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# Presence of BlaPER-1 and BlaVEB-1 Beta-Lactamase Genes among Isolates of Pseudomonas Aeruginosa from Burn and Trauma Hospital Peshawar, Pakistan

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# Presence of BlaPER-1 and BlaVEB-1 Beta-Lactamase Genes among Isolates of Pseudomonas Aeruginosa from Burn and Trauma Hospital Peshawar, Pakistan


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# PRESENCE OF BLAPER-1 AND BLAVEB-1 BETA-LACTAMASE GENES AMONG ISOLATES OF PSEUDOMONAS AERUGINOSA FROM BURN AND TRAUMA HOSPITAL PESHAWAR, PAKISTAN

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## ABSTRACT

*Pseudomonas aeruginosa spp* are the most prevalent bacteria that cause nosocomial infections in hospitals. Most antibiotics, including novel new β-lactams, are already resistant to them, and they can become resistant during treatment, which can make the treatment fail. *P. aeruginosa* isolates from ICU patients who had Per-1 and VEB-1 were the main focus of this study. These two ESBLs are the two most common in ICU patients who had them. 50 isolates were gathered from Peshawar's LRH ICU facilities in the year 2021. The antibiotic susceptibility test was conducted in accordance with the Clinical and Laboratory Standards Institute's standards (CLSI). The combination disc test used to identify isolates that produce ESBLs. Ceftazidime MIC was determined using the agar dilution method using particular primers, the PER-1 and VEB-1 genes were detected using polymerase chain reaction (PCR). Fifty-six percent patients (n=40) male, whereas forty percent (n=25) were female. Augmentin (96.6%, n=61) and cefpodoxim (86.7%, n=55) resistance was found in the majority of ICU isolates. Fifty isolates (77%) tested positive for ESBL, with 94 percent (n=47) carrying the PER-1 gene and VEB-1 gene 52 percent (n=26). Ten isolates had blaPER1 and blaVEB1 present at the same time, and seven of them amplified all three genes. ESBL producers were found in a large number of ICU *P. aeruginosa* isolates. Although blaVEB1 and blaPER1 were found in a small number of isolates, their frequency was very high. Furthermore, carbapenem resistance was negligible. Because of drug-resistant *P. aeruginosa* isolates, it is vital to monitor ICU centers.

**Keywords:** blaPER1, blaVEB1, *P. aeruginosa* isolates, *P. aeruginosa*. ESBL producer.

## INTRODUCTION

Although produced infrequently, the acquired beta-lactamase enzymes blaPER-1 and blaVEB-1 are clinically important because they confer resistance to oxyimino beta-lactams (Kumar et al. 2012). Due to its wide spectrum of activity against gram-positive and gram-negative isolates, carbapenem resistance is also a severe concern (Ghasemian et al.

2018). Carbapenem resistance is common in *Pseudomonas aeruginosa* as well as *Klebsiella pneumoniae* and *Acinetobacter spp*. The goal of the study is to find the genes that make the Class A ESBLs blaPER-1 and blaVEB-1 in *P. aeruginosa* clinical isolates from ICU patients (Nojoomi et al., 2016). *Pseudomonas aeruginosa* is a common cause of infections in hospitals, such as pneumonia, burn infections, urinary tract infections, meningitis, and bacteremia (Kohlenberg et al., 2010). In immune-compromised patients, infections can quickly escalate to a dangerous level. In people who don't have a strong immune system, infections can quickly get out of hand and become dangerous (Fernandes et al., 2013).

Antibiotics have been widely used for many years, but resistance genes have only lately emerged and become widespread (Saderi et al., 2008). Extended-spectrum-lactamases cause resistance to broad-spectrum cephalosporins such as cefotaxime, ceftriaxone, ceftazidime, and aztreonam (ESBLs). It used to be known that *Klebsiella pneumoniae* and *Escherichia coli* had some of these enzymes, but now they have been found in other pathogens as well. Carbapenem antibiotics have become more common, which has led to more carbapenem-resistant *P. aeruginosa* clinical isolates that are hard to treat. This has cut down on the treatment options for this disease (Saleh et al., 2016). Between the years 2021 and 2022, 50 samples of *P. aeruginosa* isolates from intensive care unit (ICU) patients of LRH hospitals in Peshawar, and the results were published in the journal mBio. It was done with hydrogen sulphide (H2S) tests, indole tests, catalase and oxidase tests, motility tests, triple sugar iron agar, urease, and Macconkey agar tests (Gupta et al., 2010).

## MATERIALS AND METHODS

### *Bacterial Isolates*

Between 2021 and 2022, 50 samples of *P. aeruginosa* obtained from patients admitted to (ICUs) at LRH hospitals in Peshawar. All of the strains of bacteria were identified using hydrogen sulphide (H2S) tests, indole, motility, triple-sugar iron agar (TSI) catalase and oxidase tests, urease, and Macconkey agar (Tavajjohi et al., 2011).

### *Antibiotic Susceptibility Test*

It was done in accordance with CLSI guidelines when the antibiotic susceptibility test was done, Antibiotics that were used: Levofloxacin (5µg), gentamycin (100 µg), cefepime (25µg), cepodoxime (15µg), ciprofloxacin (10µg), tobramycin (10µg), ofloxcine (5µg), cefepime (25µg), amikacin (30µg), augmentin (25µg), tetracycline (50µg), ceftriaxone (30µg) (Tavajjohi et al., 2011).

### *Phenotypic Detection of ESBL Producers*

The isolates that developed ESBLs were found using the combine disc test. We tested the efficacy of two different antibiotics, ceftazidime and cefotaxime discs, one with and one without clavulanic acid and Clampulanic acid was added, and when it was, a disc separation of more than 5 mm was seen as good (Hosseini et al., 2007).

### *DNA Extraction*

Polymerase chain reaction using specific primers was used to identify the VEB-1, PER-1 and encoding genes (Table 1).

**Table 1: Primers used in this study**

| Primer | Sequence 3' to 5' | Product size | Reference |
|---|---|---|---|
| ***bla*PER-*1*** | F: ATA GGA CTG TTA ATA TTT TCG<br>R: ATT ATC GGT TCG GAT CG | 634 | **1,12** |
| ***bla*VEB-*1*** | **F: AGC TTC ACC TAT CGC GTT GC**<br>**R: GCA CAC TAC AGC GGA TGC TC** | **925** | **9,12** |

The reaction combination for these genes as follows: 10XPCR buffer equals 2.5 L, MgCl2 (50 mM) equals 1.5 L, dNTP (10 Mm) equals 0.75 L, forward primer (100 M) equals 2.5 L, reverse primer (100 M) equals 2.5 L, Taq DNA polymerase (5 U/l) equals 0.2 L, template (DNA) equals 1 L, and nuclease-free H2O equals 14.05 L.10XPCR buffer equals 2.5 L (Szabó et al., 2008).

### *Polymerase Chain Reaction (PCR)*

Polymerase chain reaction (PCR) was used to look for the PER-1, VEB-1, and genes that make them with specific primers (Table 1). 10XPCR buffer: 2.5 mL; MgCl2: 1.5 mL; DNTPs: 10 mL; Forward and reverse primers: 2.5 mL each; Taq DNA polymerase: 0.25 U/mL; 0.2 mL; and H2O: 14.05 mL (Spagnolo et al., 2014).

### *Data Analysis*

SPSS 20 was used to analyses the data t-test was used, and P value of < 0.05 was used to determine the significance of the result.

## RESULTS

Results indicate that 46.1 percent (n = 30) of the 50 isolates were found in urine, 15.9 percent (n = 11) in pneumonia, and 10.8 percent (n = 10) in blood and other locations. Most of the patients were male, with 56% (n = 39) and 44% (n = 25) of them.

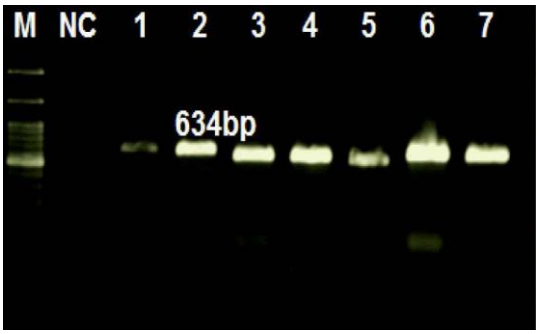


**Figure 1: *Bla* PER1 gene with 634 bp size.**

### *Antibiotic Susceptibility Test*

The overall isolates (96.8 percent, n=62) and cefpodoxime (86.7 percent, n=57) resistant to Augmentin. CAZ (65 percent, n=40), CPM (48.6 percent, n=30), AMP (47.6, n=28), CEP (66.6 percent, n=41), AMC (59 percent, n=38) CTX (68.4 percent, n=38), CIP (46.07 percent, n=29), OFX (36.2 percent, n=18), LEV (59.3 percent, n=31), GEN (36.9 percent, n=24) (Fooladi et al., 2016).

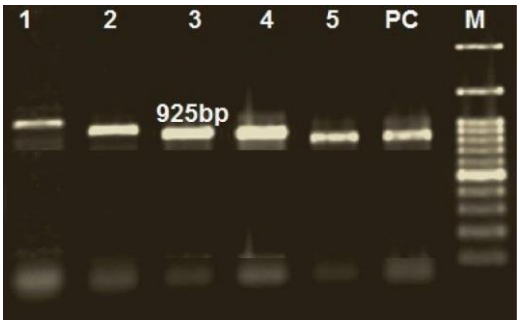


**Figure 2: *Bla* VEB1 gene with 925 bp size.**

***Phenotypically detection of ESBL producers***

*P. aeruginosa* isolates were ESBL positive in 50 (75%) of the cases. Urine infection (63 percent, n=40), wound (25 percent, n=16), sputum was (16.2 percent, n=11) were the most common ESBL positive isolates (Davodian et al., 2015).

***Detection of PER-1 VEB-1 and encoding genes***

Fifty isolates (75%) tested positive for ESBL, with 92 percent (n=48) carrying the blaPER-1 gene and 52 percent (n=26) carrying the blaVEB-1 gene (Figures 1 and 2). Ten isolates had blaPER-1 and blaVEB1 present at the same time, and seven of them amplified all three genes. Resistance showed toward ceftriaxone, ceftazidime, and cefotaxime was linked to the presence of these genes (Yusuf et al., 2017).

## DISCUSSION

*Pseudomonas aeruginosa spp* is a common bacterium responsible for a broad spectrum of nosocomial infection. Antibiotic resistance *P. aeruginosa* isolates is a result of a combination of innate and acquired (chromosome or plasmid-mediated) mechanisms. Majority of the *P.aeruginosa* isolates in our investigation came from infections of the urinary tract. Our findings are backed up by a number of earlier investigations (Shacheraghi et al., 2010). Because the principal route of antibiotic excretion from the body is through the urine, the bulk of the resistant isolates were discovered there. Many tested isolates were noticed to resistant augmentin, and cefotaxime and also third-generation cephalosporins. It is a big concern that antibiotic resistance is on the rise in gram-negative rods. *P. aeruginosa* has a small number of antibiotic resistance genes (approximately 0.3 percent of the total number of genes in the organism). ESBL producers made up about 77 percent of ICU isolates, which is similar to prior investigations. The presence of Amp C resistance to clavulanic acid suggests the presence of maybe additional gene that are unaffected by this inhibitor.

## CONCLUSION

To the best of our knowledge, this is the first study in Peshawar, Pakistan, to detect metallo-β-lactamase genes in *Pseudomonas aeruginosa* isolates. A high number isolates of *P. aeruginosa* from ICU were ESBL producer's mechanism (Davodian et al., 2016). The frequency of blaVEB1 and blaPER1 were relatively high, while blaPSE1 was detected among a low number of isolates. ICU wards are of main sources for infections with drug-resistant strains. Continuous and prolonged antibiotic periods, hospitalization and misuse are pivotal factors in the selection of highly resistant strains. Combination therapy (usually with a β-lactam and an aminoglycoside) is important to treat *Pseudomonas* infections.

## AUTHORS' CONTRIBUTION

MS conceived the idea, SM, SK, ST, performed the experiment. SK, SA, MQ wrote the manuscript. AS, BT and RS, reviewed the manuscript and SK, AU collected samples .All authors read and approved the final version

## CONFLICTS OF INTEREST

There is no conflict of interest regarding the publication of this paper.

## ETHICAL CONSIDERATIONS

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

## ACKNOWLEDGMENT

The findings of this study highlight the importance of establishing a surveillance network to track trends and the emergence of new resistance mechanisms in *P. aeruginosa* from various geographical regions. To avoid the development of such resistant pathogenic organisms, improvements in antibiotic prescribing practices and infection control programs are critical.

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