



(RESEARCH ARTICLE)



EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING GRAM-NEGATIVE BACTERIA ISOLATED FROM OCULAR INFECTIONS: PREVALENCE AND PATTERNS OF ANTIBACTERIAL RESISTANCE

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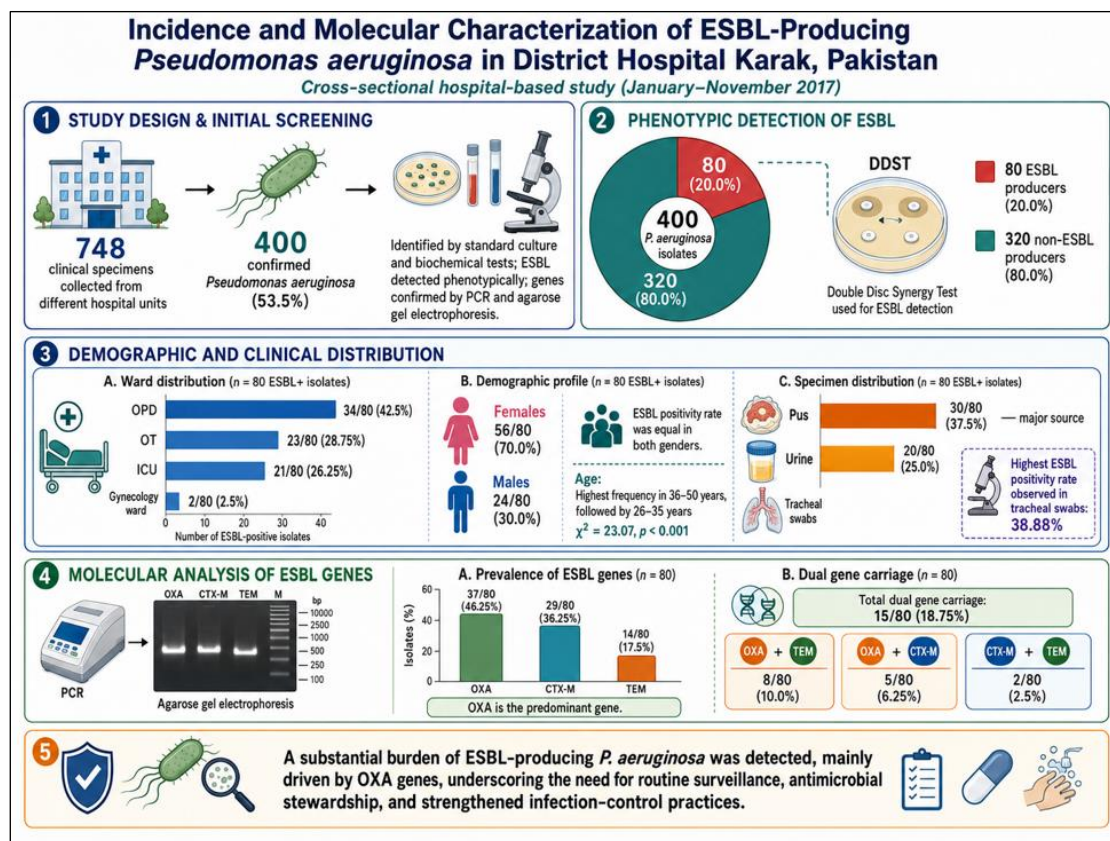
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ABSTRACT

Pseudomonas aeruginosa is a gram-negative rod-shaped bacterium occurs in soil, water, sinks, and medical equipment. It causes 559,000 deaths all over the world each year. The emergence of extended-spectrum beta-lactamases (ESBLs) further limits beta-lactam treatment options due to increased antibiotic resistance. In this study, we looked at the main genes associated with ESBL from clinical isolates at District Hospital Karak in Khyber Pakhtunkhwa, Pakistan, as well as the frequency of isolates that produce *Pseudomonas aeruginosa* (ESBL). A cross-sectional hospital-based investigation was performed from January to November 2017. Overall, 748 clinical specimens were collected. *P. aeruginosa* was identified by standard culture and biochemical methods; ESBL production genes were confirmed by PCR and agarose gel electrophoresis. Out of 748 clinical specimens, 400 (53.5%) were confirmed as *Pseudomonas aeruginosa*. Among these, 80 isolates (20.0%) were ESBL producers, while 320 (80.0%) were non-ESBL producers. ESBL-positive isolates were mainly recovered from the OPD (34/80; 42.5%), followed by the OT (23/80; 28.75%), ICU (21/80; 26.25%), and gynecology ward (2/80; 2.5%). Age-wise, the highest frequency was observed in patients aged 36–50 years, followed by 26–35 years. We noticed that the Females contributed 56/80 (70.0%) ESBL-positive isolates and males 24/80 (30.0%), although the ESBL positivity rate was equal in both genders. Specimen-wise, pus samples (30/80; 37.5%) were the major source, followed by urine samples (20/80; 25.0%), while tracheal swabs showed the highest ESBL positivity rate (38.88%). Molecular analysis showed OXA as the predominant gene (37/80; 46.25%), followed by CTX-M (29/80; 36.25%) and TEM (14/80; 17.5%). Dual gene carriage was found in 15 isolates (18.75%), mainly OXA + TEM (8/80; 10.0%), followed by OXA + CTX-M (5/80; 6.25%) and CTX-M + TEM (2/80; 2.5%). This study confirms a significant burden of ESBL-producing *P. aeruginosa*, mainly driven by OXA genes, highlighting the need for routine surveillance, stewardship, and infection control.

Keywords: *Pseudomonas aeruginosa*; ESBL; antimicrobial resistance; PCR; OXA; CTX-M; TEM.

Graphical abstract



An illustration of the molecular characterization of *Pseudomonas aeruginosa* extended-spectrum beta-lactamases (ESBLs)

1 INTRODUCTION

One of the biggest risks to contemporary clinical care is antimicrobial resistance (AMR), which lowers the efficacy of necessary antibacterial medications and raises the risk of treatment failure, extended hospital stays, and mortality. Bacterial AMR was directly responsible for 1.27 million deaths in 2019 and linked to 4.95 million deaths, according to a global systematic review, which highlights the need for pathogen-specific surveillance (Fernandez, 2022). *Pseudomonas aeruginosa* is one of the most significant resistant Gram-negative bacteria because of its high genomic adaptability, intrinsic resistance, and ability to acquire resistance determinants through mutation and horizontal gene transfer (Poole, 2011).

Pseudomonas aeruginosa is an aerobic, non-fermenting, gram-negative opportunistic pathogen widely present in hospital environments, moist surfaces, medical devices, soil, and water (Qin et al., 2022). Bloodstream infections, urinary tract infections, burn infections, wound infections, surgical site infections, ventilator-related pneumonia, and persistent respiratory infections in immunocompromised individuals are only a few of the ailments associated with healthcare (Qin et al., 2022). Its clinical relevance is further demonstrated by its placement in the World Health Organization bacterial priority diseases list, where carbapenem-resistant *P. aeruginosa* continues to be a key issue for antimicrobial research, surveillance, and infection-control methods (Sati et al., 2025). This bacterium exhibits numerous overlapping resistance pathways that make treatment of *P. aeruginosa* infections problematic (Poole, 2011). Its intrinsic resistance is mainly attributed to reduced outer-membrane permeability, inducible chromosomal AmpC β -lactamase production, multidrug efflux pumps such as MexAB-OprM, and biofilm-related adaptive alterations (Pang et al., 2019; Poole, 2011). β -lactam resistance may also result from porin loss, particularly OprD alterations, modification of penicillin-binding proteins, AmpC overexpression, and acquisition of plasmid- or integron-mediated β -lactamase genes (Glen & Lamont, 2021). Multidrug-resistant (MDR), extensively drug-resistant (XDR), or difficult-to-treat resistant phenotypes are frequently the result of several mechanisms working in concert (Glen & Lamont, 2021).

Enzymes known as extended-spectrum β -lactamases (ESBLs) catalyze the hydrolysis of monobactams and extended-spectrum cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, cefepime, and aztreonam. On the other hand, clavulanic acid, sulbactam, and tazobactam block certain traditional ESBLs (Bush & Jacoby, 2010; Paterson & Bonomo,

2005). In *P. aeruginosa*, ESBL detection is more complex than in Enterobacterales because phenotypic expression may be masked by inducible AmpC cephalosporinase, efflux activity, reduced permeability, and co-production of metallo- β -lactamases or other carbapenemases (Laudy et al., 2017). Therefore, routine susceptibility testing alone may underestimate the true burden of ESBL-producing *P. aeruginosa*, especially in laboratories with limited molecular diagnostic facilities (Laudy et al., 2017).

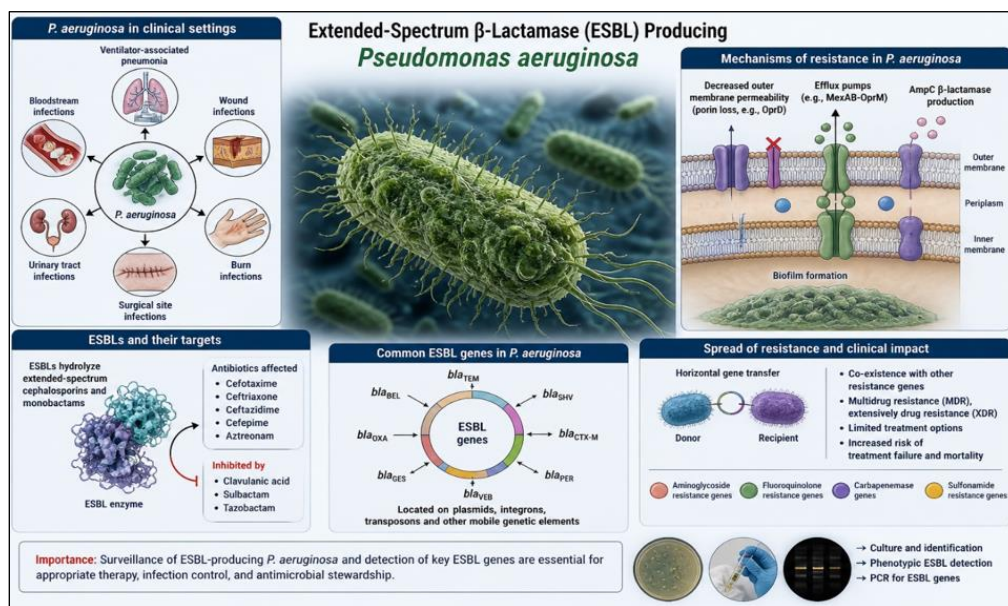


Figure 1: Extended-spectrum Beta-lactamase-producing

Molecularly, ESBL-producing *P. aeruginosa* may carry diverse β -lactamase genes, including class A enzymes such as *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*, *bla_{PER}*, *bla_{VEB}*, *bla_{GES}*, and *bla_{BEL}*, as well as class D extended-spectrum oxacillinases such as *bla_{OXA}* variants, as shown in Fig. 1 (Hosu et al., 2021; Laudy et al., 2017). A hospital-based study from Warsaw reported VEB, GES, OXA-2-like, and OXA-10-like genes in ESBL-positive *P. aeruginosa* isolates and emphasized that tests designed for Enterobacteriaceae are not always reliable for this organism (Laudy et al., 2017). Similarly, a molecular study from South Africa found a high frequency of *bla_{TEM}*, *bla_{SHV}*, and *bla_{CTX-M}* among resistant *P. aeruginosa* isolates, supporting PCR-based surveillance for defining local resistance epidemiology (Hosu et al., 2021).

The spread of ESBL genes is strongly linked to mobile genetic elements such as plasmids, integrons, insertion sequences, and transposons, which facilitate the movement of resistance determinants within and between bacterial populations (Bush & Jacoby, 2010; Poole, 2011). This mobility is clinically relevant as ESBL genes may co-exist with genes that confer resistance to aminoglycosides, fluoroquinolones, sulfonamides, and carbapenems, and so limit therapeutic options and increase the chance of treatment failure (Paterson & Bonomo, 2005). In *P. aeruginosa*, co-production of ESBLs with AmpC or metallo- β -lactamases can lead to resistance to numerous antipseudomonal β -lactams, such as ceftazidime, cefepime, aztreonam, piperacillin-tazobactam, and carbapenems (Glen & Lamont, 2021; Laudy et al., 2017). In low- and middle-income nations, including Pakistan, the prevalence of ESBL-producing Gram-negative bacteria is increased by high antibiotic consumption, over-the-counter antimicrobial availability, poor antimicrobial stewardship, overcrowded hospitals, and inconsistent infection-control practices (Abrar et al., 2018; Idrees et al., 2022). According to a molecular study conducted in Pakistan, the most common ESBL gene was *bla_{CTX-M}*, which was followed by *bla_{TEM}*, *bla_{OXA}*, and *bla_{SHV}*. This suggests that clinically important β -lactamase genes are circulating in local healthcare settings (Idrees et al., 2022). Although many Pakistani studies have focused on Enterobacterales, regional data on ESBL-producing *P. aeruginosa* remain limited, creating a need for focused prevalence and molecular characterization studies (Din et al., 2023).

Recent results from Pakistan also reveal the rise of highly resistant *P. aeruginosa* bearing ESBL and carbapenemase-associated genes in hospital settings (Din et al., 2023). Clinical isolates have included MDR and XDR strains, with phenotypic ESBL production and resistance genes such as *bla_{SHV}*, *bla_{IMP}*, and *bla_{NDM-1}* among extensively resistant isolates (Din et al., 2023). These findings are concerning because ESBL production can compromise third- and fourth-generation cephalosporins, while co-existing carbapenemases may further reduce the effectiveness of carbapenems, which are often reserved for severe MDR Gram-negative infections (Din et al., 2023; Paterson & Bonomo, 2005).

Despite the global recognition of *P. aeruginosa* as a critical antimicrobial-resistance pathogen, local molecular surveillance remains essential because resistance gene distribution varies by geography, hospital environment, antibiotic pressure, Sample source, and patient population (Laudy et al., 2017; Sati et al., 2025). Determining the prevalence of ESBL-producing *P. aeruginosa* and characterizing genes such as bla_TEM, bla_SHV, bla_CTX-M, bla_OXA, bla_PER, bla_VEB, and bla_GES can provide useful evidence for empirical therapy, infection-control interventions, and antimicrobial stewardship programs (Haghighi & Goli, 2022; Hosu et al., 2021). Therefore, the present study of the phenotypic occurrence of ESBL production and the molecular distribution of key ESBL genes among clinical *P. aeruginosa* isolates. This approach will help clarify the local burden of ESBL-mediated resistance and support evidence-based decisions for diagnosis, treatment, and prevention of MDR *P. aeruginosa* infections.

2 METHODOLOGY

2.1 Design of the study and collection of specimens

A hospital-based cross-sectional study was conducted at District Hospital Karak in Khyber Pakhtunkhwa, Pakistan, between January 2017 and November 2017, following the published report (Vandenbroucke et al., 2014; Von Elm et al., 2007). A total of 748 samples were collected from the intensive care unit, operation theatre, outpatient department, medical ward, and gynecology ward and consisted of pus/wound swabs, urine, sputum, tracheal swabs, high vaginal swabs, synovial fluid, blood culture samples, effusions, and other clinically relevant samples (Von Elm et al., 2007). The whole experimental work as shown in schematic diagram of the methodology Fig 2.

2.2 Isolation and identification of *P. aeruginosa*

Clinical specimens were inoculated on MacConkey agar, blood agar, cetrimide agar, and Mueller–Hinton agar and incubated aerobically at 37°C for 18–24 hours. Presumptive *P. aeruginosa* isolates were chosen based on their non-lactose fermentation on MacConkey agar, distinctive colony morphology, pigment synthesis, and growth on cetrimide agar. Cetrimide agar was employed because it is a selective medium that allows growth and pigment formation of *P. aeruginosa*, which facilitates presumptive identification of this species (Leber & Burnham, 2024). Selected colonies were Gram-stained and further verified to be Gram-negative rods by oxidase positivity, catalase positivity, and oxidative utilization of glucose. Antimicrobial susceptibility testing, phenotypic ESBL detection, and molecular characterization were performed only on verified *P. aeruginosa* isolates.

2.3 Test for antimicrobial susceptibility and categorization of resistance

Antimicrobial susceptibility was tested using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute guidelines (Humphries et al., 2021). Mueller-Hinton agar plates were uniformly inoculated with bacterial suspensions calibrated to 0.5 McFarland turbidity. Plates were incubated at 37°C for 16–18 hours after the antimicrobial discs were added. The CLSI performance criteria for antimicrobial susceptibility testing were utilized to evaluate inhibition zone sizes (measured in millimeters) (Bakthavatchalam et al., 2024). Mueller-Hinton agar plates were uniformly inoculated with bacterial suspensions calibrated to 0.5 McFarland turbidity. Plates were incubated at 37°C for 16–18 hours after the antimicrobial discs were added. The CLSI performance criteria for antimicrobial susceptibility testing were utilized to evaluate inhibition zone sizes (measured in millimeters) (Bakthavatchalam et al., 2024), (Magiorakos et al., 2012) established universally accepted standards for defining multidrug resistance, widespread drug resistance, and pan drug resistance. XDR was defined as not being susceptible to at least one agent in all but two or fewer antimicrobial categories, PDR as not being susceptible to all agents tested, and MDR as not being susceptible to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

2.4 ESBL production phenotypic detection

ESBL generation was examined in confirmed *P. aeruginosa* isolates that exhibited decreased sensitivity to extended-spectrum cephalosporins. The combination disc method and/or double-disk synergy test were used to confirm phenotypic ESBL, employing both β -lactamase inhibitors and extended-spectrum cephalosporins alone. Enhancement of inhibition zones around cephalosporin–inhibitor combinations, or clear synergy toward an inhibitor-containing disk, was interpreted as suggestive of ESBL production (Laudy et al., 2017).

Phenotypic ESBL detection in *P. aeruginosa* was interpreted cautiously because AmpC β -lactamase expression, efflux-pump activity, reduced outer-membrane permeability, OprD loss, and carbapenemase co-production may interfere with routine ESBL detection (Glen & Lamont, 2021; Laudy et al., 2017). Therefore, phenotypic findings were interpreted together with antimicrobial susceptibility profiles and PCR-based detection of ESBL-associated genes (Laudy et al., 2017).

2.5 Genomic DNA extraction and PCR amplification

Using the phenol–chloroform extraction method, genomic DNA was extracted from *P. aeruginosa* isolates that were either phenotypically confirmed or suspected of producing ESBL. Prior to DNA extraction, fresh colonies were inoculated in nutritious broth and cultivated for 18–24 hours at 37°C. Centrifugation was used to pellet the bacterial cells, which were then resuspended in Tris-EDTA buffer, lysed with sodium dodecyl sulfate and proteinase K, and purified with phenol-chloroform-isoamyl alcohol and chloroform-isoamyl alcohol to reduce the carryover of polysaccharides and cellular contaminants. Cold isopropanol was used to precipitate the DNA, followed by a 70% ethanol wash, air drying, resuspension in TE buffer, and storage at -20°C until PCR amplification. For the extraction of bacterial genomic DNA and the synthesis of PCR-compatible DNA, phenol-chloroform-based extraction is frequently employed (Wright et al., 2017).

PCR amplification was used to identify certain ESBL-associated genes (*bla*_TEM, *bla*_CTX-M, *bla*_OXA-1-like, *bla*_OXA-2-like, and *bla*_OXA-10-like). For every 25 µL reaction, there are 12.5 µL of PCR master mix, 0.5 µL of forward primer, 0.5 µL of reverse primer, 1 µL of template DNA, and 10.5 µL of nuclease-free water. A Veriti 96-well thermal cycler or similar PCR apparatus was used for amplification. Initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes were used to amplify *bla*_CTX-M. For the *bla*_TEM and *bla*_OXA targets, the annealing temperature was 60°C, and the other cycle parameters remained the same. PCR-based detection is typically used to molecularly characterize β-lactamase genes in Gram-negative bacteria (Dallenne et al., 2010). The primers lists have been documented in Table 1.

Table 1: Primers used for PCR identification of ESBL-related genes in *Pseudomonas aeruginosa*

Target gene/group	Primer	Sequence 5'–3'	Size of Product
<i>bla</i> _OXA-10-like	OXA-10F	TCTTTCGAGTACGGCATTAGC	760 bp
	OXA-10B	CCAATGATGCCCTCACTTTC	
<i>bla</i> _OXA-2-like	OXA-2F	GCCAAAGGCACGATAGTTGT	700 bp
	OXA-2B	GCGTCCGAGTTGACTGCCGG	
<i>bla</i> _OXA-1-like	OXA-1A	AGCCGTAAAAATTAAGCCC	908 bp
	OXA-1B	CTTGATTGAAGGGTTGGGCG	
<i>bla</i> _TEM	TEM-A	GAGTATTCAACATTTCCGTGTC	851 bp
	TEM-B	TAATCAGTGAGGCACCTATCTC	
<i>bla</i> _CTX-M group	CTX-MA	CGCTTTGCGATGTGCAG	550 bp
	CTX-MB	ACCGCGATATCGTTGGT	

2.6 Quality control and agarose gel electrophoresis

While PCR products were separated on a (2%) agarose gel made in TBE buffer, extracted genomic DNA was initially examined on a (1%) agarose gel. Using the proper DNA molecular size ladder, amplicons were combined with loading dye and run on a gel. When bands of the anticipated amplicon size appeared under ultraviolet light, positive isolates for a target gene were identified using a gel documentation system. One common technique for separating DNA fragments and estimating amplicon size after PCR amplification is agarose gel electrophoresis. (Green & Sambrook, 2019; Lee et al., 2012).

Quality control procedures were applied during specimen processing, culture identification, antimicrobial susceptibility testing, ESBL screening, DNA extraction, PCR setup, and gel electrophoresis. The antimicrobial susceptibility testing was performed using *P. aeruginosa* (ATCC 27853) as the quality control strain, wherever applicable, according to CLSI quality-control criteria (Bakthavatchalam et al., 2024). Each PCR was run with a no-template negative control to monitor for contamination, and positive-control DNA from known β-lactamase-producing isolates was utilized where available. Pre-PCR and post-PCR activities were carried out in separate workspaces utilizing nuclease-free water, aerosol-resistant pipette tips, and particular PCR chemicals to minimize carry-over contamination.

2.7 Statistical analysis and outcome measures

SPSS, GraphPad Prism, and Excel were used to analyze the data. We divided the total number of verified *P. aeruginosa* isolates by the number of isolates that tested positive for ESBL to get an estimate of the prevalence of this bacterium.

We used chi-square tests or Fisher's exact tests to look for correlations between ESBL production and categorical factors (where they were available).

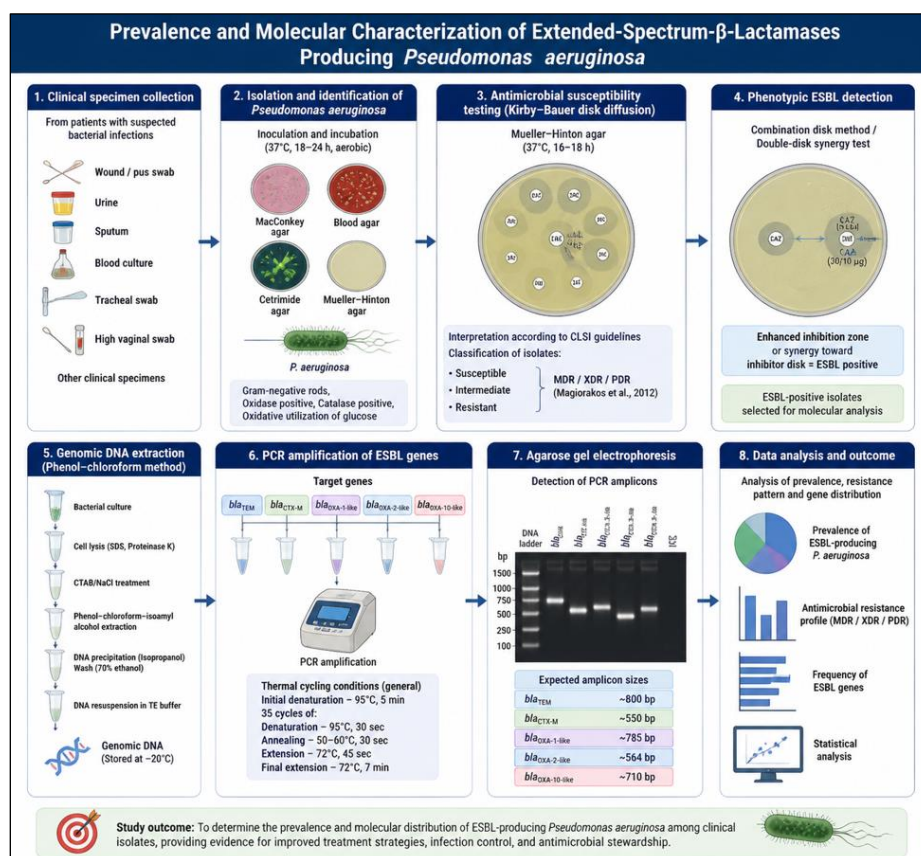


Figure 2: The schematic diagram of the methodology.

3 RESULTS

3.1 Prevalence of *Pseudomonas aeruginosa* isolates

A total of 748 clinical specimens were processed from patients presenting with suspected bacterial infections at District Hospital Karak, Khyber Pakhtunkhwa, Pakistan, between January and November 2017. Among these, *Pseudomonas aeruginosa* was isolated from 400 isolates (53.5%), showing the substantial occurrence of this pathogen in the research population. The isolates were obtained from several clinical sites, such as the Outpatient Department (OPD), the Operation Theatre (OT), the Intensive Care Unit (ICU), and the Gynecology unit.

3.2 Production of ESBLs in *Pseudomonas aeruginosa*

Out of 400 verified *P. aeruginosa* isolates, 80 (20.0%) were found to be ESBL producers by phenotypic testing. The other 320 isolates (80.0%) were confirmed as negative for ESBL. The present investigation shows that a clinically meaningful fraction of isolates of *P. aeruginosa* are resistant to extended-spectrum cephalosporins, raising the issue of antimicrobial resistance in this hospital context.

3.3 Antimicrobial susceptibility test

ESBL development was further confirmed in those organisms' exhibiting cefotaxime and ceftazidime susceptibility. Mueller-Hinton agar was used to identify ESBL phenotypically. Ceftazidime (CAZ30 µg), aztreonam (ATM30 µg), Ceftriaxone (CRO30 µg), Cefotaxime (CTX, 30 µg), Amoxicillin (20 µg), and Clavulanic acid-AMC (10 µg) are the five antibiotics. Third-generation antibiotics, such as cephalosporins (ceftazidime-CAZ 30 µg, ceftriaxone-CRO 30 µg, cefotaxime-CTX 30 µg) and aztreonam (ATM 30 µg), were positioned 15 mm (center to center) away from the amoxicillin clavulanic acid disc in the center of the plate. The test plate was incubated at 37° C for a whole day. The presence of extended-spectrum beta-lactamases is demonstrated by the extension of the zone of inhibition of any one of these medication discs towards amoxicillin-clavulanic acid as shown in Fig 3.

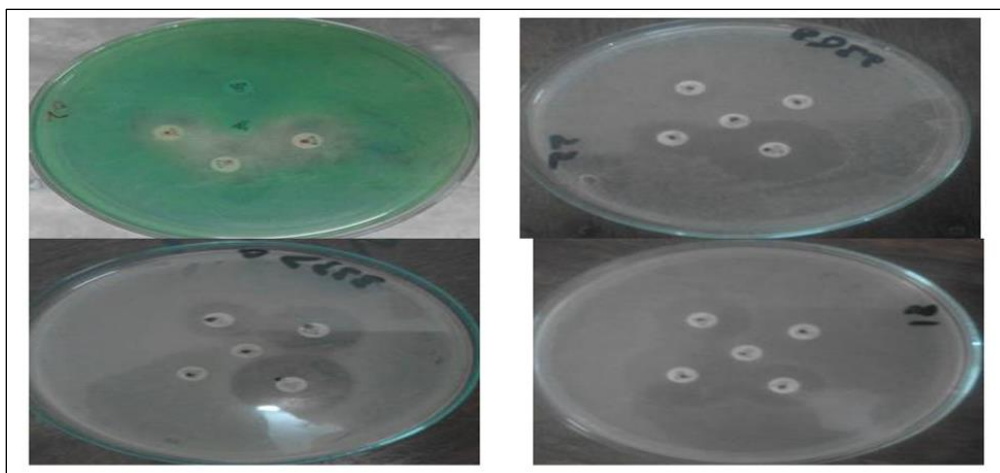


Figure 3: Antimicrobial susceptibility test of different antibiotics against *Pseudomonas aeruginosa*

4 DISTRIBUTION OF ESBL-POSITIVE ISOLATES BY WARD

The incidence of ESBL-producing *P. aeruginosa* isolates was considerably different in different wards of the hospital. The highest number of ESBL-positive isolates was acquired from the Outpatient Department (OPD), with 34 isolates (28.33%), followed by the Operation Theatre (OT), contributing 23 isolates (21.90%). The Intensive Care Unit (ICU) yielded a total of 21 isolates (24.13%), and the Gynecology Ward yielded 2 isolates (2.20%). Results reveal ESBL-positive isolates are more common in more critical care settings.

5 AGE DISTRIBUTION OF ESBL-POSITIVE ISOLATES

ESBL-positive isolates were discovered in all age groups, with the largest number of isolates in the 36-50 years age group (28, 27.45%), followed by the 26-35 years age group (23, 32.85%). Interestingly, the lowest number of ESBL-positive isolates was detected in persons beyond the age of 60 years, with only 2 isolates (3.44%) being identified.

The age distribution of ESBL-positive isolates indicated a significant variation in prevalence, with a significantly high number of ESBL-positive isolates in the age group of 26-50 years ($\chi^2 = 23.07$, $p < 0.001$). This implies that middle-aged individuals could be more susceptible to infections with ESBL-producing *P. aeruginosa*.

5.1 Gender Distribution of ESBL-Positive Isolates

The distribution by sex of ESBL-positive isolates showed that 56 isolates (70%) were isolated from female patients and 24 isolates (30%) were obtained from male patients. The ESBL-positive rate was the same in both genders and was 20.0% in males and 20.0% in females, indicating that gender was not a significant factor impacting the prevalence of ESBL-producing *P. aeruginosa*.

5.2 Specimen-wise Distribution of ESBL-Positive Isolates

ESBL-producing *P. aeruginosa* isolates were recovered from a variety of specimen types, with the highest number of isolates being recovered from pus samples ($n = 30$, 20.68%), followed by urine samples ($n = 20$, 25.97%). The greatest ESBL-positive rate was observed in tracheal swabs (38.88%, 7 isolates), but the number of isolates from this source was modest.

This means that ESBL-producing *P. aeruginosa* might lead to respiratory tract infections, especially in hospitalized patients. Pus and urine samples also contributed considerably to the ESBL-positive isolates, showing their importance as sources of infection in this cohort.

6 MOLECULAR CHARACTERIZATION OF ESBL-ASSOCIATED GENES

ESBL-linked genes were present in 80 ESBL-positive isolates as determined by PCR molecular research. The most detected gene was OXA-type β -lactamase in 37 isolates (46.25%), followed by CTX-M-type genes in 29 isolates (36.25%) and TEM genes in 14 isolates (17.5%) as shown in Fig 4.

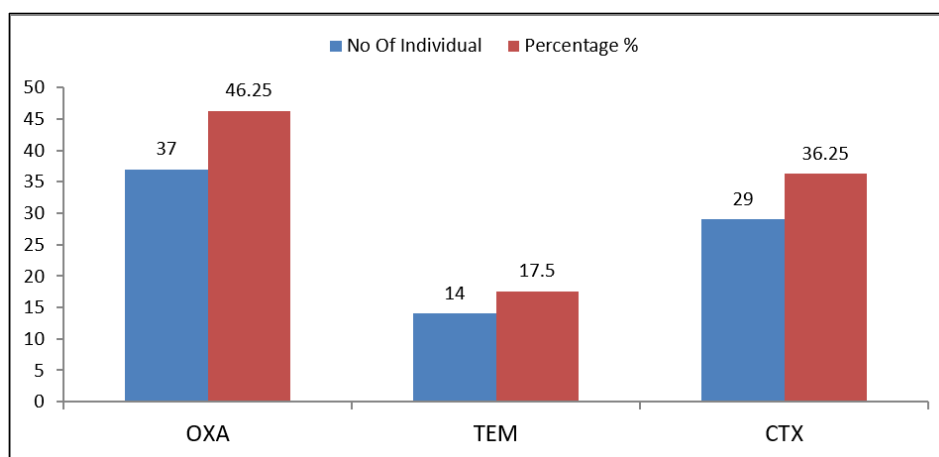


Figure 4: Results of OXA, TEM, and CTX

The results of this work demonstrate that the OXA-type β -lactamases are the principal molecular contributors to ESBL production in *P. aeruginosa*. CTX-M type β -lactamases are also essential. The presence of the TEM-type genes, although less numerous, confirms the heterogeneity of ESBL-producing pathways in this hospital environment.

6.1 Gene Combinations in ESBL-Positive Isolates

Fifteen (18.75%) *P. aeruginosa* isolates showed dual gene combinations. The most common gene pairs were:

- **OXA + CTX-M** (5 isolates, 6.25%)
- **OXA + TEM** (8 isolates, 10%)
- **CTX-M + TEM** (2 isolates, 2.5%)

These findings suggest that dual gene carriage in *P. aeruginosa* is not uncommon and may contribute to higher resistance profiles, complicating treatment strategies.

7 AGAROSE GEL ELECTROPHORESIS

The PCR results were visualized by 2% agarose gel electrophoresis, and the bands corresponded to the anticipated amplicon sizes of OXA, CTX-M, and TEM genes as shown in Fig 5.

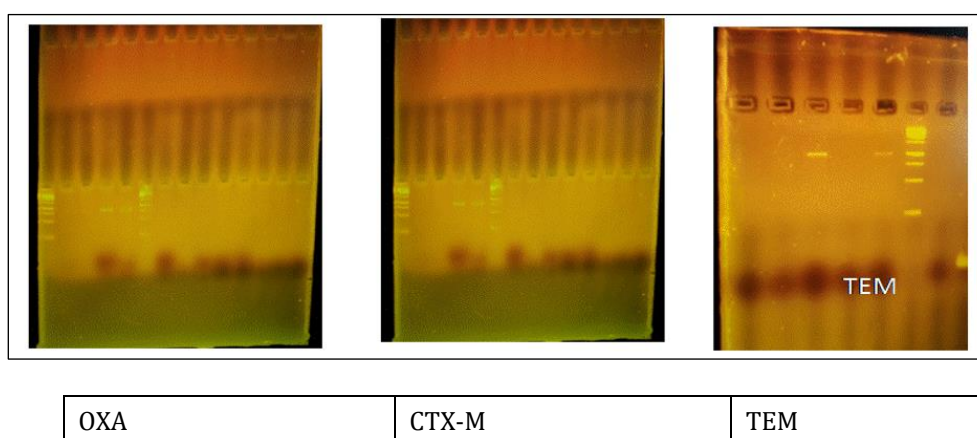


Figure 5: Amplicon sizes of OXA, CTX-M, and TEM genes

The bands for OXA (760 bp), CTX-M (550 bp), and TEM (851 bp) were in agreement with the expected molecular weights, demonstrating the presence of these ESBL-related genes in the respective isolates. The findings further support the molecular characterization of ESBL-producing *P. aeruginosa* isolates in this investigation. Shown in (Figure 5), Extended-spectrum Beta-lactamase-producing.

8 DISCUSSION

Pseudomonas aeruginosa is an opportunistic infection, particularly resistant to antibiotics in the hospital environment, and poses a serious threat to public health. The prevalence of *P. aeruginosa* is on the rise globally, with significant morbidity and death, primarily due to multidrug-resistant (MDR) strains, especially in immunocompromised and critically ill persons (Wilson & Pandey, 2023). The present work aimed to identify and describe *P. aeruginosa* from clinical specimens collected from District Hospital Karak, Khyber Pakhtunkhwa, Pakistan, and to evaluate its resistance pattern in connection with the synthesis of extended-spectrum β -lactamases (ESBLs).

The results showed a considerable rate of the incidence of *P. aeruginosa*, where 400 of 748 clinical specimens (53.5%) were positive. Of them, 80 (20%) were ESBL producers, therefore indicating that ESBL-mediated resistance is a significant challenge in this institution. Our results are comparable with other regional studies and emphasize the global issue of infection management of MDR *P. aeruginosa* (Ullah et al., 2017). The advent of ESBLs has substantial therapeutic implications. It reduces the effectiveness of cephalosporins and other beta-lactam antibiotics, and it causes physicians to use alternate treatments that may be less effective or more hazardous.

Some significant trends in the distribution of ESBL-positive *P. aeruginosa* isolates in the different hospital wards were observed. The maximum number of ESBL-positive isolates was obtained from OPD, followed by OT and ICU. The Gynecology Ward contributed the least in terms of ESBL-positive isolates across the wards. This trend may be suggestive of the clinical setting where surgical interventions and critical care are associated with higher antibiotic usage and invasive procedures, which are known risk factors for the development and spread of resistant pathogens (Elfadadny et al., 2024; Zhao & Lohans, 2025).

The examination of the age distribution revealed that *P. aeruginosa* infections, notably ESBL-expressing, were more prevalent in the age group of 26-50 years. This finding is in agreement with earlier research that has shown middle-aged people are more susceptible to acquiring resistant *P. aeruginosa* infections, especially those with comorbidities or those who often visit hospitals and undergo invasive procedures (Arun, 2015). Interestingly, the incidence of ESBL-positive isolates was decreased in the older population (≥ 60 years). This could be due to several reasons, such as improvements in clinical care in older patients or a lower prevalence of specific forms of disease in this age range (Tümmeler, 2019).

The gender distribution of ESBL-positive isolates showed no significant change in susceptibility between males and females; 70% of isolates were from females and 30% of isolates from males. The rate of ESBL positivity was equal in both genders (20%). The greater number of female isolates could be explained by the rising number of female patients or a larger clinical sample. This finding shows that gender is not a significant factor in susceptibility to the ESBL-producing *P. aeruginosa* infections in this case (Patel).

Further analysis by specimen type again reiterated the clinical variability of *P. aeruginosa* infections, with pus being the source of most ESBL-positive isolates. These data suggest that soft tissue and wound infections may be a key reservoir of resistant *P. aeruginosa* in hospitalized patients. The significant occurrence of ESBL-positive isolates in urine and tracheal swabs further stresses the importance of monitoring respiratory and urinary tract infections, especially in critically sick patients and those with invasive devices (Ramos et al., 2025).

Molecular analysis of ESBL-producing *P. aeruginosa* isolates demonstrated the presence of certain beta-lactamase genes; the most common were of OXA-type (46.25%), followed by CTX-M (36.25%) and TEM (17.5%). These results are in agreement with previous research, which reported the prevalence of OXA and CTX-M beta-lactamases in *P. aeruginosa* isolates. (Edward et al., 2024; Lin et al., 2022). This is especially relevant because OXA-type enzymes are commonly associated with resistance to beta-lactams such as penicillins and cephalosporins, and are often found in combination with other resistance mechanisms such as efflux pumps or biofilm development (Lin et al., 2022).

Interestingly, dual gene combinations (e.g., OXA + TEM and OXA + CTX-M) were detected in 18.75 % of ESBL-positive isolates. The co-transmission of numerous beta-lactamase genes can lead to resistance to a wider spectrum of antibiotics, which complicates therapy (Abdelwahab et al., 2021). We uncover evidence of dual gene combinations that suggest *P. aeruginosa* bacteria are acquiring resistance via horizontal gene transfer and that plasmids and other mobile genetic elements may be important in the dissemination of resistance in this hospital environment.

The resistance patterns identified in this experiment provided strong molecular evidence of beta-lactamase production by PCR-based techniques. This method allows more accurate detection of ESBL-producing bacteria and identification of some resistance genes that cannot be detected by phenotypic testing. Additional resistance mechanisms, such as porin mutations or the generation of additional beta-lactamases (e.g., metallo-beta-lactamases), were not studied in this

paper. Thus, future studies should focus on a wider spectrum of resistance genes to better understand the genetic variety of *P. aeruginosa* resistance in this area.

From a therapeutic point of view, the results of the present investigation underline the need for more stringent antibiotic stewardship initiatives, especially in the hospital environment where *P. aeruginosa* is common. Empiric therapy with cephalosporins is not recommended for suspected *P. aeruginosa* infections in locations with high resistance rates. Hence, routine culture and susceptibility testing are required to identify the most effective antibiotics. Dissemination of ESBL-producing *P. aeruginosa* in this institution highlights the necessity of infection control measures, including contact precautions and antibiotic surveillance, for the prevention of future spread of resistant strains (Pakistan).

This study reveals a high prevalence of *Pseudomonas aeruginosa* (53.5%) at District Hospital Karak, with 20% of these isolates confirmed as ESBL producers. The highest infection burden occurs in the OPD (42.5%), followed by the OT and ICU, with pus swabs acting as the primary reservoir and tracheal swabs showing the highest individual ESBL rate (38.88%). Genotypically, resistance is primarily driven by *bla*_{OXA} (46.25%), followed by *bla*_{CTX-M} (36.25%) and *bla*_{TEM} (17.5%), with 18.75% of strains carrying dual resistance genes. These results highlight an urgent need to restrict empirical cephalosporin use, enforce strict ward disinfection, and establish active antimicrobial stewardship programs to control the spread of these multidrug-resistant bacteria.

9 CONCLUSION

The high frequency of OXA genes in ESBL-producing *P. aeruginosa* leads to high antibiotic resistance, necessitating regular surveillance, antibiotic stewardship, and infection management.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

All authors declared no conflict of interest.

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