabetic ulcer foot patients visiting the OPD of diabetes and endocrinology at City Hospital, Peshawar. These were aseptically collected and transported to the laboratoryites in order to carry out microbiological analysis on them.

The collected samples were processed using various media, including Mueller Hinton Agar (MHA), CLED, MacConkey's agar, and thioglycolate broth, and incubated at 37°C for 24-48 hours. Colonies were identified using standard biochemical tests such as catalase, coagulase, and TSI tests. Antibiotic sensitivity was tested using the Kirby-Bauer disc diffusion method, where bacterial cultures were exposed to antibiotic discs to determine susceptibility. Data were then analyzed using SPSS version 25, with descriptive statistics like mean, median, and mode, and the results were graphically represented using bar charts.

Results

A total of 500 wound specimens were collected from individuals who had undergone amputations due to as a result of type II diabetes. Among these specimens, 200 samples showed positive growth upon culture. The samples were gathered from various regions of Khyber Pakhtunkhwa, including Peshawar (35%, n=70), Bannu (25%, n=50), Mardan (10%, n=20), Swabi (15%, n=30), and DI Khan (15%, n=30), as shown in Table 1. The study consisted of 84 males, which accounted for 42% of the whole sample, and 116 females, which accounted for 58% of the total sample. This distribution is visually represented in Figure (1). Each patient's incision was sterilized using normal saline solution to eradicate indigenous bacteria and other contaminants, while also excising necrotic tissue. The aseptic swab sticks were placed into a sterile transport medium composed of gel, and thereafter sent to the microbiology research laboratory for additional analysis.

Table 1. Compilation of samples from various regions of Khyber Pakhtunkhwa.

S.NO	Sample Area	Number of samples	Percentage (%)
1	Peshawar	70	35
2	Bannu	50	25
3	Marden	20	20
4	Sawabi	30	30
5	DI khan	30	30%
	Total	200	100%

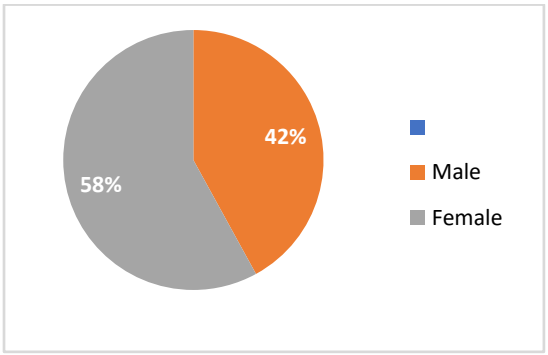

Figure 1 Gender Distribution of Diabetic foot ulcer

The specimens were collected from patients with diabetic foot ulcers who attended the outpatient department (OPD) for diabetes and endocrinology. The age range of the patients was between 20 and 80 years. The distribution of patients within different age groups was as follows: 20-40 years (12.5%, n=25), 41-60 years (68%, n=136), and 61-80 years (19.5%, n=39), as shown in Figure (2).

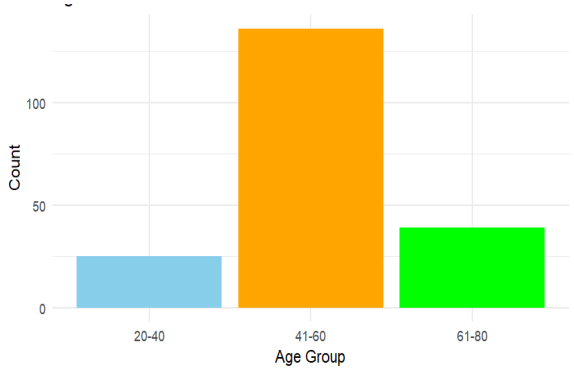

Figure 2 Age wise distribution

The predominant bacteria found in the five zones are *Staphylococcus aureus* and *Klebsiella,* based on their distribution among bacterial colonies. *Staphylococcus aureus* exhibits the highest levels of occurrence in every region, specifically in Peshawar 27, Bannu 20, and D.I. Khan 14. *Klebsiella* exhibits notable prevalence, particularly in Bannu 15 and Peshawar 13. The abundance of these bacteria regularly surpasses that of others in various places, showing their widespread occurrence as depicted in Figure (3).

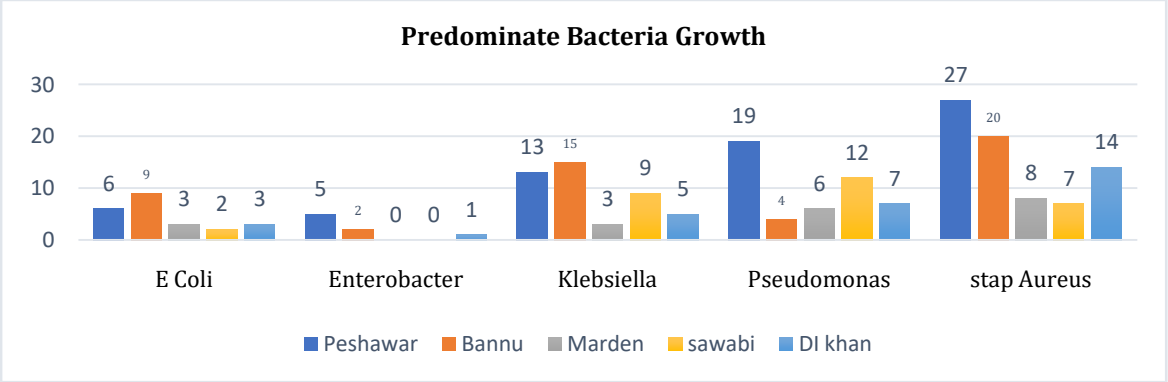

Figure 3 Most common Bacteria found in KP that cause diabetic foot ulcer

Five bacteria species were found to be present in diabetic foot ulcers –*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,Staphylococcus aureus* and *Enterobacter*. Each species had unique biochemical characteristics with *Staphylococcus aureus* emerging as most common. Most isolates were catalase-positive and cofagulase, oxidase, indol, and motilitas show in table (2).

Table 2. Biochemical test Apply on different bacterial isolated from diabetic foot ulcer

No of isolates	Bacteria	Catalase	Coagulase	oxidase	Indole	Motility
23	*Escherichia coli*	+ve	−ve	−ve	+ve	+ve
40	*Pseudomonas aeruginosa*	+ve	−ve	+ve	−ve	+ve
48	*Klebsiella pneumoniae*	+ve	−ve	−ve	−ve	−ve
76	*Staphylococcus aureus*	+ve	+ve	−ve	−ve	−ve
08	*Enterobacter*	+ve	−ve	−ve	−ve	+ve

Antibiotic Susceptibility Profile on the basis of different region in KP Peshawar, Bannu, Mardan, Sawabi, and DI Khan

Amikacin is the predominant antibiotic in the Peshawar region, exhibiting a sensitivity rate of 91%. Gentamicin demonstrates a sensitivity rate of 84% and is considered effective. Ciprofloxacin exhibits a notable efficacy, boasting a sensitivity rate of 88%. The resistance rates for Imipenem and Cotrimoxazole are particularly high. Imipenem has a resistance rate of 98%, while 92% of bacteria show resistance to cotrimoxazole, as show in the figure (4).The primary antibiotic that shows sensitivity in the Bannu region is Gentamicin, with a sensitivity rate of 89%, followed by Amikacin with a sensitivity rate of 86%. The resistance rates for Cotrimoxazole and penicillin are notably elevated at 92%. The resistance rate of imipenem is 88% show in the figure (5). In the Sawabi region, the most effective antibiotics with a high sensitivity rate are Amikacin at 86% and Gentamicin at 84%. The resistance rate for Imipenem is 93%. The effectiveness of cotrimoxazole with penicillin is notably high, reaching 91% show in the figure (7). In the DI Khan region, the most effective antibiotic is Amikacin, with a sensitivity rate of 91%. Gentamicin and vancomycin follow closely behind with sensitivity rates of 84%. However, Cotrimoxazole and penicillin have significant resistance rates of 92% and 88% respectively. Imipenem also has a resistance rate of 84% show in the figure (8).

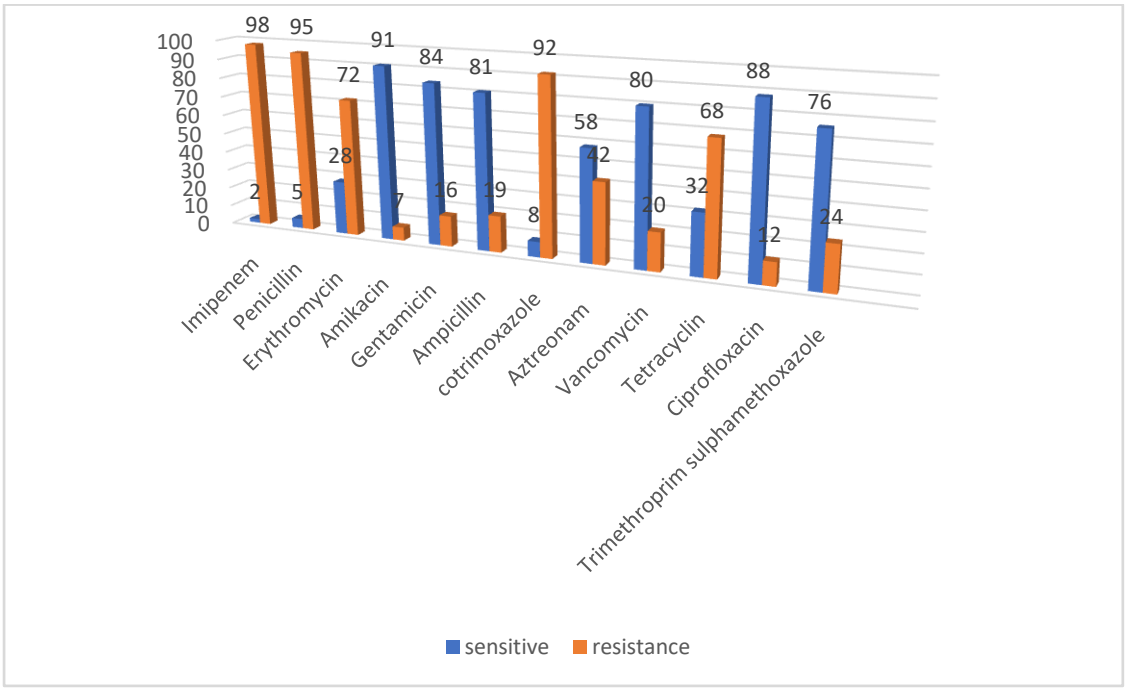

Figure 4 Antibiotic Susceptibility in Peshawar Region

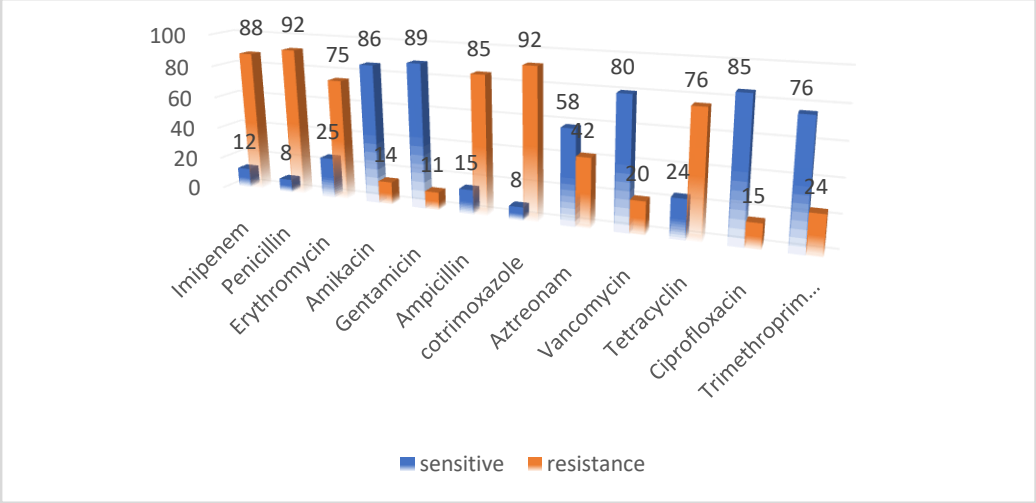

Figure 5 Antibiotic Susceptibility in Bannu Reg

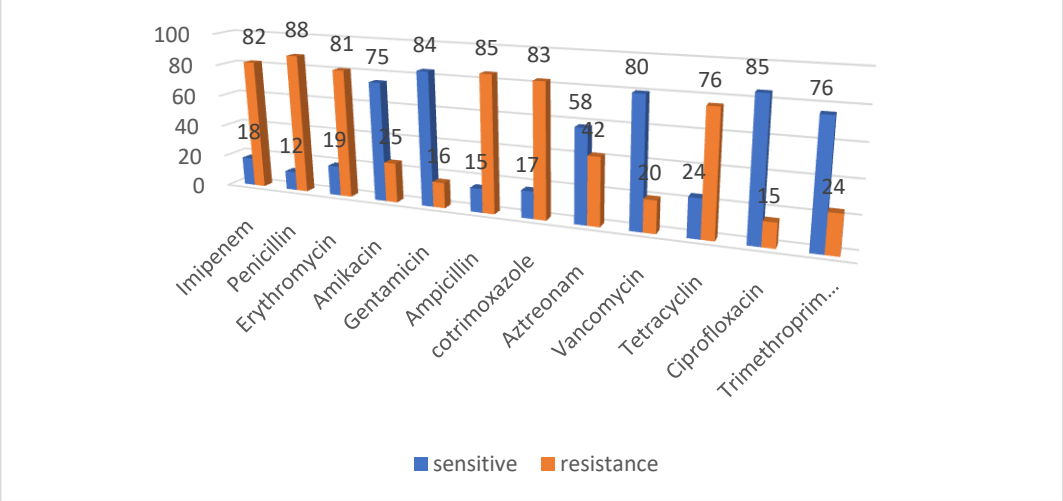

Figure 6 Antibiotic Susceptibility in Mardan Region

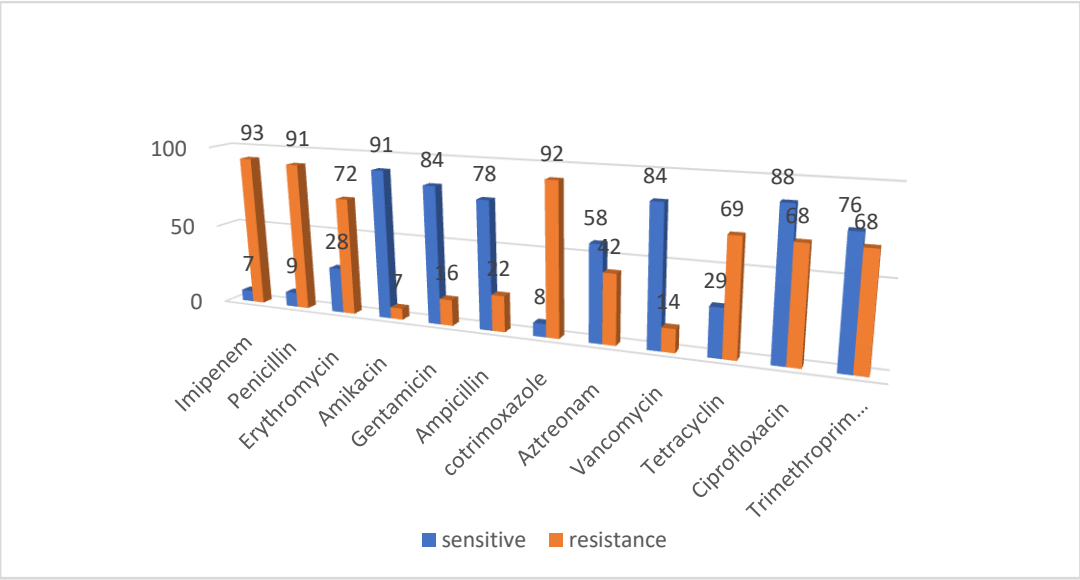

Figure 7 Antibiotic Susceptibility in sawabi Region

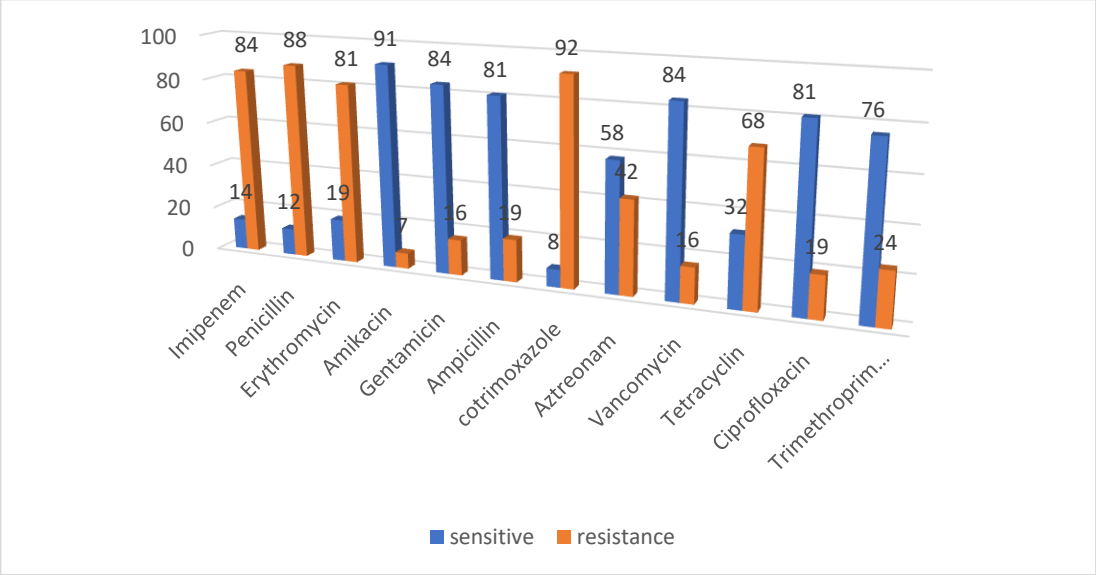

Figure 8 Antibiotic Susceptibility in DI khan Region

Discussion

Infection plays an important part in putting a diabetic foot at danger of amputation. Effective antibiotic therapy against the bacteria that cause diabetic foot infections is a critical component of treatment [17].The samples were gathered from several areas in Khyber Pakhtunkhwa, with Peshawar accounting for 35% (n=70) of the samples that tested positive. The study sample consisted of 84 males (42% of the total) and 116 females (58% of the total), with the majority of participants (68%, n=136) falling within the age range of 41-60 years. Staphylococcus aureus and Klebsiella were the most common bacteria in all regions, with Staphylococcus aureus being particularly widespread in Peshawar, Bannu, and D.I. Khan. The results of the antibiotic susceptibility tests showed that amikacin had a high sensitivity rate of 91% and gentamicin had a sensitivity rate of 84%. On the other hand, imipenem and cotrimoxazole had high resistance rates of 98% and 92% respectively.

In this particular study, it was observed that there was a notable predominance of female participants, which starkly contrasts with the findings of prior research conducted in Indonesia and India [18,19]. Likewise, it was observed that a significant portion of the subjects afflicted with Diabetic Foot Ulcer (DFU) infections fell within the demographic bracket of individuals aged between 51 and 60 years, a trend that resonates with analogous research findings conducted within the geographical contexts of India and Indonesia [20].

Overall, it was observed that the prevalence of gram-negative bacteria, accounting for 71.6% of the total isolates (86 out of 120), was significantly higher than that of gram-positive isolates, which constituted 34.16% of the isolates [21].

Diabetic populations are distinguished by a set of characteristics that distinguish them from other groups in the context of antibiotic resistance, thus posing a distinctive and specific challenge. This challenge is particularly significant due to the intricate and multifaceted relationship that exists between metabolic dysregulation and compromised immune responses within this population [22]. Our research results serve to strengthen the existing correlation, indicating that the issue of antibiotic resistance in *Staphylococcus aureus* strains found in diabetic individuals poses a noteworthy and widespread challenge that demands focused and coordinated efforts, a point that is further underscored by [23]. Most of the Staphylococcus aureus isolates in this investigation were susceptible to amikacin, oxacillin, ciprofloxacin, clindamycin, and vancomycin, but resistant to gentamicin, doxycycline, erythromycin, and trimethoprim. Likewise, all enterococci exhibit resistance to trimethoprim, erythromycin, doxycycline, and gentamicin. While all Enterococci and Staphylococcus aureus were found to be 100% sensitive to chloramphenicol [24].

Overall, the discoveries we have made serve to place into context the intricate interactions that exist between antibiotic resistance and populations affected by diabetes within the larger scope of research focused on resistance. This highlights the urgent need for interventions that are multifaceted in nature and require collaborative endeavors to effectively tackle the increasingly concerning public health challenge posed by antibiotic resistance. Moreover, it is crucial to emphasize the significant socio-economic consequences associated with antibiotic resistance, as they play a pivotal role in shaping the impact and scope of this pressing issue[25].The findings underscore the regional differences in the occurrence of bacteria and resistance to antibiotics, underscoring the importance of treatment protocols tailored to specific regions and continuous monitoring to control infections in patients with diabetic foot ulcers effectively.

Conclusion

Diabetic foot ulcers can be infested with a wide range of infections, including a substantial number of multidrug resistant bacteria. In our study a 200 wound Positive specimens were collected from individuals diagnosed with type II diabetes who had undergone amputations as a result of bacterial infection. The study emphasizes the notable occurrence of Staphylococcus aureus and Klebsiella in wound infections among individuals diagnosed with type II diabetes in the Khyber Pakhtunkhwa region. Significant regional differences were noted in the incidence of bacteria and patterns of antibiotic resistance. There was a high susceptibility to amikacin and gentamicin, but concerning resistance to imipenem and cotrimoxazole. These findings emphasize the need for antibiotic stewardship programs and customized treatment options to successfully control diabetic foot infections and reduce the emergence of antibiotic-resistant bacteria in specific regions.

Recommendation

Future studies should focus on increasing the scope of sampling to include a variety of populations, using longitudinal research to observe how resistance dynamics change over time, and utilizing genomic analysis to gain a more in-depth understanding of resistance mechanisms in diabetic individuals.

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Conflict of Interest

Not Declared

Informed Consent

Taken from the participants.

Ethical Statement

The approved by the research and ethical scientific board City Hospital, Peshawar.

Author Contribution

All authors contributes, equally in writing and editing the manuscript

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