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Comparative Evaluation of Phenotypic Methods for Testing Ceftazidime-Avibactam and Aztreonam Synergy in Carbapenem-Resistant Enterobacterales from a Tertiary Care Center in Eastern India

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Abstract

Background

Metallo-β-lactamase (MBL) producing carbapenem-resistant Enterobacterales (CRE) pose significant therapeutic challenges in clinical practice. The combination of ceftazidime-avibactam with aztreonam demonstrates promise against MBL-producing organisms, but standardized synergy testing methods remain inadequately defined.

Objective

To evaluate and compare the performance of different phenotypic methods for testing ceftazidime-avibactam and aztreonam synergy in clinical CRE isolates.

Methods

Fifty consecutive non-duplicate CRE isolates were collected from various clinical specimens at a tertiary care center in Bihar, India. Four phenotypic synergy testing methods were evaluated: E-test/disc method, double disc diffusion (discs placed 15mm apart), disc stacking method, and disc elution method. Carbapenemase genes were detected using multiplex PCR. Statistical analysis was performed using Fisher's exact test to compare method performance.

Results

Among 50 CRE isolates (26 E. coli, 24 K. pneumoniae), 37 (74%) demonstrated resistance to ceftazidime-avibactam. Synergy detection rates varied significantly between methods: E-test/disc method 27% (10/37), disc stacking method 78% (29/37), and disc elution method 81% (30/37). All isolates harbored NDM genes, with 30 (60%) co-producing OXA-48. The disc elution method showed significantly superior synergy detection compared to the E-test/disc method ($p < 0.001$).

Conclusions

The high prevalence of MBL genes among CRE isolates explains the limited efficacy of ceftazidime-avibactam monotherapy. The disc elution method demonstrated the highest synergy detection rate and offers a practical, reproducible approach for clinical microbiology laboratories.

INTRODUCTION

The global emergence of carbapenem-resistant Enterobacterales (CRE) represents one of the most pressing challenges in contemporary infectious disease management [1]. These organisms, characterized by resistance to at least one carbapenem antibiotic (ertapenem, meropenem, or imipenem), have become increasingly prevalent in healthcare settings worldwide. According to the Indian Council of Medical Research (ICMR) antimicrobial resistance surveillance network, carbapenem resistance rates have reached alarming levels, with imipenem resistance observed in 28% of E. coli, 55% of K. pneumoniae, and 80% of A. baumannii isolates [2].

The therapeutic management of CRE infections is particularly challenging due to the limited availability of effective treatment options. Traditionally, colistin has been considered a last-resort agent for CRE infections; however, its use is associated with significant nephrotoxicity and the emergence of colistin-resistant strains further complicates treatment decisions [3].

Ceftazidime-avibactam, a novel β-lactam/β-lactamase inhibitor combination, has emerged as a promising alternative for treating CRE infections. This agent demonstrates excellent activity against class A carbapenemases (such as KPC) and class D enzymes (including OXA-48-like) but lacks efficacy against class B metallo-β-lactamases (MBLs) [4]. The high prevalence of MBL-producing organisms, particularly NDM variants, in the Indian subcontinent significantly limits the utility of ceftazidime-avibactam monotherapy [5].

Aztreonam, a monobactam antibiotic, maintains stability against MBL hydrolysis but is susceptible to inactivation by extended-spectrum β-lactamases (ESBLs) and AmpC enzymes that frequently co-exist in MBL-producing isolates. The combination of ceftazidime-avibactam with aztreonam theoretically provides synergistic activity by protecting aztreonam from ESBL and AmpC hydrolysis through avibactam inhibition, while aztreonam retains activity against MBL-producing organisms [6].

Despite the theoretical rationale for this combination, practical laboratory methods for testing ceftazidime-avibactam and aztreonam synergy remain poorly standardized. Various approaches have been described in the literature, but comparative evaluations of these methods in clinical isolates are limited [7]. This study aimed to evaluate and compare different phenotypic synergy testing methods to identify the most practical and reliable approach for routine clinical laboratory use.

MATERIALS AND METHODS

Study Design and Setting This diagnostic accuracy study was conducted in the Department of Microbiology at a tertiary care center in Bihar, India. The study protocol was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Patna (Approval No.: AIIMS/Pat/IEC/2023/1177). All methods were performed in accordance with the relevant guidelines and regulations. As the study involved bacterial isolates obtained as part of routine diagnostic procedures and did not include any identifiable patient information, the requirement for informed consent was waived by the ethics committee.

Bacterial Isolates Fifty consecutive non-duplicate CRE isolates were collected from various clinical specimens including blood, pus, urine, and respiratory samples processed between [study period]. All isolates were obtained from patients with healthcare-associated infections including catheter-related bloodstream infections (CRBSI), catheter-associated urinary tract infections (CAUTI), hospital-acquired pneumonia (HAP), and surgical site infections (SSI).

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. Isolates resistant to meropenem (10 µg) and imipenem (10 µg) were selected for the study.

Ceftazidime-Avibactam Susceptibility Testing All CRE isolates were tested for ceftazidime-avibactam susceptibility using disc diffusion method with ceftazidime-avibactam discs (30/20 µg) according to CLSI

interpretive criteria. Isolates demonstrating resistance to ceftazidime-avibactam were selected for synergy testing.

Synergy Testing Methods Four different phenotypic synergy testing methods were evaluated on ceftazidime-avibactam resistant isolates, based on previously described methodologies [9, 10]:

Method 1: E-test/Disc Method A ceftazidime-avibactam E-test strip (bioMérieux) was placed on Mueller-Hinton agar plates inoculated with the test organism. An aztreonam disc (30 µg, HiMedia) was positioned 15mm from the center of the E-test strip. Plates were incubated at 37°C for 18–24 hours. Synergy was interpreted as enhancement of the inhibition zone around the aztreonam disc in the presence of the ceftazidime-avibactam gradient.

Method 2: Double Disc Method Ceftazidime-avibactam discs (30/20 µg, Oxoid) and aztreonam discs (30 µg, HiMedia) were placed 15mm apart (center to center) on Mueller-Hinton agar plates inoculated with the test organism. Synergy was interpreted as enhancement of inhibition zones between the two discs.

Method 3: Disc Stacking Method Aztreonam discs (30 µg) were placed on inoculated Mueller-Hinton agar plates, followed by ceftazidime-avibactam discs (30/20 µg) placed directly on top. Zone diameters were measured after 18–24 hours of incubation and compared to aztreonam alone controls.

Method 4: Disc Elution Method Two milliliters of sterile Mueller-Hinton broth were dispensed into test tubes. Individual tubes received either aztreonam discs alone, ceftazidime-avibactam discs alone, or both discs together. After 30 minutes of elution at room temperature, 12 µL of standardized bacterial inoculum (0.5 McFarland) was added to each tube. Tubes were incubated at 37°C for 16–20 hours. Synergy was interpreted as absence of visible turbidity in tubes containing both discs, while tubes with single discs showed growth.

Molecular Detection of Carbapenemase Genes DNA extraction was performed using standard boiling method. Multiplex PCR was performed to detect common carbapenemase genes including bla_NDM, bla_KPC, bla_OXA-48, bla_VIM, bla_IMP, and bla_SPM using previously described primer sets [11]. PCR amplification was carried out in 25 µL reaction volumes with the following thermal cycling conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. PCR products were analyzed by agarose gel electrophoresis.

Statistical Analysis Categorical variables were expressed as frequencies and percentages. Fisher's exact test was used to compare synergy detection rates between different methods. Statistical significance was defined as $p < 0.05$. All analyses were performed using appropriate statistical software.

RESULTS

Isolate Characteristics Fifty CRE isolates were included in the study, comprising 26 (52%) E. coli and 24 (48%) K. pneumoniae isolates. The distribution by specimen type was as follows: blood samples 16 (32%), pus samples 16 (32%), urine samples 10 (20%), and respiratory samples 8 (16%).

Ceftazidime-Avibactam Susceptibility Overall, 37 of 50 (74%) CRE isolates demonstrated resistance to ceftazidime-avibactam. The resistance rates varied by specimen type: blood samples 13/16 (81.25%), pus samples 10/16 (62.5%), respiratory samples 5/8 (62.5%), and urine samples 9/10 (90%). When analyzed by organism, 21/26 (80.7%) E. coli isolates and 16/24 (66.7%) K. pneumoniae isolates were resistant to ceftazidime-avibactam.

Synergy Testing Results Among the 37 ceftazidime-avibactam-resistant isolates, synergy testing was performed using all four methods. The results are summarized in Table 1.

Table 1
Synergy Testing Results by Method and Specimen Type

Method	Overall Synergy Rate	Blood (n = 13)	Pus (n = 10)	Urine (n = 9)	Respiratory (n = 5)
E-test/Disc	10/37 (27%)	4/13 (30.7%)	3/10 (30%)	2/9 (22.2%)	1/5 (20%)
Double Disc (15mm)	10/37 (27%)	4/13 (30.7%)	3/10 (30%)	2/9 (22.2%)	1/5 (20%)
Disc Stacking	29/37 (78%)	10/13 (77%)	7/10 (70%)	7/9 (78%)	5/5 (100%)
Disc Elution	30/37 (81%)	11/13 (84.6%)	8/10 (80%)	6/9 (66.7%)	5/5 (100%)

Statistical Comparison of Methods Statistical analysis revealed significant differences between synergy testing methods. When comparing disc stacking to E-test/disc methods, the p-value was 0.018. The comparison between disc elution and E-test/disc methods yielded $p < 0.001$, indicating statistical significance. However, the comparison between disc stacking and disc elution methods was not statistically significant ($p = 0.135$).

Molecular Characterization PCR analysis revealed that all 50 CRE isolates (100%) harbored NDM genes. Additionally, 30 isolates (60%) co-produced OXA-48 genes. No isolates tested positive for KPC, IMP, VIM, or SPM genes in the conventional PCR assay.

DISCUSSION

This study provides important insights into the performance characteristics of different phenotypic methods for testing ceftazidime-avibactam and aztreonam synergy in clinical CRE isolates. The finding that 74% of CRE isolates were resistant to ceftazidime-avibactam aligns with the high prevalence of MBL-producing organisms in our setting, as confirmed by the universal presence of NDM genes in our isolate collection.

The superior performance of disc elution and disc stacking methods compared to the E-test/disc approach suggests that these techniques may be more suitable for routine clinical laboratory use. The disc elution method, in particular, demonstrated the highest synergy detection rate (81%) and offers several practical advantages including standardized inoculum preparation, objective endpoint interpretation, and minimal requirement for specialized equipment.

Our findings are consistent with recent studies examining ceftazidime-avibactam and aztreonam combinations. Rawson et al. reported 81% concordance between broth dilution and E-test/disc methods in NDM-producing Enterobacterales, with broth dilution showing 74% synergy and E-test/disc method showing 53% synergy [12]. While we did not include broth dilution as a reference standard, our disc elution method achieved 81% synergy detection, comparable to the reference method in their study.

The universal presence of NDM genes in our isolate collection reflects the epidemiological pattern of carbapenem resistance in the Indian subcontinent, where NDM variants predominate [13]. The co-production of OXA-48 in 60% of isolates further emphasizes the complex resistance mechanisms present in these organisms and supports the rationale for combination therapy approaches.

The clinical implications of our findings are significant. Given the high prevalence of MBL-producing CRE in our setting, ceftazidime-avibactam monotherapy would be expected to fail in the majority of cases. The demonstration of synergy between ceftazidime-avibactam and aztreonam in 78–81% of isolates using practical laboratory methods provides clinicians with valuable information for therapeutic decision-making. However, this high synergistic activity failed to translate into clinical practice, with poor patient outcomes and high mortality rates.

It is noteworthy that during the course of this study, the Clinical and Laboratory Standards Institute (CLSI) published updated guidelines in the M100 34th edition (2024), which includes recommendations for aztreonam and ceftazidime-avibactam synergy testing using the disc elution method [8]. Our methodology closely parallels these recommendations, lending additional validity to our findings.

Clinical and Laboratory Implications The results of this study have important implications for clinical microbiology laboratories in resource-limited settings. The disc elution method offers a practical, cost-effective approach for synergy testing that can be readily implemented in laboratories without specialized equipment. The method's objective endpoint (presence or absence of turbidity) reduces subjectivity in result interpretation compared to zone measurement techniques.

For clinical practice, the demonstration of synergy in approximately 80% of MBL-producing CRE isolates suggests that the ceftazidime-avibactam and aztreonam combination may represent a viable therapeutic option for patients infected with these challenging organisms. However, clinical studies correlating laboratory synergy findings with treatment outcomes remain necessary to fully validate this approach.

CONCLUSION

This study demonstrates that the disc elution method provides superior performance for testing ceftazidime-avibactam and aztreonam synergy in CRE isolates, followed by the disc stacking method. The high prevalence of MBL genes among CRE isolates in our setting explains the limited efficacy of ceftazidime-avibactam monotherapy and supports the need for combination approaches. These findings provide practical guidance for clinical microbiology laboratories seeking to implement synergy testing for CRE isolates and align with recent CLSI recommendations for this testing approach.

Further studies with larger sample sizes, inclusion of reference methods, and clinical outcome correlation are needed to fully validate these laboratory approaches and establish their clinical utility in guiding therapeutic decisions for CRE infections.

Study Limitations

Several limitations should be acknowledged. First, the relatively small sample size (n=50) limits the statistical power of our comparisons. Second, we did not include broth microdilution as a reference standard, which would have provided more definitive assessment of method accuracy. Third, the study was conducted at a single center, which may limit the generalizability of findings to other geographic regions with different resistance patterns. Fourth, clinical outcome correlation was not performed, precluding assessment of the clinical relevance of laboratory synergy findings.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Patna (Approval No.: AIIMS/Pat/IEC/2023/1177). As this study involved bacterial isolates obtained as part of routine diagnostic procedures, the requirement for informed consent was waived by the ethics committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

D.S. and P.K conceptualized the study and designed the methodology. D.S., P.D performed the laboratory investigations and data collection. D.S and A.A contributed to the formal analysis and software validation. A.K. and B.T. provided resources and administrative support. D.S. wrote the original draft of the manuscript. P.K., and B.T. reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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