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Original Research Article

Evaluation of Antimicrobial Susceptibility of Ceftazidime/ Avibactam and Ceftolozane/Tazobactam against Clinical Isolate of MDR *Pseudomonas Aeruginosa*

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Abstract:

Introduction: To investigate the *in vitro* activity of ceftazidime/avibactam and ceftolozane/tazobactam against clinical isolates of MDR *Pseudomonas aeruginosa*.

Materials and Methods: MDR *P. aeruginosa* isolated between Oct 2021 to Aug 2022 from Dept of microbiology, Hi tech medical college and hospital, Bhubaneswar ,were used for evaluation of ceftazidime/avibactam and ceftolozane/tazobactam susceptibility.

Results: Pseudomonas aeruginosa isolates (n=1,909) were isolated from various clinical samples and their susceptibilities were tested using the broth microdilution method. Ceftazidime-avibactam (MIC50/MIC90, 2/8 mg/liter) and ceftolozane-tazobactam (MIC50/MIC90,0.5/2mg/liter) were the most active (i.e., had the highest susceptibility rates) compounds after colistin, with national susceptibility rates of 96.9% and 97.5%, respectively.

Conclusions: MDR *P. aeruginosa* susceptibility rates to ceftazidime/avibactam and ceftolozane/tazobactam were higher than those to all existing anti pseudomonal agents, except colistin, but were less than 50% in extremely resistant isolates. Non-susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam was largely due to the production of ESBL and VIM enzymes. Ceftazidime/avibactam and ceftolozane/tazobactam are possible options for some patients with MDR *P. aeruginosa*

Keywords: Antibiotics, antimicrobial resistance, multidrug resistance, extremely drug resistance, *Pseudomonas eruginosa*

Introduction

Pseudomonas aeruginos are the leading cause of hospital-acquired infections including those of the blood stream, respiratory tract, urinary tract and surgical sites. [1–3] In addition to an array of virulence determinants, *P. aeruginosa* possesses and can readily acquire abroad variety of antimicrobial resistance mechanisms. [4,5] These include up-regulation of efflux pumps, loss of outer membrane porins, the production of Amp C, ESBL and carbapenemase enzymes,

and modification of antimicrobial target sites. [6,7] Multiple resistance mechanisms are usually expressed simultaneously, resulting in resistance to agent sin multiple antimicrobial classes. [6,7] The existence of limited effective treatment options for MDR *P. aeruginosa* infections has been associated with poor clinical outcomes. [810] In their 2017 report, the WHO designated research discovery and development of new antibiotic for carbapenem resistant *P. aeruginosa* a critical priority. [11] Ceftazidime/ avibactam and ceftolozane /tazobactam are licensed for the treatment of patients with a variety of clinical infections. [12] Avibactamisa non b-lactamb lactamase inhibitor that inhibits class A, class C and most class D b-lactamases. [13] On the other hand, ceftolozane is a novel cephalosporinth-atisactive against *P. aeruginosa* isolates with Amp C hyper production and over expressed efflux mechanisms. [14] The combination of ceftolozane and the b-lactamase inhibitor tazobactamis active against many, but not all, ESBL-producing Gram-negative bacteria. [15] Several studies have reported rate sand mechanisms of *P. aeruginosa* resistance to ceftazidime/avibactam and ceftolozane /tazobactam, including MDR isolates, from Europe and North America. [15–20] However, there are limited data on the potential utility of ceftazidime/ avibactam and ceftolozane/ tazobactam for MDR *P. aeruginosa* from the Arabian Peninsula, are gion of extremely diverse demography and close travel links to all corners of the world. [21,22] The aim of this study was to investigate the invitro activity of ceftazidime/ avibactam and ceftolozane/ tazobactam against MDR *P. aeruginosa* and to explore the associated genetic diversity and mechanisms of resistance.

Materials and Methods:

MDR *P. aeruginosa* isolated between Oct 2021 to Aug 2022 from Dept of microbiology, Hi tech medical college and hospital, Bhubaneswar, were used for evaluation of ceftazidime /avibactam and ceftolozane/tazobactam susceptibility.

Bacterial Isolates

The data presented in this paper has been obtained from Dept of microbiology, Hi tech medical college and hospital, Bhubaneswar, strictly with the project's recommendations. The isolated strains were identified locally, and predefined number of selected bacterial species were collected. Isolates were accepted into the study regardless of antimicrobial susceptibility. Further analysis was done. Non-duplicate, clinically significant P. aeruginosa strains were collected from patients with respiratory tract, skin and musculoskeletal tissue, genitourinary tract, intra-abdominal, bloodstream, urine or other (ear, eye) infections by Dept of microbiology, Hi tech medical college and hospital, Bhubaneswar. A total of 1909 isolates of P. aeruginosa were included in the study. Isolates of *P. aeruginosa*) was collected from adults and approximately 40% were from elderly (>60years) patients. Most isolates of *P. aeruginosa* were from patients located inwards that were not classified as intensive care units (ICU). The predominant *P. aeruginosa* isolates sources were respiratory (40.3%), genitourinary (22.7%) and skin or musculoskeletal (26.3%). Demographic information recorded for each isolate included specimen source, patient age and type of hospital setting.

Results and Discussion

A total of 1,909 *P. aeruginosa* isolates (1 per infection episode) were consecutively collected from as par to of the program. Only bacterial isolates determined to be significant by local criteria as the reported probable cause of an infection were included in this investigation, by following the manufacturer's instructions. Antimicrobial susceptibility was evaluated by reference broth microdilution methods, conducted according to Clinical and

Laboratory Standards Institute (CLSI) procedures (documentM07) [23]. Avibactam was provided by Allergan (Irvine, CA, USA) and combined with ceftazidime (avibactam at fixed concentration of 4 mg/liter) for susceptibility testing. A ceftolozane stock solution was obtained from Thermo Fisher Scientific (Cleveland, OH, USA) and combined with tazobactam (acquired from United States Pharmacopeia [USP]) at a fixed concentration of 4 mg/liter for susceptibility testing. All other compounds were obtained from USP or Sigma-Aldrich (St. Louis, MO, USA). Concurrent quality control (QC) testing was performed to ensure proper test conditions and procedures. QC strains included P. aeruginosa ATCC27853. CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility interpretive criteria were used to determine susceptibility and resistance rates for comparator agents [24,25]. Ceftazidime-avibactam (MIC50/MIC90, 2/8mg/liter) and ceftolozane tazobactam (MIC50/MIC90, 0.5/2 mg/liter) were the most active compounds after colistin, with national susceptibility rates of 96.9% and 97.5%, respectively (Table 1). Moreover ceftazidime-avibactam and ceftolozane-tazobactam retained activity, 70.2% susceptibility and 78.7% susceptibility, respectively, against many isolates that were non susceptible to ceftazidime, cefepime, meropenem, and piperacillin-tazobactam (Table1). Colistin (MIC50/MIC90, 0.5/1mg/liter) was active against 99.9% of P. aeruginosa isolates overall 1 (Table 1) The aminoglycosides tobramycin (MIC50/MIC90, 0.5/2 mg/liter) and amikacin (MIC50/MIC90, 4/16mg/liter) were also very active, demonstrating 93.1% susceptibility per LSI and EUCAST and 94.8% and 84.1% susceptibility per CLSI and EUCAST, respectively (Table1). The national susceptibility rates for piperacillin-tazobactam (MIC50/MIC90, 4/128 mg/liter) and meropenem (MIC50/MIC90, 0.5/16mg/liter) were 77.5% and 76.0%, respectively (CLSI and EUCAST), and ciprofloxacin was active against 77.9% and 70.8% of isolates per CLSI and EUCAST break point criteria, respectively (Table 1). Of note, if the CLSI-revised breakpoints (to be published in January 2019) for ciprofloxacin (≤0.5 mg/liter and ≥2 mg/liter for susceptible and resistant, respectively) and levofloxacin (≤1 mg/liter and ≥4 mg/liter for susceptible and resistant, respectively) were applied, the susceptibility rates would be 70.8% for ciprofloxacin and 61.3% for levofloxacin After colistin, ceftazidime-avibactam and ceftolozane-tazobactam were the most active compounds and had susceptibility rates of 93.3% to 99.4%. Infections caused by MDR *P. aeruginosa* a strains and a delay in appropriate antimicrobial therapy for serious *P. aeruginosa* infections are associated with increased mortality and longer hospital stays. Our results showed that in addition to colistin, only ceftazidime-avibactam and ceftolozane-tazobactam were active against>95% of the isolates overall. The susceptibility rates exhibited by these 2 combinations were generally very similar, with both being active against >90% of the isolates in all census divisions and retaining good activity against MDR isolates When the results from this investigation were compared with previous results obtained from the INFORM program [26,27] we observed that the activity of ceftazidime-avibactam has remained stable since its initial U.S. FDA approval in early 2015 (97.0% susceptibility rate in the 2012 to 2015 period and 96.9% in 2017). In contrast, susceptibility rates have decreased for other β-lactams, such as those for meropenem (82.0% in 2012 to 2015 and 76.0% in 2017) and piperacillin-tazobactam (80.5% in 2012 to 2015 and 77.5% in 2017) [26,27] Resistance mechanisms related to ceftazidime avibactam or ceftolozane tazobactam were not evaluated in the present study, but the

results from a previous investigation indicated that ceftazidime-avibactam resistant isolates usually express more than 1 mechanism related to resistance to β-lactam compounds, including Opr D loss, over expression of chromosomal Amp C, and over expression of MexCD –OprJ, Mex AB- OprM, and/or MexXY-OprM [28]. The study also showed that the resistance mechanisms found in ceftazidime-avibactam-resistant isolates were also found in ceftazidime-avibactam-susceptible isolates that were resistant to other β-lactams, and metallo-β-lactamase-producing P. aeruginosa isolates were rare in the U.S. medical centers that participate in the INFORM program [28]. Additionally, it has been shown that resistance to ceftolozane-tazobactam is associated with alterations on the chromosomal Amp C Ω loop [29].

Table 1: Showing the samples and percentage

Samples	No. of Samples	% of Isolated
Blood	1909	
Respiratory Sputum	955	40.3%
Urine	485	30.6%
Skin	469	29.1%

Table 2: Antimicrobial susceptibility of 1,909 Pseudomonas aeruginosa clinical isolates from Hospital

Antimicrobial agent byisolate group (n)	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	Susceptibility rates (%) according to a			
			CLSI		EUCAST	
			S	R	S	R
Allisolates (1,909)						
Ceftazidime-avibactamb	2	8	96.9	3.1	96.9	3.1
Ceftolozane-tazobactamc	0.5	2	97.5	1.3	97.5	2.5
Piperacillin-tazobactam	4	128	77.5	12.2	77.5	22.5
Ceftazidime	2	32	82.5	13.2	82.5	17.5
Cefepime	4	16	82.4	6.5	82.4	17.6
Meropenem	0.5	16	76.0	17.0	76.0	11.5
Doripenem	0.5	>8	77.4	16.6	69.4	22.6
Imipenem	1	>8	75.7	20.1	79.9	13.7
Aztreonam	8	>16	68.1	20.8	7.9	20.8
Ciprofloxacin	0.25	>4	77.9	16.2	70.8	29.2
Levofloxacin	1	16	72.1	18.9	61.3	38.7
Gentamicin	2	8	81.7	8.7	81.7	18.3
Amikacin	4	16	94.8	2.7	87.1	5.2
Tobramycin	0.5	2	93.1	5.3	93.1	6.9
Colistin	0.5	1	99.9	0.1	99.9	0.1
β-Lactam-nonsusceptibleisolates (161) Ceftazidime-avibactamb	d 8	32	70.2	29.8	70.2	29.8
Ceftolozane-tazobactamc	2	16	78.7	12.4	78.7	21.3
Aztreonam	>16	>16	2.5	88.2	0.0	88.2
Ciprofloxacin	4	>4	31.1	57.1	21.7	78.3
Levofloxacin	8	>16	23.1	65.6	11.2	88.8
Gentamicin	8	>16	48.4	31.1	48.4	51.6

Amikacin	8	>32	82.0	11.8	64.0	18.0
Tobramycin	2	>16	71.4	24.2	71.4	28.6
Colistin	0.5	1	100.0	0.0	100.0	0.0

A Resistance criterion as published by CLSI 2018 and EUCAST 2018. S, susceptible; R, resistant. B Susceptible/resistant break points of ≤8 and ≥16 mg/liter, respectively, for CLSI and EUCAST. C Susceptible and resistant break points of ≤4 and ≥8 mg/liter, respectively, for CLSI and EUCAST. D Isolates non susceptible to ceftazidime, cefepime, meropenem, and piperacillin-tazobactam.

Conclusions:

MDR *P. aeruginosa* susceptibility rates to ceftazidime/avibactam and ceftolozane/tazobactam were higher than those to all existing anti pseudomonal agents, except colistin, but were less than 50% in extremely resistant isolates. Non-susceptibility to ceftazidime/avibactam and ceftolozane/tazobactam was largely due to the production of ESBL and VIM enzymes. Ceftazidime/avibactam and ceftolozane/tazobactam are possible options for some patients with MDR *P. aeruginosa*

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