The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 3, 2026

PHENOTYPIC CHARACTERIZATION OF CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATES FROM BURN PATIENTS AT AYUB TEACHING HOSPITAL, ABBOTTABAD

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DOI: https://doi.org/10.5281/zenodo.21975102

Keywords

Article History
Received: 28 January 2026
Accepted: 13 March 2026
Published: 27 March 2026

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Abstract

The purpose of this study was to identify the incidence, antibiotic susceptibility, and phenotypic expression of carbapenemases of Pseudomonas aeruginosa isolated from burn cases in Ayub Teaching Hospital (ATH), Abbottabad. From the total number of 180 burn wound specimens that were cultured on conventional microbiological media and biochemical analysis, 151 (83.9%) showed pure bacterial growth; P. aeruginosa was found to be the most dominant bacteria (n=90), followed by S. aureus (n=50), Klebsiella spp. (n=15), and A. baumannii (n=6). Antimicrobial susceptibility was tested using the disk diffusion method. All isolates were highly susceptible to levofloxacin (100%), followed by a moderately susceptible level to ciprofloxacin (50%) and gentamicin (45%). However, there was an extremely high resistance level of isolates against carbapenems; all P. aeruginosa isolates showed resistance to meropenem (100%), and 50% showed resistance to imipenem. Phenotyping of carbapenemase production through the Carbapenem Inactivation Method (CIM) showed that 54 isolates (60%) were carbapenemases, while the rest 36 resistant or intermediate isolates (40%) were negative carbapenemases. This is further emphasized by the important burden of CRPA among patients with burns in this region's burn management facilities, making it imperative that there be adequate antimicrobial surveillance, proper infection control strategies, and appropriate empirical therapy to reduce hospital-acquired infections.

INTRODUCTION

Microbiology is the branch of biology that studies microorganisms, including bacteria, viruses, fungi, protozoa, and algae. These microorganisms play essential roles in environmental processes, biotechnology, industry, and human health. Among them, bacteria are highly adaptable organisms, with some species causing diseases in humans, animals, and plants. The rapid emergence of antibiotic-resistant bacteria has become a major global public health concern, making the study of pathogenic bacteria and their resistance mechanisms increasingly important (1).

Pseudomonas aeruginosa is a Gram-negative, aerobic, motile, non-spore-forming bacterium belonging to the family *Pseudomonadaceae*. It is widely distributed in soil, water, plants, sewage, and hospital environments due to its remarkable metabolic versatility. Microscopically, it is a rod-shaped bacterium possessing a single polar flagellum that facilitates motility and host colonization. *P. aeruginosa* produces characteristic pigments, including pyocyanin, pyoverdine, pyorubin, and pyomelanin, which aid in laboratory identification and contribute to its virulence. It is also recognized by its distinctive fruity or grape-like odor (2).

Pseudomonas aeruginosa is an oxidase-positive, catalase-positive, non-fermentative Gram-negative bacterium that derives energy through oxidative metabolism. It grows optimally at 37°C and can also grow at 42°C, a characteristic that distinguishes it from many other non-fermenting bacteria (3). Due to its minimal nutritional requirements, *P. aeruginosa* readily survives on medical equipment, disinfectants, catheters, ventilators, and other moist hospital environments. As an opportunistic pathogen, *P. aeruginosa* primarily infects immunocompromised individuals, burn patients, intensive care unit (ICU) patients, and those with chronic illnesses. It is responsible for a wide range of infections, including burn wound infections, pneumonia, urinary tract infections, bloodstream infections, surgical site infections, meningitis, and chronic respiratory infections in cystic fibrosis patients (3). Its pathogenicity is attributed to numerous virulence factors, including exotoxin A, elastases, proteases, phospholipases, rhamnolipids, biofilm formation, and specialized secretion systems that promote tissue damage, immune evasion, and host colonization.

A major clinical concern is its intrinsic and acquired resistance to multiple antibiotics, mediated by low outer membrane permeability, efflux pumps, β-lactamase production, target-site modifications, and biofilm formation. These mechanisms have led to the emergence of multidrug-resistant (MDR) and carbapenem-resistant strains, posing a serious global health challenge (4). Burn patients are particularly vulnerable to *P. aeruginosa* infections because thermal injury disrupts the skins protective barrier and induces local and systemic immunosuppression, including impaired neutrophil and macrophage function and dysregulated cytokine responses. Furthermore, *P. aeruginosa* readily adheres to damaged tissues and forms biofilms, which protect the bacteria from host immune defenses and antimicrobial agents, contributing to persistent infections and delayed wound healing (4).

In burn patients, *P. aeruginosa* can invade damaged tissues and enter the bloodstream, leading to severe complications such as bacteremia, sepsis, and multiple organ dysfunction syndrome. The risk of infection is particularly high in patients with extensive burns, prolonged hospitalization, and invasive medical procedures, making *P. aeruginosa* a major cause of morbidity and mortality in burn units (5). The treatment of *P. aeruginosa* infections has become increasingly challenging due to the emergence of multidrug-resistant (MDR) and carbapenem-resistant strains. Resistance mechanisms include β-lactamase production, efflux pumps, reduced outer membrane permeability, target-site modifications, and biofilm formation, all of which limit antibiotic efficacy (6). Consequently, resistant infections are associated with prolonged hospitalization, increased healthcare costs, treatment failure, and higher mortality rates. Owing to its environmental persistence, extensive virulence factors, biofilm-forming ability, and multidrug resistance, *P. aeruginosa* remains one of

the leading pathogens responsible for burn wound infections worldwide (7).

Antimicrobial resistance (AMR) has emerged as one of the greatest global public health challenges of the 21st century, reducing the effectiveness of antibiotics used to treat infectious diseases. *P. aeruginosa* is a major multidrug-resistant pathogen due to its intrinsic and acquired resistance mechanisms, including low outer membrane permeability, multidrug efflux pumps, biofilm formation, and the acquisition of resistance genes through horizontal gene transfer and genetic mutations (8). Of particular concern is the increasing prevalence of **carbapenem-resistant** *P. aeruginosa* **(CRPA)**. Carbapenems, such as imipenem, meropenem, and doripenem, are considered last-line β-lactam antibiotics for treating severe multidrug-resistant Gram-negative infections; however, their effectiveness has been significantly compromised by the emergence of resistant strains. Resistance is mediated through multiple mechanisms, including carbapenemase production, overexpression of efflux pumps, loss of the OprD porin, and the production of AmpC and extended-spectrum β-lactamases (ESBLs), often acting simultaneously to confer high-level resistance (9). Carbapenem-resistant *P. aeruginosa* infections are associated with prolonged hospitalization, increased healthcare costs, treatment failure, and higher morbidity and mortality, particularly among burn patients and those admitted to intensive care units (ICUs). Consequently, the World Health Organization (WHO) recognizes carbapenem-resistant *P. aeruginosa* as a priority pathogen requiring continuous surveillance, effective infection control strategies, and the development of novel therapeutic approaches (10).

The growing prevalence of multidrug-resistant (MDR) and carbapenem-resistant *P. aeruginosa* has become a major public health concern, particularly in developing countries where healthcare resources, infection control practices, and antimicrobial stewardship programs are often limited. In Pakistan, the increasing misuse of broad-spectrum antibiotics, prolonged hospitalization, overcrowded healthcare facilities, and inadequate surveillance have contributed to the rapid emergence of resistant Gram-negative pathogens, with *P. aeruginosa* being a leading cause of healthcare-associated infections, especially in intensive care units (ICUs) and burn wards (11).

Ayub Teaching Hospital (ATH), Abbottabad, is one of the largest tertiary care hospitals in Khyber Pakhtunkhwa, serving patients from Abbottabad and surrounding regions. Its burn unit manages a large number of burn victims annually, where prolonged hospitalization, invasive procedures, frequent antibiotic exposure, and compromised immunity create favorable conditions for *P. aeruginosa* colonization and infection. The emergence of carbapenem-resistant *P. aeruginosa* in this setting poses a significant therapeutic challenge by limiting treatment options and increasing the risk of wound deterioration, septicemia, prolonged hospital stays, healthcare costs, and mortality (12). Therefore, continuous surveillance of antimicrobial resistance patterns and phenotypic characteristics of clinical isolates is essential for effective infection control and evidence-based antimicrobial therapy.

Despite the clinical importance of carbapenem-resistant *P. aeruginosa*, limited local data are available regarding its prevalence and phenotypic characteristics among burn patients in Abbottabad and the surrounding regions. Most available studies have focused on larger metropolitan areas, while comparatively little information exists regarding the epidemiological situation in healthcare facilities serving northern Pakistan. The absence of local surveillance data may hinder the development of targeted infection prevention strategies and appropriate empirical treatment guidelines. Therefore, investigating the phenotypic characteristics of carbapenem-resistant *P. aeruginosa* isolates recovered from burn patients at Ayub Teaching Hospital is both timely and necessary.

RESEARCH METHODOLOGY

Study Setting

The current research study titled "Phenotypic Characterization of Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates in Burn Patients" was performed at the Microbiology Research Lab,

Abbottabad University of Science and Technology (AUST) in collaboration with the Ayub Teaching Hospital, Abbottabad. One hundred and eighty samples were collected from burn patients, out of which 90 isolates of *Pseudomonas aeruginosa* were obtained and then subjected to phenotypic characterization.

Collection of samples

Cultures of *P. aeruginosa* were obtained from Ayub Teaching Hospital in Abbottabad for the current investigation. A total of 180 samples were taken from hospitalised burn patients.

Processing of Clinical Samples and Preparation of Culture Media

The samples underwent additional processing by being inoculated into MacConkey agar and nutrient agar. The maker's instructions were followed for preparing the media. In short, one litre of distilled water was used to dissolve a certain quantity of lyophilized powder, which was then autoclaved at 121°C for fifteen minutes at 15 Psi (pound per square inch). The experiment used the nutrient broth for stock production and enrichment. *P. aeruginosa* was confirmed using a variety of biochemical tests, including oxides, catalase tests, and Gram staining. Multidrug-resistant bacterial strains were confirmed using antibiotic susceptibility assays.

Preparation of Bacterial Stock Cultures

Stock cultures of *P. aeruginosa* were initially sub-cultured on MacConkey agar plates and allowed to grow for 24 hours at 37 °C. The resulting actively growing stock cultures were prepared by sub-culturing a well-separated colony in sterile nutrient broth and allowed to incubate overnight at 37 °C. The overnight stock cultures were mixed with sterile glycerol to prepare cultures having a 15% (v/v) final concentration of glycerol. Subsequently, aliquots of approximately 400 µL were dispensed into sterile cryovials and stored at −20 °C.

Assessment of Antimicrobial Susceptibility

The antimicrobial susceptibility profile of the *P. aeruginosa* isolates was evaluated against six antibiotics: tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem. Susceptibility testing was performed using the Kirby–Bauer disk diffusion method, a standardized and widely accepted technique for assessing bacterial susceptibility to antimicrobial agents. Fresh bacterial colonies were inoculated onto Mueller–Hinton agar (MHA) plates and incubated at **37 °C for 18–24 hours**. A bacterial suspension equivalent to the **0.5 McFarland turbidity standard** was subsequently prepared from the overnight culture. Sterile cotton swabs were used to evenly spread the standardized inoculum over the surface of fresh MHA plates to obtain a uniform bacterial lawn. Commercial antibiotic discs containing predetermined concentrations of tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem were then aseptically placed onto the inoculated agar surface before incubation for susceptibility assessment. Following incubation, the zones of growth inhibition surrounding each antibiotic disc were measured in millimeters using a calibrated ruler. The measured inhibition zone diameters were interpreted in accordance with the interpretive criteria established by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Based on these standardized breakpoints, each *Pseudomonas aeruginosa* isolate was categorized as susceptible (S), intermediate (I), or resistant (R) to the antimicrobial agents evaluated.

Phenotypic Detection of Carbapenem-Resistant *Pseudomonas aeruginosa*

Phenotypic detection of carbapenem resistance in *P. aeruginosa* isolates was achieved using the carbapenem inactivation method (CIM), which is a proven method used to determine carbapenemase production. The method was carried out according to the following steps. First, *P. aeruginosa* isolates were grown and identified microscopically. A standardized bacterial suspension was prepared and imipenem and meropenem antibiotic discs were dipped into the suspension and left to incubate at 37 °C for 2 hours to enable hydrolysis of the antibiotics, in case of any carbapenemases. To detect

carbapenemase activity, the treated antibiotic discs were further inoculated onto Mueller–Hinton agar plates that had been pre-inoculated with the test organism *E. coli* ATCC 25922. The plates were further incubated at 37 °C for 24 hours and observed for zones of inhibition. The formation of an inhibition zone around the carbapenem discs showed absence of carbapenemase activity while lack of an inhibition zone showed carbapenemase production in the tested *P. aeruginosa* isolates.

RESULTS

Isolation and Identification of *P. aeruginosa*

In order to isolate, identify, test for antibiotic susceptibility, and screen for carbapenem-resistant *P. aeruginosa*, 180 samples were taken from burn patients. As shown in Figure 1, of the 180 samples cultured, 151 (83.9%) showed pure bacterial growth, 10 (5.6%) showed mixed bacterial growth, and 19 (10.6%) showed no growth on the culture media. Pure isolates were obtained by repeatedly subculturing samples that showed mixed growth. The pure isolates were then examined under a microscope and biochemically characterised to confirm and identify *P. aeruginosa*.

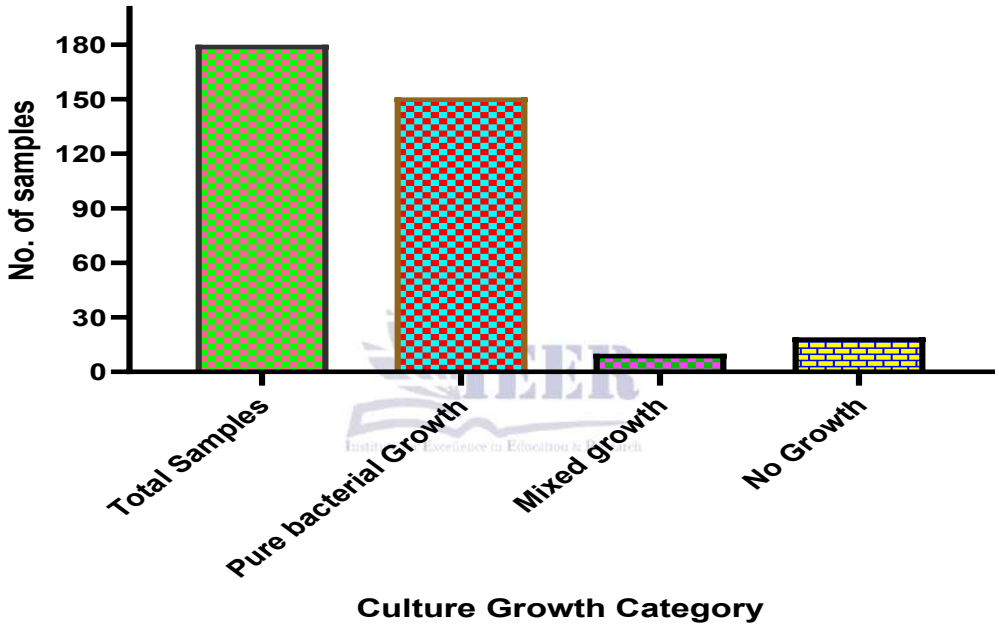


Figure 1. Distribution of positive and negative samples

Microscopic examination and morphological characterization of bacterial isolates

All bacterial isolates recovered from burn wound samples were subjected to microscopic examination and biochemical characterization. Gram staining identified 50 isolates as Gram-positive cocci arranged in grape-like clusters, which were confirmed as *Staphylococcus aureus* based on positive catalase and coagulase tests. The remaining isolates were Gram-negative rods. Among these, 90 isolates were identified as *Pseudomonas aeruginosa*, characterized by positive catalase, oxidase, and motility tests and a negative indole reaction. Additionally, 15 isolates were identified as *Klebsiella* spp., showing positive urease, citrate, and catalase tests, with negative indole and non-motile characteristics. Six isolates were identified as *Acinetobacter baumannii* based on their Gram-negative coccobacillary morphology and biochemical characteristics. The distribution of bacterial isolates identified by microscopic examination and biochemical testing is presented in Table 1.

Table 1. Microscopic and biochemical characterization of bacterial isolates

Biochemical Test	*Staphylococcus aureus*	*Pesudomonas aeruginosa*	*Klebsiella spp.*	*Acinetobacter baumannii*
Gram Staning	+ive, cocci in clusters	- ive, rods	- ive, rods	- ive, rods
Catalase test	+ ive	+ ive	+ ive	+ ive
Coagulase test	+ ive	- ive	- ive	- ive
Oxidase test	- ive	+ ive	- ive	- ive
Motility test	- ive	+ ive	- ive	- ive
Indole test	- ive	- ive	- ive	- ive
Urease test	Variable	- ive	+ ive	- ive
Citrate test	-ive	+ive	+ive	+ ive
No. of isolates	50	90	15	6

Identification of *Pseudomonas aeruginosa* by microscopic and biochemical tests

A number of biochemical tests and microscopic examination were used to identify and confirm the *P. aeruginosa* isolates. It was discovered that all isolates were oxidase-positive, catalase-positive, and Gram-negative rods; nevertheless, they did not produce indole. Table 2 displays the outcomes of these microscopic and molecular traits. The isolates identity was confirmed since the observed phenotypic profile matched the typical *P. aeruginosa* identifying traits.

Table 2. Biochemical identification of *Pseudomonas aeruginosa* isolates

S. No.	Biochemical Characteristics	Reaction of *P. aeruginosa*
	Gram Staining	Negative (-)
	Catalase test	Positive (+)
	Oxidase test	Positive (+)
	Indole Test	Negative (-)

Colony Morphology

After observing the colony morphology of *P. aeruginosa*, colorless, circular, smooth, and moist colonies were observed on MacConkey agar, as shown in Figure 2.



Figure 2. Colony morphology of *Pseudomonas aeruginosa* on MacConkey agar

Microscopic Examination



Gram staining was performed for all isolates of *P. aeruginosa*. Upon microscopic examination, all isolates appeared as Gram-negative rods.

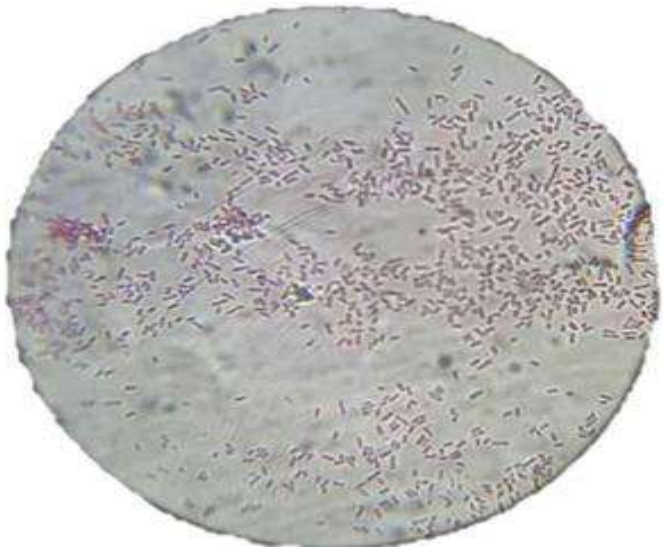


Figure 3. Gram staining showed gram negative and rad shape bacteria

Catalase test

The catalase test was performed on all isolates of *P. aeruginosa*, and all isolates exhibited positive catalase activity.

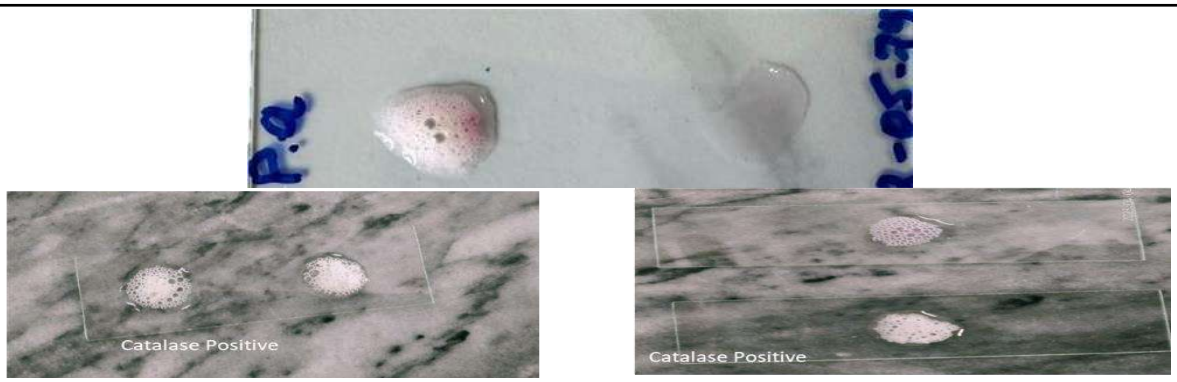


Figure 4. Catalase test showed *P. aeruginosa* is catalase positive

Oxidase Activity Assessment

The oxidase assay was conducted to evaluate the oxidase activity of the all *P. aeruginosa* isolates, while *Escherichia coli* was used as a negative control. All tested *P. aeruginosa* isolates exhibited a positive oxidase reaction, confirming their characteristic biochemical profile.

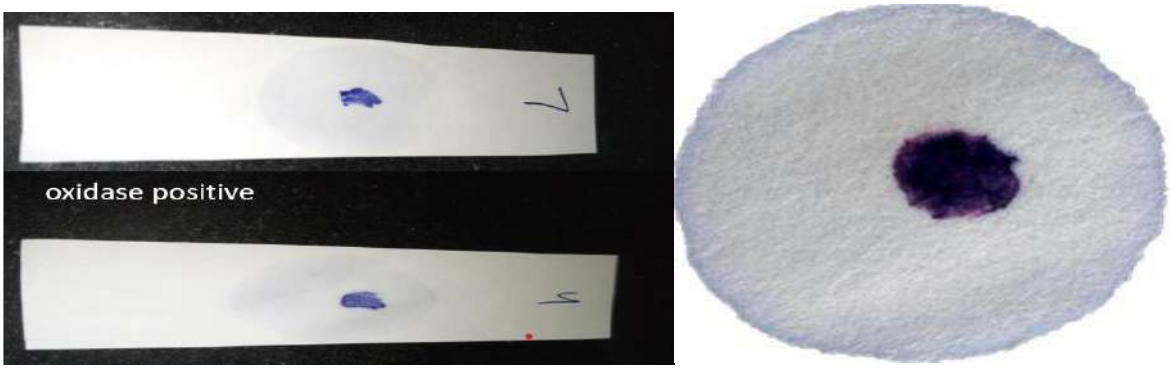


Figure 5. *P. aeruginosa* showed Oxidase positive result

Coagulase test

The coagulase test was performed on the given strain of *P. aeruginosa*. *P. aeruginosa* did not show coagulation within 5-10 seconds, indicating the absence of the coagulase enzyme. Hence, the result for *P. aeruginosa* was negative.

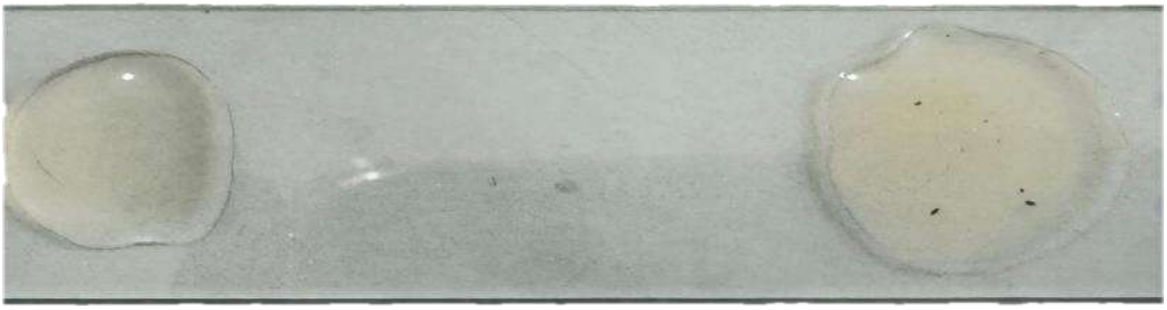


Figure 6. *P. aeruginosa* showed negative results for coagulase test

Indole test

Indole test was performed for the given isolates of *P. aeruginosa*. *E. coli* was used as a positive control which indicates that all the isolates of *P. aeruginosa* were indole negative.



Figure 7. Indole test showed *P. aeruginosa* is indole negative

Antibiotic Susceptibility Pattern of *P. aeruginosa*

Six commonly used drugs have been used in the experiment to check the sensitivity of the antibiotic to the *P. aeruginosa* bacteria, which is a common infection of burn patients: tobramycin, gentamicin, levofloxacin, ciprofloxacin, imipenem, and meropenem. It has been found that there was variation in the zones of inhibition of bacterial growth for the different types of antibiotics used in the experiment, as well as the samples tested using the same antibiotic (Figure 8), as illustrated in Table 3 below. The interesting thing about the isolated bacterium is that it was 100% sensitive to levofloxacin, indicating high susceptibility. Meropenem was the least sensitive (100%). But 50% of the bacteria were resistant to imipenem, whereas 50% and 45% of the bacteria were moderately sensitive to gentamicin and ciprofloxacin, respectively. Such results provide insight into the different susceptibility profiles of *P. aeruginosa* towards the frequently prescribed therapeutic drugs. It is crucial to ascertain the susceptibility profile of this pathogen in order to make an appropriate choice regarding the administration of effective antimicrobials and prevent antibiotic resistance among burn patients. This helps a great deal with proper antimicrobial stewardship strategies.

Table 3. Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates

		***Pseudomonas aeruginosa* (n=90)**		
	Antibiotics	**Susceptible (S) (%)**	**Intermediate (I) (%)**	**Resistant (R) (%)**
	Tobramycin	35	35	30
	Ciprofloxacin	50	30	20
	Levofloxacin	100	0	0
	Gentamicin	45	30	25
	Imipenem	25	25	50
	Meropenem	0	0	100

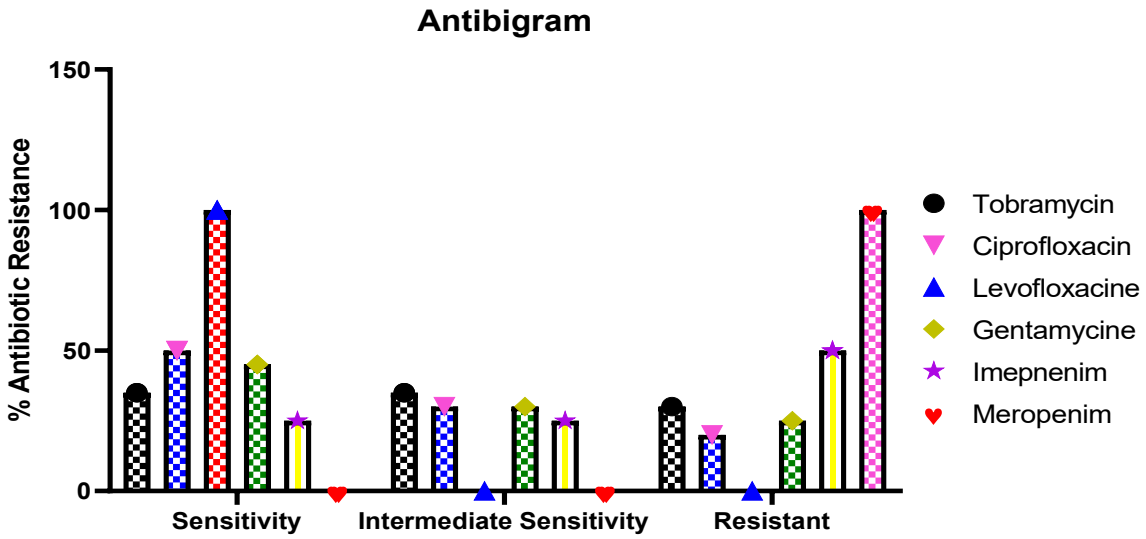


Figure 8. The drug sensitivity profile of *P. aeruginosa* has been illustrated in the above-attached figure. It is worth mentioning that all isolated strains were resistant to 100% meropenem and sensitive to 100% levofloxacin.

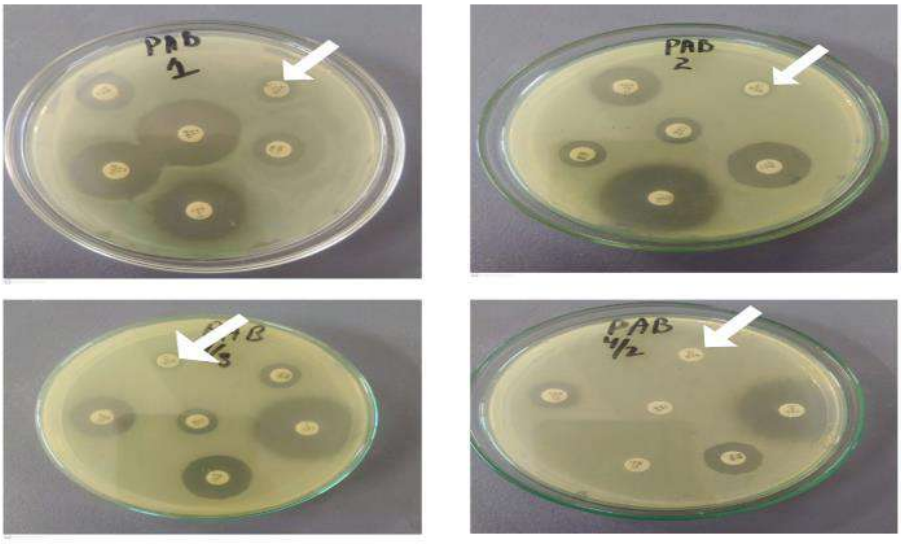


Figure 9. Antibiotic zones of inhibition of *P. aeruginosa* on Mueller–Hinton agar (MHA) following antimicrobial susceptibility testing. The susceptibility profile of *P. aeruginosa* is shown in the figure. Notably, all isolates exhibited 100% resistance to meropenem and 100% susceptibility to levofloxacin, indicating marked differences in susceptibility to the tested antibiotics.

Frequency of Carbapenemase producing *P. aeruginosa*

Our findings provide important insights into the prevalence of carbapenem resistance among clinical isolates of *Pseudomonas aeruginosa*. Of the 90 isolates examined, 54 (60%) produced inhibition zones of <5 mm in the Carbapenem Inactivation Method (CIM) assay, indicating the presence of carbapenemase-producing isolates. This high prevalence of carbapenemase production suggests that carbapenem resistance is widespread among *P. aeruginosa* isolates recovered

from burn patients. These findings highlight the urgent need for continuous surveillance, strict infection prevention and control measures, and effective antimicrobial stewardship programs to limit the spread of carbapenem-resistant *P. aeruginosa* in clinical settings.

However, in 36 (40%) cases, the samples tested negative for carbapenemase enzyme. This shows that the samples do not contain *P. aeruginosa* that produce the carbapenemase enzyme. However, since the absence of the carbapenemase enzyme does not always mean that the bacteria are susceptible to carbapenems, it is important to be cautious about interpreting these negative findings. The bacteria could be resistant to carbapenem through other means, such as modification of outer membrane permeability and up-regulation of the efflux pump. Further studies are therefore needed to understand the dynamics of antibiotic resistance of *P. aeruginosa* isolated from burn wounds.

In general, the results obtained during this study confirm the complicated mechanism of antibiotic resistance in *P. aeruginosa* and highlight the necessity to ensure proper monitoring and infection prevention to tackle the problems caused by the presence of carbapenem-resistant bacteria. In order to successfully treat multiple drug resistant infections, further studies are necessary to determine the molecular mechanisms responsible for carbapenem resistance in *P. aeruginosa*.

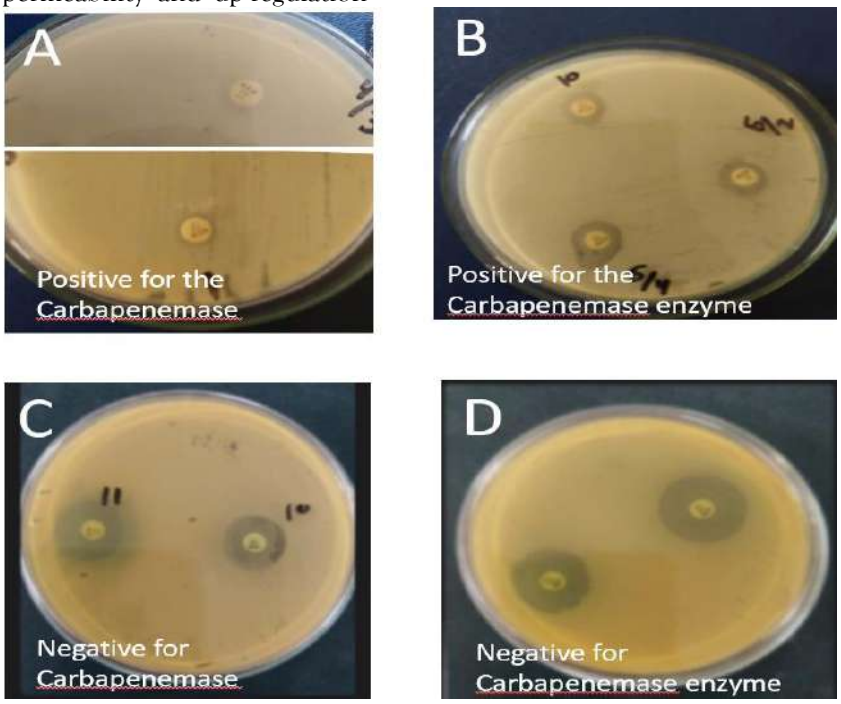


Figure 10. Representative images showing the phenotypic detection of carbapenemase production among P. aeruginosa isolates using the modified carbapenem inactivation method. Panels A and B demonstrate carbapenemase-positive isolates, while panels C and D show carbapenemase-negative isolates based on the observed inhibition zones around the antibiotic discs

Discussion

The present study was conducted to isolate and phenotypically characterize *Pseudomonas aeruginosa* isolated from burn patients admitted to Ayub Teaching Hospital, Abbottabad. Burn wound infections remain one of the leading causes of morbidity and mortality among hospitalized patients due to the destruction of the skin barrier, which provides an ideal environment for microbial colonization and proliferation (13). Among the various bacterial pathogens associated with burn wound infections, *P. aeruginosa* is

considered one of the most clinically significant because of its remarkable environmental adaptability, diverse virulence factors, biofilm-forming ability, and intrinsic resistance to multiple antimicrobial agents (14). In the present study, a total of 180 clinical samples were collected, of which 151 (83.9%) yielded pure bacterial growth, 10 (5.6%) showed mixed bacterial growth, and 19 (10.6%) exhibited no growth on culture media. The high proportion of culture-positive samples reflects the increased susceptibility of burn wounds to microbial colonization, as the loss of skin integrity, presence of necrotic tissue, and impaired immune response create favorable conditions for bacterial growth. Similar findings have been reported by Lee *et al.,* (2015), who described burn wounds as highly vulnerable to bacterial colonization due to tissue damage and immunosuppression (15). Likewise, Ibrahem *et al.,* (2017) reported a high frequency of positive bacterial cultures among burn patients, emphasizing that prolonged hospitalization and exposure to the hospital environment significantly increase the risk of wound infection (16). The occurrence of mixed bacterial growth observed in the present study further supports the polymicrobial nature of burn wound infections and is consistent with the findings of Roy *et al.,* (2024), who reported that mixed infections are common in burn units and contribute to delayed wound healing and increased disease severity (17). The culture-negative samples observed in this study may be attributed to prior antibiotic therapy, inadequate bacterial load, or the presence of fastidious organisms that fail to grow under routine laboratory conditions, as similarly suggested by (18).

Microscopic examination and biochemical characterization revealed that *P. aeruginosa* was the predominant bacterial pathogen isolated from burn wound samples, followed by *Staphylococcus aureus*, *Klebsiella* spp., and *Acinetobacter baumannii*. The predominance of *P. aeruginosa* is in agreement with the findings of Guo *et al.,* (2017) and Azimi et al. (2017), who identified this organism as the most frequently isolated Gram-negative pathogen in burn units due to its ability to survive in moist hospital environments and resist adverse conditions (19, 20). The isolation of *Staphylococcus aureus* as the second most common pathogen is also consistent with reports by Raquid *et al.,* (2018), who demonstrated that this organism commonly colonizes damaged skin and burn wounds during the early stages of infection (21). Furthermore, the recovery of *Klebsiella* spp. and *Acinetobacter baumannii* highlights the polymicrobial and nosocomial nature of burn wound infections, findings that are supported by Weber et al. (2016) (22). The predominance of Gram-negative bacteria observed in the present study may be attributed to prolonged hospitalization, extensive antibiotic exposure, invasive procedures, and environmental contamination within healthcare settings, all of which favor the selection and dissemination of multidrug-resistant organisms.

The identification of *P. aeruginosa* in this study was confirmed through conventional microscopic examination and biochemical tests, with all isolates demonstrating Gram-negative rod morphology, positive catalase and oxidase reactions, and negative indole test results. These findings are in accordance with the standard biochemical characteristics of *P. aeruginosa* described by Forbes et al.(2018), who recognized these tests as reliable methods for the routine identification of this organism (23). Similarly, Iheukwumere *et al.,* (2024) emphasized that Gram staining combined with catalase, oxidase, and indole testing provides a simple, economical, and accurate approach for the identification of *P. aeruginosa*, particularly in resource-limited laboratories (24). The oxidase-positive reaction serves as an important characteristic distinguishing *P. aeruginosa* from members of the family *Enterobacteriaceae*, while catalase production enables the organism to neutralize reactive oxygen species generated by host immune cells, thereby enhancing its survival during infection. Overall, the findings of the present study confirm that conventional microbiological methods remain effective for the isolation and identification of *P. aeruginosa* and reinforce the organism's prominent role as a major etiological agent of burn wound

infections. These observations highlight the necessity for continuous microbiological surveillance and accurate laboratory diagnosis to facilitate timely antimicrobial therapy and effective infection control measures in burn care settings.

Antimicrobial susceptibility testing plays a pivotal role in the effective management of *Pseudomonas aeruginosa* infections, particularly among burn patients were delayed or inappropriate therapy can lead to severe complications, septicemia, prolonged hospitalization, and increased mortality. In the present study, the susceptibility pattern of *P. aeruginosa* isolates demonstrated considerable variation against the six antibiotics tested. Among all antibiotics, levofloxacin exhibited the highest antimicrobial activity, with all isolates (100%) being susceptible, whereas meropenem showed complete resistance (100%), indicating a worrying increase in carbapenem resistance among burn wound isolates. Moderate susceptibility was observed for ciprofloxacin (50%), gentamicin (45%), and tobramycin (35%), while imipenem demonstrated only 25% susceptibility, with half of the isolates exhibiting resistance. These findings suggest that resistance among *P. aeruginosa* isolates is increasing and that the effectiveness of several commonly prescribed antibiotics has declined significantly.

The excellent activity of levofloxacin observed in the present study is comparable to the findings of Rashad *et al.,* (2022) who reported high susceptibility of *P. aeruginosa* to fluoroquinolones in clinical isolates from burn patients (25). Similar observations were also documented by **Khan *et al.,***(2023) who found levofloxacin to be one of the most effective therapeutic agents against *P. aeruginosa* due to its excellent tissue penetration and inhibition of bacterial DNA gyrase and topoisomerase IV (26). In contrast, the moderate susceptibility to ciprofloxacin and aminoglycosides observed in this study may reflect the widespread empirical use of these antibiotics in hospital settings, resulting in selective pressure and gradual development of resistance. Comparable reductions in susceptibility to ciprofloxacin and gentamicin have been reported by **Sader et al.** and **Livermore**, who attributed this phenomenon to chromosomal mutations, target site alterations, and the overexpression of multidrug efflux pumps.

The most alarming finding of the present study was the complete resistance of all isolates to meropenem and the high level of resistance observed against imipenem. Similar trends have been reported by Meletis and Karastergiou., (2025) who identified carbapenem resistance in *P. aeruginosa* as one of the greatest challenges facing modern healthcare due to the organism's remarkable ability to acquire and express multiple resistance mechanisms (27). Carbapenem resistance may arise through the production of carbapenemase enzymes, loss or modification of the OprD porin channel, overexpression of multidrug efflux pumps, AmpC β-lactamase hyperproduction, or a combination of these mechanisms. The emergence of carbapenem-resistant isolates is of particular concern because carbapenems are often regarded as last-line agents for treating severe infections caused by multidrug-resistant Gram-negative bacteria. Consequently, infections caused by resistant strains are associated with increased therapeutic failure, longer hospital stays, greater healthcare costs, and higher mortality rates.

Phenotypic screening for carbapenemase production further demonstrated that 54 (60%) of the 90 *P. aeruginosa* isolates were positive for carbapenemase production using the Carbapenem Inactivation Method (CIM), whereas 36 (40%) isolates were negative. The high prevalence of carbapenemase-producing isolates observed in the present study is consistent with reports by Nordmann and Poirel., (2019), who documented the global emergence of carbapenemase-producing *P. aeruginosa* as a major public health concern (28). Likewise, McMullen *et al.,* (2017) emphasized that carbapenemase enzymes are among the most important mechanisms responsible for resistance to carbapenem antibiotics and facilitate the rapid dissemination of resistance genes within hospital environments through mobile genetic elements (29). The detection of carbapenemase-producing isolates in more than half of the isolates analyzed indicates the substantial burden of carbapenem

resistance among burn patients and highlights the urgent need for continuous microbiological surveillance and strict infection prevention strategies.

Interestingly, 40% of isolates were phenotypically negative for carbapenemase production despite exhibiting reduced susceptibility to carbapenems. This observation suggests that mechanisms other than carbapenemase production may contribute to carbapenem resistance, including reduced outer membrane permeability, OprD porin loss, overexpression of efflux pumps, and AmpC β-lactamase overproduction. Similar findings have been reported by Diorio-Toth *et al.,* (2022) who concluded that carbapenem resistance in *P. aeruginosa* is multifactorial and often results from the coexistence of several resistance mechanisms within a single isolate (30). Overall, the findings of the present study demonstrate the increasing burden of multidrug-resistant and carbapenem-resistant *P. aeruginosa* among burn patients and emphasize the importance of routine antimicrobial susceptibility testing, phenotypic screening for carbapenemase production, antimicrobial stewardship programs, and stringent infection control measures to limit the spread of resistant strains and improve patient outcomes.

Conclusion

The present study investigated the phenotypic characteristics and carbapenem resistance of *Pseudomonas aeruginosa* isolated from burn patients admitted to Ayub Teaching Hospital, Abbottabad. Among the 180 burn wound samples analyzed, 151 (83.9%) showed bacterial growth, with *P. aeruginosa* identified as the predominant pathogen, highlighting its major role in burn wound infections. Conventional microbiological methods, including Gram staining and biochemical tests, successfully identified the isolates, demonstrating that these techniques remain reliable for routine diagnosis in resource-limited laboratories. Antimicrobial susceptibility testing revealed marked variations in antibiotic efficacy. All isolates were susceptible to levofloxacin, whereas complete resistance was observed against meropenem. Moderate susceptibility was noted for ciprofloxacin, gentamicin, and tobramycin, while imipenem exhibited reduced activity. These findings indicate a concerning increase in carbapenem resistance, limiting the effectiveness of one of the most important treatment options for multidrug-resistant Gram-negative infections.

Phenotypic detection using the Carbapenem Inactivation Method (CIM) showed that **54 (60%)** isolates were carbapenemase producers, confirming carbapenemase production as a major mechanism of resistance. The remaining **36 (40%)** carbapenem-resistant isolates were carbapenemase-negative, suggesting the involvement of additional resistance mechanisms such as efflux pump overexpression, reduced outer membrane permeability, porin loss, and AmpC β-lactamase production.

Overall, this study demonstrates that carbapenem-resistant *P. aeruginosa* represents a significant threat to the effective management of burn wound infections at Ayub Teaching Hospital. The high prevalence of carbapenem-resistant and carbapenemase-producing isolates emphasizes the urgent need for routine antimicrobial susceptibility testing, continuous surveillance, antimicrobial stewardship, and strict infection prevention and control practices to reduce the spread of multidrug-resistant strains.

Furthermore, this study provides valuable baseline epidemiological data for Abbottabad and surrounding regions, where information on carbapenem-resistant *P. aeruginosa* remains limited. These findings may assist clinicians in selecting appropriate empirical therapy and support healthcare authorities in developing evidence-based infection control strategies. Future studies employing molecular characterization of carbapenem resistance genes and other resistance determinants are recommended to better understand the epidemiology, transmission dynamics, and evolution of carbapenem-resistant *P. aeruginosa*.

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