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SYNERGY TESTING OF CEFTAZIDIME- AVIBACTAM WITH AZTREONAM ON CARBAPENEMASE PRODUCING KLEBSIELLA SPECIES IN A TERTIARY CARE HOSPITAL

Clinical Microbiology

Dr. Sumedha Thanvi Resident, Department of Microbiology, S.M.S Medical College, Jaipur
Dr. Vithika Vyas M.B.B.S, Kasturba Medical College, Manipal
Dr. Pranzal Garg Senior Resident, Department of Pulmonary Medicine, S.M.S Medical College, Jaipur
Dr. Aruna Vyas Senior Professor, Department of Microbiology, S.M.S Medical College, Jaipur

ABSTRACT

Resistance in Gram-negative bacteria has become a major concern in recent times. To overcome the challenge posed by metallo-beta-lactamases, which are not adequately countered by the combination of a beta-lactamase inhibitor and a beta-lactam antibiotic, the addition of a third antibiotic has been employed to restore susceptibility. One of the recent combinations used is ceftazidime-avibactam in combination with aztreonam. However, the effectiveness of this combination, along with the role of rapid synergy reporting, has not been extensively evaluated. **Methods**: Phenotypic synergy testing of 100 multidrug-resistant isolates identified as carbapenem resistant *Klebsiella* species was performed by two phenotypic synergy methods for ceftazidime-avibactam with aztreonam (Disc-E strip method, Disc- Disc method) for rapid reporting to clinicians. **Results**: Out of 100 isolates of carbapenem resistant *Klebsiella* species, synergy was seen in 50 isolates (50%) by Disc-E strip and Disc- Disc method. **Conclusions**: Ceftazidime–avibactam and aztreonam rapid synergy testing will be highly beneficial and help the clinicians to take decisions for antibiotic selection.

KEYWORDS

Beta-lactamase, ceftazidime-avibactam, aztreonam, phenotypic, synergy

INTRODUCTION

Multi Drug Resistance (MDR) is a common occurrence among ESKAPE pathogens in tertiary healthcare centers, encompassing Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, and Enterobacter species[1]. These pathogens, particularly the gram-negative bacteria, including extended spectrum β-lactamases (ESBL)-producing and carbapenemase- producing Enterobacteriaceae, have become increasingly prevalent worldwide, resulting in substantial medical burdens and mortality rates.

Klebsiella pneumoniae, in particular, with its carbapenemase (KPC), poses significant therapeutic challenges. Although carbapenem antibiotics have traditionally been highly effective against ESBL-producing Enterobacteriaceae, there has been a dramatic rise in carbapenem-resistant Enterobacteriaceae (CRE), necessitating the urgent development of new antibiotics targeting carbapenemase-producing Enterobacteriaceae on a global scale[2].

Although we have some antibiotic options against Gram-positive pathogens, there are few antibiotic options left for Gram-negative pathogens. Only colistin, tigecycline and fosfomycin are some of the options but toxicity of these agents (especially colistin) is a limiting factor for use in critically ill patients[3].

Beta lactam (especially cephalosporins like ceftazidime and ceftriaxone agents are excellent against Gram negative pathogens because of their broad-spectrum activity, safety and well tolerability. However, their role in MDR pathogens are diminishing due to very high prevalence of enzymes like extended spectrum beta lactamases (ESBL), Metallo beta lactamses (MBLS), AmpC beta lactamses and OXA-48 producing pathogens[1].

INTRODUCTION

Ceftazidime–avibactam is combination of cephalosporin (ceftazidime) and betalactamase inhibitor (avibactam). Avibactam inhibits ESBLS, AmpC betalactamses, OXA-48 and serine carbapenmases like *Klebsiella pneumoniae* carbapenemase (KPC). However, it cannot inhibit metallobetalactamases like New Delhi Metallo Betalactamases (NDM). Aztreonam is a monobactam antibiotic. The metallobetalactames like NDM cannot destroy aztreonam. But ESBLs and other enzymes inactivate the antibiotic[1].

So, the combination of Ceftazidime-avibactam and aztreonam can act against all betalactamase producing Gram-negative pathogens. MDR Gram-negative bacteria isolates typically contain multiple resistance mechanisms and genes. So, it becomes imperative to use multiple antibiotics in combination.

It is prudent to know the synergistic, additive or antagonistic activity of these antibiotics. It also helps the clinicians in choosing the right combination of antibiotics. Traditional methods of antibiotic synergy testing are time consuming, exhaustive, costly and impractical in day to day reporting to clinicians.

Hence, this study was designed to understand the efficacy of ceftazidime–avibactam; ceftazidime–avibactam with aztreonam by different laboratory phenotypic methods in clinical bacterial isolates.

MATERIALAND METHODS

A total of 100 multidrug-resistant isolates identified as carbapenem resistant *Klebsiella* species were collected in a tertiary care centre, SMS Medical College, Jaipur, Rajasthan over a period of 1 year and included in the study. These isolates were collected from different sources such as blood, pus, respiratory specimens like sputum, bronchoalveolar lavage fluid, and other body fluids. The Kirby-Bauer disk diffusion method along with MIC determination using the Vitek 2 system revealed resistance to beta-lactams,cephalosporins, and carbapenems like imipenem and meropenem. Identification of the bacterial isolates was confirmed by Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF MS) method (VITEK MS, Biomerieux, France).

Detection of Carbapenamase

Isolates showing resistance to imipenem & meropenem (inhibition zone <19mm) by disc diffusion method were considered potential MBL producers and further tested by mCIM test.

For each isolate to be tested 1µl loopful of growth is emulsified from an overnight blood agar plate in 2 mL trypticase soya broth (TSB). It was vortexed for 10-15 seconds. 10 µg meropenem disc was added to each tube using sterile forceps; it wasensured that the entire disc is immersed in suspension. After incubation at 35^{0}C for 4 hours,a lawn culture of ATCC *Escherichia coli* 25922 of turbidity of 0.5 McFarland on Muller Hinton agar plate was prepared, allowed to dry for 3-10 minutes before adding the meropenem disk.

The meropenem disc was removed from each TSB meropenem disc suspension using a 10 µl loop by placing the flat edge of the disk and using surface tension to pull the disk out of liquid,carefully dragged and loop was pressed along the inside edge of tube to expel excess liquid from the disk. The disk was removed from the tube and placed on MHA plate previously inoculated with meropenem susceptible *Escherichia coli* ATCC 25922 indicator strain. Inoculated at 35^{0}C for

18-24 hours , zones of inhibition were measured.

An inhibition zone size of 6–15mm in mCIM was considered as indicative of the production of carbapenemase in the test isolate, proving that the meropenem disk had been hydrolyzed by the carbapenemase produced by the test isolate. An inhibition zone diameter of ≥19mm was interpreted as susceptible and negative for carbapenemase production and between 16–18mm as indeterminate.[4]

Synergy Testing

Testing of Ceftazidime–Avibactam and Aztreonam synergy was done by different phenotypic methods.

1. Disc-E strip synergy testing[5] : Mueller–Hinton agar plates (150mm diameter) were inoculated with suspensions of study isolates grown to an optic density of 0.5 McFarland units. E test strip of Ceftazidime –Avibactam (Pfizer, India) was put on the plate. Then, Aztreonam disc (30 mg) was put near the E test strip such that centre of disc remained parallel to the cut-off sensitivity mark of ceftazidime-avibactam (8 mg/mL). The distance between the strip and disc was 30mm (centre to centre). The plate was incubated for 18h. The reduction of MIC by 3-fold was reported as synergy.

2. Disc – disc synergy testing- Mueller–Hinton agar plates (150mm diameter) were inoculated with suspensions of study isolates grown to an optic density of 0.5 McFarland units. Disc of Ceftazidime–Avibactam (Pfizer, India) was put on the plate. Then, Aztreonam disc (30 mg) was put near the CZA disc. The distance between the discs was 30mm (centre to centre). The plate was incubated for 18h. Formation of D zone or ghost zone between the discs was reported as synergy.

Fig.1: E strip synergy test showing synergy between Ceftazidime-Avibactam Aztreonam.

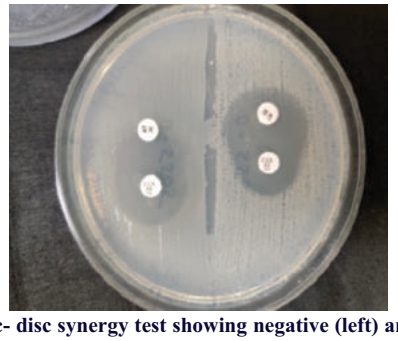


Fig 2. Disc- disc synergy test showing negative (left) and positive (right) results.

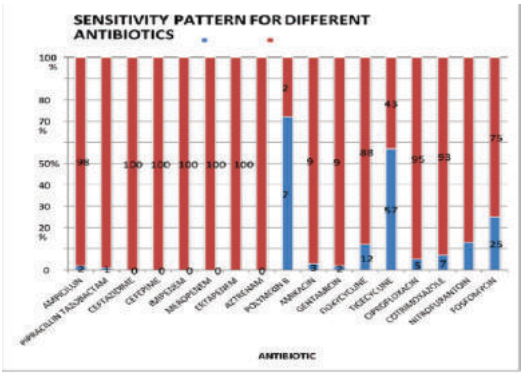
RESULTS

One hundred isolates of carbapenemase producing *Klebsiella spp.* received in Department of Microbiology, SMS Medical College, Jaipur (Rajasthan) were included in the study. The age group most commonly affected was 61 - 70 years (20%).Mean age was 43.87 ± 23.19 years and median range 48(0.01-80) years. Almost two third of the patients were males (69%) and the rest of the patients were females (31%).

Out of all the *Klebsiella* isolates, maximum number were isolated from pus samples (43%), followed by urine (31%). Both comprised almost three-fourth of the total isolated samples (74%). 14% were isolated from ET swabs. Central line tip, blood, sputum and wound swab together comprised of only 12% of the isolates. These isolates were found to be resistant to most antibiotics. Highest susceptibility was seen with Polymyxin B (72%) , followed by Tigecycline (57%), Fosfomycin (25%), nitrofurantoin (13%), doxycycline (12%). Peniciilins, Cephalosporins, Carbapenems, monobactam and aminoglycosides showed almost 100% resistance pattern.

Table 1 : Susceptibility Pattern For Different Antibiotics

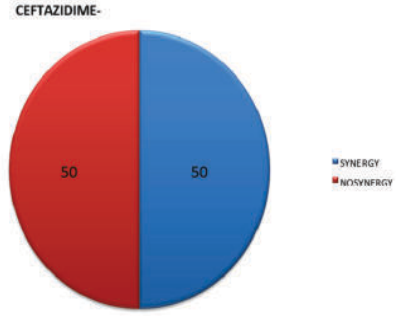
ANTIBIOTICS	SENSITIVE		RESISTANT	
	N	%	N	%
AMPICILLIN	2	2	98	98
PIPRACILLIN TAZOBACTAM	1	1	99	99
CEFTAZIDIME	0	0	100	100
CEFEPIME	0	0	100	100
IMIPENEM	0	0	100	100
MEROPENEM	0	0	100	100
ERTAPENEM	0	0	100	100
AZTREONAM	0	0	100	100
POLYMIXIN B	72	72	28	28
AMIKACIN	3	3	97	97
GENTAMICIN	2	2	98	98
DOXYCYCLINE	12	12	88	88
TIGECYCLINE	57	57	43	43
CIPROFLOXACIN	5	5	95	95
COTRIMOXAZOLE	7	7	93	93
NITROFURANTOIN	13	13	87	87
FOSFOMYCIN	25	25	75	75



After routine antimicrobial susceptibility testing, these isolates were subjected to synergy test between ceftazidime- avibactam with aztreonam. Fifty (50%) of the isolates showed synergy of ceftazidime-avibactam and aztreonam by Disc-E strip method & Disc-Disc method.

Table 2 : Ceftazidime- Avibactam With Aztreonam Synergy

SYNERGY	NUMBER	PERCENTAGE
Yes	50	50
No	50	50
Total	100	100



Graph 2. Ceftazidime - Avibactam With Aztreonam Synergy

DISCUSSION

Antimicrobial resistance in gram negative bacteria is a growing concern in health care as there is lack of viable antimicrobial therapeutic alternatives. Resistance to safer drugs like carbapenems often pose challenge in antibiotic therapy especially in hospitals having high load of metallobeta-lactamase-producing bacteria.

CZA is a newer combination comprising of a third-generation

cephalosporin and a novel beta-lactamase inhibitor. The role of Avibactam is to re-establish the in vitro activity of ceftazidime against Ambler class A, class C, and some class D beta-lactamase-producing pathogens . It inhibits beta-lactamase-producing Gram-negative pathogens, ESBLs, AmpC -lactamases, and KPC-producing *K. pneumoniae* and MDR P. *aeruginosa* . This beta -lactam– beta -lactamase inhibitor combination is currently FDA approved for the treatment of various complicated infections like complicated urinary tract infections, hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia.[6]

The present study reveals that, there is a predominance of males (69%) over females (31%) ,more common age group 61-70 years, highest percentage of *Klebsiella* pneumonia were isolated from pus (43%) followed by urine (31%), which is also documented in other studies like Mohammad et al[7,] Jaggi et al[8,]and Gill et al[9].

High level of resistant is observed to ampicillin, fluoroquinolones and aminoglycosides. Ciprofloxacin was found resistant in 95% whereas amikacin resistance was 97% and gentamicin 98% respectively. 100% isolates showed resistance to cephalosporins like cefepime and ceftazidime . Among carbapenems, imipenem , ertapenem and meropenem, 100% resistance was reported. Similar studies by Li Y et al[10,] Jaggi et al[8], Gill et al [9],Asha et al[11] have also reported high resistance to these groups of antibiotics. Unfortunately, in the present study significant resistance to tigecycline (43%) as well as polymyxin B (28%) was also observed.

So, combined antibiotic strategy helps in overcoming antibiotic resistance and improving efficacy.

In our study, by the E-strip/disc qualitative method and disc-disc method, 50% of the isolates have shown synergy. Similar results were observed in a study by Pankey et al[12], Rawson et al[13] where synergy was demonstrated in 23 of 43(53%) and 42% *Klebsiella pneumonia* isolates respectively.

The study by Pankey et al[12] demonstrated that the Etest synergy method is a potential method for evaluating in vitro antimicrobial synergy that could be performed easily in a clinical microbiology laboratory.

Limitations

The enhanced activity observed with the combination of Aztreonam and Ceftazidime Avibactam is encouraging and deserves further consideration in the era of diminishing treatment options for carbapenem-resistant pathogens although additional data is needed to evaluate the in vivo safety and efficacy of this regimen since the in–vitro susceptibility cannot always predict the in-vivo activity of the drugs in the patient because other factors like pharmacodynamics and host immune response also play a role in the clinical outcome of the patient.

In our study, samples were collected from patients attending a single tertiary care centre. Therefore, the results of this study cannot be generalized for all the organisms and for all hospital settings as the rate of carbapenem resistance and types of betalactamases vary in different geographical areas. Further studies with a substantial number of isolates are needed to evaluate this antibiotic combination of ceftazidime- avibactam and aztreonam to guide the clinicians in treating MDR infections with suitable therapeutic options and to formulate antibiotic policy.

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