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IN VITRO SUSCEPTIBILITY COMPARISON OF AZTREONAM ALONG WITH CEFTAZIDIME-AVIBACTAM SYNERGY TESTING METHODS AND AZTREONAM-AVIBACTAM AGAINST CARBAPENEM RESISTANT ORGANISMS IN ISOLATES OBTAINED FROM CANCER PATIENTS

Clinical Microbiology

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ABSTRACT

Introduction: The global rise of multidrug-resistant (MDR) Gram-negative pathogens, particularly carbapenem-resistant Enterobacterales (CRE) and Pseudomonas aeruginosa, presents a critical therapeutic challenge. Resistance is often driven by the co-expression of metallo-β-lactamases (MBLs) like NDM, along with serine β-lactamases (e.g., ESBLs, AmpC). Aztreonam (ATM) retains intrinsic activity against MBLs but is hydrolyzed by serine β-lactamases. Avibactam, a novel non-β-lactam β-lactamase inhibitor, inhibits class A, C, and some class D enzymes but lacks efficacy against MBLs. The combination of drugs like aztreonam and avibactam (ATM-AVI) offers a promising strategy to overcome such dual resistance. **Objective:** To evaluate the in vitro efficacy of the aztreonam-avibactam combination against carbapenem-resistant, NDM-producing Gram-negative bacilli and to compare it with Ceftazidime-Avibactam along with Aztreonam. **Method:** A total of 100 non-duplicate clinical isolates of Carbapenem Resistant Organisms (CRO) were recovered from cancer patients in a tertiary care hospital. Resistant genotype was confirmed and those isolates showing NDM genotype with or without other resistant genotypes were included in the study. Synergy between Aztreonam and Ceftazidime-avibactam was assessed using three techniques: ICMR recommended strip and disc method, Broth Disc Elution (BDE) method with 5ml of broth (CLSI recommended) and 7.5ml broth (Inhouse modified) method. The isolates were also tested by Aztreonam-Avibactam E-strips. **Results:** Of the 100 isolates, susceptibility of ATM-AVI was observed in 79% isolates. Isolates were most susceptible to Ceftazidime Avibactam along with Aztreonam by broth disc elution method (CLSI-75% and inhouse modification-79%). **Conclusion:** The use of broth disk elution method proved to be practical and reliable approaches for synergy testing in routine clinical microbiology laboratories. Aztreonam-avibactam (ATM-AVI) exhibits robust in vitro activity against NDM-producing Gram-negative bacilli, representing a promising therapeutic option in the context of increasing metallo-β-lactamase (MBL) prevalence. These findings underscore the potential clinical utility of ATM-AVI in managing infections caused by multidrug-resistant, NDM-producing pathogens, particularly in high-burden settings.

KEYWORDS

Aztreonam-Avibactam, Ceftazidime-avibactam-Aztreonam synergy testing, Carbapenem resistance

INTRODUCTION

The global rise in carbapenemase-producing Gram-negative bacilli, particularly within Enterobacterales and Pseudomonas aeruginosa, poses a significant therapeutic challenge due to limited treatment options and high associated morbidity and mortality. Carbapenem resistance among Enterobacterales is primarily mediated by the production of carbapenem-hydrolyzing β-lactamases, or carbapenemases, which inactivate a wide spectrum of β-lactam antibiotics, including carbapenems. These enzymes are classified under the Ambler scheme into class A (e.g. KPC), class B (metallo-β-lactamases [MBLs] such as NDM, VIM, IMP), and class D (e.g. OXA-48-like) carbapenemases (7).

Several novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations—including ceftazidime-avibactam (CZA), meropenem-vaborbactam, and imipenem-relebactam—have shown substantial activity against class A, class C, and certain class D enzyme-producing organisms. However, they exhibit minimal to no activity against class B MBLs (5,7,11). The therapeutic challenge is further compounded by the frequent co-expression of MBLs with serine β-lactamases such as extended-spectrum β-lactamases (ESBLs), AmpC, KPC, and OXA-48 within the same clinical isolate (7).

Aztreonam, a monobactam, remains stable in the presence of MBLs but is highly susceptible to degradation by serine β-lactamases (7,12). Avibactam, a non-β-lactam diazabicyclooctane inhibitor, effectively inhibits class A (e.g. KPC, CTX-M, SHV), class C (AmpC), and certain class D enzymes (e.g. OXA-48-like), but lacks inhibitory activity against class B MBLs (4). Therefore, the combination of aztreonam with avibactam (aztreonam-avibactam) represents a mechanistically rational approach. In this pairing, avibactam protects aztreonam from hydrolysis by co-produced serine β-lactamases, while aztreonam retains its intrinsic activity against MBLs (2,7,11).

In Ceftazidime avibactam aztreonam combination, Avibactam, from the ceftazidime-avibactam combo, inhibits the co-produced serine β-lactamases (e.g., ESBLs, AmpC). This protects aztreonam from degradation, allowing it to act freely against the MBL-producing pathogen. Ceftazidime has minimal direct effect but provides the vehicle for avibactam.

Large international surveillance programs, including ATLAS and SENTRY, have demonstrated excellent in vitro potency of aztreonam-avibactam against CRE isolates, including those co-producing MBLs and serine β-lactamases. Between 2019 and 2021, these programs reported that approximately 99.9% of Enterobacterales isolates were inhibited, with MIC50/90_{50/90}50/90 values of 0.03/0.12 µg/mL overall and 0.25/0.5 µg/mL among CRE isolates. Notably, 99.7% of isolates were inhibited at concentrations ≤8 mg/L (10). Similarly, a separate dataset comprising 24,924 Enterobacterales, including 1,098 CRE isolates from 36 countries, showed that 99.6% of CRE were inhibited at ≤8 mg/L, including 98.9% of CZA-resistant isolates (9).

In comparative studies, aztreonam-avibactam retained activity against 100% of meropenem-vaborbactam-non-susceptible strains, 99.5% of imipenem-relebactam-non-susceptible strains, and 90% of CZA-non-susceptible strains, highlighting its unique and consistent efficacy against multidrug-resistant organisms (6). These findings underscore the therapeutic potential of aztreonam-avibactam in managing infections caused by CRE, particularly those producing both MBLs and serine β-lactamases (2,6,9,10).

Time-kill assays and pharmacodynamic modeling support the synergistic interaction between aztreonam and avibactam, with MIC values reduced below clinical breakpoints when tested against isolates co-producing MBLs and serine β-lactamases (3,4). The REJUVENATE Phase IIa trial demonstrated favorable pharmacodynamic target attainment using prolonged infusion regimens, with >90% of the dosing interval spent above the mutant prevention concentration (fT > MPC), contributing to suppression of resistance (3).

Given the widespread distribution of carbapenemases globally—especially KPC, NDM, and OXA-48, which together account for approximately 90.2% of CRE isolates—aztreonam-avibactam fulfills a critical unmet therapeutic need (10). Current guidelines from the

Infectious Diseases Society of America (IDSA) recommend the use of aztreonam in combination with CZA in regions or settings where standalone aztreonam-avibactam is not yet available (10). Observational studies and real-world clinical data further support this strategy, with reduced mortality, shorter hospital stays, and improved outcomes reported in bloodstream infections due to MBL-producing Klebsiella pneumoniae and Escherichia coli (2,8).

In light of these findings, aztreonam-avibactam represents a significant advancement in the treatment of infections caused by carbapenemase-producing Gram-negative bacilli. Further clinical trials and resistance monitoring are warranted to validate its efficacy and preserve its utility.

MATERIALS AND METHODS

The study was conducted on isolates obtained from cancer patients at a tertiary care centre, Mumbai, from December 2024 to April 2025. Positive blood cultures, sterile body fluids, pus and urine samples which showed growth of Carbapenem resistant Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae and Pseudomonas aeruginosa, which showed presence of NDM genotype, with or without other genotype, were included in the study.

The isolates were tested for susceptibility test to Ceftazidime-Avibactam along with Aztreonam by three methods-ICMR recommended strip and disc method, Broth Disc Elution (BDE) method with 5ml of broth (CLSI recommended) and 7.5ml broth (Hospital modified) method. The isolates were also tested by Aztreonam-Avibactam strips (by Liofilchem).

Interpretation

For synergy test between Ceftazidime-Avibactam along with Aztreonam, by ICMR method, formation of inverted D shaped zone between the disc and the strip was considered as synergy positive. For BDE method, absence of turbidity in the tube was considered as susceptible. For Aztreonam-Avibactam strip test, the zone of inhibition was determined and compared to the cut off.

RESULTS

Table 1

Synergy	ICMR Disc-strip method	CLSI 5ml broth	Hospital modified 7.5ml broth
Positive	67	75	79
Negative	33	25	21

P value=0.146 > 0.05, the difference in positive and negative detection between methods is not statistically significant at the 5% level.

In the study, it was observed that majority of the isolates 79% (79/100) were susceptible Aztreonam, when tested in combination with Ceftazidime Avibactam. 67% (67/100) and 75% (75/100) of the isolates were susceptible to the combination by ICMR recommended disc and E strip method and broth disc elution method of CLSI, respectively.

Table 2

	Aztreonam-Avibactam		
Organism	Sensitive	Resistant	Total
Pseudomonas aeruginosa	2	10	12
Klebsiella pneumoniae	42	2	44
Escherichia coli	30	9	39
Enterobacter cloacae	5	0	5

Majority of the isolates in the study were Klebsiella pneumoniae 44% (44/100), followed by Escherichia coli 39% (39/100), Pseudomonas aeruginosa 12% (12/100) and Enterobacter cloacae 5% (5/100).

In the study, 79% (79/100) of the total isolates were susceptible to Aztreonam-Avibactam, tested by E strip method. 95.45% (42/44) of Klebsiella pneumoniae isolates were susceptible to Aztreonam-Avibactam. Amongst 39 of total Escherichia coli isolates, 30, 76.92% (30/39), were susceptible to the combination drug. Susceptibility of Pseudomonas aeruginosa was found to be only 16.66% (02/12), whereas all the 5 isolates of Enterobacter cloacae 100% were susceptible to the antibiotic combination.

DISCUSSION

The present study evaluated and compared the performance of three methods—Synergy test (as per ICMR protocol), the CLSI-recommended broth disc elution method, and an in-house modified broth-based screening method (5 ml and 7.5 ml volumes). The positivity rates observed were 67% for the Synergy method, 75% for the CLSI broth based method, and 79% for the Inhouse modified broth-based method. While the broth-based technique yielded the highest number of positive isolates, statistical analysis did not reveal a significant difference among the three methods.

The chi-square test of independence revealed a p-value of 0.146. This indicates that the differences in detection rates across the methods are not statistically significant, suggesting that all three methods may perform similarly in identifying resistant isolates in this cohort.

Although not statistically significant, the broth-based method demonstrated 100% sensitivity in detecting positive isolates as compared to the CLSI disc-strip method, which was considered the reference standard in this analysis. Its specificity, however, was lower (~84%), indicating a potential for false-positive results. On the other hand, the Synergy method showed excellent specificity (100%) but a lower sensitivity (~89.3%), suggesting a higher likelihood of false negatives. These findings are consistent with earlier studies that have noted variability in the sensitivity and specificity of phenotypic methods depending on the resistance mechanism and organism involved [13-15].

From a practical standpoint, the in-house broth-based method offers several advantages, including ease of preparation, cost-effectiveness, and higher yield. This makes it particularly attractive for laboratories in resource-limited settings, where access to commercial confirmatory kits or molecular testing may be constrained [16,17]. However, due to the observed over-detection, results from the broth method should ideally be corroborated with molecular or standardized phenotypic methods, especially for critical or invasive isolates [18].

It is important to acknowledge the limitations of this study, notably the sample size (n=100 per method) and the absence of genotypic correlation to confirm the presence of resistance genes. Future studies with larger sample sizes and inclusion of molecular assays such as PCR or sequencing are essential to validate and refine the role of in-house broth-based methods in clinical microbiology diagnostics.

Our data demonstrate that aztreonam–avibactam exhibits strong in vitro activity against Klebsiella pneumoniae, Escherichia coli, and Enterobacter cloacae, while its activity against Pseudomonas aeruginosa is significantly limited. These findings align with emerging clinical and surveillance reports.

A large global surveillance study (2019–2021) involving regions across Latin America, Asia, Eurasia, and the Middle East reported >99 % susceptibility of Enterobacterales to aztreonam–avibactam, including meropenem-nonsusceptible and MBL-producing strains (19). Similarly, a multicenter study from the United States reported 99.9–100 % susceptibility of Enterobacterales and carbapenem-resistant Enterobacterales (CRE) to aztreonam–avibactam (20). These findings correspond well with our observed high sensitivity rates of 95.5 % in K. pneumoniae, 76.9 % in E. coli, and 100 % in E. cloacae.

P. aeruginosa remains poorly susceptible to aztreonam–avibactam, as reflected in our sensitivity rate of only 16.7 %. A recent U.S. study found susceptibility rates of 78.0–81.9 % among P. aeruginosa isolates, significantly lower than for Enterobacterales (20). The limited activity is likely due to intrinsic resistance mechanisms, including chromosomally encoded AmpC, overexpression of efflux pumps, and the presence of non-classical β-lactamases such as GES and OXA (21).

Our findings reaffirm the potential role of aztreonam–avibactam as a key agent against CRE and MBL-producers among Enterobacterales. However, its reduced activity against P. aeruginosa highlights the necessity of susceptibility testing and suggests a need for combination regimens or alternative agents, such as cefiderocol, in resistant P. aeruginosa infections.

CONCLUSION

The comparative analysis of Synergy (ICMR), CLSI disc-strip, and in-house broth-based methods revealed no statistically significant difference in diagnostic performance (p = 0.146). While the broth-based method is easy to perform, gives minimal errors and well-suited for low-resource or routine hospital labs, it demonstrated the highest positivity and sensitivity, its specificity was comparatively lower. Given its simplicity and low cost, it holds potential as a screening tool

in resource-limited settings, but should ideally be followed by confirmatory testing. Larger, multicentric, and genotypically supported studies are recommended to further assess the diagnostic utility and reproducibility of the broth-based approach.

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