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Original Research Article

In vitro assessment of ceftazidime-avibactam combined with aztreonam for mitigating antimicrobial resistance in clinical isolates of multidrug-resistant Gram-negative bacilli

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ABSTRACT

Background: Antimicrobial resistance (AMR) represents a major global public health threat, with increasing MDR infections caused by Gram-negative such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter spp*. The limited antibiotic pipeline and ineffective treatments have necessitated the development of novel drug combinations. Among these, the combination of ceftazidime-avibactam with aztreonam has shown promise, particularly against serine-β-lactamase and metallo-β-lactamase producing strains that are resistant to conventional therapeutics.
Methods: Identification of isolates was done by routine biochemical testing; AST was determined by Kirby-Bauer disc diffusion, interpreted by CLSI guidelines. MDR, XDR, PDR were characterized. ESBL producers and carbapenem resistant strains were detected phenotypically using CLSI guidelines. In vitro synergy of ceftazidime-avibactam plus aztreonam was assessed by broth disc elution, following CLSI recommendations.
Results: Of 183 isolates of gram-negative bacilli, *Escherichia coli* (n=67), *Klebsiella pneumoniae* (n=72), *Pseudomonas aeruginosa* (n=29) and *Acinetobacter spp.* (n=15) in which MDR 68.65% was reported in *Escherichia coli* and 66.66% of XDR was reported in *Klebsiella pneumoniae*. ESBL was detected in 68 of 80 Enterobacterales, while 90 of 150 tested GNB were carbapenem resistant; *Klebsiella pneumoniae* contributed highest numbers. The combination of ceftazidime-avibactam with aztreonam yielded synergistic activity in 83.4% of all GNB isolates. Within carbapenem-resistant Enterobacterales, susceptibility to the combination was 97.2%, contrasting with only 15.7% susceptibility among carbapenem-resistant non-fermenters. Resistance to the combination was especially high among XDR and PDR *Acinetobacter spp*. and *Pseudomonas aeruginosa*.
Conclusions: The combination of ceftazidime-avibactam with aztreonam demonstrates strong in vitro synergy and enhanced susceptibility against MDR and carbapenemase-producing Enterobacterales especially *Klebsiella pneumoniae* suggesting clinical promise where conventional drugs fail. However, limited efficacy was observed against non-fermenter groups, underscoring the need for continuing resistance surveillance and further therapeutic innovation in multidrug-resistant non-fermenters.

Keywords: AMR, Avibactam/Aztreonam, Aztreonam, Carbapenem-resistant, Ceftazidime-avibactam, Ceftazidime-synergy testing, Enterobacterales, ESBL, Gram-negative bacilli, MDR, Non-fermenters

INTRODUCTION

Antimicrobial resistance (AMR) is now a major threat to the public health; the recent rise in the antimicrobial resistance possesses challenges with shrinking antibiotic pipeline leading to limited effective treatments.[1] Antimicrobial resistance is at critical point and in the 21st century it is referred to as "silent pandemic" making it as the top three major public health threats by World Health Organization (WHO).[2] Forming drug resistance is a natural evolving process, when enormous amount of selective pressure applied to the bacteria or other microorganism antimicrobial resistance accelerates gradually making them grow within the presence of the antibiotics.[1]

There are many mechanisms of antimicrobial resistance which can develop at any point of time. Resistance mechanisms involve diverse strategies including natural genetic mutation leading to intrinsic resistance and acquired resistance mechanisms like drug inactivation by enzymes, reduced permeability, efflux pump overexpression, altered binding sites, target site modification and biofilm formation,[3,4] In the recent era human errors makes the major reason for the pressing antibiotic resistance especially their misuse and overuse of antimicrobial agents. Prolonged use of antibiotics in the hospital setting and over the counter medicine prescriptions adds to the pile of rising antimicrobial resistance.[4] A great resistance increase is noted among the gram-negative bacterial pathogens making their treatment increasingly challenging. The limited availability of antibiotics with the rising antimicrobial resistance makes the treatment even more challenging.[5,6] After 1987 there has been a void in the discovery of new antibiotics.[7] Rising AMR made the resistance to the first line drugs which lead to the heavy usage of last line drugs like carbapenems. Over use of last line agents allow bacteria to adapt and develop mechanisms to resist them, further compromising their effectiveness as a result treatment options fail and thereby silently leading to global resistance pandemics. According to the latest report by WHO in 2024 "critical priority group" includes Carbapenem-resistant *Acinetobacter baumannii* (CRAB), 3rd generation cephalosporin-resistant Enterobacterales and carbapenem-resistant Enterobacterales (CRE).[8]

The control of this AMR is now a great challenge faced by the health sectors especially when the discovery of antibiotics slowed down which led to the attention of nationwide researchers to develop a rational design of drug combination therapies to combat antibiotic resistance. With limited antibiotics available researchers started repurposing the existing antibiotics into new combinations, clubbing activities of combined drug together and making effective treatment. One such combination available for treatment is ceftazidime-avibactam in combination with aztreonam.[9] This combination could treat serine-β-lactamase and metallo-β-lactamase strains (MBL). Combining these drugs showed significant synergistic activity in most strains, the strains showed individual resistance to these drugs when used separately.[5] The present study is designed to address in vitro efficacy of the combination of ceftazidime-avibactam and aztreonam in combating antimicrobial resistance among clinical isolates of multi-drug resistant (MDR) Gram-negative bacilli.

METHODS

The present cross-sectional study was conducted between June 2024 and June 2025, performed at School of Medical Education (SME), Kerala, India. 183 isolates of Gram-negative bacilli were collected including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter spp*. were collected from the Microbiology Laboratory of St. Mary's Hospital, Thodupuzha, Kerala, India. The bacterial isolates were further identified by routine biochemical tests and antibiotic susceptibility was determined.

Antimicrobial susceptibility testing (AST)

AST was performed by the Kirby-Bauer disk diffusion as prescribed by Clinical and Laboratory Standards Institute (CLSI) M02-A1310. Antibiotic discs used common for GNB were gentamicin (10 µg), amikacin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), piperacillin-tazobactam (100/10 µg), cefoperazone-sulbactam (75/30 µg). cefuroxime (30 µg), cefoxitin (30 µg), amoxicillin-clavulanate (20/10 µg), tigecycline (15 µg) (Enterobacterales). Aztreonam (30 µg) (*E. coli*, *K. pneumoniae* and *P. aeruginosa*). tetracycline (30 µg), cefotaxime (30 µg), cotrimoxazole (1.25/23.75 µg) (*E. coli*, *K. pneumoniae* and *Acinetobacter spp.*). Colistin (*E. coli*, *Acinetobacter spp*. and *P. aeruginosa*). Cefepime (30 µg) (*K. pneumoniae*, *Acinetobacter spp*. and *P. aeruginosa*). Ampicillin (10 µg), nitrofurantoin (300 µg), Cefixime (5 µg) (*E. coli*). meropenem (10 µg), ceftazidime-avibactam (30/20µg) (*K. pneumoniae*). ampicillin-sulbactam (10/10 µg) (*Acinetobacter spp*.). They were analysed using interpretive standards of CLSIM100™ 34th edition and are categorized into multi-drug resistance (MDR), non- multidrug-resistance (non-MDR), extensively drug resistance (XDR) and pan drug resistance (PDR) groups based on Centers for Disease control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC) guidelines.[11] Isolates were termed as MDR if it shows non-susceptibility to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (bacterial isolates remain susceptibility to only one or two categories). (non-MDR) is defined as susceptibility to all the agents in all antimicrobial categories. PDR is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism). All the antibiotic disc, culture media were purchased from HiMedia Laboratories, Mumbai, India.

Detection of extended-spectrum β-lactamases (ESBL) in Enterobacterales and carbapenem-resistant (CR) isolates from Gram-negative bacilli (GNB) isolates

Detection of ESBL production in Enterobacterales was done phenotypically by disc combination method and CR isolates in GNB was detected phenotypically by modified carbapenem inactivation method (mCIM) and modified carbapenem inactivation method (eCIM) as per the recommended guidelines of CLSIM100TM 34th edition.[11]

Determining the in vitro synergistic activity ceftazidime-avibactam in combination with aztreonam against MDR, ESBL and CR isolates by disc elution test

To determine the in vitro synergistic activity of ceftazidime-avibactam (CZA) in combination with aztreonam (ATM) broth elution test as prescribed by CLSIM100TM 35th edition was used with minor modifications.[12] Briefly 5 MHB tubes were labelled as ATM, CZA, ATM+CZA, GC (growth control) and NC (negative control). A disc of aztreonam (30 µg) was added to the tube labelled "ATM", one ceftazidime-avibactam (30/20 µg) disc to the "CZA" tube, and both discs were added aseptically to the "ATM+CZA" tube. The tubes were vortexed allowing the antibiotics to elute from the discs for a minimum of 30 minutes and a maximum of 60 minutes at room temperature. A standardized bacterial inoculum of 25 µl was then added to all the tubes including GC. The tubes were tightly capped and vortexed again slowly to mix the contents, ensuring that the discs did not adhere to the cap or the upper glass surface. The caps were slightly loosened while incubating at 37ºC for 18 to 24 hours for the assessment of turbidity.

Statistical analysis

All data and graphs were processed using Microsoft Excel and appropriate statistical analysis were performed. The study was approved by the institutional ethical committee (IEC) at the School of Medical Education, Kerala, India.

RESULTS

183 isolates of Gram-negative bacilli were collected including *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter spp*. were collected. Among 183 isolates *E. coli* (n=67), *K. pneumoniae* (n=72), *P. aeruginosa* (n=29) and *Acinetobacter spp*. (n=15) is given in the Figure 1.

In the current study AST of GNB species, *E. coli* exhibited 0% sensitivity and 100% resistance to ampicillin, 28.35% sensitivity and 71.64% resistance to amoxicillin-clavulanate, 59.70% sensitivity and 40.29% resistance to piperacillin-tazobactam, 64.17% sensitivity and 35.82% resistance to cefoperazone-sulbactam, 0% sensitivity and 100% resistance to cefuroxime, 64.17% sensitivity and 35.82% resistance to cefoxitin, 0% sensitivity and 100% resistance to cefixime, 0% sensitivity and 100% resistance to ceftazidime, 0% sensitivity and 100% resistance to cefotaxime, 67.16% sensitivity and 32.83% resistance to aztreonam, 59.70% sensitivity and 40.29% resistance to gentamicin, 59.70% sensitivity and 38.80% resistance to amikacin, 68.65% sensitivity and 31.34% resistance to imipenem, 56.71% sensitivity and 43.28% resistance to nitrofurantoin, 20.89% sensitivity and 79.10% resistance to ciprofloxacin, 44.77% sensitivity and 55.22% resistance to tetracycline, 71.64% sensitivity and 28.35% resistance to tigecycline, 40.29% sensitivity and 59.70% resistance to cotrimoxazole, and 77.61% sensitivity and 22.38% resistance to colistin. *K. pneumoniae* exhibited 4.10% sensitivity and 95.83% resistance to amoxicillin-clavulanate, 1.30% sensitivity and 98.61% resistance to cefuroxime, 12.50% sensitivity and 87.50% resistance to ciprofloxacin, 16.66% sensitivity and 83.33% resistance to cotrimoxazole, 16.66% sensitivity and 83.33% resistance to tetracycline, 0% sensitivity and 100% resistance to ceftazidime, 0% sensitivity and 100% resistance to cefotaxime, 1.30% sensitivity and 98.61% resistance to cefepime, 20.83% sensitivity and 79.16% resistance to amikacin, 26.38% sensitivity and 73.61% resistance to gentamicin, 27.77% sensitivity and 72.22% resistance to piperacillin-tazobactam, 29.16% sensitivity and 70.83% resistance to cefoperazone-sulbactam, 27.77% sensitivity and 72.22% resistance to imipenem, 27.77% sensitivity and 72.22% resistance to meropenem, 29.16% sensitivity and 70.83% resistance to aztreonam, 25% sensitivity and 75% resistance to cefoxitin, 33.33% sensitivity and 66.66% resistance to ceftazidime-avibactam, and 44.44% sensitivity and 55.55% resistance to tigecycline. P. aeruginosa exhibited 58.62% sensitivity and 41.37% resistance to piperacillin-tazobactam, 62.06% sensitivity and 37.93% resistance to cefoperazone-sulbactam, 41.37% sensitivity and 58.62% resistance to ceftazidime, 65.51% sensitivity and 34.48% resistance to cefepime, 58.62% sensitivity and 41.37% resistance to aztreonam, 24.13% sensitivity and 75.86% resistance to amikacin, 58.62% sensitivity and 41.37% resistance to imipenem, 51.72% sensitivity and 48.27% resistance to ciprofloxacin, and 96.55% sensitivity and 3.44% resistance to colistin. *Acinetobacter spp*. exhibited 13.33% sensitivity and 86.66% resistance to piperacillin-tazobactam, 33.33% sensitivity and 66.66% resistance to cefoperazone-sulbactam, 53.33% sensitivity and 46.66% resistance to ampicillin-sulbactam, 6.66% sensitivity and 93.33% resistance to ceftazidime, 13.33% sensitivity and 86.66% resistance to cefotaxime, 33.33% sensitivity and 66.66% resistance to cefepime, 33.33% sensitivity and 66.66% resistance to amikacin, 33.33% sensitivity and 66.66% resistance to imipenem, 33.33% sensitivity and 66.66% resistance to ciprofloxacin, 33.33% sensitivity and 66.66% resistance to cotrimoxazole, and 100% sensitivity with 0% resistance to colistin.

Based on the antimicrobial susceptibility profiles of the 183 isolates of Gram-negative bacilli, among the 67 isolates of *E. coli* showed 68.65% MDR strains, 31.34% XDR strain. In 72 isolates of *K. pneumoniae* it showed 33.33% MDR strains, 66.66% XDR strains. In 29 isolates of *P. aeruginosa* it showed 44.82% (non-MDR) strains,

20.68% MDR strains, 31.03% XDR strains and 3.44% PDR strains. In 15 isolates of *Acinetobacter spp*., it showed 13.33% (non-MDR) strains, 20% MDR strains, 66.66% XDR strains is given in Figure 2.

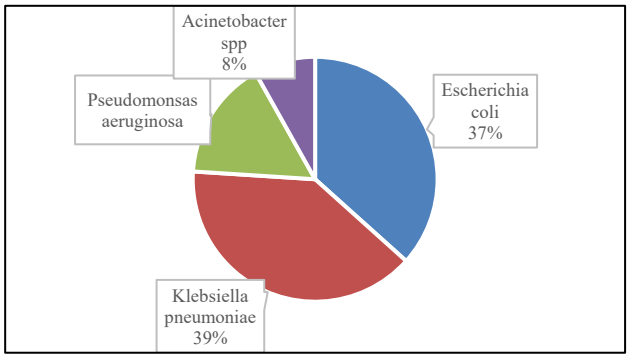

Figure 1: Distribution of Gram-negative bacilli isolate in the present study.

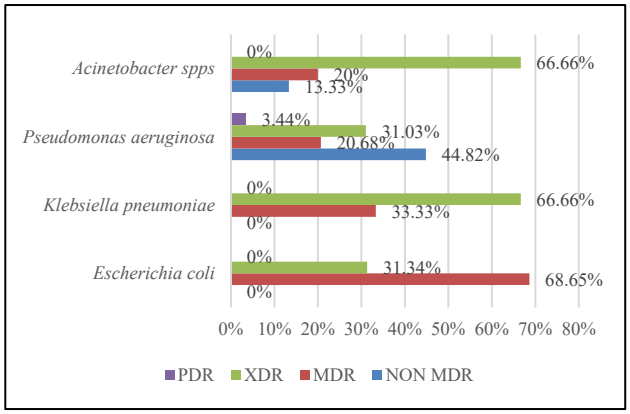

Figure 2: Distribution of antimicrobial resistance pattern of gram-negative bacilli.

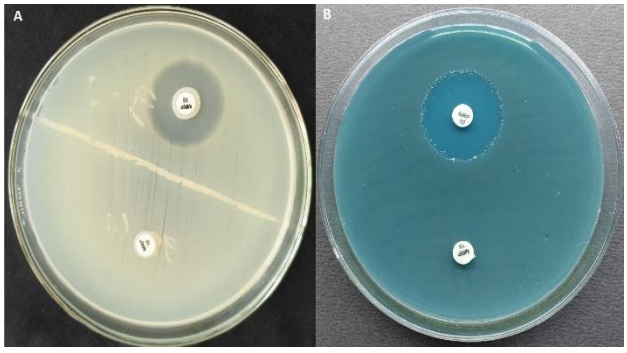

Figure 3: Production in A) *Escherichia coli*; B) *Pseudomonas aeruginosa* isolates using modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM).

ESBL production in Enterobacterales was 85% (n=68). Among these 68 positive isolates *K. pneumoniae* exhibited 29.41% (n=20), *E. coli* showed 70.59%, (n=48). Carbapenem-resistant Gram-negative bacilli were identified in 90 of the 150 isolates tested. Among these resistant isolates, *K. pneumoniae* exhibited 57.78% (n=52), *E. coli* showed 21.11%, (n=19), *P. aeruginosa* showed 10% (n=9) and *Acinetobacter spp*. showed 11.11% (n=10), as shown in Figure 3.

In-vitro synergistic activity of ceftazidime-avibactam in combination with aztreonam against MDR, ESBL and CR isolates by disc elution method

In this study, a total of 183 isolates of Gram-negative bacilli were obtained, among which Enterobacterales constituted 139 isolates and non-fermenters was 44 isolates. In GNB exhibited 83.42% susceptibility towards the combination ceftazidime-avibactam + aztreonam (Figure 4) and 14.75% non-susceptibility were non-susceptible (Figure 5). Comparing the CR strains of gram-negative bacilli, including CR Enterobacterales and CR non-fermenters, a distinct difference in response was observed. Among CR Enterobacterales isolates, combining ceftazidime-avibactam with aztreonam resulted in a marked improvement in activity, with 97.2% of isolates susceptible and only 1.38% non-susceptibility. In contrast, for CR non-fermenters, the combination produced only a modest improvement, with 15.7% of isolates susceptible and 84.21% non-susceptible, showed in the Table 1 and Figure 6.

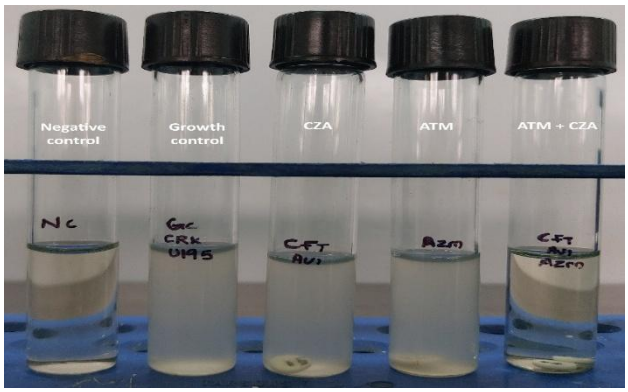

Figure 4: Ceftazidime-avibactam + aztreonam broth disc elution method demonstrating susceptibility of *Klebsiella pneumoniae*.

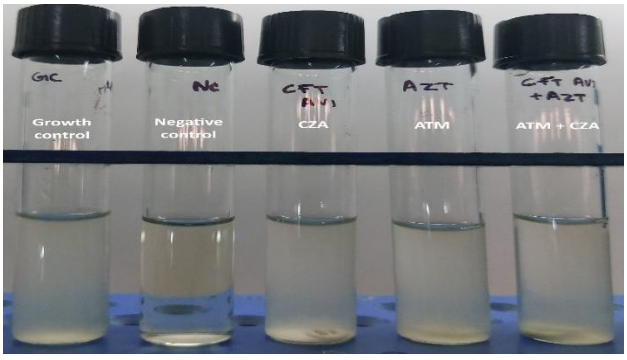

Figure 5: Ceftazidime-avibactam + aztreonam broth disc elution method demonstrating non-susceptibility of *Klebsiella pneumoniae*.

Table 1: Comparison of ceftazidime-avibactam + aztreonam broth disc elution result between CR Enterobacterales and CR non-fermenters.

Antibiotics	Enterobacterales susceptible (%)	Enterobacterales non-susceptible (%)	Non-fermenters susceptible (%)	Non-fermenters non-susceptible (%)
Ceftazidime-Avibactam	12.5	86.11	10.52	89.47
Aztreonam	12.5	86.11	0	100
Ceftazidime-Avibactam + Aztreonam	97.2	1.38	15.7	84.21

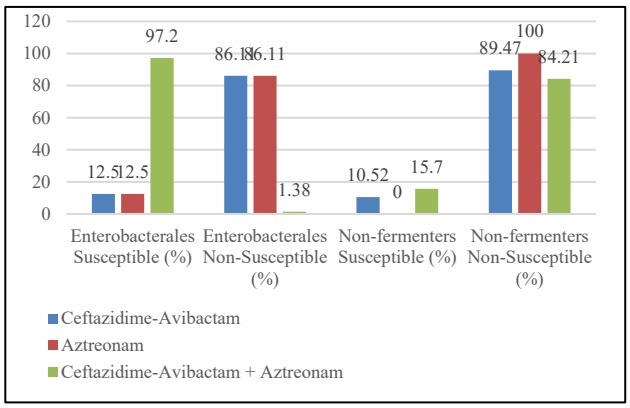

Figure 6: Comparative susceptibility to ceftazidime-avibactam, aztreonam and ceftazidime-avibactam + aztreonam detected by broth disc elution method in carbapenem resistant Enterobacterales and carbapenem resistant non-fermenters.

DISCUSSION

The development of new antimicrobial drugs is limited and the resistance particularly among the GNB is steadily increasing. Treating the MDR organisms with the existing antibiotics is often challenging especially when highly drug-resistant bacteria present and few options of novel antibiotics being discovered. As a result, researchers have made combinations of existing antibiotics, allowing each drug's individual mechanism to work synergistically to combat MDR bacteria. The in vitro synergy between ceftazidime-avibactam and aztreonam, as assessed by the disk elution method, is recognized as a standard procedure by the CLSI. This combination is particularly effective against MDR Gram-negative bacilli, especially MBL producers. So, this study tested the synergy investigating the efficacy of ceftazidime-avibactam with aztreonam for ESBL and CR strains.

In the case of AST done in Enterobacterales, complete resistance (100%) was observed to 3rd generation cephalosporins and high resistance was seen in 2nd generation cephalosporin. In the study conducted by Lal et al the high resistance was also seen in 2nd and 3rd generation around 60 to 70%.[13] A study conducted by Sivadas et al also exhibited high resistance to 3rd generation cephalosporins around 60 to 65% in Enterobacterales.[14] The difference in percentage is because in our study less isolates used compared to theirs and this study concentrates more on the selectively picked MDR strains. In P. aeruginosa high resistance was seen towards amikacin (75.86%) and ceftazidime (58.62%) meanwhile the study conducted by Shahid et al also showed as a striking feature as the resistance towards amikacin and ceftazidime in P. aeruginosa.[15] In the case of *Acinetobacter spp*. high susceptibility was noted against colistin (100%) similarly in a study conducted by Tewari et al non-*Acinetobacter baumannii* species showed 100% susceptibility to colistin.[16]

In the present study, 68.65% of *E. coli* were MDR (n=67), meanwhile *K. pneumoniae* showed 66.66% (n=72) XDR strains. A study conducted by Lal et al in *K. pneumoniae* showed 41.66% (n=120) XDR and *E. coli* exhibited 66% MDR (n=100), in both the test highest rate of XDR strains shown by *K. pneumoniae* and highest rate of MDR strains shown by *E. coli*.[13] In our study, *P. aeruginosa* showed 3.44% PDR strains (n=29) meanwhile a study conducted by Shokri et al 1.1% PDR (n=96). This is because their study was highly concentrated on finding the PDR strains only compared to this study.[17] Similarly in *Acinetobacter spp*., it showed 13.33% (non-MDR) strains, 66.66% XDR strains (n=15) meanwhile a study conducted by Pattnaik et al showed 71.63% MDR, 50.35% XDR and 2.84% PDR.[18] Pattnaik et al study was highly concentrated on tertiary care centre hospitals where highly multidrug resistant strains are visible.

In this study, the ESBL producing strains in Enterobacterales among the 80 isolates tested in which 68 isolates showed positive ESBL reaction by combination disk test of cephalosporin/clavulanic acid. Among these 68 positive isolates *E. coli* exhibited 70.59% (n=48) and *K. pneumoniae* showed 29.41% (n=20). A similar study conducted by Sivadas et al showed 79% in *E. coli* (n=107) and 72% for *K. pneumoniae* (n=108).[15] Other study conducted by Kumar et al revealed a total of 34.9% positive ESBL producers among 107 isolates tested, in which *E. coli* showed 34.42% (n=79) and *K. pneumoniae* showed 37.31% (n=25).[19] This difference in the percentages is because, in this study the main focus point was MDR strains, so after general sensitivity only MDR strains was used to check the ESBL production whereas in other studies they took all the strains irrespective of multidrug resistant strains.

The prevalence of CR strains was 90 out of 150 gram-negative bacilli isolates tested. Among these 90 isolates *E. coli* exhibits 21.11% (n=19), *K. pneumoniae* showed 57.78% (n=52), *P. aeruginosa* 10% (n=9) and *Acinetobacter spp*. 11.11% (n=10). A study conducted by Verma G *et al*. exhibited *K. pneumonia* 60.4%, *E. coli* 17.6%, and *P. aeruginosa* 12.4%.[20] In both works *K. pneumoniae* is having high number of CR strains. The slight difference in the percentage of CR strains due to the larger sample size tested by the other researcher and the inclusion of samples from both ICU and patient wards. Patient wards often have more highly virulent strains with multiple resistance mechanisms, whereas in this study only MDR strains identified through AST were included.

The synergistic activity of ceftazidime-avibactam combined with aztreonam against MDR GNB in the present study revealed an overall susceptibility of 83.42%, while 14.75% of isolates were non-susceptible. In a similar study conducted by Rajshekar et al an overall of 67.74% synergism to the combination of ceftazidime-avibactam with aztreonam and 32.25% non-susceptibility to the combination of ceftazidime-avibactam with aztreonam was seen.[21] In the present study within Enterobacterales (n=139; *E. coli*- 67, *K. pneumoniae*- 72), the combination exhibited a very high activity, with 99.28% susceptibility and only 0.71% non-susceptibility, observed in a single isolate of *K. pneumoniae* (1.3% resistance). All *E. coli* isolates demonstrated 100% synergy to the drug combination. Comparable findings have been reported in previous studies. Rajshekar et al observed synergy in *K. pneumoniae* (n=30), with 93.33% susceptibility and 6.67% non-susceptibility, while in *Escherichia coli* (n=3), the susceptibility was 66.67% with 33.33% non-susceptibility.[21] The higher *E. coli* susceptibility seen in the present study can be attributed to differences in study design. Rajshekar et al evaluated only ceftazidime-avibactam resistant strains, whereas the present study considered all MDR isolates, thus demonstrating a broader spectrum of synergy. Similarly, Biswal et al reported 90.9% susceptibility in *E. coli* with 9.1% non-susceptibility, while Rajan et al had 71.43% susceptibility and 28.57% non-susceptibility in *K. pneumoniae.*[22,24] These variations likely reflect differences in isolate selection, local epidemiology, and resistance mechanisms. Although ceftazidime-avibactam with aztreonam is primarily employed for the treatment of MBL producing strains, non-susceptibility to this combination has been documented across multiple studies, including the present one. Importantly, non-susceptibility is frequently associated with XDR and PDR isolates, particularly *K. pneumoniae* (based on the present study and other studies), suggesting a concerning trend of rising resistance even against this last-line therapeutic option.

When non-fermenters were analysed, 59.09% of isolates demonstrated non-susceptibility to the ceftazidime-avibactam plus aztreonam combination. Among these, *P. aeruginosa* accounted for 37.93% resistance. Rajan et al similarly reported resistance in a single *P. aeruginosa* isolate; however, their study population largely consisted of Enterobacterales and *Proteus spp.*, limiting direct comparison.[23] In contrast, Rajshekar et al reported much higher resistance in *P. aeruginosa* (n=29), with 58.62% non-susceptibility and 41.38% susceptibility similarly in Biswal et al showed 87.5% (n=8) non-susceptibility to the combination of ceftazidime-avibactam with aztreonam.[21] The higher resistance observed in *P. aeruginosa* is likely due to its chromosomally encoded AmpC β-lactamase, production of multiple resistance enzymes, and adaptive mechanisms such as efflux pumps and porin loss, which collectively contribute to reduced susceptibility compared to Enterobacterales. In the present study, one PDR *P. aeruginosa* isolate also failed to respond, further supporting this trend.

For *Acinetobacter spp.*, synergy testing revealed complete resistance in 34.09% of isolates. This lack of activity may be explained by the inability of avibactam to enhance ceftazidime activity against *Acinetobacter spp.*, as it does not adequately penetrate the bacterial outer membrane. Furthermore, the intrinsic resistance of *Acinetobacter spp*. To aztreonam contributes significantly to the absence of synergy, making this pathogen particularly difficult to manage with this combination.

The primary limitations of this study are its exclusive in vitro design, which restricts the direct translation of results to clinical practice, as patient pharmacokinetics, pharmacodynamics and toxicity may differ significantly from laboratory conditions. Although the study demonstrated marked effectiveness against MDR Enterobacterales, the representation of non-fermenters such as *P. aeruginosa* and *Acinetobacter spp.* was comparatively lower, potentially limiting the generalizability of findings to these pathogens. The selection process for isolates may have also introduced bias by emphasizing highly resistant strains, which may not fully reflect the diversity encountered in clinical settings. Notably, the study did not correlate in vitro findings with clinical outcomes, side effect profiles, or pharmacodynamic data, making it difficult to ascertain the real-world therapeutic efficacy or safety of the drug combination.

CONCLUSION

In conclusion, this study demonstrated that the combination of ceftazidime-avibactam with aztreonam provides significant in vitro synergistic activity against MDR GNB, particularly Enterobacterales. The markedly enhanced susceptibility observed compared with either agent alone in MBL producing Enterobacterales especially *K. pneumoniae* however limited efficacy observed against non-fermenter group among GNB. These results highlight the promise of ceftazidime-avibactam plus aztreonam in addressing difficult to treat Enterobacterales infections, while also emphasizing the need for further research, ongoing resistance surveillance and exploration of

alternative therapeutic options for non-fermenter infections.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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