RESEARCH PAPER

Evaluation of Disc Diffusion, E test and Vitek 2 Method For Detection of Antimicrobial Susceptibility of Ceftazidime–Avibactam Against Enterobacterales

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ABSTRACT

Background: Ceftazidime–avibactam (CZA) is a key β lactam/β lactamase inhibitor combination for treating infections caused by multidrug resistant Enterobacterales. Reliable antimicrobial susceptibility testing (AST) is essential to guide therapy and stewardship, yet routine laboratory methods vary in performance and feasibility across settings.

Objective: To compare the diagnostic performance, categorical agreement, and operational feasibility of disc diffusion, E test, and VITEK 2 automated testing for CZA susceptibility among clinical Enterobacterales isolates in a tertiary care microbiology laboratory.

Methods: A cross sectional laboratory study was conducted over 12 months. Two hundred sixty four non duplicate clinical Enterobacterales isolates were identified by VITEK 2. AST for CZA was performed by VITEK 2 (reference), disc diffusion (30/20 µg), and E test. CLSI interpretive criteria were applied. Quality control used ATCC strains. Primary outcomes included categorical agreement (CA), very major errors (VME), major errors (ME), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Secondary outcomes assessed organism specific patterns and operational considerations (turnaround time, cost).

Results: Klebsiella pneumoniae (61.0%) and Escherichia coli (35.6%) predominated. VITEK 2 classified 150/264 (56.8%) isolates as resistant and 114/264 (43.2%) as susceptible. Categorical agreement with VITEK 2 was 98.1% for disc diffusion and 98.9% for E test. Disc diffusion sensitivity and specificity were 98.7% and 97.4%, respectively; E test sensitivity and specificity were 99.3% and 98.3%, respectively. ME and VME rates were low for both methods. K. pneumoniae showed significantly higher non susceptibility and elevated MIC distributions compared with E. coli.

Conclusions: Disc diffusion and E test demonstrate excellent concordance with VITEK 2 for CZA AST in Enterobacterales. Disc diffusion is a reliable, cost effective screening tool for resource limited settings; E test and VITEK 2 provide precise MIC data useful for critical care and stewardship.

Keywords: *Ceftazidime–avibactam, Carbapenem resistant Enterobacterales, E-Test, VITEK, Minimum Inhibitory Concentration, β lactam-β lactamase inhibitor*
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INTRODUCTION

Carbapenem-resistant Enterobacterales (CRE) have emerged as a major global public-health threat, causing infections with high morbidity and mortality and severely limiting therapeutic options.[1] The global epidemiology of carbapenemase-producing Enterobacterales demonstrates rapid dissemination of diverse resistance determinants, complicating empirical therapy and infection control.[2] Newer β-lactam/β-lactamase inhibitor combinations, notably ceftazidime–avibactam (CZA), have expanded

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treatment choices by restoring activity against many class A (KPC), class C (AmpC), and some class D (OXA-48-like) β-lactamases; however, activity against class B metallo-β-lactamases (MBLs) such as NDM and VIM is limited.[3] Clinical guidance and reviews support CZA's role in treating complicated intra-abdominal infections, complicated urinary tract infections, and hospital-acquired/ventilator-associated pneumonia, and real-world data indicate favourable outcomes compared with older, more toxic agents.[4]

Accurate antimicrobial susceptibility testing (AST) is essential to ensure appropriate use of CZA and to limit the spread of resistance.[5] Broth microdilution (BMD) is the reference method but is resource-intensive and impractical for many routine laboratories.[6] Disc diffusion is widely used for its simplicity and low cost, while gradient diffusion (E-test) yields MICs with moderate resource requirements. Automated systems such as VITEK-2 offer rapid, standardized MIC reporting and high throughput but require validation for novel agents and periodic performance checks.[7–8] Differences in interpretive breakpoints between CLSI and EUCAST further complicate inter-laboratory comparability and clinical decision-making.[9] In resource-limited settings, method selection is often driven by cost, availability, and technical capacity rather than analytical superiority.[10]

Given these challenges, comparative evaluations of routine AST methods for CZA are necessary to inform laboratory practice and antimicrobial stewardship. This study compares disc diffusion, E-test, and VITEK-2 for CZA susceptibility testing of clinical Enterobacterales isolates in a tertiary-care Indian laboratory, assessing categorical agreement, error rates, organism-specific patterns, and operational feasibility to guide practical diagnostic strategies.[11–12]

MATERIALS AND METHODS

Study design and setting

A cross-sectional laboratory study was performed in the Department of Microbiology at a tertiary-care teaching hospital over 12 months (May 2024–April 2025). The institutional ethics committee approved has been obtained; individual patient consent was waived for anonymized laboratory isolates.

Isolate selection and inclusion criteria

Two hundred sixty-four non-duplicate clinical Enterobacterales isolates were included. Isolates originated from routine clinical specimens (blood, urine, pus, sputum, endotracheal aspirates, and other body fluids). Only the first isolate per patient was considered; repeat isolates and non-Enterobacterales organisms were excluded.

Identification

Preliminary identification used colony morphology and Gram stain. Definitive species identification was performed using the VITEK-2 Compact system (bioMérieux) according to manufacturer instructions.[13]

Antimicrobial susceptibility testing

AST for ceftazidime–avibactam (CZA) was performed by three methods:

- **VITEK-2 automated testing (reference):** AST-N cards; inoculum standardized to 0.5 McFarland; MICs and categorical interpretations generated by instrument software and interpreted using CLSI breakpoints current during the study.[14]
- **Disc diffusion:** Mueller–Hinton agar inoculated with a 0.5 McFarland suspension; CZA disc (30/20 µg) placed; plates incubated at 35±2°C for 16–18 hours; zone diameters measured and interpreted per CLSI (S ≥21 mm; I 18–20 mm; R ≤17 mm).[15]
- **E-test:** Mueller–Hinton agar inoculated as above; CZA E-test strip applied; plates incubated 16–20 hours; MIC read at ellipse intersection and interpreted per CLSI MIC breakpoints (S ≤8/4 µg/mL; I 16/4 µg/mL; R ≥32/4 µg/mL).[16]

Disc diffusion and E-test were performed on the same plate for each isolate to ensure uniform incubation conditions.

Quality control

Quality control used ATCC control strains tested with each new batch of media, discs, E-test strips, and VITEK cards. Control results were accepted only when values fell within CLSI-specified ranges.[17]

Data collection and analysis

Data recorded included species identification, specimen source, VITEK-2 MIC and categorical result, disc diffusion zone diameter and categorical result, and E-test MIC and categorical result. Using VITEK-2 as the laboratory reference, the following metrics were calculated: categorical agreement (CA), very major errors (VME; false susceptible), major errors (ME; false resistant), minor errors (discrepancies involving intermediate category), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. Statistical analyses used chi-square tests for categorical comparisons; $p < 0.05$ considered significant.

RESULTS

Isolate and specimen characteristics

A total of **264** Enterobacterales isolates were analysed. Patient demographics showed a predominance of male patients (65.9%). Specimen distribution reflected a high proportion of critically ill patients: endotracheal aspirates (25.8%), blood cultures (22.7%), urine (18.2%), pus/wound swabs (16.3%), and other fluids (17.0%).

Species distribution

Klebsiella pneumoniae comprised 161/264 (61.0%) of isolates and *Escherichia coli* 94/264 (35.6%); remaining species (Enterobacter spp., Proteus spp., Serratia spp., Providencia spp.) accounted for the remainder.

VITEK-2 MIC distribution and categorical results
VITEK-2 categorized 150/264 (56.8%) isolates as resistant and 114/264 (43.2%) as susceptible to CZA. *K. pneumoniae* represented 104/150 (69.3%) of resistant isolates. MIC distributions showed *K. pneumoniae* clustering at higher MICs (≥16 µg/mL common), while *E. coli* had a broader distribution with many isolates at low MICs (≤0.12 µg/mL).

Disc diffusion and E-test results (Table-1,2)
Disc diffusion zone diameters correlated with MIC distributions: *K. pneumoniae* frequently produced small zones (<20 mm), whereas *E. coli* more often produced larger zones (≥25 mm). E-test MICs aligned closely with VITEK-2 MICs; most discrepancies occurred near clinical breakpoints.

Method agreement and diagnostic performance (Table 3,4,5,6)
Using VITEK-2 as the reference:

- Disc diffusion vs VITEK-2: Categorical agreement 98.1%; sensitivity 98.7%; specificity 97.4%; PPV 98.0%; NPV 98.2%. ME = 3; VME = 2.
- E-test vs VITEK-2: Categorical agreement 98.9%; sensitivity 99.3%; specificity 98.3%; PPV 98.7%; NPV 99.1%. ME = 2; VME = 1.

Discrepancies were concentrated among isolates with MICs or zone diameters near CLSI breakpoints. Chi-square testing showed a significant association between species and resistance (p = 0.003), with *K. pneumoniae* more likely to be non-susceptible.

OPERATIONAL OBSERVATIONS
VITEK-2 provided MIC results within 6–8 hours and integrated reporting, advantageous for rapid clinical decisions. Disc diffusion required minimal resources and personnel time but necessitated overnight incubation and manual measurement. E-test produced MICs with good concordance but incurred higher per-test costs.

DISCUSSION
This comparative evaluation demonstrates high concordance among disc diffusion, E-test, and VITEK-2 for CZA susceptibility testing in Enterobacterales isolates from a tertiary-care setting. The predominance of *Klebsiella pneumoniae* and its disproportionate contribution to CZA non-susceptibility reflect regional surveillance and prior reports identifying *K. pneumoniae* as a major driver of multidrug resistance in hospital settings.[18–20]

Both disc diffusion and E-test showed excellent categorical agreement with VITEK-2 (98.1% and 98.9%, respectively) and low VME/ME rates. These findings align with multicentre evaluations and prior single-centre studies reporting high agreement for CZA across routine methods, while noting that discrepancies typically occur near interpretive breakpoints or in isolates with complex resistance mechanisms.[21–23] The E-test's marginally higher sensitivity and specificity likely reflect its quantitative MIC output and closer alignment with growth-based reference methods, whereas disc diffusion's robust performance supports its role as a pragmatic screening tool in resource-limited laboratories.[22]

Organism-specific differences were notable: *K. pneumoniae* exhibited higher MICs and smaller inhibition zones compared with *E. coli*, suggesting a heavier burden of carbapenemase production and combined resistance mechanisms (e.g., KPC variants, porin loss, AmpC overexpression) in *K. pneumoniae* within this cohort.[24,25] The clinical implication is that empirical use of CZA in settings with high *K. pneumoniae* prevalence should be guided by local antibiograms and rapid confirmatory testing to avoid ineffective therapy and preserve stewardship goals.[4]

From a practical standpoint, disc diffusion offers a cost-effective, reliable screening approach with minimal infrastructure needs an important consideration for peripheral laboratories in low- and middle-income countries.[10,15] However, when MIC data are clinically important such as in severe sepsis, ICU patients, or when borderline results are obtained ,E-test or automated systems like VITEK-2 provide actionable MICs and faster turnaround.[14,21] The VITEK-2 system's automation and speed support high-throughput workflows but require capital investment, maintenance, and periodic validation against reference methods.[22,26]

Limitations - This study include the single-centre design and the absence of parallel broth microdilution (BMD) testing and molecular characterization of resistance determinants (e.g., *bla_KPC*, *bla_NDM*, *bla_OXA-48*). BMD remains the reference standard and would strengthen validation; molecular data would clarify the genetic basis of resistance and explain specific discordances between phenotypic methods.[27,28] Future work should integrate genotypic assays and clinical outcome correlations to better link laboratory susceptibility with therapeutic success and to monitor the emergence of CZA resistance during therapy.[29]

Overall, the high categorical agreement observed supports the pragmatic use of disc diffusion for routine CZA screening and E-test or VITEK-2 for confirmatory MIC determination in critical cases. These findings inform laboratory strategy and antimicrobial stewardship in high-resistance environments where CZA remains a valuable therapeutic option.

Conclusions
Disc diffusion and E-test demonstrate excellent concordance with VITEK-2 for CZA susceptibility testing in Enterobacterales, with E-test providing slightly superior MIC precision. *Klebsiella pneumoniae* showed a higher burden of non-susceptibility and elevated MICs compared with *E. coli*.

Recommendations:

- Use disc diffusion as a primary, cost-effective screening method for CZA in routine laboratories; reserve E-test or VITEK-2 for isolates with borderline results, critical infections, or when MICs will influence therapy.
- Maintain periodic verification of automated systems and adhere to current CLSI interpretive criteria.
- Integrate phenotypic carbapenemase detection and, where feasible, molecular testing to elucidate resistance mechanisms and guide stewardship.
- Develop and update local antibiograms to reflect organism-specific resistance patterns.

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Table 1: Distribution of Disc Diffusion Zone Sizes for Ceftazidime-Avibactam (CZA) across Enterobacterale Species

S.No	Organisms	Zone Diameter (mm)			
		≥25 mm n (%)	20–24 mm n (%)	<20 mm n (%)	No Zone n (%)
1	Enterobacter Cloacae Complex	1 (1.3)	1 (3)	0 (0)	0 (0)
2	Escherichia Coli	41 (51.2)	14 (42.4)	37 (26.6)	2 (16.7)
3	Klebsiella Pneumoniae	38 (47.5)	17 (51.7)	97 (69.8)	9 (75)
4	Proteus Mirabilis	0 (0)	1 (3)	3 (2.2)	0 (0)
5	Providencia Rettgeri	0 (0)	0 (0)	1 (0.7)	0 (0)
6	Serratia Fonticola	0 (0)	0 (0)	1 (0.7)	0 (0)
7	Serratia Marcescens	0 (0)	0 (0)	0 (0)	1 (8.3)

Table 2: Distribution of E-Test MIC Values for Ceftazidime-Avibactam across Enterobacterale Species

S.No	Organism	CZA- MIC			
		≥8 n (%)	2–8 n (%)	<2 n (%)	No Zone
1	Enterobacter Cloacae Complex	2 (2.2)	0 (0)	0 (0)	0 (0)
2	Escherichia Coli	45 (48.4)	8 (61.5)	37 (25.5)	4 (30.8)
3	Klebsiella Pneumoniae	46 (49.5)	5 (38.5)	102 (70.3)	8 (61.5)
4	Proteus Mirabilis	0 (0)	0 (0)	4 (2.8)	0 (0)
5	Providencia Rettgeri	0 (0)	0 (0)	1 (0.7)	0 (0)
6	Serratia Fonticola	0 (0)	0 (0)	1 (0.7)	0 (0)
7	Serratia Marcescens	1 (7.7)	0 (0)	0 (0)	1 (7.7)

Table 3: Comparison of VITEK-2 and Disc Diffusion (DD) method for Ceftazidime–Avibactam (CZA) among Enterobacterale Isolates

S.No	Organisms	VITEK VS DD		
		Categorical Agreement (N=259) n (%)	Major Error (ME) (N=3) n (%)	Very Major Error (VME) (N=2) n (%)
1	Enterobacter Cloacae Complex	2 (0.8)	0 (0)	0 (0)
2	Escherichia Coli	93 (35.9)	0 (0)	1 (50)
3	Klebsiella Pneumoniae	159 (61.4)	2 (66.7)	0 (0)
4	Proteus Mirabilis	3 (1.2)	0 (0)	1 (50)
5	Providencia Rettgeri	1 (0.4)	0 (0)	0 (0)
6	Serratia Fonticola	1 (0.4)	0 (0)	0 (0)
7	Serratia Marcescens	0 (0)	1 (33.3)	0 (0)

Table 4: Comparison of VITEK-2 and E-Test method for Ceftazidime–Avibactam (CZA) among Enterobacterale Isolates

S.No	Organism	VITEK VS E-Test		
		Categorical Agreement (N=261) n (%)	Major Error (ME) (N=2) n (%)	Very Major Error (VME) (N=1) n (%)
1	Enterobacter Cloacae Complex	2 (0.8)	0 (0)	0 (0)
2	Escherichia Coli	93 (35.9)	0 (0)	1 (100)
3	Klebsiella Pneumoniae	160 (61.3)	1 (50)	0 (0)
4	Proteus Mirabilis	4 (1.5)	0 (0)	0 (0)
5	Providencia Rettgeri	1 (0.4)	0 (0)	0 (0)
6	Serratia Fonticola	1 (0.4)	0 (0)	0 (0)
7	Serratia Marcescens	0 (0)	1 (50)	0 (0)

Table 5: Diagnostic Accuracy of Disc-Diffusion for Ceftazidime-Avibactam with VITEK-2 as the Reference Standard

Statistic	Value	95% CI
Sensitivity	98.67%	95.27% to 99.84%
Specificity	97.37%	92.50% to 99.45%
Disease prevalence	56.82%	50.61% to 62.88%
Positive Predictive Value	98.01%	94.17% to 99.34%
Negative Predictive Value	98.23%	93.34% to 99.55%
Accuracy	98.11%	95.64% to 99.38%

Table 6: Diagnostic Accuracy of E-Test for Ceftazidime-Avibactam with VITEK-2 as the Reference Standard

Statistic	Value	95% CI
Sensitivity	99.33%	96.34% to 99.98%
Specificity	98.25%	93.81% to 99.79%
Disease prevalence	56.82%	50.61% to 62.88%
Positive Predictive Value	98.68%	94.96% to 99.66%
Negative Predictive Value	99.12%	94.07% to 99.87%
Accuracy	98.86%	96.72% to 99.77%