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ANTIMICROBIAL RESISTANCE TRENDS IN WOUND PATHOGENS: A STUDY AT A TERTIARY CARE HOSPITAL IN PESHAWAR, PAKISTAN

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ABSTRACT

Objective: This study aimed to investigate prevalent bacterial pathogens causing wound infections and their antibiotic resistance patterns among patients at a Tertiary care hospital in Peshawar, Pakistan. The primary focus was to identify geographic-specific resistance trends and clinical implications for effective management strategies.

Methodology: Samples collected from patients presenting with microbiological infection signs at Tertiary care hospital from February 23rd, 2022, to August 23rd, 2023, included 243 plus specimens. The selection of Peshawar as the study site factored in its geographical specificity, potentially impacting bacterial prevalence and resistance patterns. The collection utilized cotton swabs, and subsequent processing employed the streak plate method and inoculation onto Blood agar, chocolate agar, and MacConkey agar for optimal bacterial growth. Microbiological assays and specialized tests were performed, including Triple Sugar Iron (TSI) Test, Motility Indole Urease (MIU) Test, Simmons Citrate Test, Bile Esculin Agar, Catalase Test, Coagulase Test, Oxidase Test, and Antibiotic Susceptibility Test via disc diffusion method.

Results: Analysis revealed prevalent pathogens: *Staphylococcus aureus* (30%), *Escherichia coli* (24%), *Pseudomonas* (24%), *Klebsiella spp.* (10%), *Streptococcus* (4%), *Proteus spp.* (3%), *Acinetobacter spp.* (3%), and *Enterococcus* (2%). Notably, Gram-positive bacteria displayed resistance to Cephalosporins but sensitivity to Carbapenems. Conversely, Gram-negative bacteria exhibited substantial antibiotic resistance. Antibiotic Resistance: The study found high resistance rates in *Staph. aureus* (92% to Cefixime and 94% to Ceftazidime). One potential reason for the increased antibiotic resistance observed, as indicated in this study, could be the overuse or inappropriate use of antibiotics.

Conclusion: Our study highlights the need for specific strategies to reduce antibiotic resistance in pus samples. Resistance percentages and prevalence statistics, analyzed, have yielded significant findings. These findings underscore the critical need for tailored treatments, stringent antibiotic management, and coordinated policy actions

KEYWORDS: Wound infection, Bacteria, MDR, Staphylococcus aureus, Bacterial pathogens, Pakistan

INTRODUCTION

Throughout the world, MDR occurrence has revolutionized the way we treat bacterial infections. Due to constant bacterial resistance development, we must adjust and develop new and more long-lasting solutions [1] [2]. Antibiotic resistance is a major health concern in Pakistan. The urgency of the circumstance has driven the WHO to back key measures worldwide. As anti-microbial resistance may be a developing peril around the world, Pakistan is not only facing its own immediate challenges, but besides playing a vital portion in around the world setting [3].

This study focused on AMR in Pakistan, with a special focus on Pakistan's unique circumstances. An interaction between environment and socio-economic elements is at the center of wound contaminations, and understanding these elements is key to battling AMR in Pakistan [4] [5]. Wounds commonly harbor bacteria such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Proteus species, Acinetobacter species,* and *Candida albicans* [6]. Each of these bacteria has its own unique characteristics, which play a part in their association in wound contaminations. The risk postured by STA particularly its ability to develop resistance mechanisms, emphasizes the need for further research to develop effective treatment strategies. Understanding and tending to the interesting characteristics of these microbes is basic for creating viable approaches to battle wound diseases [7].

Complex biological mechanisms are involved in antimicrobial resistance functions, which include changes in cell wall synthesis, production of nucleic acid, activity of ribosome, synthesis of protein, and function of cell membrane. This multifaceted progress highlights the complexity of the challenge and underscores the basic for comprehensive methodologies to successfully address these issues [8] [9] [10]. In Pakistan, antibiotics use without prescription from the doctors is the major cause of increase in antibiotic resistance. To protect the health of the public, proper measures, and public awareness are needed [11]. Antimicrobial use has become a huge problem in Pakistan. The misuse of antibiotics is responsible for resistance. Public awareness and regulatory interventions are the need of the hour in order to tackle the antimicrobial resistance problem [12].

Comparing the current findings on multidrug resistance of bacteria with patterns seen with wound infections is a challenge. In this study multidrug resistance patterns in bacteria isolated from wound infections of Pakistani patients is the purpose to be investigated. The aim is to know, in detail, these patterns that are going to lead to the implementation of the most efficacious interventions, give the most specific treatment recommendations, and realize the most targeted healthcare policies. Therefore, this strategy can contribute greatly to the decrease of antimicrobial resistance.

METHODOLOGY

Sample Collection and Research Details: Research was conducted at Tertiary care hospital, which is situated in Peshawar, Pakistan, from February 2023 to August 2023. Microbiological cultures for the study were taken from patients, showing clinically relevant signs of infections. The location of hospital added to the complexity of our choice because of its setting which might affect the spread of resistant bacteria in the community. Specimen Type and Quantity: The clinical isolates amounted to a total of 243 in the entire study, sampling the wound infections particularly.

Sampling and Microbiological Techniques: As reported by the literature, aseptic conditions were satisfied with pus samples obtained from individuals with wound infections using cotton swabs.

Hence, these samples were put on Blood agar, chocolate agar, and MacConkey agar and the results were documented. The incubation period was provided to the above samples which were kept for 24 to 48 h at 37°C temperature to form a favorable environment for bacteria growth. The rigorous process, along with the high reliability and accuracy, were an integral part of the study.
Microbial assays and typical diagnostics were applied to wound material to identify the pathogens involved. The TSI Test, the MIU Test, the Simmons Citrate Test, the Bile Esculin Agar Test, the Catalase Test, the Coagulase Test, the Oxidase Test, and the Antibiotic Susceptibility Test were among the tests that were conducted, just to name a few. This hence led to the development of a sophisticated battery of tests that were necessary to have a refined picture of the microbial profile in wounded infections. Using the disc diffusion method, synthesizing antibiotic-infused discs on sensitive media was shown. The zones around the disks determined antibiotics that acted upon specific bacterium species without bacteria on the growth. Clinical Relevance and Significance: In Peshawar, Pakistan, patient selection criteria and pathogen identification were rigorously conducted to highlight the clinical relevance and importance of the identified microorganisms.

RESULTS

In the microbiology section of the Tertiary care hospital in Peshawar, we examined 243 samples to determine how resistant patients were to various medications. The results, when broken down by age, gender, and multidrug resistance (MDR), paint a varied picture. To understand how different factors, affect resistance levels among patients, it's like peeling back layers.

Table 1 Results of biochemical tests performed on isolated bacteria

Organisms	Citrate Test	Oxidase Test	MIU Medium			TSI		
			Motility	Indole	Urea	Slant	Butt	H2S
E. coli	-	-	+	+	-	A	A	+
Klebsiella spp.	+	-	-	-	+	A	A	+
Pseudomonas spp.	+	+	+	-	-	A	A	+
Staphylococcus aureus	-	-	-	-	-	K	K	-
Acinetobacter spp.	+	-	-	+	+	A	A	-

In **Table 1** is the data from biochemical tests done with microbial samples. The tests include the Triple Sugar Iron (TSI) test, the Oxidase activity test, the Citrate utilization test, and the Motility-IndoleUrea (MIU) Medium assessment. The interpretations for these tests are as follows: During the Citrate Test an organism that indicates citrate as its carbon source will show a positive result however, one that is unable to use citrate will give a negative result. The Oxidase test gives a positive result in having cytochrome c oxidase produced in the organism. A negative outcome shows there is no production of this enzyme. Assessment of MIU Medium is signified by (+) and (-). The (+) sign denotes the presence of motility and indole, while the (-) sign rather indicates the absence of these substances. Furthermore, the (-) sign shows hydrolysis of urea, while the (de) sign refers to the lack of it. Corresponded to acidity, alkalinity shown as A and K, respectively. The (+) sign at the top of H2S column indicates that hydrogen sulfide has been detected.

Percentage of Gram-positive & negative organisms of total positive samples

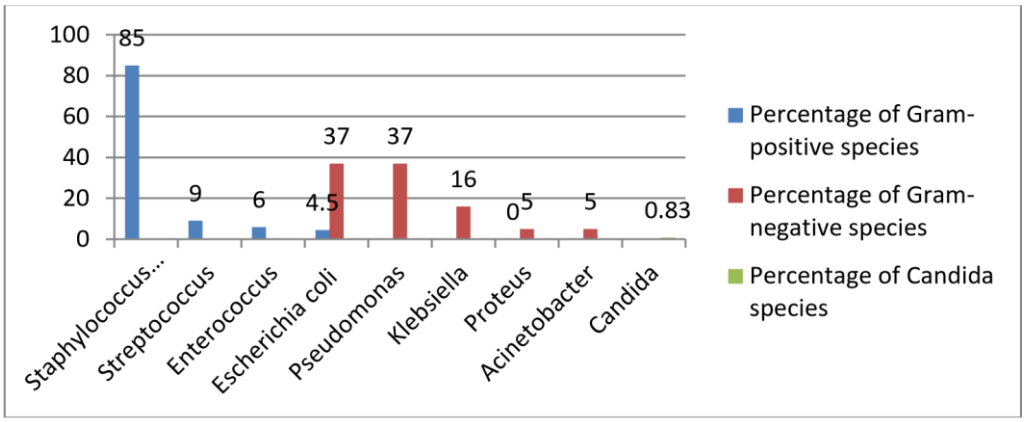

Figure 1

In the examined dataset of wound pus samples, Gram-positive organisms constituted a total of 85 samples, with *Staphylococcus aureus* representing 85%, *Streptococcus* comprising 9%, and *Enterococcus* constituting 6%. Gram-negative species totaled 156 samples, with *Escherichia coli* and *Pseudomonas* together accounting for more than 20%, while *Klebsiella, Proteus*, and *Acinetobacter* ranged from 2-10%. *Candida species* were observed in 2 samples, amounting to 0.83%. These figures delineate the prevalence of various pathogens in wound infections, highlighting the dominance of specific bacteria like *S. aureus* and the significance of *E. coli*, *Pseudomonas*, and *Candida species*, emphasizing the need for tailored treatment strategies to address these diverse microbial profiles effectively.

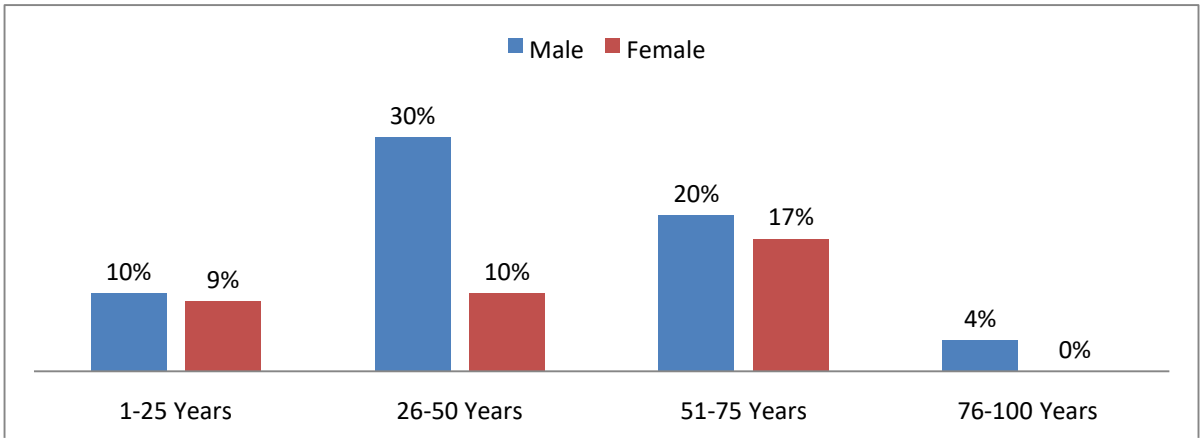

Figure 2 Percentages of positive patients according to age and sex groups

The age group of 26-50 years has a higher prevalence of positive patients, estimated at 30%. The age group of 51-75 years has a prevalence of 20%, while the age group of 76-100 years has a prevalence

of 4%. In contrast, female patients are more prevalent in the age range of (51-75) years and gradually decrease to 10% in the (26-50) age group and 9% in the (1-25) age group.

Antibiotic resistance patterns are shown by different organisms.

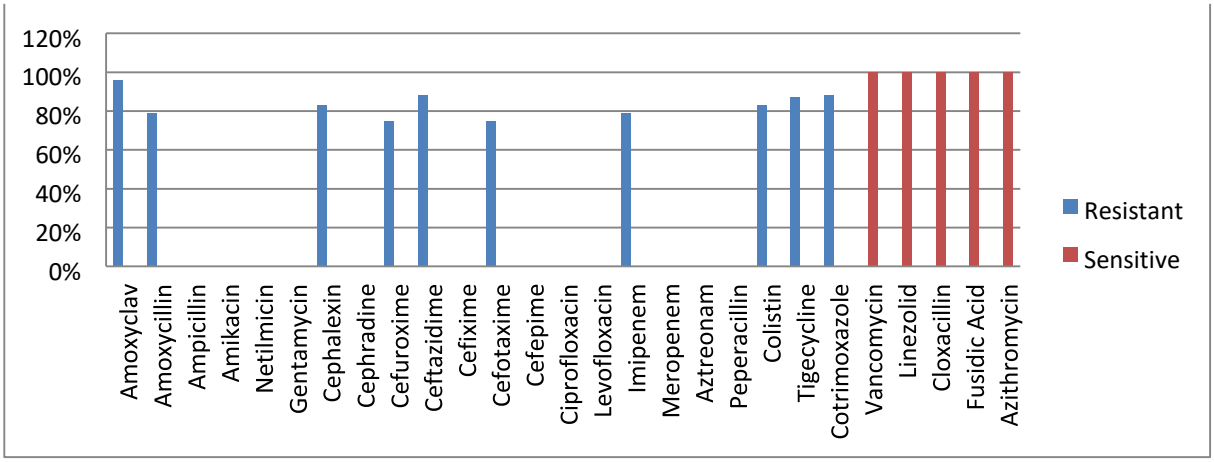

Figure 3 Antibiotic resistance pattern of *Klebsiella spp.*

The results of our investigation indicate that *Klebsiella spp.* shown a high level of susceptibility (100%) to Linezolid, Vancomycin, Cloxacillin, Fusidic Acid, and Azithromycin. These antibiotics have the potential to be efficacious in the treatment of Klebsiella spp. infections. Significantly, Amoxiclav had a 96% efficiency rate, whereas Imipenem, Colistin, Amoxycillin, and Tigecycline exhibited effectiveness rates ranging from 79% to 87%. Nevertheless, Cefotaxime had a resistance rate of 75%, whereas Cotrimoxazole, Ceftazidime, Cephalexin, and Cefuroxime showed resistance rates of 88%, 88%, 83%, and 75% respectively.

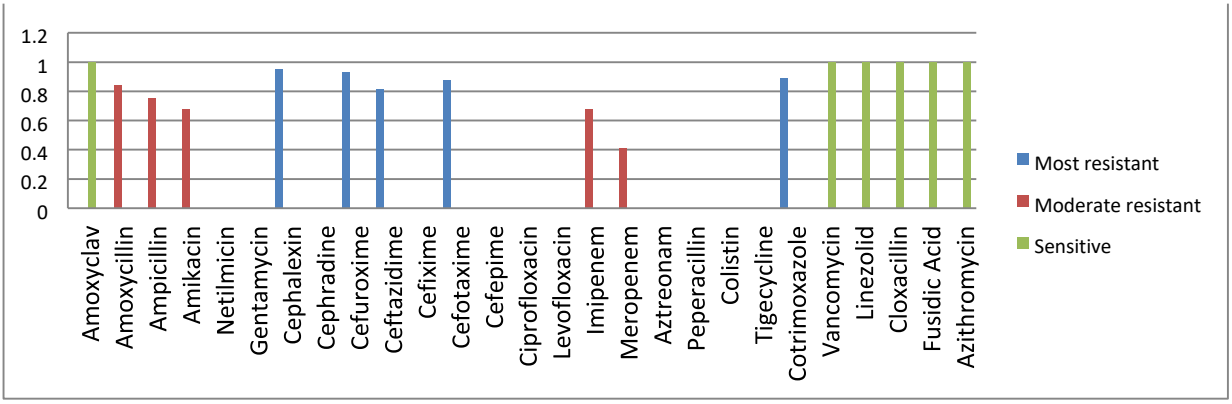

Figure 4 Antibiotic resistance pattern of *Pseudomonas spp.*

The findings of our study showed that *Pseudomonas species* exhibited significant resistance to Cephalexin (95%), Cefuroxime (93%), Ceftazidime (81%), Cefotaxime (88%), and Cotrimoxazole (89%). This pathogen exhibited reduced susceptibility to antibiotics belonging to the Cephalosporin category. On the other hand, Amoxycillin exhibited a resistance rate of 84%, Ampicillin had a resistance rate of 75%, Amikacin showed a resistance rate of 68%, while both Imipenem and Meropenem had a moderate resistance rate of 68% and 41% respectively. Nevertheless, Pseudomonas spp. exhibited complete sensitivity (100%) to Vancomycin, Linezolid, Cloxacillin, Fusidic Acid, Azithromycin, and Amoxyclav.

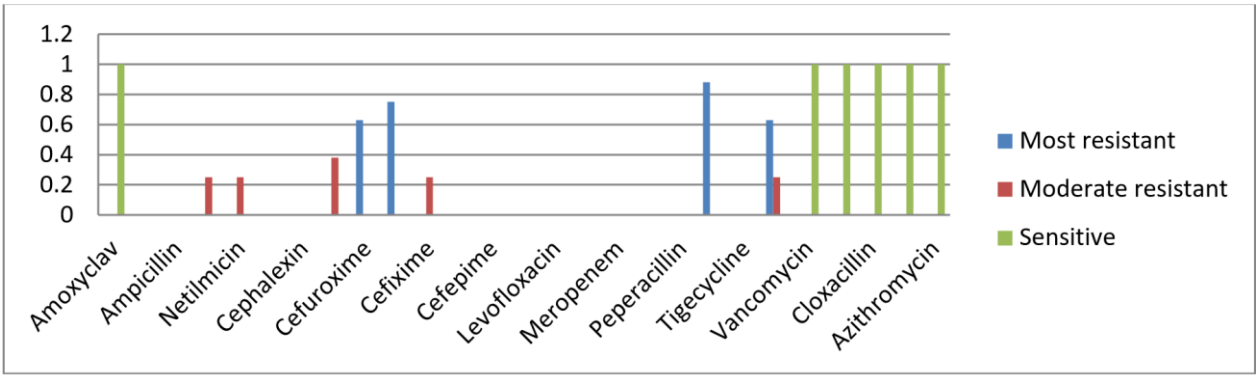

Figure 5 Antibiotic resistance pattern of *Proteus spp.*

The *Proteus species* showed 100% susceptibility to Amoxyclav, Vancomycin, Linezolid, Cloxacillin, Fusidic acid, and Azithromycin, indicating their potential for successful therapeutic intervention. Notable resistance was seen in Cephalexin (88%), Cefuroxime (63%), Ceftazidime (75%), Collistin (88%), and Cotrimoxazole (63%). Antibiotics include Amikacin (25%), Netilmicin (25%), Cephradine (38%), Cefixime (25%), Ceftriaxone (25%), and Imipenem and Azteronam shown intermediate resistance or susceptibility.

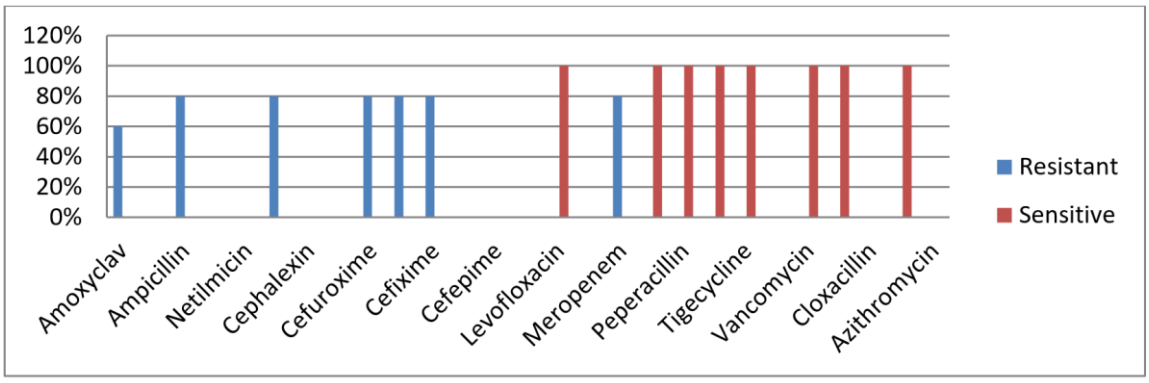

Figure 6 Antibiotic resistance pattern of *Enterococcus spp.*

Enterococcus species have shown resistance to Amoxyclav (60%), Ampicillin (80%), Gentamycin (80%), Cephalexin (80%), Cefuroxime (80%), Ceftazidime (80%), Cefixime (80%), and Cefotaxime (80%) among the tested antibiotics. Nevertheless, Piperacillin-tazobactam, Levofloxacin, Azteronam, Colistin, Tigecycline, Vancomycin, Linezolid, and Fusidic Acid have shown complete sensitivity.

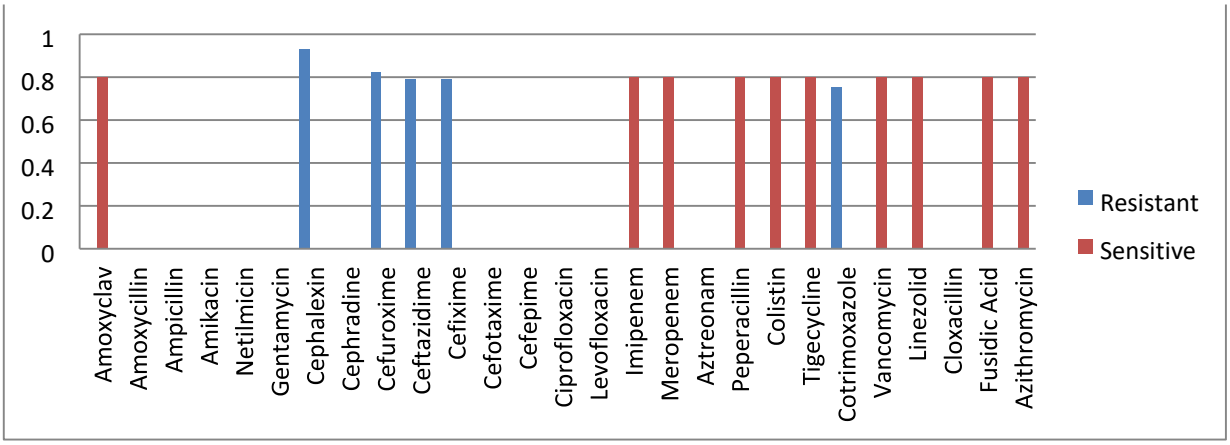

Figure 7 Antibiotic resistance pattern of *E. coli*

Escherichia coli showed sensitivity rates over 80% for Meropenem, Piperacillin-tazobactam, Amoxyclav, Imipenem, Colistin, Linezolid, Cloxacillin, Tigecycline, Vancomycin, Fusidic acid, and Azithromycin. On the other hand, Cephalexin exhibited a resistance rate of 93%, while Cefuroxime, Ceftazidime, Cefixime, and Cotrimoxazole showed resistance rates of 82%, 79%, 79%, and 75% respectively.

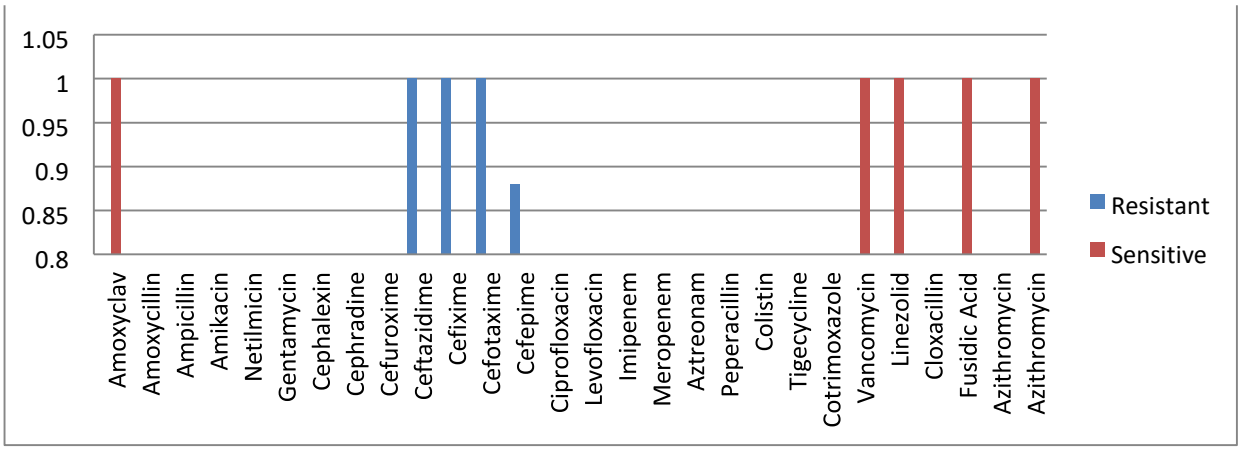

Figure 8 Antibiotic resistance pattern of *Acinetobacter*

Our research discovered that *Acinetobacter* exhibited a significant level of resistance (100%) to Cephalosporin antibiotics such as Ceftazidime, Cefixime, Ceftriaxone, and Cefotaxime. Nevertheless, Meropenem, Azteronam, and Piperacillin-tazobactam showed no resistance. Amoxyclav, Vancomycin, Linezolid, Cloxacillin, Fusidic Acid, and Azithromycin showed complete sensitivity, with a rate of 100%.

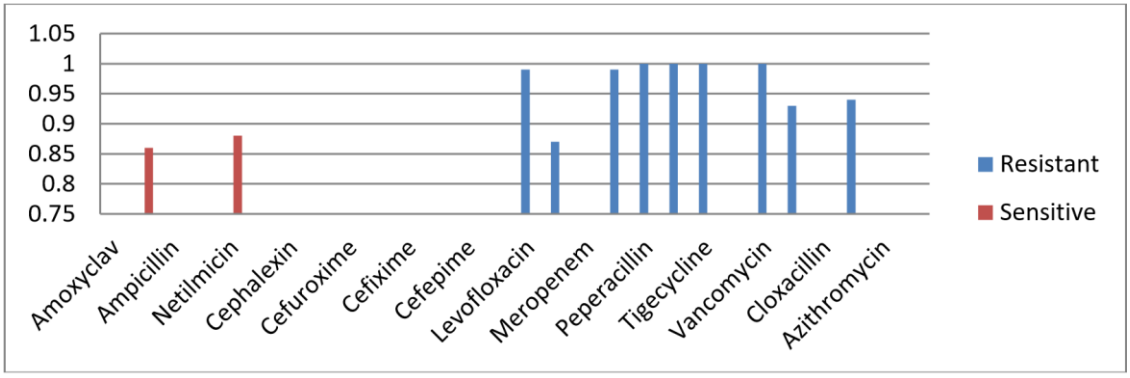

Figure 9 Antibiotic resistance pattern of *S. aureus*

Staphylococcus aureus exhibited significant susceptibility to Levofloxacin (99%), Imipenem (87%), Azteronam (99%), Piperacillin-tazobactam (100%), Colistin (100%), Tigecycline (100%), Vancomycin (100%), Linezolid (93%), and Fusidic Acid (94%). The bacterium exhibited the highest resistance rates against Ampicillin (93%), Ceftazidime (94%), and Cefixime (92%).

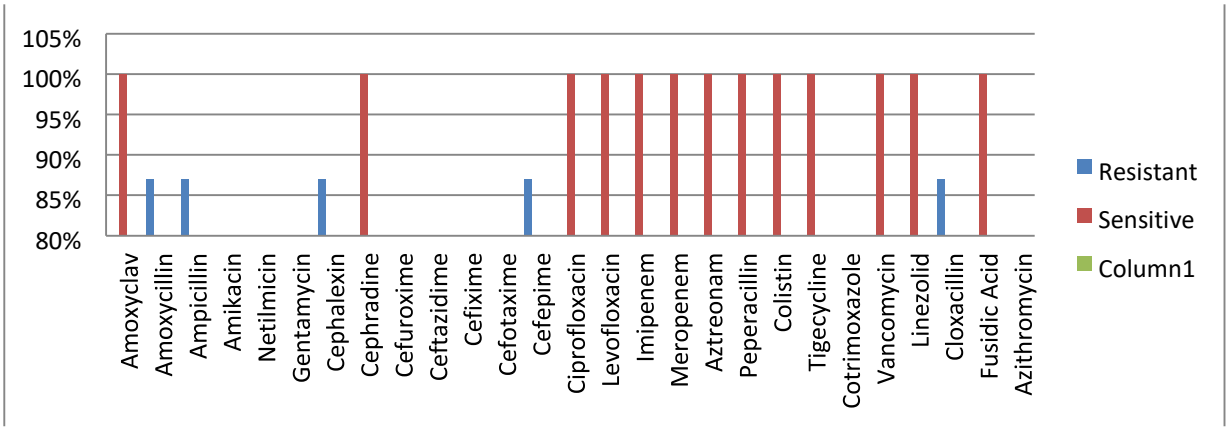

Figure 10 Antibiotic resistance pattern of *Streptococcus*

Streptococcus exhibited complete susceptibility to a range of antibiotics, such as Cephradine, Ciprofloxacin, Amoxyclav, Levofloxacin, Imipenem, Meropenem, Azteronam, Piperacillintazobactam, Colistin, Tigecycline, Vancomycin, Linezolid, and Fusidic Acid, with a sensitivity rate of 100%. Ampicillin, Amoxycillin, Cephalexin, Cefepime, and Cloxacillin had a sensitivity rate of around 87%, indicating a modest level of effectiveness.

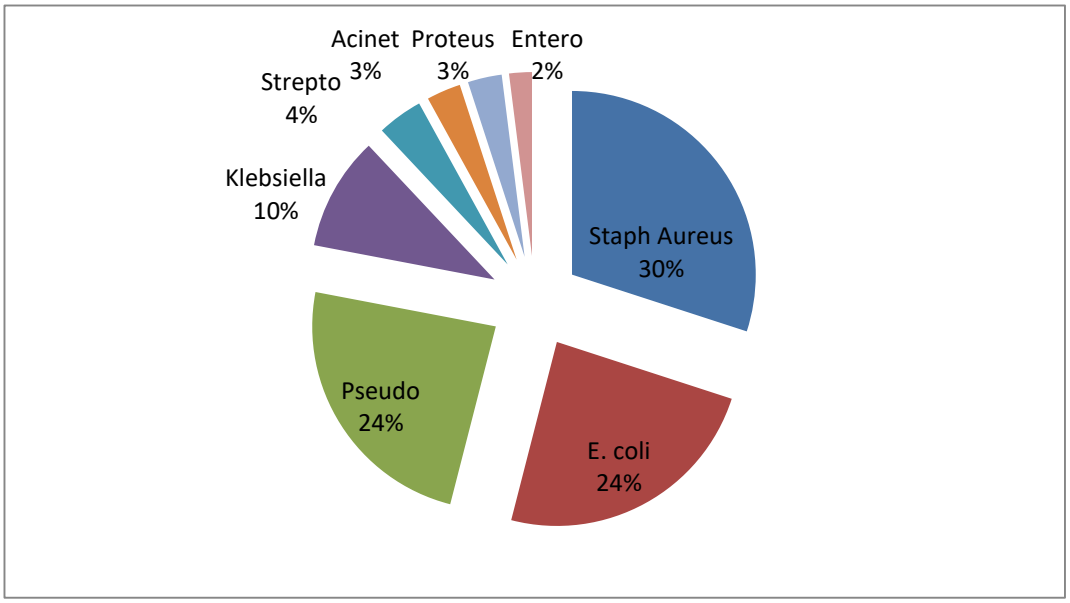

Figure 11 Percentages of etiological agents of bacterial infection

Figure 11 presents the percentages of various bacterial etiological agents contributing to infections. *Staphylococcus aureus* accounts for 30% of cases, while *Escherichia coli* and *Pseudomonas aeruginosa* both represent 24% of the total infections. *Klebsiella* follows at 10%, and the prevalence decreases further for *Streptococcus* (4%), *Acinetobacter* (3%), *Proteus* (3%), and *Enterococcus* (2%). These percentages offer a comprehensive overview of the distribution of bacterial pathogens implicated in infections, highlighting the varying degrees of prevalence among different species. The findings of this study carry significant clinical implications. There is a concern about treating wound infections caused by multiple bacteria, both gram-positive and gram-negative. It is extremely difficult to choose a treatment option for Gram-positive bacteria due to the substantial prevalence of antibiotic resistance, especially against commonly used antibiotics like penicillin, ampicillin, and oxacillin, and for Gram-negative bacteria due to ampicillin, cotrimoxazole, and doxycycline resistance. It is possible that these resistance patterns lead to poor first treatment, longer recovery periods, and difficulty in selecting empirical treatments resulting from these resistance patterns.

Many bacteria are multidrug-resistant (MDR), which poses a challenge for healthcare workers. Treating infections caused by MDR bacteria, especially *Staphylococcus aureus*, is heightened. Infections caused by MDR bacteria cause longer hospital stays, higher morbidity rates, and higher mortality rates. Staphylococcus aureus, an MDR bacteria, is particularly difficult to treat because of the lack of antibiotics.

According to this study, multidrug resistance is now common in both gram-positive and gramnegative bacteria. Similar resistance has been observed in Ethiopia in previous researches. New findings show increase in multidrug resistance among species of *Proteus* and some strains of *Staphylococcus aureus*. This underlines a concern over these species. While the results have similarities with previous studies, the differences in resistance rates proposes geographical differences or local resistance profile changes. Antibiotic resistance is a dynamic and evolving phenomenon, requiring ongoing vigilance and adaptability in treatment approaches.

DISCUSSION

An assessment of antibiotic susceptibility, multidrug resistance prevalence, and bacterial reports in 243 pus samples was conducted in a microbiology section of a Tertiary care hospital in Peshawar. These results can be utilized to advise clinical practice and public health interventions.

It was very susceptible to certain antibiotics, such as Vancomycin (100%), Linezolid (93%), and Imipenem (87%), but very resistant to Ceftazidime (94%), and Cefixime (92%). As for Escherichia coli, Meropenem and Piperacillin-tazobactam showed more than 80% sensitivity, but Cephalexin (93%), Cefuroxime (82%), and Cotrimoxazole (75%) showed significant resistance.

Compared with previous research [13], our findings indicate shifts in bacterial frequency and resistance patterns. There is, however, a need for more research and individualized treatment procedures as a result of differences in resistance rates, which suggest possible geographical variances or emerging resistance mechanisms.

As the prevalence of antibiotic resistance increases, individualized therapies are becoming more important alongside antibiotic stewardship, local resistance-based treatment recommendations, and enhanced infection control procedures.

As Sweden and the Netherlands have demonstrated, political dedication is essential in the fight against antibiotic resistance. [14] [15]. Through its Global Action Plan on Antimicrobial Resistance, the World Health Organization addresses this worldwide health issue. [16].

CONCLUSION

Our study highlights the need for specific strategies to reduce antibiotic resistance in pus samples. Resistance percentages and prevalence statistics, analyzed, have yielded significant findings. These findings underscore the critical need for tailored treatments, stringent antibiotic management, and coordinated policy actions. It's crucial to uphold a strong commitment to reducing antibiotic resistance, evidenced by the impactful legislation enacted globally. Implementing these measures is vital to safeguard public health and sustain the efficacy of antibiotics for future generations.

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