Molecular detection of blaPER-1, blaVEB-1, and blaPSE-1 β-lactamase genes from P.aeruginosa Severe Urogenital UTI Infection

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Abstract

Background: Nosocomial infections are mostly caused by Pseudomonas aeruginosa. Burn and wound infections are primarily caused by multidrug-resistant P. aeruginosa isolates. Nosocomial isolates often withstand several antibiotics, including new β-lactam antibiotics. Detecting multidrug-resistant strains might improve medication administration by (Davodian et al., 2015). Objectives: The objective of this study was to see whether P. aeruginosa isolates from people with urinary tract infections (UTIs) included the genes for class A ESBLs such PER-1, VEB-1, and PSE-1 by (Davodian et al., 2016). Methodology: In 2021 and 2022, 50 isolates were taken from the department of pathology's urine section at five different hospitals in Peshawar Pakistan. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to do the antibiotic susceptibility test. Agar dilution method was used to find the MIC of ceftazidime by (Fernandes et al., 2013). ESBL-producing isolates were looked for with the combine disc test. PCR (polymerase chain reaction) was used with specific primers to find the PER-1, VEB-1, and PSE-1 genes. Result: 60% (n=30) of the patients were male and 40% (n=20) were female. Most of the bacteria found in urine samples were resistant to the antibiotics augmentin (98.8%) and cefpodoxime (86%). Eighty percent of the isolates tested positive for ESBLs, with 94% (n=47) including the PER-1 gene, 55% (n=30) containing the VEB-1 gene, and 18% (n=10) containing the PSE-1 gene by (Fooladi et al., 2016). Conclusion: 10 isolates had blaPER1 and blaVEB1, and 7 amplified all three genes. ESBL-producing urine P. aeruginosa was common. BlaVEB1 and blaPER1 were common, but blaPSE1 was rare by (Fazeli, et al., 2015).

INTRODUCTION

Carbapenem resistance was moderate.Microbial resistance to extended-spectrum beta-lactamase (ESBL) was initially observed in Europe in the early 1980s, and then in the United States promptly after third-generation cephalosporins were introduced into clinical use by (Ghasemian, et al., 2018). Gram-negative bacteria resist betalactam drugs due to the enzyme β-lactamase. ESBL enzymes hydrolyze and induce resistance to oxyimino-cephalosporins and aztreonam by (Ghasemian, et al., 2018). Infections are caused by a wide variety of microorganisms, but P.aeruginosa is one of the most prevalent of them. Since these strains have an innate resistance to the majority of medications, including new β-lactam antibiotics, treatment with them often fails, which leads to a greater death rate by (Davodian et al., 2015). P. aeruginosa strains are becoming more resistant to antimicrobial agents, and the prevalence of multidrug resistance is also rising. Many studies have recorded outbreaks that were caused by carbapenem- and multidrug-resistant isolates by (Davodian et al., 2015). The downregulation of membrane porins (OprD) and upregulation of multidrug efflux pumps (MexAB-OprM) promote intrinsic drug resistance. Antimicrobial resistance in gram-negative isolates has been linked to the emergence of new beta-lactamases around the globe. These beta-lactamases include AmpC beta-lactamases, extended-spectrum beta-lactamases (ESBLs), and metallo-beta-lactamases (MBLs) by (Gupta et al., 2013). The plasmids and integrons that code for the ESBL enzymes were first discovered in Klebsiella pneumonia isolates found in Germany. This country reportedly was the initial one to confirm their presence by (Davodian et al., 2015). In P. aeruginosa, various kinds of enzymes have been found. They include class A ESBLs, which are made up of blaPER-1 and blaVEB-1, as well as GES/IBC and BEL types. These enzymes were first described in Turkey, south Asia, and France by (Hosseini-Mazinani et al., 2007) . It has been proven that ESBL enzymes are derivations of TEM- and SHV-lactamases, with just a slight genetic mutation in the active site. Since it is responsible for resistance to oxyimino beta-lactams, the acquired beta-lactamase enzyme known as blaVEB-1 is the acquired beta-lactamase enzyme with the highest therapeutic value by (Johnson, et al., 2015). The blaPER mutation is seen less often, but it is clinically significant since it confers resistance to oxy-imino-beta-lactams by (Khan, S et al., 2021). Consequences for health care, medical research, and medical training Nosocomial pathogen P. aeruginosa is a major reason to be concerned, as is the rising prevalence of antibiotic resistance among the bacteria it inhabits. To use just one example, extended-spectrum beta-lactamase (ESBL) enzymes give resistance to a wide variety of medicines in this class. Better monitoring of this pathogen may be achieved by the detection of ESBLs and a greater understanding of their significance and prevalence by (Khan, S., et al., 2022).

Objectives:

The purpose of this research was to identify P. aeruginosa isolates from patient urine that included genes for the production of class A ESBLs PER-1, VEB-1, and PSE-1.

Materials and Methods:

Bacterial isolates: In the mid-year of 2021 and early in 2022, a total of 50 clinical isolates of P. aeruginosa were obtained from urine samples at several hospitals. These isolates were identified by catalase and oxidase tests, hydrogen sulphide (H2S), indol, motility (SIM medium), triple sugar iron agar (TSI), and methyl red

(MR), as well as urease, oxidative/fermentative (OF), and Macconkey agar blood agar by (Kohlenberg A, et al., 2010).

Antibiotic susceptibility pattern: Antibiotic susceptibility was determined using the CLSI method. Three Families of Antibiotics Used in his Study Beta-lactams, µg, Fluoroquinolones, µg Aminoglycosides, µg. The antibiotic discs used were: aztreonam (30), augmenten (30), amikaciin (30), piperacellin (100), ofloxaccin (5), ceftriaxne (30), cefperazone (7), cefotaaxime (30), cefpoodoxime (10), ciprrofloxacin (5), carbenicilin (100), levofloxacin (5), meropenem (10), netilmicin (30), Amikacin (30), tobrramycin (10), genatamicin (120). Azatreonam (30), pipaeracillin (100), carabenicillin (100), meropenm (10), neitilmicin (30), ticaarcillin (75), pipearacillin-tazoobactam (110), cefooperazone (75), imiipenem (10), ceftriaaxone (30), ceftaziidime (30) and cefepeime (30) Ofloxaacin (5) by (Kumar et al., 2012).

ESBL producer phenotyping

The isolates that contain ESBLs were identified using the combine disc test. Therefore in specific experiment, the ceftaazidime and cefoataxime discs, either with or without clavulaniic acid, were used. Together with the presence of clavulanic acid, a difference of more than 5 mm between these discs was judged to be positive by (Davodian et al., 2015).

Extraction of DNA

After an overnight incubation at 37 ° C. in 10 ml of Luriea Berrtani (LB) broth medium for each bacterial isolate, the tubes were centrifuged for 10 min at a speed of 4000 rpm. The precipitate that was obtained was then re-suspended in sterile water and DNA was extracted from it In addition, the method of isolating DNA included both the step of boiling the sample as well as the utilisation of a DNA Extraction kit (DIAtom DNA Prep 100) by (Momenah et al., 2023).

Amplification of polymerase chain reaction (PCR).

Table 1 shows the primers used for blaPER-1, blaVEB-1, and blaPSE-1 ESBL-encoding gene PCR.Gene reaction mixture: 10X PCR buffer, dNTP (10 Mm), MgCl2 (50 mM), forward and reverse primer (100 µM), template (DNA), Taq DNA polymerase (5 U/µL), and nuclease-free H2O total 14.05 µL by (Nojoomi F, et al., 2016).

Statistical Analysis

The Student's t-test was used for analysis, and P values < 0.05 were deemed significant SPSS 20 analyzed data.

Table 1. The primers that were used for this study

Primer	Sequence 3' to 5'	Product Size	Reference
blaPER-1	F: GTA TAA CTG TTA TTA ATT TCG R: CAT ATT GAC AAT AAT TAA AGC	927	10
blaPSE-1	F: CGG AGC GTC AGT TGT AAT R: GCC TCG CAG TCA ACA TTA	699	9
blaVEB-1	F: AGG CAA AGC AAA GGG ACG GG R: TCC GTT TCG TTT CCC TGC CC	624	9,1

Results:

P. aeruginosa wound isolates were obtained from six hospitals in Peshawar, Pakistan (IKD), (LRH), (KTH), (HMC), and (BPSC).These isolates were identified using standard biochemical testing .Among the total 50 isolates, 70.1% (n=30) were obtained from urine samples , 15.9% (n=20) from wound , and 15.8% from other clinical sites. 60% percent (n=30) of the patients were men, while 40% (n=20) were women. Evaluation of the patient's resistance to antibiotics. The majority of the isolates showed resistant characteristics by (Saderi H, et al., 2008). As shown in table no 2.

Table 2: The Extended Spectrum B-Lactamases Antibiotic Susceptibility Test Pattern for Positive and Negative Urine *Pseudomonas aeruginosa* Isolates

Disks/Isolates	ESBL-Positive (Resistance %), n = 37	**ESBL-Negative (Resistance %), n = 13**
Augmeentin	98	**95**
Cefepiime	94	**71**
Ceftaziidime	90	**69**
Cefpodoxiime	86	**60**
Carbeniciillin	89	**68**
Ceftriiaxone	96	**70**
Piperaacillin	87	**50**
Aztreeonam	89	**66**
Cefopeerazone	90	**50**
Cefotaaxime	99	**70**
Ticarciillin	65	**60**
Imipeneem	25	**20**
Meropeenem	26	**21**
Ciproflooxacin	94	**69**
Levoflooxacin	82	**49**
Ofloxaacin	80	**59**
Netilimicin	70	**49**
Amikacin	86	**47**
Gentamicin	72	**47**
Tobramycin	72	**49**
Piperacillin	**60**	**40**

Detection phenotypically of ESBL producers A vast number of P. aeruginosa isolates, 50 (70%) tested positive for ESBL production (Table 2). Urine infections represented 70 % (n=30) of the ESBL-positive isolates by (Tasleem, S., et al., 2022) , followed by wound infections (15 %; n=11), and blood infections (15 %; n=9).

Gene detection for VEB-1, PER-1, and PSE-1 94% (n=47) of ESBL-positive isolates had blaPER-1, 53% (n=27) had blaVEB-1, and 15% (n=9) had blaPSE-1 (Fig 1, 2 and 3), 7 isolates amplified all three genes by (Tavajjohi Z, et al., 2011). While 10 isolates had blaPER-1 and blaVEB1 together (Table 3). Ceftazidime, ceftriaxone, cefpodoxime, and cefotaxime resistance was linked to these genes.

Table 3. Third-generation cephalosporin resistance and beta-lactamase genes in ESBL-positive isolates F: female, M: male, CTXE: cefotaxiime, CAZE: ceftaezidime, CROE: ceftraiaxone, CPME: ceafpodoxime

Isolate	Gender	3rd generation, cephaalosporin resistance	sets of genes
1	M	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1
2	M	CTXE, CAZE, CROE	BlaPER-1, blaVEB1, blaPSE1
3	F	CTXE, CAZE, CPME	BlaPER-1, blaVEB1
4	F	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
5	M	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
6	F	CTXE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
7	F	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
8	F	CTXE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
9	M	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1
10	F	CTXE, CAZE, CROE, CPME	BlaPER-1, blaVEB1, blaPSE1

Figure 1: PCR product of 720-bp blaVEB1 gene.

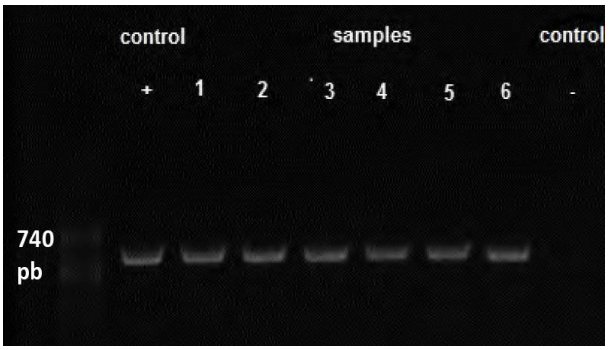


Figure 2: PCR product of 720-bp PER1 gene.

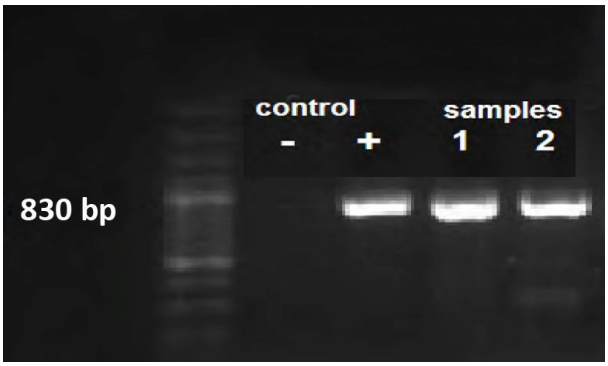
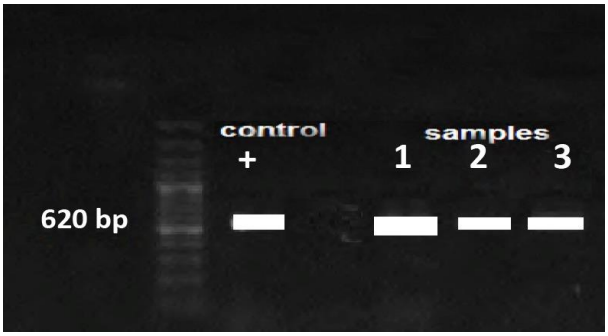


Fig: 3 PCR product of 620-bp PSE1 gene.



Discussion and Conclusion

Pseudomonas aeruginosa causes several nosocomial and opportunistic infections. Antibiotic resistance in P. aeruginosa isolates is both innate and acquired (either chromosomal or plasmid) by (Yusuf E, et al., 2017).All of the isolates used here were from patients with urinary tract infections. Our findings have been confirmed by many similar study. In most cases, resistant isolates were found in urine since that is where antibiotics are mostly eliminated from the body by (Davodian et al., 2015). Most of the isolates in this investigation showed resistance to the antibiotics augmentin/co-amoxiclav and cefpodoxime. Additionally, the majority of the patients showed resistance to cephalosporins of the third generation (aztreonam, ceftriaxone and cefotaxime) by (Tasleem, et al., 2022). Antibiotic resistance is a major problem, especially since these drugs are so effective against gram-negative rods. P. aeruginosa contains around 0.3% of all genes, and these genes are responsible for antibiotic resistance. Similar to previous studies, 80% of urine isolates produced ESBL by (Khan. et al., 2022). Clavulanic acid susceptibility diagnoses. AmpC and other enzymes that resist clavulanic acid are probably involved. In this research, 96% and 55% of ESBL producer amplified blaPER1 and blaVEB1, implicating additional enzymes such Amp-C and MBLs or efflux pump interference. In the research by (Davodian et al., 2015) 100% of ESBL-positive isolates had blaVEB-1 and 68.3% had blaPER-1 (18). In another study, 5.9% of imipenem-resistant isolates had blaPER-1 by (Khan et al., 2022) found that 90% and 88% of wound isolates were resistant to augmmentin and cefpoedoxime, with 40% blaVEB1 positive (15). An overwhelming majority of the P. aeruginosa isolates obtained from urine sections were ESBL producers by (Davodian et al., 2015). Although blaVEB1 and blaPER1 were found in a very high percentage of the isolates, blaPSE1 was only found in a small fraction of the samples. A critical role in the development of highly resistant bacteria is played by variables such as continuous and prolonged antibiotic use, hospitalizations, and incorrect use by (Ghasemian et al., 2018). Combination therapy, often consisting of a beta-lactam and an aminoglycoside, is essential for the treatment of Pseudomonas infections.

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