Original Article

EMERGENCE OF CEFTAZIDIME-AVIBACTAM RESISTANCE IN *ENTERO-BACTERALES* AND *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objective: The objective of this study is to determine the emergence of resistance to Ceftazidime-Avibactam (CAZ-AVI) by *Enterobacterales* and *Pseudomonas aeruginosa* in clinical isolates.

Material and Methods: In this cross-sectional study (6-months) March-August 2022 Carbapenem resistant *Enterobacterales* were tested for Ceftazidime-Avibactam (30/20 µg, Oxoid Pvt Ltd) using Disk diffusion technique and enzymes were identified in resistant strains by Carbapenem Inactivation Method (mCIM, eCIM) as per CLSI M100 Guidelines 2022.

Results: CAZ-AVI effectiveness has greatly decreased among *Enterobacterales* and *P. aeruginosa* isolates in recent past. Antimicrobial susceptibility testing results were interpreted using CLSI M100 document. Resistance against CAZ-AVI in *Enterobacterales* was found to be 80.8 % in *E. coli* and 72.1% in *Klebsiella pneumonia* isolates. This higher emergence is associated with CRE isolates majorly comprising MBLs in our country. Moreover, it has been observed that Metallo- β-lactamases mediated enzyme resistance is one of the major resistance patterns followed by serine carbapenemases.

Conclusion: The high frequency of resistance 77% was observed against CAZ-AVI in CRE and in CRPA, the resistance is 80.1% respectively. In our country this tremendously increase in CAZ-AVI resistance is attributed to the existence of NDM in the region.

Keywords: Carbapenem Resistant *Enterobacterales* (CRE), Metallo beta lactamases (MBLs), Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA) Ceftazidime-Avibactam (CAZ-AVI), Modified Carbapenem Inactivation Method(mCIM), EDTA-modified Carbapenem Inactivation Method(eCIM), Multiple Drug Resistance (MDR)

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INTRODUCTION

Over the last few years, the prompt spread of Carbapenem resistant *Enterobacterales* has tremendously affected universal public health. Despite being a part of Human Normal flora, *Enterobacterales* can become resistant to carbapenems by acquiring certain resistant mechanisms like production of carbapenemases, cell permeability changes/ expression of efflux pumps, chemical modification of antibiotic target, and also by mobile genes on plasmids that could spread through bacterial populations [1].

Ceftazidime (CAZ) being a third-generation cephalosporin has extensive activity (cell-wall synthesis inhibitor) against gram negative bacilli. Ceftazidime-avibactam (CAZ-AVI), FDA approved (2015) is a newly combined 3rd generation cephalosporin with beta lactamase inhibitor being clinically available for only last few years. But resistance to CAZ-AVI is being observed as a serious concern. As per Global Surveillance program (Latin America) CRE isolates, 24.4% (139/570) had MBLs [2]. IDSA Guidelines (2022) recommends Ceftazidime-avibactam for infections due to CRE, while Aztreonam combined with CAZ-AVI for MBL producing CRE or Cefiderocol as a monotherapy. Knowledge about CRE isolate with specific carbapenemase production is of utmost importance towards guiding treatment decisions [3].

Avibactam (AVI) increases the antibacterial activity of CAZ against AmpC-, Extended spectrum beta lactamases (ESBL) and CRE [4]. CRE/ CRPA Spread is a crucial nosocomial issue, because only few antimicrobial agents are susceptible. Also, not many new drugs are under research to treat these pathogens. A recently published study (Pakistan, 2023) demonstrated the prevalence of CR to be 42.1% (913) in 2170 clinical isolates. The 82.2% of CR isolates were found to have Carbapenemases enzymes: NDM-1(41.1%), OXA-48(32.6%), KPC-2(5.5%), NDM-1/oxa-48(11.4%) respectively [5].

Recent increase in CAZ-AVI resistance particularly in our region, is possibly due to the presence of metallo- β-lactamases as their activity is not inhibited by avibactam [6,7]. High emergence of resistance emphasizes strict infection control to prevent spread of these organisms [8].

The main purpose of our study is to determine the emergence of resistance to Ceftazidime-Avibactam (CAZ-AVI) by *Enterobacterales* and *Pseudomonas aeruginosa* in clinical isolates.

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MATERIAL AND METHODS

A cross-sectional study of 6 months duration (March-August 2022) was done after getting IRB approval reference No CIP/IRB/1100. This study was carried out to determine the frequency of Ceftazidime-Avibactam resistance in *Enterobacterales* and *Pseudomonas aeruginosa* in Microbiology Department, Chughtai Institute of Pathology, Lahore Pakistan. Sampling was done by non-probability convenience sampling technique.

Enterobacterales and *Pseudomonas aeruginosa* isolates determined to be carbapenem resistant by standard disk diffusion method were included. All duplicate isolates of patients with Gram negative rods within one month time period were excluded.

From a total of 14,457 isolates (*Enterobacterales* and *P. aeruginosa*) identified from patient samples, 1882 were found Carbapenem resistant on routine antimicrobial susceptibility testing (AST) following the CLSI Guidelines 2022.All of the Carbapenem resistant isolates (CRE, CRPA) were further tested for CAZ-AVI Susceptibility by standard Kirby-Bauer disk diffusion method. The interpretation was made according to the given breakpoints in CLSI (For *Enterobacterales* and *P. aeruginosa*, zone diameters of inhibition S: ≥21 mm and R: ≤20 mm using 30/20 µg a disk content of CAZ-AVI) and compared with *E. coli* ATCC 35218. These plates were incubated at 37C for18-24 hours and zone of inhibition was measured. Isolates with borderline inhibitory zones (18-20) were not included in the study (requires confirmatory testing) as per CLSI Guidelines. Only 1 isolate was available of both *Citrobacter spp, Providencia spp. hence,* removed from the analysis.

CRE and CRPA isolates were then tested for enzyme identification (type of beta lactamase) through carbapenem inactivation method (CIM) where mCIM detects Carbapenemases in *Enterobacterales* and *P.aeruginosa* and eCIM together with mCIM differentiates metallo-beta lactamases from serine carbapenemases in *Enterobacterales*. Inhibitory zones of inhibition mCIM, eCIM were interpreted on plates inoculated Meropenem-susceptible indicator strain *E. coli* ATCC 25922 as mentioned in CLSI 2022 (Figure-I).

Figure-I: Identifying Carbapenemases by Carbapenemase Inactivation Method (CIM).

Final identification of the clinical isolates was done by the VITEK MS system using direct deposit from bacterial colonies in agreement with manufacturer's guidelines [9].

RESULTS

Out of the Total 1445 Carbapenem resistant *Enterobacterales (CRE)* isolates,1111 were 76.89% CAZ-AVI resistant (Figure-II). Total 780 isolates of *Klebsiella pneumoniae* were tested against CZA-AVI and 562(72.1%) were found to be resistant. Whereas resistance frequency in *Escherichia coli* 80.8%, *Enterobacter spp.* 86.8%, *Serratia marcescens* 90.6% was observed (Table-I). Allocation of the tested isolates based on gender (female 42.3%, male 57.7%) and specimen type is shown in the (Table-II).

Total 437 P. aeruginosa isolates were tested for Carbapenem (Meropenem)in which 350/437 were found resistant to CAZ-AVI with overall resistance rate of 80.1%.

Out of CAZ-AVI resistant isolates 100 were tested by mCIM and eCIM for determination of beta lactamases as per (CLSI M100 Guidelines). Among *Enterobacterales*, *Klebsiella pneumoniae* showed 93.3% MBL based resistance followed by 53.3% in E. coli, whereas Serine Carbapenemases related resistance was identified as 14.6% in E.coli isolates (Figure-III). In P. aeruginosa isolates resistance due to Metallo beta lactamases were 62.5% and Serine Carbapenemases 37.5% respectively while 31.7% of the E. coli isolates tested inconclusive for Carbapenemase detection.

Table-I: *CRE* isolates and *Pseudomonas aeruginosa* showing CAZ-AVI resistance percentage.

Group	Organism	n	CAZ-AVI Resistance (%)
Enterobacterales	*Enterobacter spp.*	53	86.8%
	Escherichia coli	527	80.8%
	Klebsiella pneumoniae	780	72.1%
	Serratia marcescens	85	90.6%
	Total	**1445**	**77%**
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	437	80.1%
	Total	**437**	**80.1%**

Table-II Showing gender, specimen-based distribution of the *CRE* isolates.

Specimen Type	Female	Male	Grand Total
Enterobacter spp.			
Blood Culture	8	16	24
Wound/PUS/Fluid	10	19	29
Escherichia coli			
Blood Culture	16	18	34
Urine C/S	190	140	330
Wound/PUS/Fluid	72	91	163
Klebsiella pneumoniae			
Blood Culture	40	80	120
NTX	7	22	29
Urine C/S	169	153	322
Wound/PUS/Fluid	122	187	309
Serratia marcescens			
Blood Culture	22	49	71
Wound/PUS/Fluid	8	6	14
Pseudomonas aeruginosa			
Blood Culture	2	8	10
Respiratory Samples	5	9	14
Urine C/S	48	151	199
Wound/PUS/Fluid	78	136	214
Grand Total	**797(42.3%)**	**1085(57.7%)**	**1882**

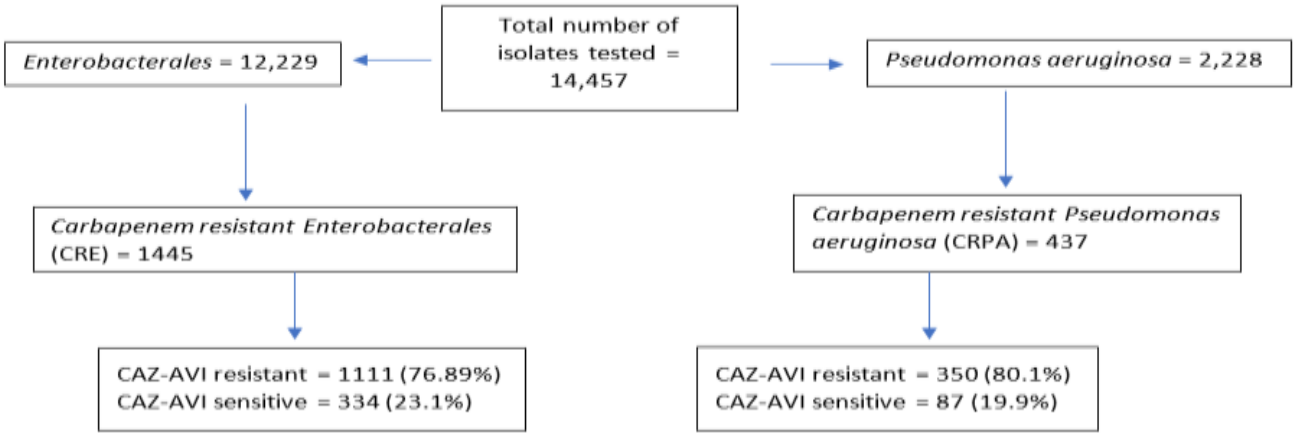

Figure-II: Flow chart showing categorization of isolates.

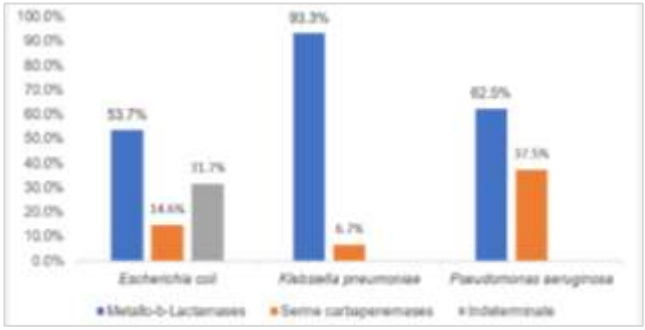

Figure-III: Enzyme based resistance (Carbapenemases) in *Enterobacterales* and *P. aeruginosa.*

DISCUSSION

Extensive drug resistance has been reported in past few decades with increased morbidity and mortality rates (CDC 2019) [10]. This study compiles the brief description of CAZ-AVI resistance in various sample. Avibactam is a non-β-lactam, β-lactamase inhibitor that acts particularly against Ambler class A, C, D beta lactamases, moreover its addition to Ceftazidime improves activity against CRE and *P. aeruginosa* [11].

Commonly reported Carbapenemases from Pakistan include NDM-1, NDM-7, VIM, IMP [5,12], NDM-1, OXA-48 [13], NDM-1, KPC-2 [14]. Carbapenem-resistant *Enterobacterales* (CRE) are micro-organisms especially resistant to carbapenems, thus difficult to treat [15]. A similar study which was done in China (2020) 103 out of total 120 CRE isolates were found to have Carbapenemases and when further CAZ-AVI susceptibility testing done on these isolates, showed 25% resistance, and all of these resistant isolates were MBL producers [16].

Even though CAZ-AVI usage is recent in Pakistan, still in our study considerable resistance pattern has been observed in *Enterobacterales* (77%), *Pseudomonas aeruginosa* (80.1%) against it.

Globally in the past few years, Clinical use of CAZ-AVI had lowered the burden to a major extent brought by XDR and MDR Gram negative bacteria. An article published in 2016 reports CAZ-AVI resistance in 30.1% of *Enterobacterales,* and this was attributed to their extended use for relatively longer times in

critically ill patients, moreover it was also suggested to further validate its effectiveness in CRE and CRPA [17]. An year later, a study published (2017) in USA shows beta lactam resistant *Pseudomonas aeruginosa* to be less susceptible (50%) to CAZ-AVI, when compared to *Enterobacteriaceae* (99%), and thus suggested CAZ-AVI, a good treatment option for beta lactam resistant gram-negative bacteria, if used cautiously [18]. As per global surveillance program (INFORM 2015-17), CRE isolates showed reduced susceptibility to CAZ-AVI, among which *E. coli* and *P. aeruginosa* were the major ones [19].

In another study which was done as part of (INFORM) global surveillance program (2014 to 2016) reports 2.3% resistance to CAZ-AVI in Colistin resistant *Enterobacteriaceae* with (MIC50) 0.25 µg/mL, and MIC of 2 µg/mL (MIC Criteria per FDA), this emphasizes on the fact that resistance has tremendously increased in recent few years of CAZ-AVI use [20]. However, in Carbapenemase resistant (CR) strains some previously done reports show marked increase in CAZ-AVI resistance reaching up to 24.7% [20,21,22]. In India, a Surveillance study was conducted on in-vitro activity of CAZ-AVI and its comparators tested against CRE (2018-2019) which showed 49% resistance to CAZ-AVI among *Klebsiella pneumonia (CRE)* isolates, and 76% in *E. coli (CRE)* isolates and 68% of these *E. coli* isolates had NDM (New Delhi Metallo-beta lactamases), whereas 24% had OXA-48 [23]. This means high resistance rates have emerged due to NDM, since last 3-4 years in India. In another study done in Iran [2020] shows that P. aeruginosa isolates from UTI patients which were resistant to CAZ-AVI, were predominantly found to have Metallo beta lactamases (75%) [24].

A study in Taiwan (Liao *et al*.,2019) has also shown resistance rate of 21% to CAZ-AVI against carbapenem resistant *P. aeruginosa* isolates in ICU admitted patients, before the drugs were officially launched and used regularly [25]. According to a meta-analysis review published in China (Li *et al*.,2021), there is no markable difference associated with CAZ-AVI either used singly or in combination in post treatment patients with carbapenem resistant Gram-negative pathogens (26). On the other hand, a study in India published 2021 which focused on clinical outcome of ICU patients on CZA, have reported 21% mortality rate with CAZ-AVI when used in combination with azithromycin, polymyxin, Fosfomycin [27].

There are few limitations of this study. Firstly, we used standard disk diffusion method for susceptibility testing, as per CLSI Guidelines (2022) however MICs could be performed along with Standard disk diffusion testing for further validation and interpretation in future. Secondly molecular characterization to identify exact underlying resistance mechanism along with epidemiological surveillance needs to be considered.

CONCLUSION

This study shows an increased resistance to CAZ-AVI in CRE (77%) possibly due to MBLs and in CRPA (80.1%) through Serine Carbapenemases and AmpC. Shortage of health care facilities and common practice of self-medication are few of the root causes of emerging antimicrobial resistance in our country. Laws and SOPs are imperative to prioritize antibiotics usage and to prevent its future critical outcomes. In this era of peak antibiotics resistance this study suggests that effective Antimicrobial Stewardship programs, infection prevention and control established in health care systems, and national action plan implementation is the need of time. All of these collectively can play significant role in combatting this problem. A high quality, multicenter study with larger sample size, based on CAZ-AVI MIC testing is suggested to confirm our findings.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTION

Alina Mehwish: Substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work

Irim Iftikhar: Revision, final approval

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