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PHENOTYPIC SCREENING OF CARBAPENEM RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES FROM BURN PATIENTS

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen and causes variety of infections, including respiratory tract infections and skin associated infections. Antimicrobial resistance, mainly multidrug resistant P. aeruginosa (MDRPA), is a pressing concern related to P. aeruginosa infections. The present study aims at screening carbapenemase producing P. aeruginosa isolates from burn patients. Altogether 200 swab samples were collected from the burn and care unit of Pakistan Ordnance Factory (POF) hospital, Wah Cantt. Samples were processed for biochemical and microbiological identification. Antibiotic susceptibility testing was done by disc diffusion method. Carbapenemase detection was performed by carbapenem inactivation method (CIM). Overall, the results confirmed 80 (42%) of the isolates as P. aeruginosa, followed by E. coli (23.8%). K. pneumoniae and S. aureus (each 14.28%). The results also revealed that P. aeruginosa exhibited high susceptibility to levofloxacin (100%), while highest resistance was observed against meropenem (100%) followed by ciprofloxacin (50%), imipenem (50%) and gentamicin (45%). Further, CIM method revealed that 48 out of 80 (60%) of the isolates were positive for carbapenemase production. These findings underscore the varying susceptibility patterns of P. aeruginosa to different antibiotics commonly used in clinical practice. The results also highlight the notable presence of carbapenem resistant P. aeruginosa further endorsing the need of novel therapeutic drugs to treat such infections.

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INTRODUCTION

Pseudomonas aeruginosa, primarily identified in 1882 by Gessard and recognized as a pathogen in 1890 by Charrin, has arose as a significant threat, particularly among susceptible populations such as weakened, burned, cystic fibrosis, and immunocompromised individuals (1, 2). It is pervasive in numerous environmental positions including soil, water, plants, and animals. The bacterium is known for producing unique pigments, pyoverdine and pyocyanin, and produces a distinctive fruity odor due to the production of 2-aminoacetophenone(3-5). Colonies on sheep blood agar plates typically exhibit beta-hemolysis and a greenish metallic sheen. P. aeruginosa is non-fermentative, obligatory aerobic, and capable in making biofilms, which pose challenges in antimicrobial treatment due to their resistance to conventional treatments (4, 6).

In the last two decades, P. aeruginosa has arose as a significant pathogen, contributing to about 10-20% of infections within hospital settings. Mostly dominant among individuals with burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous drug use, Pseudomonas infections pose a significant risk (7). Frequently found as a nosocomial contaminant, outbreaks have been connected to various items in hospital settings. Prolonged hospital stays increase the probability of colonization by P. aeruginosa, increasing the risk of infection. Severe infections associated with this pathogen include malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (8).

Burn wounds are a major public health concern globally, often resulting in death or serious difficulties due to infection (9). The compromised skin barrier following burns allows for the entry of harmful microorganisms, with P. aeruginosa being a prominent cause of colonization and severe wound infections. Such colonization can escalate to sepsis, increasing morbidity and mortality rates among burn patients. Furthermore, the prevalence of multi-drug resistant strains poses extra tasks in managing these infections (10). The prevalence of P. aeruginosa infections in burn patients presents significant challenges in treatment and management. This bacterium is notorious for its intrinsic resistance to multiple antimicrobial agents and its ability to develop acquired resistance mechanisms rapidly. Moreover, P. aeruginosa can form biofilms on wound surfaces, further exacerbating treatment difficulties and increasing the risk of chronic infections.

P. aeruginosa is a ubiquitous Gram-negative bacterium known for its opportunistic pathogenicity, particularly in immunocompromised individuals and those with burn injuries. Burn wounds provide an ideal environment for P. aeruginosa colonization and infection due to compromised skin barriers and impaired immune responses. Burn patients are at heightened risk of P. aeruginosa infections, which can lead to severe complications and increased mortality rates. The prevalence of P. aeruginosa infections in burn patients presents significant challenges in treatment and management. This bacterium is notorious for its intrinsic resistance to multiple antimicrobial agents and its ability to develop acquired resistance mechanisms rapidly. Moreover, P. aeruginosa can form biofilms on wound surfaces, further exacerbating treatment difficulties and increasing the risk of chronic infections.

Empirical therapy for P. aeruginosa wound infections in burn patients often involves the use of broad-spectrum antibiotics, including carbapenems, due to their efficacy against Gram-negative bacteria. However, the rising prevalence of carbapenem-resistant P. aeruginosa strains poses a serious therapeutic challenge, necessitating alternative treatment approaches.

Understanding the epidemiology and antibiotic susceptibility patterns of P. aeruginosa infections in burn patients is essential for guiding appropriate therapeutic interventions and infection control measures. Surveillance studies play a crucial role in monitoring trends in antimicrobial resistance and informing empirical treatment protocols to optimize patient outcomes and minimize the spread of resistant strains within healthcare settings.

MATERIALS AND METHODS

Study Location and Sample Collection

This Cross-Sectional Laboratory-Based Observational Study was conducted at the Department of

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Microbiology, Hazara University, Mansehra. Clinical isolates were collected from Pakistan Ordnance Factory (POF) Hospital, Wah Cantt, Taxila. A total of 200 samples were obtained from hospitalized burn patients, yielding 80 P. aeruginosa isolates. Ethical approval was secured from the POF Hospital Ethics Committee prior to sample collection.

Sample Processing

Following collection, the clinical samples were immediately transported to the Department of Microbiology, Hazara University, Mansehra, under aseptic conditions and processed within two hours of arrival. Each sample was cultured on general purpose, selective and differential media, including Nutrients Agar and MacConkey agar, and incubated at 37°C for 24 - 48 hours. Colonies showing characteristic morphology of Pseudomonas aeruginosa such as green pigmentation and grape-like odor were further subjected to Gram staining and standard biochemical tests (oxidase, catalase, citrate, indole, and coagulase) for preliminary identification. Isolates presumptively identified as P. aeruginosa were preserved on nutrient agar slants and stock culture were prepared and stored at 4°C for further antimicrobial susceptibility testing and phenotypic screening.

Stock culture preparation

P. aeruginosa was streaked onto MacConkey agar media, and the plates were incubated at 37 °C for 24 hours. Following incubation, optimum growth was observed on the agar plate. To prepare a stock culture, a loopful of bacterial growth was transferred from the MacConkey agar plate into a sterilized liquid broth. Subsequently, this liquid broth was incubated overnight at 37 °C. The next day, 15% glycerol was added to the bacterial culture, and it was stored in 400 µl aliquots at -20 °C for preservation.

Antimicrobial susceptibility testing.

Antimicrobial susceptibility testing (AST) was done to evaluate the response of P. aeruginosa isolates to six different antibiotics: tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem. The selected methodology for this evaluation involved the commonly employed disc diffusion method, offering a reliable and uniform approach. Bacterial isolates of P. aeruginosa were primarily subculture onto Mueller-Hinton agar (MHA) plates, followed by an incubation period at 37 °C for 18-24 hours. Then, a standardized bacterial suspension was prepared to achieve a specific turbidity in accordance with the McFarland standard. This suspension was then uniformly spread across the surface of Mueller-Hinton agar plates. Discs saturated with recognized concentrations of tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem were aseptically placed on the inoculated agar plates. The plates were then incubated under standardized conditions, and after the incubation period, the diameter of the resulting inhibition zones around each antibiotic disc was accurately measured using a standardized ruler.

The recorded measurements were interpreted based on recognized interpretative standards provided by authoritative bodies such as the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These standards categorized the isolates as susceptible, intermediate, or resistant, forming the basis for the determination of the strains' susceptibility profiles to the tested antibiotics.

Phenotypic screening of carbapenem-resistant P. aeruginosa.

The phenotypic screening of carbapenem-resistant P. aeruginosa was undertaken through the use of the Carbapenem Inactivation Method (CIM), a vital method for identifying carbapenemase production. The methodology involved some systematic steps. Initially, P. aeruginosa isolates were cultured and their identities confirmed (11). Then, a standardized bacterial suspension of P. aeruginosa was prepared, and imipenem and meropenem discs were immersed in the bacterial suspension, undergoing incubation at 37 °C for two hours. To check carbapenemase activity, a second step involved spreading Escherichia coli (ATCC 25922) on Mueller-Hinton agar plates, onto which the previously incubated discs were then placed. They were subsequently incubated at 37°C for 24 hours. The presence of a visible zone of growth inhibition around the carbapenem discs indicated susceptibility, while the absence of such inhibition strongly suggested the production of

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carbapenemase. This sequential approach provided a robust method for confirming carbapenem resistance mechanisms in P. aeruginosa isolates.

RESULTS:

Isolation and Biochemical Identification of Pseudomonas aeruginosa

A total of 200 clinical samples were collected from burn patients for the isolation, antimicrobial susceptibility testing, and phenotypic screening of carbapenem-resistant P. aeruginosa. Of these, 168 samples (84%) yielded positive bacterial growth on selective media, while 32 samples (16%) showed no growth as shown in figure 1. Identification was confirmed through standard biochemical assays, revealing Gram-negative, rod-shaped bacteria that were catalase-positive, oxidase-positive, indole-negative, and coagulase-negative. These biochemical characteristics facilitated the accurate identification of P. aeruginosa and differentiation from other Gram-negative pathogens, aiding in effective clinical management and infection control.

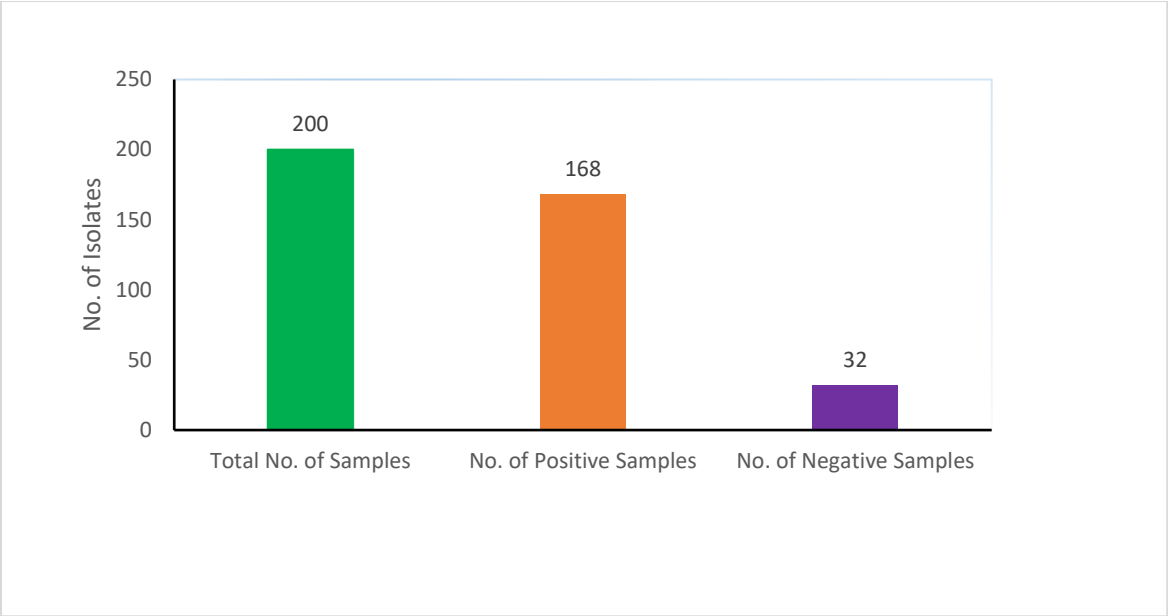

Figure 1: Distribution of positive and negative samples

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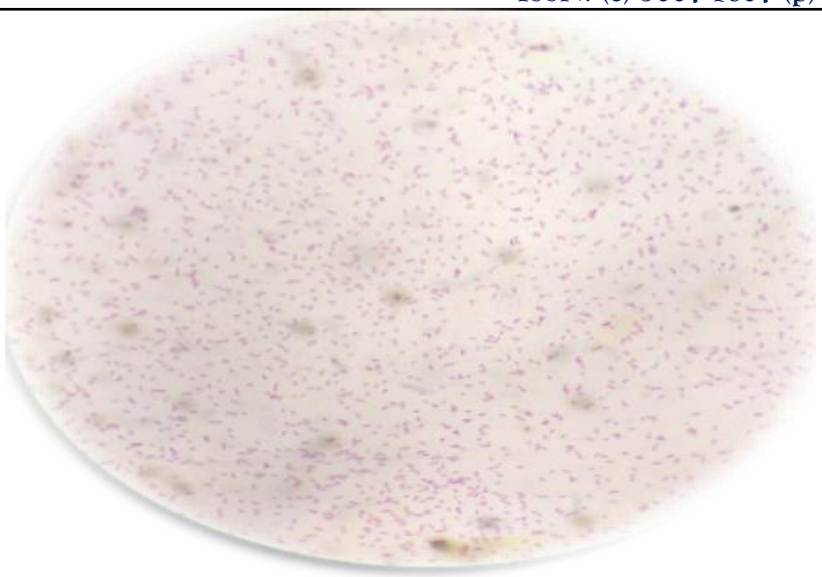
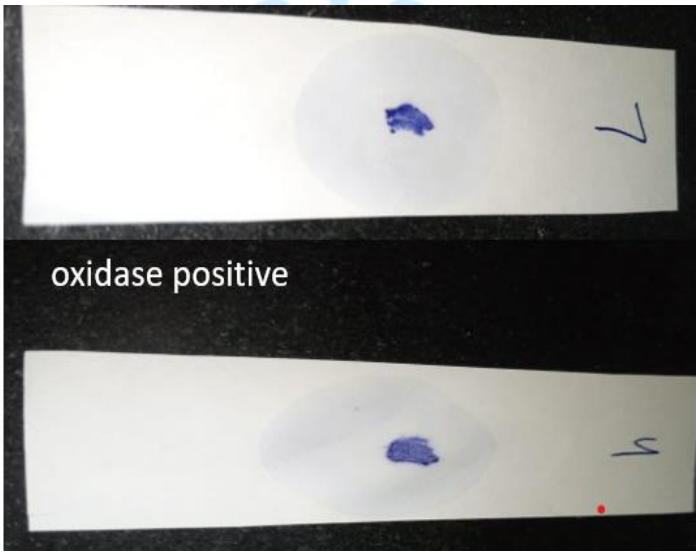


Gram-negative rod



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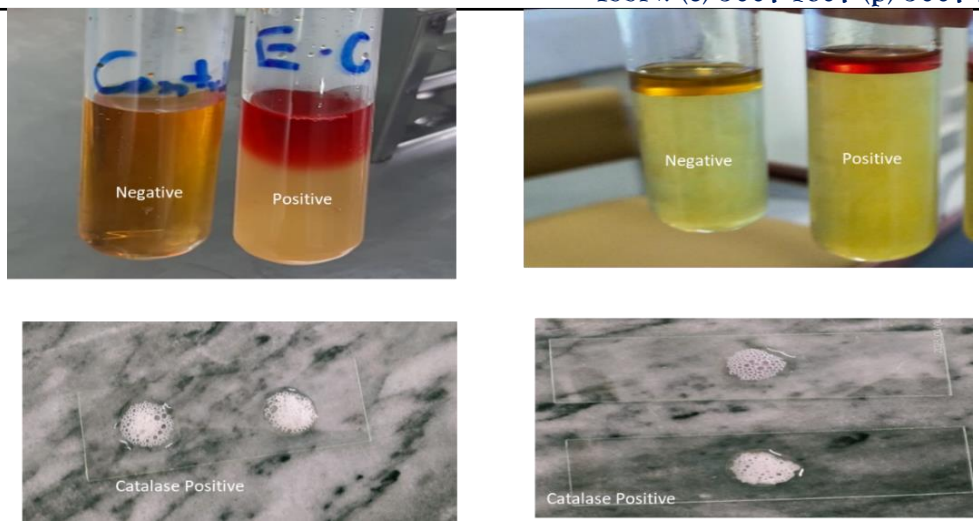

Figure 2: The figures show Gram-negative rods, oxidase positive, indole negative and catalase positive results for P. aeruginosa.

Specie wise distribution of bacteria isolated from burn patients

In the current study, a comprehensive analysis of bacterial isolates from burn patients revealed the presence of both gram-positive and gram-negative bacteria. Gram-positive bacteria were identified as Staphylococcus, accounting for 14.28% of the total isolates. On the other hand, gram-negative bacteria comprised a diverse range of species, with P. aeruginosa being the most prevalent, constituting 47.61% of the isolates. Escherichia coli accounted for 23 .8% of isolates, while Klebsiella species comprised 14.28% as shown in Figure 3. Species wise distribution of bacterial isolates from the given study underscores the predominance of P.aeruginosa, followed by Escherichia coli, in the bacterial flora of burn patients. Staphylococcus and Klebsiella species were also identified but in relatively lower proportions compared to P. aeruginosa and Escherichia coli. This detailed analysis provides valuable insights into the spectrum of bacterial pathogens colonizing burn wounds in the studied population.

Table 1: Distribution of species wise bacteria isolated from burn patients.

Isolated organisms	No. of positive Samples	Percentage
Pseudomonas aeruginosa	80	47.61%
Escherichia coli	40	23.8%
Staphylococcus	24	14.28%
Klebsiella spp.	24	14.28%

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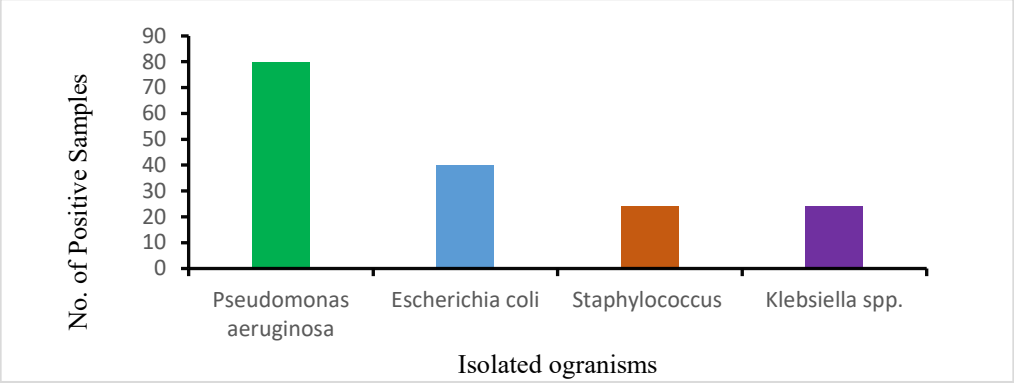

Figure 3: Specie wise distribution of bacteria isolated from burn patients.

Antibiotic susceptibility pattern of P. aeruginosa

In this study, the antibiotic sensitivity testing against P. aeruginosa, a common pathogen in burn patients, was conducted using six commonly used antibiotics: tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem. The results, as depicted in Table 2. revealed variations in the zone of inhibition of bacterial growth among the different antibiotics tested, as well as within samples treated with the same antibiotic (Figure 3). Notably, P. aeruginosa exhibited high susceptibility to levofloxacin, with 100% sensitivity observed in the isolated strain. Conversely, the bacterium displayed the highest resistance to meropenem, with 100% of strains exhibiting resistance. Ciprofloxacin and gentamicin demonstrated moderate sensitivity, with 50% and 45% sensitivity, respectively. However, Imipenem exhibited 50% resistance in the tested strains. Ciprofloxacin and gentamicin displayed moderate sensitivity, with 50% and 45% sensitivity, respectively, while Imipenem exhibited 50% resistance. These findings underscore the varying susceptibility patterns of P. aeruginosa to different antibiotics commonly used in clinical practice.

Table 2. Antibiotic Susceptibility Pattern P. aeruginosa

	Antibiotics	**Pseudomonas aeruginosa (n=20)**		
		Sensitivity (%)	**Intermediate Sensitivity (%)**	**Resistance (%)**
1.	Tobramycin	35	35	30
2.	Ciprofloxacin	50	30	20
3.	Levofloxacin	100	0	0
4.	Gentamicin	45	30	25
5.	Imipenem	25	25	50
6.	Meropenem	0	0	100

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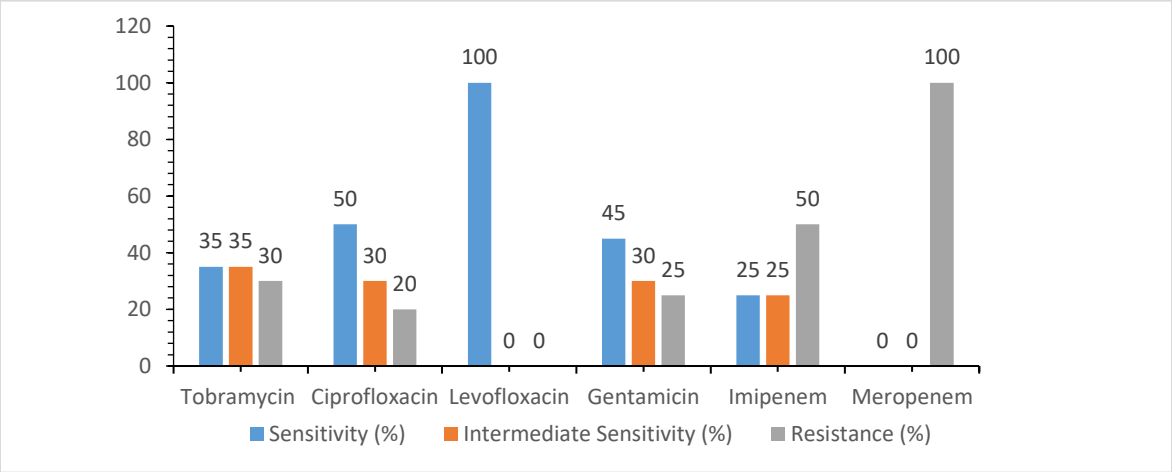

Figure 4. The given figure illustrated antibiotic susceptibility pattern of P. aeruginosa. Notably, all isolated strains displayed 100% susceptibility to levofloxacin while exhibiting 100% resistance to meropenem, highlighting significant differences in susceptibility among commonly used antibiotics.

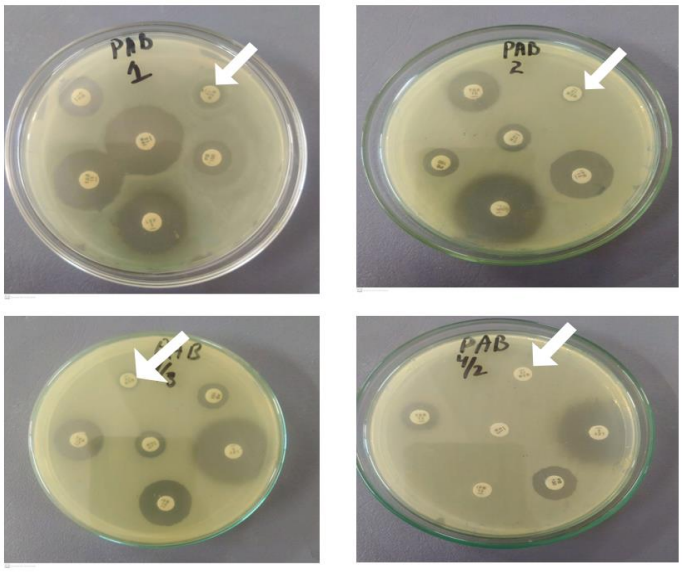

Figure 5. Zone of inhibition of different antibiotics to P. aeruginosa on MHA. The given picture illustrates antibiotic susceptibility pattern of P. aeruginosa. Notably, all isolated strains displayed 100% susceptibility to levofloxacin while exhibiting 100% resistance to meropenem, highlighting significant differences in susceptibility among commonly used antibiotics.

Frequency of carbapenemase producing P. aeruginosa

Our results give important insights into the frequency of carbapenem resistance among clinical isolates of P. aeruginosa. Out of the 80 isolates analyzed, 48(60%) samples shown zone is less than 5 mm in CIM experiment; mean they were positive for the presence of carbapenemase enzyme. This specifies the existence of carbapenemase-producing strains of P. aeruginosa within the given samples. The detection of carbapenemase enzyme in the majority of samples advises an important frequency of carbapenem resistance among P. aeruginosa isolates obtained from burn patients. This finding underlines the crucial need for heightened attentiveness and proactive measures to mitigate the spread of carbapenem-resistant P. aeruginosa in clinical settings.

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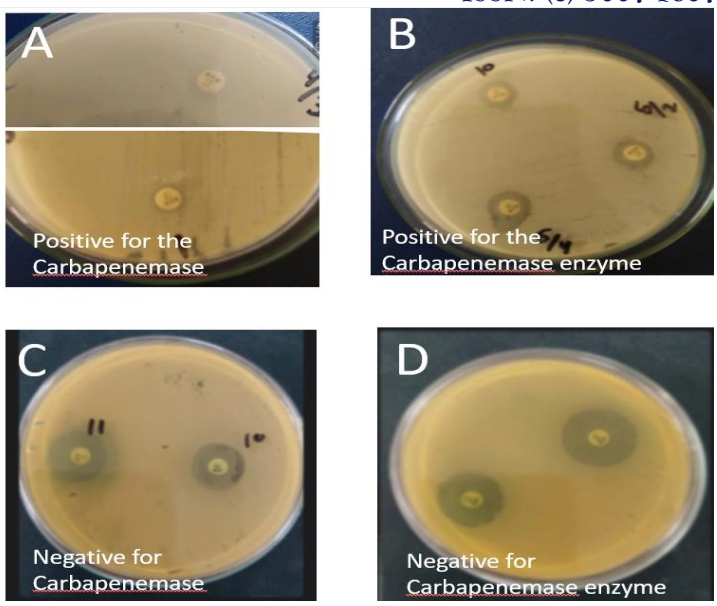

Figure 6: The figures show positive and negative isolates P. aeruginosa for carbapenemase enzyme.

Discussion

Pseudomonas aeruginosa is a Gram-negative, opportunistic bacterium that commonly affects immunocompromised and burn patients, leading to serious infections such as those of the wounds, urinary tract, respiratory system, and bloodstream (6). Its strong adaptability, ability to form biofilms, and natural resistance to many antibiotics especially carbapenems make it difficult to treat and control in healthcare settings. The rise of multidrug-resistant strains has become a global health concern. Due to the high mortality rates, particularly in hospital-acquired infections, continuous regional monitoring is essential to understand resistance trends and support the selection of effective empirical therapies (12).

P. aeruginosa is frequently isolated from burn wounds, presenting challenges in treatment and control due to its prolonged survival in the environment and resistance to multiple antimicrobial agents (13). Burn patients, particularly those exposed to carbapenems and broad-spectrum antibiotics, are at higher risk of P. aeruginosa infections, exacerbated by extended hospital stays and prior antibiotic use. The prevalence of antibiotic resistance complicates empiric therapy for P. aeruginosa wound infections (13, 14). P. aeruginosa continues to be the primary pathogen responsible for wound infections in burn centers. Studies have consistently reported a high incidence of P. aeruginosa infections in these settings (15). Notably, the ICU records the highest frequency of P. aeruginosa cases, likely due to prolonged hospitalization and extensive antibiotic usage. Moreover, multi-drug resistance is prevalent among P. aeruginosa strains associated with burn wounds (16). Similarly, other studies such as Arslan et al (17) and Naqvi et al (18) Bhatt et al (19) also showed a prevalence of P. aeruginosa infection among burn patients to be 53.97%, 59.6% and (76.8%) respectively. However, Ekrami and Kalantar (20) showed a prevalence of 37.5%.

Similarly, other studies such as Bhatt et al. (19) reported that P. aeruginosa isolated from burn patients exhibited 61% resistance to imipenem and 54% resistance to meropenem. However, Moazami-Goudarzi and Eftekhar (21) observed that 94.7% of isolates were resistant to both imipenem and meropenem in their study. Saderi et al. (22) found that 69% of isolated P. aeruginosa strains were MDR, whereas Moazami-Goudarzi and Eftekhar (21) reported 100% MDR isolates in burn patients. The high resistance rates observed in P. aeruginosa against clinically relevant antibiotics underscore the urgent need for alternative treatment approaches. However, the efficacy of single agents may be compromised when used as monotherapy,

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necessitating combination therapy for optimal outcomes.

Our results give important insights into the frequency of carbapenem resistance among clinical isolates of P. aeruginosa. Out of the 80 isolates analyzed, 48 (60%) samples shown zone is less than 5 mm in CIM experiment; mean they were positive for the presence of carbapenemase enzyme. This specifies the existence of carbapenemase-producing strains of P. aeruginosa within the given samples. The detection of carbapenemase enzyme in the majority of samples advises an important frequency of carbapenem resistance among P. aeruginosa isolates obtained from burn patients. This finding underlines the crucial need for heightened attentiveness and proactive measures to mitigate the spread of carbapenem-resistant P. aeruginosa in clinical settings.

On the other hand, 8(40%) samples tested negative for carbapenemase enzyme. This advises the lack of carbapenemase-producing P. aeruginosa among these samples. However, it is important to understand these negative results with caution, as the absence of carbapenemase enzyme does not essentially specify susceptibility to carbapenems. Other mechanisms of carbapenem resistance, such as alterations in outer membrane permeability or efflux pump overexpression, could contribute to resistance in these strains. Further examination into the mechanisms of carbapenem resistance in these samples is warranted to better understand the dynamics of antibiotic resistance in P. aeruginosa isolates from burn patients.

Overall, the results of this study highlight the complex nature of antibiotic resistance in P. aeruginosa and underline the requirement for complete surveillance and infection control measures to address the tasks posed by carbapenem-resistant pathogens in clinical practice. Further research is necessary to explain the molecular mechanisms underlying carbapenem resistance in P. aeruginosa and to advance targeted therapeutic plans to fight multidrug-resistant infections efficiently.

CONCLUSIONS

In conclusion, the study underscores the urgent need for multifaceted approaches to address P. aeruginosa infections in burn patients, emphasizing the importance of judicious antibiotic use, infection control measures, and ongoing surveillance to combat antibiotic resistance and reduce morbidity and mortality associated with these infections.

Conclusion

Despite having a high emotional intelligence, ambulance workers struggle with considerable stress and fatigue as a result of their busy schedules and poor work-life balance. Although there are links between these parameters, more study is still required. It is still essential for their performance and general well-being to prioritize sleep, breaks, and healthier work habits. In order to support the well-being and maximize the performance of ambulance staff, this study highlights the obstacles they experience and highlights the necessity for interventions that promote healthy work practices and work-life balance.

Conflict of Interest:

No potential conflict of interest relevant to this article was reported.

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