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ORIGINAL ARTICLE

Ceftazidime-avibactam activity against *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

Background and Objectives: Carbapenem-resistant Enterobacteriaceae (CRE) infections are extremely difficult to treat and have a high fatality rate. The study's primary goal was to determine the rate of ceftazidime-avibactam susceptibility using disc diffusion and E-Test, as well as to evaluate the agreement between the two methods.
Materials and Methods: A total of 124 multidrug-resistant (including carbapenem) *Escherichia coli* and *Klebisella pneumoniae* isolates were included. Kirby Bauer's disc diffusion and E-test were used as the testing methods in this study.
Results: In this study 37.5% and 33.9% of the isolates were susceptible to ceftazidime-avibactam by E test and Disc diffusion respectively. There were five isolates which produced discordant results. Among the 56 isolates there was 91% agreement between the two methods.
Conclusion: Among the discordant isolates the alarming disparity in zone size was a significant concern. Since CRE infections are very common, an economical and practical method for testing ceftazidime-avibactam susceptibility is needed in all the clinical microbiology laboratories as it is a last resort drug.

Keywords: Infection; Resistance; Susceptibility; Enterobacteriaceae; Carbapenem

INTRODUCTION

Since there are few approved treatments for carbapenem-resistant Enterobacteriaceae (CRE) infections, they have become challenging to treat. Moreover, the high mortality rate linked to CRE infections is alarming (1). There have been reports of carbapenem resistance rates in India of up to 30% for *Escherichia coli* and 50% for *Klebisella pneumoniae* (2). It is also quite concerning that *Pseudomonas aeruginosa* is becoming more resistant to carbapenems (3-6). The highest degree of worry for human health is indicated by the Centers for Disease Control and Prevention's (CDC) classification of CRE as an urgent danger.

Carbapenemases, which are divided into three classes: Class B, metallo-β-lactamases (MBLs), Class A, *K. pneumoniae* carbapenemases (KPCs), and Class D, OXA-48 type carbapenemase, are the primary cause of carbapenem resistance. Class B carbapenemases require zinc, whereas Class A and D require serine (7, 8). Other mechanisms of carbapenem resistance include the production of Ambler class C beta-lactamase, the presence of efflux pumps, or porins (9, 10). OXA-48, either by itself or in conjunction with NDM, is the resistance mechanism seen in *K. pneumoniae* while metallo betalactamase (NDM) is frequently linked to carbapenem resistance in *E.*

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coli (11, 12). The development of colistin resistance in CRE has become concerning, as colistin was the final medication utilized to treat CRE infections (13).

Commonly prescribed drugs for CRE infections include tigecycline and polymixins. In certain cases, polymixins should not be used because of its adverse effects such nephrotoxicity. Tigecycline on the other hand cannot reach sufficient plasma concentrations (14-16). The beta-lactam, beta-lactamase inhibitor ceftazidime-avibactam is one of many BL-BLIs which has shown promise in treating CRE infections (17-19). While avibactam does not have much of an impact on metallo-beta-lactamases (MBLs), it has remarkable efficacy against class A (ESBLs and KPC), class C (AmpC), and even certain class D (OXA-48 related) resistance mechanisms (20). Adults and children alike are recommended to use ceftazidime-avibactam for the treatment of various infections, such as ventilator-associated pneumonia (VAP), UTIs, and intra-abdominal infections (21, 22).

There are currently relatively few automated systems available for testing for ceftazidime-avibactam susceptibility, and most laboratories do not regularly use the reference broth microdilution technique. Here, we assess how well the disk diffusion and Etest work against isolates of *K. pneumoniae* and *E. coli* that are resistant to carbapenem.

The main objective of the study was to:

1. Estimate the rate of ceftazidime-avibactam susceptibility in CRE by disc diffusion and E-test.

2. To assess the agreement of ceftazidime-avibactam susceptibility in CRE by disc diffusion to E-test.

MATERIALS AND METHODS

With the approval from the Ethical Committee (IEC/2021/05), this prospective study was carried out at the Department of Microbiology during a one-year period, from May 2021 to May 2022. The results of a prior investigation by Sherry et al. indicated that a minimum sample size of 112 was necessary (23). During the research period, 124 CRE isolates that were multidrug resistant (including carbapenem) were collected. Acquired resistance to at least one agent in at least three antimicrobial classes was considered as multidrug resistance (24). The 124 carbapenem resistant isolates from blood, urine, pus, and respiratory specimens that were part of the investigation were all clinically significant. Using VITEK-2 (Biomerieux-Vitek) and standard biochemical reactions such as indole, citrate utilization, urea hydrolysis test, triple sugar iron test, and nitrate test, all isolates were identified.

In order to determine sensitivity, Kirby Bauer's disc diffusion and E-test were performed. Following preparation of lawn culture of the organism, 30/20 µg of ceftazidime-avibactam disc (Biomerieux, France) was added to the plate and left to incubate at 37°C for 18 to 24 hours. The additional drugs that were used for susceptibility testing were ampicillin, ceftriaxone, ciprofloxacin, nitrofurantoin (for urinary isolates), piperacillin-tazobactam, imipenem, and meropenem. The ceftazidime-avibactam E strips (CZA 0.016-256 µg/mL) were supplied by Biomerieux..After covering the lawn culture of the organism with the E-test strips, the plates were incubated at 37°C for 18 to 24 hours. MIC (Minimum Inhibitory Concentration) values can be interpreted where the ellipse meets the scale. Since it is possible to get MIC values "in-between" two-fold dilutions using the E-test strip's continuous gradient, these numbers were rounded to the nearest two-fold dilution before categorization. The zone size and MIC were evaluated using the CLSI 2021 criteria (25). Disc diffusion was performed on all organisms; however the E-test was only administered to 56 randomly selected CRE isolates.

CLSI 2021. Ceftazidime avibactam Breakpoints for Enterobacteriaceae.

MIC- ≤8/4- Susceptible, ≥16/4- Resistant

Zone size- ≥ 21 – Susceptible, ≤ 20 – Resistant

Errors in the study. Any isolate giving discordant results by E test and disc diffusion was considered as an Error.

Statistical analysis. Rate of susceptibility will be estimated for CRE & CRP by E test and by Disc Diffusion and compared using z test. Agreement of The two methods (E test and Disc Diffusion) will be assessed using Cohen’s Kappa.

RESULTS

There were 80 (59.7%) and 44 (32.8%) isolates of *K. pneumoniae* and *E. coli* respectively among the 124 carbapenem-resistant isolates in the research. The majority of isolates were from urine (30.6%) and bron-

choalveolar lavage (32.3%). The remaining isolates, however, were from blood (19.4%) and pus (17.7%) respectively. All of the isolates exhibited resistance to imipenem, meropenem, ampicillin, ceftriaxone, ciprofloxacin, nitrofurantoin, and piperacillin-tazobactam. 56 isolates in the research underwent both the E test and disc diffusion, whereas all 124 isolates underwent disc diffusion. E test and disc diffusion showed that 37.5% and 33.9% of the isolates were sensitive to ceftazidime-avibactam respectively, whereas 62.5% and 66.1% of the isolates were resistant to this antibiotic.

91.7% of the 56 isolates that underwent both the E test and disc diffusion had similar results, however, 8.3% of the isolates showed errors. Table 1 shows the distribution of zone sizes and minimum inhibitory concentrations (MICs) for the erroneous isolates. For the 56 isolates that underwent both the E test and disc diffusion, the agreement between the two techniques is shown in Table 2. Fig. 1 shows the susceptibility of ceftazidime-avibactam by the E-test, whereas Fig. 2 shows the susceptibility via disc diffusion. Table 3 shows the distribution of MIC for the isolates. We created ROC curves to see whether disc diffusion is a good predictor of ceftazidime-avibactam resistance (Fig. 3). With a zone size cut-off value of less than 21 mm, disc diffusion revealed a diagnostic sensitivity of 94% (95% CI 0.81-0.99) for predicting ceftazidime-avibactam resistance, the specificity was 86% (95% CI 0.64- 0.97), the positive predictive value was 92% (95% CI 0.78- 0.98) and the negative predictive value was 90% (95% CI 0.68- 0.99), with a zone size cut-off value of <21mm.

Table 1. Ceftazidime avibactam MIC and zone sizes of the isolates which gave errors

Sample	Identification	MIC (µg/ml)	Zone size (mm)
Bronchoalveolar lavage	*Klebsiella pneumoniae*	16- R	21-S
Urine	*Klebsiella pneumoniae*	16-R	24-S
Blood	*Klebsiella pneumoniae*	8-S	16-R
Blood	*Klebsiella pneumoniae*	8-S	16-R
Pus	*Klebsiella pneumoniae*	8-S	16-R

S- Susceptible, R- Resistant
MIC- Minimum inhibitory concentration

Table 2. Agreement Between E-Test and Disc Diffusion to detect Susceptibility to Ceftazidime avibactam in Enterobacteriaceae Infection

Susceptibility by Disc Diffusion	Susceptibility by E Test		
	Resistant	Sensitive	Total
Resistant	33 (58.9%)	3 (5.4%)	36 (64.3%)
Sensitive	2 (3.6%)	18 (32.1%)	20 (35.7%)
Total	35 (62.5%)	21 (37.5%)	56 (100.0%)
Agreement			91%
McNemar'sChisquare Test			p=0.7
Cohen's Kappa			0.81 (0.65-0.97)

E-test- Epsilometer test.

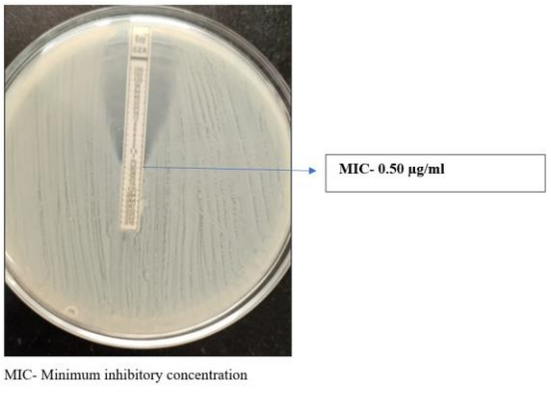

Fig. 1. Ceftazidime avibactam susceptibility by E-test

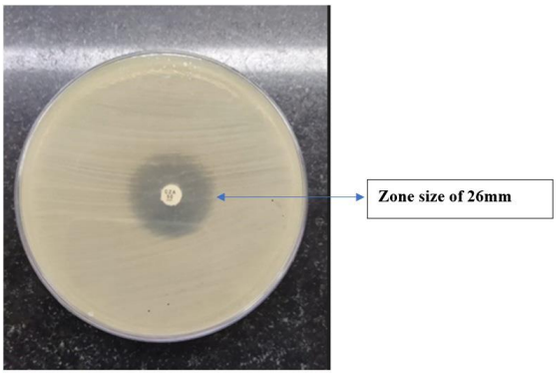

Fig. 2. Ceftazidime avibactam susceptibility by disc diffusion

Table 3. MIC distribution of the isolates.

MIC (µg/ml)	No of isolates, n (%)
0.38-0.75	14 (25)
1-2	4 (7.1)
8	3 (5.4)
16-32	13 (23.2)
>256	22 (39.3)

MIC- Minimum inhibitory concentration

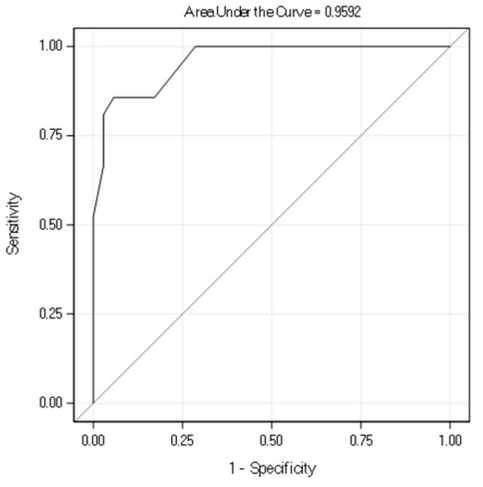

Fig. 3. ROC Curve Depicting Area Under the Curve when Disc Diffusion is used to Predict Resistance to Ceftazidime Avibactam when compared to E-Test

DISCUSSION

Insufficient treatment options and limitations associated with use of tigecycline and colistin have made CRE infections a grave concern around the world (26, 27). Another concerning finding in India is the increasing OXA-48 resistance (59%), as reported by Shankar et al. (12). The new drug ceftazidime-avibactam has excellent activity against resistance mechanisms like OXA-48 and KPC, hence it should be used as a front line option. Susceptibility testing for ceftazidime-avibactam is not done in many automated systems and the recommended method of broth microdilution is not practised in many laboratories. Testing for ceftazidime-avibactam susceptibility against Gram-negative bacilli requires a precise, cost-effective, and useful approach. This study is one of the few studies in India that compares the disc diffusion and E-test techniques for ceftazidime-avibactam susceptibility determination.

Only 37.5% of the isolates in this investigation were found to be sensitive to ceftazidime-avibactam when the E test was employed as a susceptibility testing technique. It has been observed that 87.5% of carbapenem-resistant Enterobacteriaceae in Latin America, 76.8% in Europe, 50.8% in Africa, and 48.3% in Asia are susceptible to ceftazidime-avibactam (28). Our study's low ceftazidime-avibactam susceptibility is consistent with a prior Asian research (28). The large percentage of metallo-betalactamase isolates in Asia may be one of the primary causes of the poor ceftazidime-avibactam susceptibility (28). Conversely, KPCs are the primary resistance mechanism responsible for the high ceftazidime-avibactam susceptibility in Latin America and Europe. Due to budgetary limitations, it was not possible to do the genotypic characterization of the isolates, which would have shown the distribution of NDM, OXA-48, KPC, and other genes in our research and predicted their sensitivity to ceftazidime-avibactam. Further research based on genotypic characterisation is required to supplement phenotypic data.

According to a Clinical and Laboratory Standards Institute (CLSI) guideline, isolates whose zone of inhibition is confirmed to be between 20 and 22 mm should undergo confirmatory ceftazidime-avibactam MIC testing. This will avoid any error in susceptibility testing of this new drug (25). There were 17 (13.7%) isolates in our study, which had zone sizes between 20-22mm. MIC testing was done in only seven of these isolates and the discordant results by E test and disc diffusion was seen in only one isolate. Han et al. reported in his study that only 5.2% of the isolates revealed ceftazidime-avibactam zone of inhibition between 20-22mm (29). Among the 56 isolates for which E test and disc diffusion was done, five isolates (8.9%) gave discordant results and there was 91% agreement between the two methods. Han et al. in his study found that there was an error rate of only 0.2% and agreement of more than 99% between E test and disc diffusion (29). Wang et al. on the other hand in another study reported no errors while using E test and 2.5% errors while using the disc diffusion method (30). Among the five discordant results in our study, three isolates had an MIC of 8µg/ml (Sensitive), while their zone size by disc diffusion method was 16 mm (Resistant) which is 5mm below the cut off zone size of 21mm. This alarming disparity in zone size was a significant concern in this study for the disc diffusion method. Other studies have mentioned that testing for ceftazidime-avibactam can be affected by inoculum effect and inhibitory zone measurement, particularly for isolates with zones of 20-21 mm (31-33). The presence of a single or multiple colonies within the inhibitory zone should be taken into consideration, and only the inner margin should be considered, during the zone of inhibition measurement (29). A thin veil of growth within an obvious growth inhibition zone should be ignored. When it comes to susceptibility testing, both recommendations indicate different disk contents, CLSI

(30/20 mg) and EUCAST (10/4 mg). The best way to identify the breakpoint and disk content for ceftazidime-avibactam disk diffusion requires numerous investigations because the resources needed for MIC testing are not readily available (34).

As far as we are aware, this is one of the few papers from India that compares disc diffusion and E test susceptibility testing for ceftazidime-avibactam. According to the E test and disc diffusion, 37.5% and 33.9% of the isolates in this investigation were sensitive to ceftazidime-avibactam, respectively. The low susceptibility rate is probably due to the high prevalence of metallobetalactamase in India. There were five isolates which produced discordant results. Among the 56 isolates there was 91% agreement between the two methods. The diagnostic sensitivity of disc diffusion in predicting resistance to to ceftazidime-avibactamwas 94% and the specificity was 86% with a zone size cut-off value of <21mm. Among the discordant isolates the alarming disparity in zone size was a significant concern. Since CRE infections are very common, an economical and practical method for testing ceftazidime-avibactamsusceptibility is needed in all the clinical microbiology laboratories as it is a last resort drug. Since CRE infections are on the rise, authors would like to recommend the inclusion of ceftazidime-avibactamdiscs in routine testing for samples received from patients in intensive care units and the use of E test be reserved when zone of inhibition is found to be from 16 to 22 mm.

This study had a few limitations. E test could be done for only 56 isolates. Broth microdilution method which is the gold standard could not be done due to financial limitations. Genotypic characterization of the isolates would have shown the distribution of NDM, OXA-48,KPC among our isolates, which would in turn give evidence on ceftazidime-avibactam susceptibility. However, this could not be done due to financial constraints.

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