Section: Microbiology

Original Research Article

COMPARATIVE STUDY ON CEFTAZIDIME – AVIBACTAM – AZTREONAM SYNERGY BY USING VARIOUS PHENOTYPIC METHODS AGAINST CARBAPENAMASE PRODUCING GRAM NEGATIVE ORGANISMS

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Keywords:
CP GNB - Carbapenemase-producing Gram negative bacilli, PDR - Pan-Drug Resistant, XDR - Extensive Drug-Resistant, MDR – multidrug resistance, MBL - metallo β-lactamase, BCL - beta-lactam combinations, CZA/CAZ - ceftazidime–avibactam, AZT – Aztreonam, MHA – Muller Hinton Agar.

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ABSTRACT

Background: Carbapenemase-producing gram negative bacilli (CP GNB) show rapid global dissemination and pose a significant therapeutic challenge. This study aims to compare the synergy between ceftazidime-avibactam plus aztreonam in Carbapenam Resistant Gram-Negative Bacteria. **Materials and Methods:** A total of 15 E. coli, 15 Kleb. pneumoniae and 15 Pseudo aeruginosa strains were selected from clinical samples. These 45 strains were resistant to carbapenems (imipenem, ertapenem, meropenem) tested by disk diffusion and were intermediate susceptible to Colistin by broth microdilution. We assessed the effectiveness of the tests to compare the in vitro synergy of combination of AZT and CZA. Using, Gradient Disc - E strip method, Disc diffusion method and Disc replacement method. **Result:** Out of 45 isolates 41 (91%) showed Synergy positive for combination of AZT and CZA. All 15 isolates of CR E. coli showed 100% Synergy positive for triple combination of AZT and CZA. Out of 15 isolates of CR Klebpneumoniae, 14 isolates (93%) showed Synergy positive for triple combination of AZT and CZA. Out of 15 isolates of CR Pseudo aeruginosa 12 isolates (80%) showed Synergy positive for triple combination of AZT and CZA. A comparison study of Gradient Disc - E strip method, Disc diffusion method and Disc replacement method revealed identical results. **Conclusion:** Ceftazidime-avibactam and aztreonam combination has been an effective therapeutic option against carbapenamase producing E. coli and Klebsiella isolates. There is a reduced therapeutic efficacy against Nonfermenters (Pseudomonas aeruginosa).

INTRODUCTION

Antimicrobial resistance is recognized as one of the significant global health threats by the World Health Organization.[1] E.coli and Klebsiella spp. are considered to be the most important Enterobacterales and some of nonfermentative gram negative bacilli like Pseudomonas aeruginosa that commonly cause health-associated and community-associated infections.[2] Among multidrug-resistant bacteria, carbapenemase - producing gram negative bacilli (CP GNB) show rapid global dissemination and pose a significant therapeutic challenge.[1,3] This is besides the high threat imposed by extensive drug resistant (XDR) and pan drug resistant (PDR) CP GNB. Multidrug-resistance (MDR) is defined as resistance to at least one agent in three or more antimicrobial classes, XDR is defined as resistance to at least one agent in all but sensitive with two or fewer antimicrobial categories, and PDR is defined as resistance to all agents in all antimicrobial classes.[4]

Detection of carbapenemases rapidly is crucial to ensure the management and treatment of clinical infections.[5] These carbapenemases include Ambler class A (KPC, and GES enzymes) and class D (OXA-48-like enzymes) which are serine carbapenemases, and class B (Metallocarbapenemases); (NDM, VIM, and IMP enzymes).[6] Phenotypic methods are commonly used in routine laboratory practice.

With the advent of a new effective therapeutic option; ceftazidime-avibactam, a 3rd generation cephalosporin combined with a β-lactamase inhibitor, has a wide-spectrum activity against serine β-lactamases but is hydrolyzed by metallo β-lactamases. In contrast, the aztreonam, a monobactam, is stable in the presence of metallo β-lactamases but susceptible to hydrolysis by serine β-lactamases.[7] Thus, the combination of ceftazidime-avibactam and aztreonam is considered to have a synergistic therapeutic effect against CP GNB.

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This study aims to evaluate the synergy between ceftazidime-avibactam plus aztreonam in Carbapenam Resistant Gram-Negative Bacteria.

Aim

To compare the phenotypic synergy of Ceftazidime-Avibactam with Aztreonam in Carbapenem resistant Gram-negative pathogens.

MATERIALSANDMETHODS

Study Centre: The study was conducted in the Department of Microbiology, KAP Vishwanatham government medical college, Trichy. The clinical samples were obtained from MGM government hospital, Trichy.

Study period: January 2025 to June 2025.

Study design: In this study, using a cohort of 45 isolates of Enterobacterales and P. aeruginosa. we assessed the effectiveness of the tests to determine the in vitro synergy of combination of AZT and CZA. Using,

1. Gradient Disc - E strip method
2. Disc diffusion method
3. Disc replacement method

Bacterial isolates and their selection for the experiment: The strains included in this study were initially isolated from different clinical specimens from patients admitted in MGMGH. Identification was done by conventional techniques and susceptibility testing of the isolates was carried out with Kirby-Bauer disc diffusion method. As a result of which the MDR isolates which are intermediate susceptible to Colistin were selected for this study.

A total of 15 E. coli, 15 Kleb. pneumoniae and 15 Pseudo aeruginosa strains were selected from clinical samples. These 45 strains were resistant to carbapenems (imipenem, ertapenem, meropenem) tested by disk diffusion and were intermediate susceptible to Colistin by broth microdilution

Procedure:

Inoculum Preparation: The bacterial suspensions were made by emulsifying 3 or 4 individual test strain colonies in a tube of 0.9% normal saline.

Compare turbidity to that in the 0.5 McFarland standards.

Adjust turbidity of inoculum in order to match the standard.

Inoculation into the Muller Hinton agar (MHA): Dip a sterile cotton swab in the inoculum and rotate the swab against the inside of the tube above the fluid level to remove excess liquid before swabbing.

Streak (lawn culture) the swab over the entire surface of the agar plate by rotating the plate approximately 60 ° with 3 times. Complete inoculation by running the swab around the rim of the agar plate.

Interpretation

Table 1: CLSI clinical breakpoints for ceftazidime–avibactam and aztreonam (for Enterobacterales other than salmonella and shigella).

Antimicrobial agent	Disc content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			
		S	SDD	I	R
Beta-lactam combinations					
Ceftazidime – avibactam	30/20 µg	≥ 21	-	-	≤ 20
Monobactams					
Aztreonam	30 µg	≥ 21	-	18-20	≤ 17

Table 2: CLSI clinical breakpoints for ceftazidime–avibactam and aztreonam (for Pseudomonas).

Antimicrobial agent	Disc content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm		
		S	I	R
Beta-lactam combinations				
Ceftazidime - avibactam	30/20 µg	≥ 21	-	≤ 20
Monobactams				
Aztreonam	30 µg	≥ 22	16-21	≤ 15

S, susceptible; I, intermediate; R, resistant. CLSI, Clinical and Laboratory Standards Institute.[9]

1.Testing by gradient Disc: E Strip method: To the inoculated MHA plate AZT discs (30µg) were placed 15mm away from centre of CZA strip (HiMedia Laboratories, India) near the susceptibility MIC breakpoint for Enterobacterales, i.e., ≤ 8/4µg/ml) as per CLSI criterion and incubated overnight at 37°C for 18 to 24 hours.

After incubation, Synergy by this method was interpreted by a qualitative approach of forming an inverse-D-shaped zone of inhibition.

Additionally, a quantitative approach was adopted to define synergy. AZT zone reduced towards the CAZ-AVI E-strip and opposite to the CAZ-AVI strip were measured and converted to an estimated zone of inhibition diameters for CAZ-AVI and AZT alone, respectively. Zone diameters were then compared as per CLSI zone diameter breakpoints and interpreted for synergy, based on restoration of AZT zone diameter (in the presence of AVI) crossing the CLSI susceptibility breakpoint, i.e., ≥ 21 mm.

2. Testing by Kirby Bauer disc diffusion method: The selected MDR isolates were further tested by Kirby Bauer disc diffusion tests with AZT (30µg), CAZ-AVI (30 µg /20 µg) (HiMedia Laboratories, India).

Synergy was present if there was enhancement of the zone of inhibition of the AZT disc towards the CAZ-AVI disc and if there was a characteristic

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'keyhole' shaped zone of inhibition between two discs

The results were interpreted as per zone diameter breakpoints described in the performance standards of antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI) in 2024.

3. Testing by Disc replacement method: A CAZ-AVI (30 µg /20 µg) (HiMedia Laboratories, India) disc was placed on the MHA, and the plate was incubated at 37˚C for one hour.

After that, the CAZ-AVI disc was quickly removed and replaced with an AZT (30 µg) disc at the same position on the agar surface. The plate was then further incubated overnight at 37˚C. Synergy was considered present if the zone diameter of the replacement AZT disc was ≥ 21 mm.

RESULTS

The results were interpreted as per zone diameter breakpoints described in the performance standards of antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI-M 100 Edi 35) in 2025.

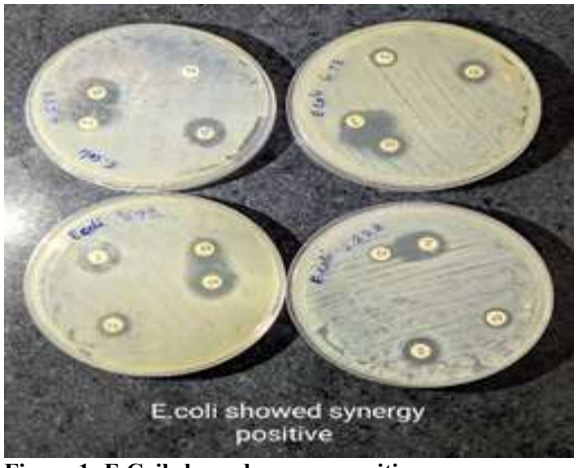

Figure 1: E.Coil showed synergy positive

Statistical analysis of the data: SPSS software package version 20.0 was employed for analyzingthe data that was fed into the computer. (IBM Corp., Armonk, NY) Numbers and percentages were used to describe the qualitative data. The results' significance was established at the 5% level ($p < 0.05$).

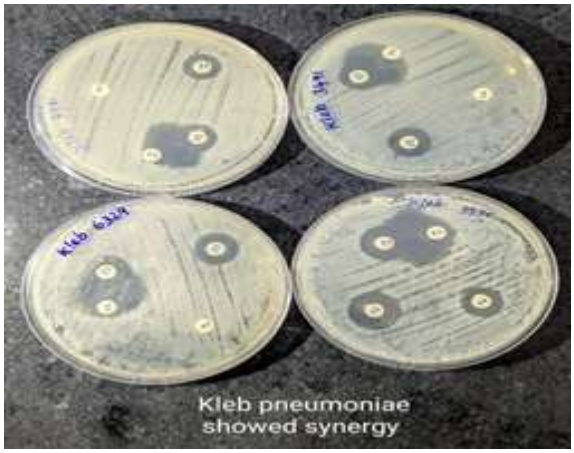

Figure 2: Kleb pneumonia showed synergy

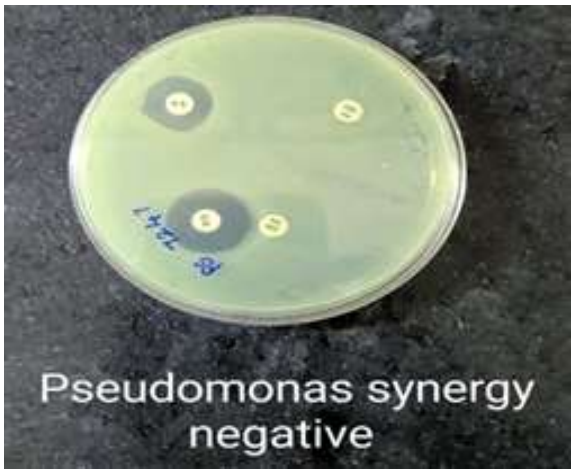

Figure 3: Pseudomonas synergy negative

Table 3: Percentage of isolates showing susceptibility to CZA and AZT

No of isolates non susceptible to CZA	No of isolates non susceptible to AZT	No of isolates susceptible to CZA+AZT	No of isolates non susceptible to CZT+AZT
39 (87%)	42(93%)	41(91%)	4(9%)

Table 4: Number of isolates showing synergy positive and negative by various methods

Method	Gradient Disc - E Strip method		Kirby Bauer disc diffusion method		Disc replacement method	
	Synergy positive	Synergy negative	Synergy positive	Synergy negative	Susceptible	Resistant
E.coli (N=15)	15 (100%)		15(100%)		15(100%)	
Klebpneumoniae (N=15)	14 (93%)	1(7%)	14(93%)	1(7%)	14 (93%)	1(7%)
Pseudo aeruginosa(N=15)	12 (80%)	3(20%)	12(80%)	3(20%)	12(80%)	1(20%)

In this study 45 isolates of Carbapenam resistant gram-negative bacteria with intermediate susceptible to Colistin (CR E. coli 15, CR Kleb. pneumoniae 15 and Pseudo aeruginosa 15) were taken.

We assessed the effectiveness of the tests to determine the in vitro synergy of triple combination of AZT and CZA. Using,

1. Gradient Disc - E strip method
2. Disc diffusion method
3. Disc replacement method

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The results were interpreted as per zone diameter breakpoints described in the performance standards of antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI) in 2024.
Out of 45 isolates 41 (91%) showed Synergy positive for combination of AZT and CZA.
All 15 isolates of CR E. coli showed 100% Synergy positive for triple combination of AZT and CZA
Out of 15 isolates of CR Klebpneumoniae showed 14 isolates (93%) showed Synergy positive for triple combination of AZT and CZA
Out of 15 isolates of CR Pseudo aeruginosa showed 12 isolates (80%) showed Synergy positive for triple combination of AZT and CZA.
A comparison study of Gradient Disc - E strip method, Disc diffusion method and Disc replacement method revealed identical results.

DISCUSSION

Ceftazidime-avibactam and aztreonam combination has been an effective therapeutic option against carbapenamase producing E. coli and Klebsiella isolates. There is a reduced therapeutic efficacy against Nonfermenters (Pseudomonas).[8-11]
A comparison study of Gradient Disc - E strip method, Disc diffusion method and Disc replacement method revealed identical results. The sensitivity and specificity of all the methods for the detection of ceftazidime-avibactam/aztreonam synergy against extensively drug-resistant Enterobacterales has also been noted the same in this study. To assess the efficacy of ceftazidime-avibactam/aztreonam combination CLSI suggests elution method. Hence, in a resource deficient setting like Government laboratories a cost-effective Disc based methods would yield the same result as of any Strip based method. Due to cost effectiveness disc-based methods can be employed as a routine diagnostic method.[12-15]

CONCLUSION

Carbapenems represent an important class of antibiotics that are still reserved for infections caused by MDR microorganisms. However, the emergence of carbapenem resistance has dramatically increased worldwide and therefore poses a serious public health threat. Several mechanisms including reduced uptake, active efflux of carbapenems, as well as inactivation via carbapenemases are involved in the bacterial resistance to carbapenems. consider the combination of CAZ/AVI and AZT as a new therapeutic option to treat infections caused by highly resistant gram-negative bacterial strains. Fosfomycin, Polymyxins (colistin), Tigecycline, Plazomicin, Cefiderocol (siderophore cephalosporin), and new members of tetracyclines such as Eravacycline are the last treatment options for the treatment of infections caused by carbapenem-resistant bacteria. Therefore, the use of these last resort antibiotics should be controlled to avoid antibiotic misuse or overuse, and their use should be limited to the intensive care units in hospitals and only prescribed under strict medical supervision.

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