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MINIMUM INHIBITORY CONCENTRATION OF CEFTAZIDIME AVIBACTAM IN EXTENDED SPECTRUM BETA LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA

Syeda Fatima Nadeem[1], Ahmad Saqib[2], Manahil Rai[3], Muhammad Bilal[4], Zainab Yousaf[*5], Fareeha Imran[6]

[1]MPhil (Microbiology), B.S.C (Hons) MLT, Medical Lab Technologist, Sheikh Zayed Medical College/ Hospital, Rahim Yar Khan, Pakistan
[2]MBBS (2nd Year), Shalamar Medical and Dental College, Lahore, Pakistan
[3]MBBS (1st Year), Al Aleem Medical College, Lahore, Pakistan
[4]B.S MLT, Department of Biological Sciences, Superior University, Lahore, Pakistan
*[*5]MPhil (Human genetics & Molecular biology), B.S.C (Hons) MLT, Lab Manager, Department of Pathology, Farooq Hospital Westwood, Lahore, Pakistan*
[6]MBBS, Senior demonstrator, Post Graduate Medical Institute, Lahore, Pakistan

[1]fatimanadeem291@gmail.com, [2]ahmadsaqib2014@outlook.com, [3]manahilrai1506@gmail.com, [4]mlt2017011@gmail.com, [*5]zainabyousaf00@gmail.com, [6]fareehaimran536@yahoo.com,

[1]0000-0002-8490-583X

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Corresponding Author: * **Zainab Yousaf**

Abstract

Background: *Antibiotic resistance has emerged as a great challenge for clinicians and researchers across the globe. Pseudomonas aeruginosa can quickly develop resistance to commonly used antimicrobial agents. In this situation, combination antibiotic plays significant role in treatment. Ceftazidime/avibactam, an emerging combination drug is particularly useful for treating gram negative infections.*

Methodology: *This cross-sectional study was conducted from November 2024 to April 2025. This study enrolled seventy patients having infection and both males and females of all age groups. The different clinical samples were collected from patients. Pseudomonas aeruginosa was obtained from the clinical specimens from patients presented to the tertiary care hospital. The ceftazidime-resistant strains were screened for extended spectrum beta lactamase using the Calibrated Dichotomous Sensitivity Test. The activity of ceftazidime/avibactam was assessed using the disc diffusion (30/20 µg) method. For the detection of minimum inhibitory concentration, ceftazidime/avibactam E strips (Liofilchem MTS) was used. The collected data was entered in a excel sheet and analyzed by the Statistical Package for the Social Sciences version 27.0 software.*

Results: *From all (n=70) patients, 42 (60.0%) were males and 28 (40.0%) were females. Most Pseudomonas aeruginosa were isolated from the medicine ward (n=31, 44.28%), and from urine samples (n=40, 57.14%). Among all Pseudomonas aeruginosa, 44 (62.85%) showed positive results for ESBL*

production while 26 (37.15%) showed no production of ESBL enzyme. Among all ESBL producing Pseudomonas aeruginosa, 41 (93.18%) isolates were sensitive, while, 03 (6.81%) were resistant to ceftazidime/avibactam. According to MIC, 41 (93.18%) extended spectrum beta lactamase producing Pseudomonas aeruginosa were sensitive, while, 03 (6.81%) were resistant to ceftazidime/avibactam.

Conclusion: *The observed minimum inhibitory concentration demonstrate that ceftazidime/avibactam retains significant in vitro activity against extended spectrum beta lactamase producing Pseudomonas aeruginosa, suggesting as a valuable therapeutic option.*

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a gram negative bacterium and well known for its pathogenicity and adaptability. It is a versatile and opportunistic pathogen that can cause a wide range of infections in humans, particularly in individuals with compromised immune systems or underlying medical conditions (1). P. aeruginosa is commonly found in various environments, and can thrive in diverse conditions and is often resistant to many environmental stresses, such as extreme temperatures and low nutrient levels (2). P. aeruginosa is a highly virulent bacterium and attributed to various factors, including toxins, enzymes, and structural components that help the bacterium evade the host immune system and cause damage to host tissues (3, 4).

P. aeruginosa is notorious for its ability to develop antibiotic resistance through various mechanisms (5). These mechanisms can be intrinsic or acquired through mutations or horizontal gene transfer. Understanding these mechanisms is essential for addressing antibiotic-resistant P. aeruginosa infections effectively. The efflux pump systems of P. aeruginosa actively remove antibiotics from the bacterial cell before they have a chance to do their damage (6). Beta-lactamase enzymes produced by P. aeruginosa can hydrolyze and inactivate beta-lactam antibiotics, such as penicillins and cephalosporins. There are multiple types of beta-lactamases produced by P. aeruginosa, including ESBLs and carbapenemases (7).

P. aeruginosa has the ability to alter antibiotic target locations. It might change the target of beta-lactam antibiotics, penicillin-binding proteins, making them less vulnerable to the medications. Certain P. aeruginosa strains are able to create enzymes that directly break down antibiotics, making them useless. Aminoglycoside-modifying enzymes have the ability to alter and make aminoglycoside antibiotics inactive. Antibiotic resistance in P. aeruginosa can arise from spontaneous DNA alterations (8). Over time, exposure to antibiotics can select for resistant mutants, leading to the development of antibiotic-resistant strains. It can acquire antibiotic resistance genes from other bacteria through processes like plasmid transfer, transduction, or conjugation. This horizontal gene transfer allows the bacterium to gain new resistance mechanisms (9). P. aeruginosa infections can be challenging to treat, particularly when the bacterium is resistant to multiple classes of antibiotics (5). Effective management often requires a combination of antimicrobial therapy, infection control measures, and understanding of resistance mechanisms is necessary.

Ceftazidime/avibactam (CZA) is a combination antibiotic medication used to treat serious bacterial infections, particularly caused by gram-negative bacteria. It is considered a combination product because it contains two active ingredients: ceftazidime and avibactam. Ceftazidime is an antibiotic belonging to the third generation of cephalosporins. Avibactam inhibits beta-lactamases. It does not have inherent antibacterial activity but is included in the combination to inhibit the action of certain beta-lactamases, including some extended spectrum beta lactamase (ESBL) and class C beta-lactamase (AmpC beta-lactamases). By inhibiting these enzymes, avibactam helps ceftazidime remain effective against beta-lactamase producing bacteria (10). CZA is particularly useful for treating infections caused by gram negative bacteria, including ESBL producing P. aeruginosa (11). The present study aimed to evaluate the minimum inhibitory concentration (MIC) of CZA in ESBL producing P. aeruginosa.

Materials & Methodology

This cross-sectional study was conducted from November 2024 to April 2025. This study enrolled seventy patients having infection and both males and females of all age groups. A performa was designed to collect the data. After obtaining verbal informed consent, different clinical samples were collected from patients. P. aeruginosa was recovered from the clinical specimens from patients presented to the tertiary care hospital. Specimens other than urine were inoculated on blood agar and MacConkey agar while urine was inoculated via a sterile calibrated loop on CLED (cysteine lactose electrolyte deficient agar) and incubated for 24 hours. Plates were incubated at 37°C for 48 hours. The P. aeruginosa were identified on the basis of its morphology (typically round with a fluorescent greenish color, and often produces a distinctive odor), gram negative rods on gram stain, and citrate positive test.

For antimicrobial sensitivity testing, the isolates were spread on Mueller hinton (MH) agar (Oxoid UK) using the standard Kirby-Bauer Disk Diffusion technique in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. The zones of inhibition were interpreted based on the breakpoints. The ceftazidime-resistant strains were screened for ESBL using the Calibrated Dichotomous Sensitivity Test (CDST). The CDST test was performed using plain ceftazidime disk (30 µg) and ceftazidime/clavulanic acid (30/10 µg) and placing other third generation cephalosporins such as cefotaxime, ceftriaxone, ceftazidime, and cefepime at 20 millimeters (mm) distance from ceftazidime/clavulanic acid disk. Strains with a synergistic zone of more than 05 mm were shown to develop ESBL. The activity of CZA was assessed using the disc diffusion (30/20 µg) method. The zone of inhibition of ≥21 mm was considered as sensitive, whereas ≤20 was considered as resistant. For the MIC detection of CZA in ESBL producing P. aeruginosa, CZA E strips (Liofilchem MTS) was used. The zone of inhibition of >21 mm was considered as sensitive, whereas < 8/4 was considered as intermediate, and >16/4 mm was considered as resistant. The collected data was entered in a excel sheet and analyzed by the Statistical Package for the Social Sciences (IBM SPSS) version 27.0 software. Descriptive statistical analysis was done. The frequencies mean and percentages of study variables were calculated.

Results

From all (n=70) patients, 42 (60.0%) were males and 28 (40.0%) were females. Most P. aeruginosa were obtained from the medicine ward (n=31, 44.28%), followed by the surgery ward (n=19, 27.14%), intensive care unit (n=7, 10.0%), urology (n=7, 10.0%), nephrology (n=3, 4.28%), and peads ward (n=3, 4.28%) respectively. Most P. aeruginosa were isolated from urine samples (n=40, 57.14%), followed by pus (n=9, 12.85%), stool (n=7, 10.0%), bronchial lavage (n= 5, 7.14%), peritoneal fluid (n=3, 4.28%), blood culture (n=3, 4.28%), and bronchial secretion (n=3, 4.82%) respectively. Among all P. aeruginosa, 44 (62.85%) showed positive results for ESBL production while 26 (37.15%) showed no production of ESBL enzyme (Figure 1).

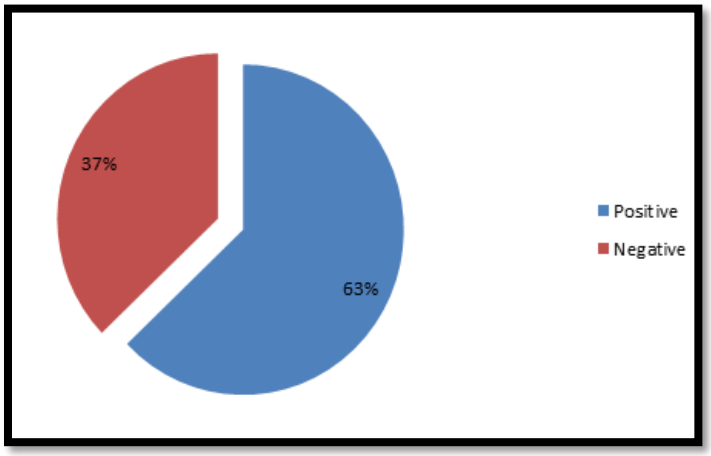

Figure 1: Percentage of ESBL producing Pseudomonas aeruginosa

The ESBL producing P. aeruginosa (n=44) showed highest sensitivity toward 3rd and 4th generation cephalosporins 65.9% (ceftazidime and cefepime) followed by imipenem/meropenem 63.6% later gentamicin and amikacin 56.8%. P. aeruginosa shows highest resistance towards piperacillin (100%), ceftriaxone (77.3%) and ciprofloxacin (63.6%) (Table 1).

Table 1: Antibiotics sensitivity pattern of ESBL producing Pseudomonas aeruginosa

Antibiotics	Resistant Frequency (%)	Sensitive Frequency (%)	Intermediate Frequency (%)
Piperacillin	44 (100%)	0 (0%)	0 (0%)
Piperacillin/tazobactam	21 (47.7%)	23 (52.3%)	0 (0%)
Ceftazidime	15 (34.1%)	29 (65.9%)	0 (0%)
Ceftazidime/Clavulanic acid	30 (68.2%)	14 (31.8%)	0 (0%)
Ceftriaxone	34 (77.3%)	6 (13.6%)	4 (9.1%)
Cefepime	15 (34.1%)	29 (65.9%)	0 (0%)
Aztreonam	21 (47.7%)	14 (31.8%)	9 (20.5%)
Imipenem/meropenem	16 (36.4%)	28 (63.6%)	0 (0%)
Gentamicin	19 (43.2%)	25 (56.8%)	0 (0%)
Amikacin	20 (45.5%)	24 (54.5%)	0 (0%)
Ciprofloxacin	28 (63.6%)	15 (34.1%)	1 (2.3%)

The antimicrobial susceptibility (disc method) of CZA was assessed against ESBL producing P. aeruginosa. Among all ESBL producing P. aeruginosa 41 (93.18%) isolates were sensitive, while, 03 (6.81%) were resistant to this combination drug (Table 2). The antimicrobial susceptibility (MIC method) of CZA was also assessed against ESBL producing P. aeruginosa. According to MIC, 41 (93.18%) ESBL producing P. aeruginosa were sensitive, while, 03 (6.81%) were resistant to this combination drug (Table 2).

Table 2: Susceptibility pattern of ceftazidime/avibactum through disc and minimum inhibitory concentration methods

Ceftazidime/avibactum	Disc method Frequency (%)	Minimum inhibitory concentration Frequency (%)
Sensitive	41 (93.18%)	41 (93.18%)
Resistant	03 (6.81%)	03 (6.81%)

Discussion

Global morbidity and mortality rates have significantly increased as a result of the development of multi-drug resistant P. aeruginosa, therefore new formulation of antibiotics for clinical application are urgently needed (12). In present study, P. aeruginosa were isolated from different clinical samples. Out of 70 samples, 57.14% P. aeruginosa were isolated from urine followed by blood cultures. A study conducted in Pakistan, showed that the P. aeruginosa were isolated form burns, wounds, blood infections, urinary tract infections, and pulmonary diseases. Further observation revealed that 36.3% P. aeruginosa were MDR (13).

In present study, resistance profile of P. aeruginosa showed highest sensitivity towards third and fourth generation cephalosporins 65.9% (ceftazidime and cefepime) followed by imipenem/meropenem (63.6%) later gentamicin and amikacin (56.8%). P. aeruginosa shows highest resistance towards piperacillin (100%), ceftriaxone (77.3%) and ciprofloxacin (63.6%). According to a study, the highest prevalence rates of MDR were identified in MRSA (86.6%), A. baumannii (36.8%), P. aeruginosa

(29.1%), and Klebsiella pneumoniae (24.4%) (14). In present study, 44 (62.85%) P. aeruginosa showed positive results for ESBL production while 26 (37.15%) showed no production of ESBL enzyme. According to Chakraborty et al., non-fermenting gram negative rods accounted for 37% of the gram negative isolates. MDR was found in 66.9% of P. aeruginosa. Phenotypic detection revealed the presence of ESBLs was found in 21.4%, of isolates (15).

The present study determined that the CZA was highly effective against ESBL producing P. aeruginosa. According to disc and MIC method of susceptibility testing it was found that the 41 (93.18%) ESBL producing P. aeruginosa were sensitive, while, 03 (6.81%) were resistant to CZA. This antibiotic showed promising results against gram negative infections. In a study conducted on Enterobacteriaceae, out of 386 isolates 54 isolates were ESBLs negative, 104 were ESBLs positive and 228 were carbapenem resistant Enterobacteriaceae. 287 isolates were susceptible to CZA and 99 isolates were resistant. CZA resistant isolates were mostly carbapenem resistant Enterobacteriaceae (16).

Carbapenemase enzyme production is becoming more common in non-fermenting gram negative rods because of frequent use of carbapenem antibiotics. So need of the hour is to limit its use and make it important to do susceptibility testing before prescribing antibiotics. In order to prevent carbapenem use we have to use other combinations drugs which can inhibit beta lactamases enzymes. CZA is combination drug which inhibit the action of beta lactamases and class C beta lactamases. Present study conducted on P. aeruginosa showed promising results of CZA on ESBL positive isolates making it's a good option for ESBL producing P. aeruginosa. Use of this drug would help clinicians to limit carbapenem use and thus preventing carbapenem resistance genes. Therefore, further studies are suggested to conduct on larger sample size and by using advanced methodology to validate these preliminary results.

Conclusion

The present study highlights the effectiveness of CZA against ESBL producing P. aeruginosa isolates. The observed MIC demonstrate that CZA retains significant in vitro activity against the resistant strains, suggesting its potential as a valuable therapeutic option in the management of infections caused by multidrug-resistant P. aeruginosa.

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