Research Article

SUSCEPTIBILITY PATTERN OF CEFTAZIDIME-AVIBACTAM AGAINST MULTI DRUG RESISTANT GRAM-NEGATIVE RODS

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ABSTRACT:

This study was conducted to evaluate the susceptibility pattern of ceftazidime avibactam against multi drug resistant gram negative rods. This prospective study cross sectional study conducted in Microbiology Section of Dow Diagnostic Reference and Research Laboratory, Dow University of Health Sciences, Karachi, Pakistan. Identification of isolates was done in accordance with the standard bacteriological technique, and were distinguished based on gram staining, colony morphology and biochemical tests. Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar by Kirby-Bauer Disk Diffusion method in accordance with the Clinical Laboratory Standard Institute (CLSI) guidelines. Good sensitivity of Lactose fermenters (*Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter*) were observed against Ceftazidime avibactam. *Pseudomonas aeruginosa* exhibited 42% resistance in all clinical samples. *Proteus species* and *Serratia* have shown high resistance in our study. Our observations showed the persistence of high ceftazidime avibactam activity against pathogenic and multi drug resistant strains of Enterobacterales and Non lactose fermenting bacteria.

Keywords: Ceftazidime-avibactam, Multi drug resistant organisms (MDROs), Enterobacterales, Pseudomonas aeruginosa

INTRODUCTION

Multi drug resistant organisms are those in which there is a development of resistance to minimum two or more classes of antimicrobial drugs (1). Numerous elements contribute in the emergence of drug resistance such as frequent use of antimicrobials for minor infections and the lack of new antibiotics development (2). These multi-drug resistant bacteria considerably increase the mortality, morbidity and length of hospital stay which also increases the cost of treatment and pose unnecessary burden on healthcare system. Gram negative rods infections are one of the major causes of nosocomial infections. These organisms are more prone to develop resistance by up regulating and acquiring genes of resistance (3). Various mechanisms involve in the development of antimicrobial resistance in gram negative rods as they produce drug inactivating enzymes, reorganization of the drug targets, accession of target by pass mechanism, decreased cell permeability and quick elimination of the drugs from cell (4). Production of extended spectrum βlactamase enzymes and carbapenemase enzymes are among the most common drug resistance mechanisms. Carbapenem resistant Enterobacterales, multi-drug resistant Pseudomonas aeruginosa are the ultimate threat for humans and amongst the most prevalent organisms that cause nosocomial infection (5). There is a need of new antibiotics for the treatment of these multi-drug resistant organisms. One of the most wide-ranging antibiotics which demonstrate activity against multi drug resistant antibiotics is Ceftazidime-avibactam. Ceftazidime is the third generation, broad spectrum cephalosporin combines with the β-lacatamase inhibitor avibactum. Avibactam efficiently inhibit sclass A β-lactamases as well as TEM, SME, PER, CTX-M, KPC, SHV, GES, chromosomal class C that is Amp C, plasmid class C like FOX,DHA, MOX, CMY, LAT, ACC, class D including OXA-48 from Klebsiella pneumoniae and OXA-24, OXA-40 and OXA-69 from Acinetobactor baumanni (6). Ceftazidime-avibactam is broad spectrum showing extensive activity against Enterobacteriaceae, Pseudomonas aeruginosa and also used for empirical treatment of nosocomial infections (7).

Emergence of resistance against Ceftazidime avibactam were also reported in many cases against gram negative rods (8-10). The goal of our study is to evaluate the efficacy of ceftazidime avibactam against pathogens in different specimens which will help physicians in designing of empirical treatment against gram negative rods.

MATERIAL AND METHODS

This prospective study with a cross sectional design was conducted in Microbiology section of Dow Diagnostic Reference and Research Laboratory, Dow University of Health Sciences, Karachi, Pakistan. Samples including urine, pus, blood, sputum, tracheal aspirate, sterile body fluids received from all patients regardless of their age and gender. Written approval was taken from the institutional review board with reference no IRB-2649/DUHS/Approval/2022/1024.All cultures were performed in Department of Microbiology, DDRRL, DUHS, according to the protocols of Clinical Laboratory Standard Institute (CLSI).

Identification of isolates was done in accordance with the standard bacteriological techniques, and were distinguished based on gram staining, colony morphology and biochemical tests, such as oxidase, urease, citrate, indole, triple sugar iron tests for gram-negative isolates (11). Analytical Profile Index-20E (API20E) was further used to distinguish gram-negative rods (GNRs). Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar by Kirby-Bauer Disk Diffusion method in accordance with the CLSI guidelines (12). For gram-negative bacteria, Ampicillin (AMP) (10µg), Ciprofloxacin (5µg), Ceftriaxone (CRO) (30µg), Ceftazidime (CAZ) (30µg), Gentamicin (10µg), Tobramycin (10µg), Amikacin (30µg), Cotrimexazole (25µg), Azithromycin (AZT), Amoxicillin-clavulanic acid (AMC) (30/10µg), Tazobactum-piperacillin (TZP) (100/10µg), and Meropenem (30µg) were used (11). The susceptibility breakpoints were interpreted according to CLSI guidelines 2022. *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923) and *Pseudomonas aeruginosa* (ATCC27853) were used as quality control strains for culture and susceptibility testing(13).

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Statistical methods

The data was analyzed by using Statistical Package for Social Sciences (SPSS version22.0). Mean was calculated for continuous variables, while frequency and percentages were computed for categorical variables including microorganism, Ceftazidime avibactam susceptibility, gender and specimen type i.e. pus, blood, urine, sputum, tracheal aspirate and sterile body fluids.

RESULTS:

A total of 348 samples of Multi drug resistant gram-negative rods were studied. Among these isolates 186 were males and 162 were females. Mean age was 44.44 years, with the youngest one of 1 day and oldest of 104 years of age. Maximum numbers of multi drug resistant organisms were noticed in the ages of 41 to 60 years (Figure 1). In our study, 186 (53%) of multi drug resistant gram negative rods were isolated from blood followed by urine 136 (39%) while remaining were isolated from pus, tracheal aspirate, sputum, different fluid fluids and tissues (Figure 2).

Escherichia coli was the dominant pathogen followed by Klebsiella pneumonia, Enterobacter and Pseudomonas aeruginosa from clinical specimens. Majority of organisms were resistant to major classes of antibiotics like Beta lactam, Beta lactamase inhibitors, Aminoglycosides, Fluroquinolones, Cotrimoxazole and Colistin. In urine samples these organisms showed resistance against Fosfomycin and Nitrofurantoin. It was observed that these organisms have shown less resistance against Tigecycline and Minocycline. Further analysis of all antibiotic sensitivity pattern including ceftazidime avibactam for different organisms presented in Table 1.

Considerable sensitivity of Lactose fermenters (*Escherichia coli* , *Klebsiella pneumoniae* and *Enterobacter*) were observed against Ceftazidime avibactam. *Pseudomonas aeruginosa* exhibited 42% resistance in all clinical samples. *Proteus species* and *Serratia* has shown high resistance in our study. Further pattern of Ceftazidime avibactam susceptibility against MDROS in different clinical specimens was elaborated in Figure 3.

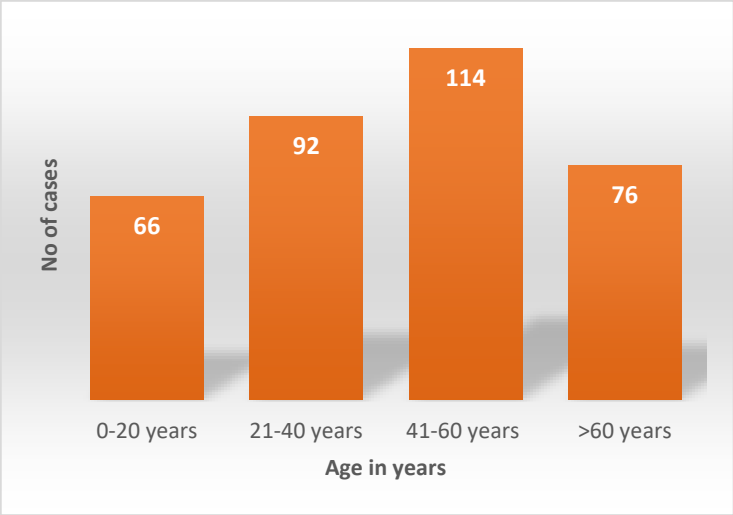

Figure 1. Age-wise distribution of specimen.

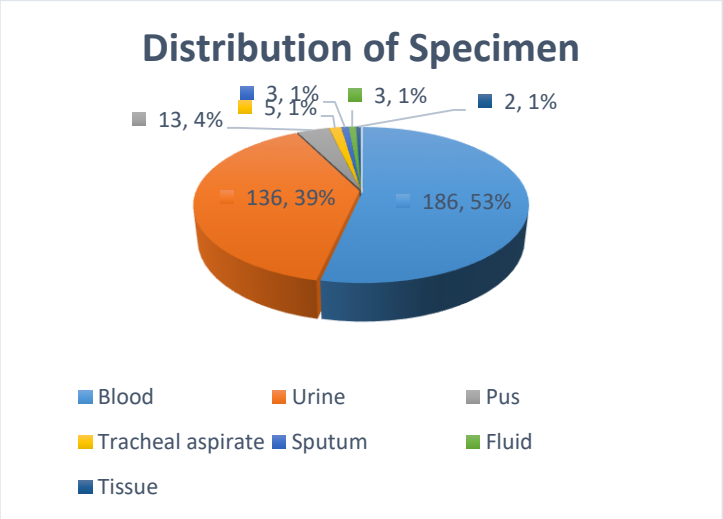

Figure 2. Distribution of specimen positive for MDROS

Table 1. Antibiotic resistance pattern of Multi drug resistant gram-negative rods

Org Name n=409 (100%)	AMP	AMC	CRO	CXM	CFM	CAZ	TZP	MEM	CT	CIP	TGC	SXT	MH	AK	CN	TOB	FOS	F	CZA
***Escherichia coli* = 159**																			
BLD=78	78	77	77	77	77		73	64	6	78	2	65	22	56	54	40	NT	NT	22
U=72	60	60	65	68	68		66	60	5	70	0	66	0	37	50	60	62	50	33
P=5	5	5	5	5	5	NT	4	5	0		3	5	0	4	3	4			2
FL=3	3	3	3	3	3		3	3	1	3	0	3	0	3	3	3	NT	NT	0
SPT=1	1	1	1	1	1		1	1	1	1	NT	1	NT	0	0	NT			1
***Klebsiella pneumoniae* = 77**																			
BLD=77		36	44	44	44		39	25	13	29	2	42	18	39	39	39	NT	NT	12
U=22		19	18	21	21		20	21	5	18	0	20	0	13	19	16	NT	17	8
P=5		2	5	5	5		5	5	0	5	1	5	0	3	4	5			1
SPT=1	NT	1	1	1	1	NT	1	1	0	1	NT	1	1	1	1	1	NT	NT	1
TA=3		3	3	3	3		3	3	0	8	0	3	1	3	3	0			2
TSU=2		2	2	2	2		2	2	3	3	0	3	1	2	2	0			0
***Enterobacter spp*=47**																			
BLD=40			40	40	40		31	34	20	36	0	35	20	32	40	31	NT	NT	9
U=3			3	3	3		3	3	0	3		3		3	3	3	NT	3	2
P= 1	NT	NT	1	1	1	NT	0	1	1	1	NT	1	NT	1	0	0			0
SPT=1			1	1	1		1	1	1	1		1	1	1	1	1	NT	NT	0
TA=2			2	2	2		3	2	2	2	0	2	0	2	2	0			0
***Pseudomonas aeruginosa*=45**																			
BLD=9						5	6	5	0	6				NT	NT	6	NT	NT	2
U=34	NT	NT	NT	NT	NT	19	23	18	0	24	NT	NT	NT	19	NT	26	NT	NT	16
P=2						1	2	2	0	2				NT	NT	2	NT	NT	1
***Klebsiella oxytoca* =10**																			
BLD=8	NT	6	8	8	8	NT	5	2	2	4	1	6	2	7	6	NT	NT	NT	1
U=2		2	2	2	2		1	2	0	2	0	2	NT	2	2	2	NT	2	2
***Serratia spp*= 6**																			
BLD=5	NT	NT	5	NT	5	NT	4	5	NT	1	0	1	0	5	5	5	NT	NT	3
U=1			1		1		0	0		1	0	1	NT	1	1	1	NT		0
***Proteus spp* = 5**																			
BLD=3	NT	NT	3	NT	3	NT	3	3	NT	3	NT	3	NT	0	0	0	NT	NT	2
U=2			2		2		1	2		2		2		2	2	2	NT		1

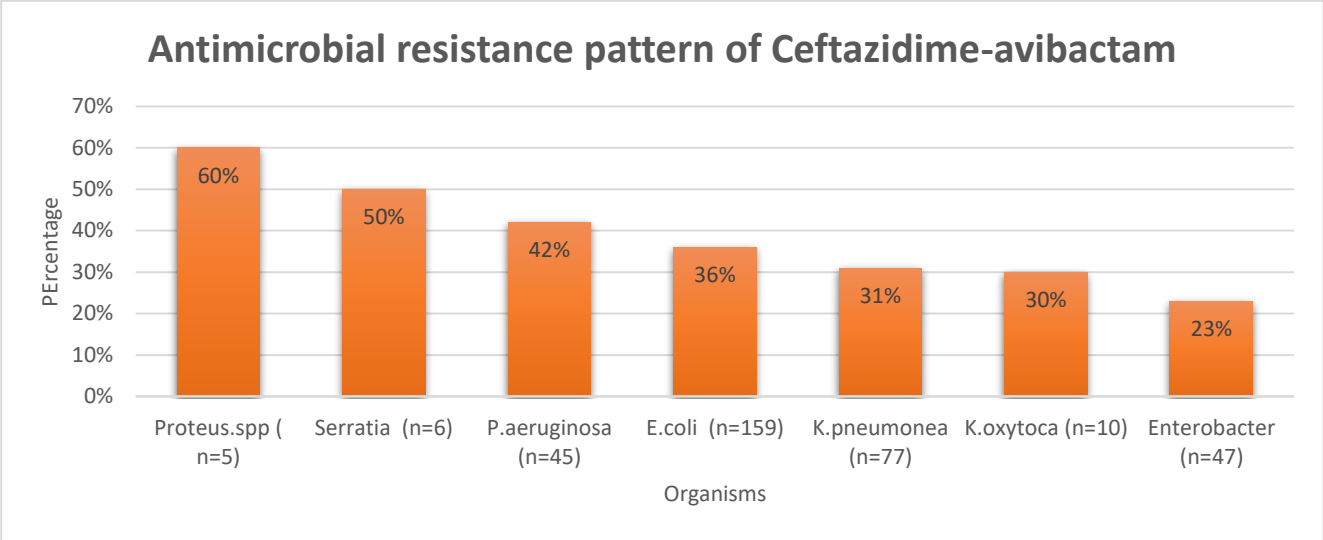

Figure 3 Antimicrobial Sensitivity Pattern of Ceftazidime-avibactam

DISCUSSION:

Infection due to resistant gram-negative bacteria is now emerging as a serious health concern globally, as they post a great challenge to healthcare providers while treating them and result in high rates of morbidity and mortality. Ceftazidime avibactam is approved recently to treat number of community and hospital acquired multi drug resistant organisms. In this study, we report antimicrobial susceptibility rates for ceftazidime avibactam and other commonly used antibiotics to a collection of clinical isolates of *Enterobacterales* and *Pseudomonas aeruginosa*.

In our study majority of patients were males and belonged to 41 to 60 years of age which might imitate variances in prior antimicrobial exposure or gender differences in acquiring healthcare facilities, fluctuate by both geographical and social influences, as well as income and literacy rate, also shown by Amanati A et al (14). Majority of drug resistant bacteria in our analysis were isolated from blood followed by urine, pus, sputum, body fluids and tissue, whereas Mshana, S. E. et al has high number of resistant bacteria from urine, wound swabs and blood (15).

Escherichia coli is the most frequently isolated organisms in all samples, also reported in many studies (11, 16). *Klebsiella pneumoniae, Enterobacter* and *Pseudomonas aeruginosa* were other common isolates which was also observed by Wu, X. et al and Yaseen, M et al (17, 18). We evaluated highest resistance against like Beta lactam, Beta lactam inhibitors, Aminoglycosides, Fluroquinolones, Cotrimoxazole and Polymyxins and in urine nitrofurantoin and Fosfomycin which is consistent with the analysis of Wangai, F. K. et al, Bitew, A. et al and Teklu, D. S. et al (19-21). A recent study stated that 28% of *Proteus mirabilis*, 56% of *Klebsiella pneumoniae,* and 78% of *Escherichia coli* isolates revealed resistance to Fluroquinolones also constant with our findings(21). In our study, Meropenem and Colistin showed resistance and susceptibility against *Pseudomonas aeruginosa*. In contrast, according to Prakash & Saxena, sparfloxacin and meropenem are the most resistant and susceptible drugs, respectively, in *Pseudomonas aeruginosa*(22). Our study shown reduce resistance against Tigecycline which is also observed in other studies also (23).

Our analysis reported high resistance of Ceftazidime avibactam in *Proteus species, Serratia spp*, *Pseudomonas aeruginosa* and other *Enterobacterial.* In contrast, Jia P et al showed high susceptibilities of ceftazidime avibactam against *Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa* (24). Ceftazidime avibactam attained the maximum activity against *E.coli* and *K. pneumoniae* isolates which were among the most abundant organisms about 53% and 42% of MDR in our analysis and the similar trend of high Ceftazidime avibactam susceptibility is maintained in the study by Kristof et al (6).

The study presents compressive data on an important health issue, with a considerable sample size, which is considered as strength of the disease.

CONCLUSION:

The current study grants important information to demonstrate and narrate with further similar studies to specify the current mode of antimicrobial susceptibility of multi drug resistant gram-negative rods and assist in determining the sensitivity pattern of ceftazidime avibactam. Our observations supports the persistence of high ceftazidime avibactam activity against pathogenic strains of *Enterobacterales* and *Pseudomonas aeruginosa*, including those carrying different types of antibiotic resistance.

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