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Original Article

An insight into Ceftazidime-avibactam activity on Carbapenem-resistant Enterobacterales causing bloodstream infections

Ekadashi Rajni*, Himanshi Galav, Kanika Bairwa

Department of Microbiology, Mahatma Gandhi University of Medical Science & Technology, Jaipur, India

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Abstract

Introduction: Carbapenem-resistant Enterobacterales (CRE) are among the biggest challenges faced by the healthcare community worldwide**.** Antibiotic treatment of these infections remains challenging, especially in developing nations. Ceftazidime-avibactam (CZA) is a β-lactam-β-lactamase inhibitor combination with potent activity against CRE. The present study was conducted to provide insight into the in vitro activity of CZA against CRE causing bloodstream infections.

Methods: This was a prospective observational study, conducted from July 2022 to June 2023 at a tertiary care teaching hospital, Jaipur, and included all non-duplicate CRE isolates obtained from bloodstream infections in adult patients admitted to the ICU. Identification and antimicrobial susceptibility testing were performed using the VITEK-2 automated system and interpreted according to Clinical and Laboratory Standards Institute guidelines. All CRE isolates resistant to CZA were further tested for synergy between Ceftazidime-avibactam and Aztreonam (AT) using the disk elution method.

Results: During the one-year study period, 104 CRE strains were isolated in the laboratory from bloodstream infections in adult patients admitted to the ICU, which included 19 (18%) *E. coli*, 80 (77%) *K.pneumoniae* and 5 (5%) *Enterobacter spp*. Susceptibility rates of 61%, 31% and, 30% were observed for amikacin, tigecycline and minocycline, respectively. Overall, 31% and, 4% CRE isolates were susceptible to CZA and AT alone, respectively, while 97% were found to exhibit synergy.

Conclusion: In the present study, 69% CRE isolates were found to be resistant to CZA. CZA + ATM combination, however, demonstrated excellent in vitro activity against CRE isolated from blood cultures in our ICU setting. Thus, this combination can be considered a suitable therapeutic option in patients with sepsis.

Introduction

Paul Ehrlich, in the early 1900s, had commented that "Drug resistance follows the drug like a faithful shadow." This is indeed true because no sooner than a drug is introduced for clinical use, issues regarding its abuse and reports of clinical failure start surfacing. Antimicrobial resistance (AMR) is a global crisis. A recent review highlighted that approximately 1.3 million deaths were directly attributable to antimicrobial-resistant bacterial pathogens in 2019.[1]

The introduction and use of third-generation cephalosporins were followed by reports of an increasing prevalence of extended-spectrum beta-lactamases (ESBLs). Following this, carbapenems were introduced in the 1980's and were considered the most reliable last resort drugs. They were rampantly used and were soon followed by growing reports of carbapenem resistance. While this phenomenon was first reported in 1991 in the USA, it is now prevalent globally and associated with significant morbidity and mortality. Carbapenem-resistant Enterobacterales (CRE) among of the biggest challenges facing the healthcare community worldwide.[2]

CRE are defined as members of the Order Enterobacterales that are resistant to at least one of the carbapenems (ertapenem, meropenem or imipenem). Antibiotic treatment of these infections remains challenging, particularly in developing countries. CZA is a 3rd generation cephalosporin combined with a β-lactamase inhibitor. It is approved by the FDA for use in intra-abdominal infections, complicated urinary tract infections (UTI), pyelonephritis and hospital-acquired pneumonia caused by multidrug-resistant Gram-negative bacteria.[3-5]A huge body of evidence exists demonstrating CZA to be more effective than tigecycline and polymyxins for combating CRE.[6-9] Several recent studies have been published analysing the activity of CZA as a potential therapeutic option.[5,10-11]

To date, there is no published report on the activity

***Corresponding Author:** Ekadashi Rajni, Email: ravajni@yahoo.co.in

Study Highlights

What is current knowledge?

• Limited data exists on the in vitro activity of Ceftazidime-avibactam against Carbapenem-resistant Enterobacterales causing bloodstream infections, particularly in the Indian context.

What is new here?

• This study provides insight into the in vitro activity of Ceftazidime-avibactam on Carbapenem-resistant Enterobacterales causing bloodstream infections in a tertiary care hospital in Jaipur, India,

• To the best of our knowledge, this is the first study presenting such data from Western India.

• The study aims to sensitize clinicians that this latest addition to their armamentarium needs to be used prudently.

of CZA against CRE causing bloodstream infections (BSI) from the study site. Hence, the present study was conducted to provide an insight into the in vitro activity of Ceftazidime-avibactam against CRE causing bloodstream infections in a tertiary care hospital in Jaipur, Western India. These data shall contribute to the formulation of empirical therapy guidelines when challenged with CRE infections in critical care units and in turn, strengthen antimicrobial stewardship practices.

Material and Methods

Study design

This was a prospective observational study, conducted from July 2022 to June 2023 at Mahatma Gandhi Medical College & Hospital, Jaipur with, 1450 beds. The study included all consecutive non-duplicate CRE isolates obtained from bloodstream infections in adult patients (>18 years of age) admitted to the ICU. All carbapenem-susceptible isolates were excluded.

Ethical Approval

Approval of the Institutional Ethics Committee was obtained prior to commencement of this study. Since the patient's information was used in a completely confidential manner, no informed consent was obtained from the participants.

Blood culture and Antibiotic susceptibility testing:

Blood culture was performed using the automated blood culture system Bactec TM FX (Becton Dickinson, Sparks, MD). Identification and antimicrobial susceptibilitytesting (AST) of CRE isolates were performed using the VITEK-2 automated system based on the broth microdilution principle (bioMèrieux, Marcy l'Étoile, France) and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[12]

Disk elution method for testing synergy in ceftazidime-avibactam and aztreonam combination

All CRE strains resistant to CZA were tested for synergy between ceftazidime-avibactam and aztreonam combination using the disk elution method. In separate test tubes containing 2ml of sterile Mueller-Hinton broth, disks of ceftazidime–avibactam (CZA, 30/20 µg), and aztreonam (AT, 30 µg) were dispensed and the antibiotic was allowed to elute from the disks into the broth for 30 min. This was then followed by adding 12 µl of a 0.5 McFarland-adjusted CRE bacterial suspension and incubating for 16-20 h. After incubation, growth of the organism was indicated by turbidity in any of the tubes and susceptibility was denoted by the clearing of the growth. The quality control strain, *E. Coli* ATCC 25922, was used with each batch of testing.[13]

Results

During the one-year study period, 104 CRE strains were isolated in the laboratory from bloodstream infections in adult ICU patients. The mean age of the patients was 53.07 years; while the median age was 47 years, with a range of 20 to 85 years; 75% (78/104) of the patients were male, and 25% (26/104) were female. Of the 104 patients, 75 (72%) were immunocompromised due to various underlying conditions such as diabetes mellitus, chronic kidney disease, chronic liver disease, malignancy, sepsis and other systemic illnesses. The majority of patients (78%) were admitted to the medical ICU followed by Neuro-ICU (11%), SICU (8%) , and liver transplant ICU (3%).

Among the 104 CRE, 19 (18%) were *E. coli*, 80 (77%) were *Klebsiella pneumoniae (K.pneumoniae)*, and 5 (5%)

Table 1. Antibiotic susceptibility profile of 104 CRE isolates

Antibiotics	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Cefuroxime	0	0	104(100%)
Ceftriaxone	0	0	104(100%)
Cefepime	0	0	104(100%)
Amoxicillin-clavulanate	1(1%)	0	103(99%)
Cefoperazone-sulbactam	2(2%)	0	102(98%)
Piperacillin-tazobactam	0	0	104(100%)
Amikacin	63(61%)	0	41(39%)
Gentamicin	17(16%)	0	87(84%)
Minocycline	31(30%)	0	73(70%)
Tigecycline	32(31%)	0	72(69%)
Ciprofloxacin	2(2%)	0	102(98%)
Co-trimoxazole	11(11%)	0	93(89%)
Colistin	0	91(88%)	13(12%)
Aztreonam	4(4%)	0	100(96%)
Ceftazidime-avibactam	32(31%)	0	72(69%)
Ceftazidime-avibactam + aztreonam	70/72(97%)	0	2/72(3%)

Table 2. Species-wise susceptibility to aztreonam, ceftazidime avibactam and synergy between ceftazidime avibactam + Aztreonam

Organisms	No. of isolates	Aztreonam		Ceftazidime avibactam		Ceftazidime avibactam and Aztreonam synergy	
		Susceptible	Resistant	Susceptible	Resistant	Present	Absent
E.coli	19	1/19(5%)	18/19(95%)	7/19(37%)	12/19(63%)	11/12(92%)	1/12(8%)
K. pneumoniae	80	3/80(4%)	77/80(96%)	25/80(31%)	55/80(69%)	54/55(98%)	1/55(2%)
Enterobacter spp	5	0	5/5(100%)	0	5/5(100%)	5/5(100%)	0
Total	104	4/104(4%)	100/104(96%)	32/104(31%)	72/104(69%)	70/72(97%)	2/72(3%)

Table 3. Colistin Resistance among different species of CRE

Organism (n)	Colistin resistance profile among different *spp* of CRE	
	Colistin	
	Intermediate	Resistance
E.coli (19)	18 (95%)	1 (5%)
K. pneumoniae(80)	68(85%)	12(15%)
Enterobacter spp (5)	5(100%)	0
Total (104)	88%	12%

were *Enterobacter spp*.

Amongst the 104 CRE isolates tested, 61% were susceptible to amikacin, 31% to tigecycline and, 30% to minocycline. Overall, 31% and 4% CRE were susceptible to CZA and AT alone, respectively, and 97% exhibited synergy between the two (Table 1).

Species-wise susceptibility to Ceftazidime-avibactam, Aztreonam and the synergy between the two is depicted in Table 2. Synergy was observed in 100%, 98% and 92% of *Enterobacter spp, K pneumoniae* and *E coli* strains respectively.

Overall, 88% of the CRE isolates demonstrated intermediate susceptibility to colistin, while 12% were resistant. Species-wise, 95 % *E coli* and 85% *K pneumoniae* exhibited intermediate susceptibility to colistin in contrast to *Enterobacter spp wherein no resistance was observed.* (Table 3).

The broth dilution test used to demonstrate **s**ynergy between ceftazidime-avibactam and aztreonam is depicted in Figure 1. Tube 1 serves as the control. Tubes containing ceftazidime-avibactam (tube 2) and aztreonam (tube 3) show turbidity, indicating growth of the organism (resistance). Clearance of growth in the tube containing both ceftazidime-avibactam and aztreonam (tube 4) indicates synergy.

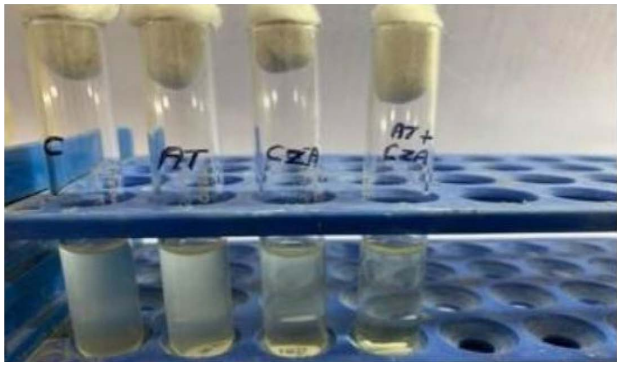

Figure 1. Broth disc elution test: Synergy testing of ceftazidime-avibactam with aztreonam. Tube 1 is the control. Tubes containing aztreonam (tube 3) and ceftazidime-avibactam disc (tube 2) show turbidity, indicating growth of the organism (resistance). Clearance of growth in the tube containing ceftazidime-avibactam and aztreonam (tube 4) indicates synergy between the two

Discussion

The increasing frequency of infections caused by Carbapenem-resistant Enterobacterales is a serious threat to public health.[2] Likewise, bloodstream infections (BSIs) are a major challenge for healthcare institutions. There is a 9% increment in mortality for each hour that passes between the presentation and administration of antibiotics for such critically ill patients.[14] CREs causing bloodstream infections are associated with a high risk of mortality of up to 30%, thus constituting a major therapeutic challenge faced by clinicians.[15] Keeping these points in mind, the current study was designed to address this ongoing epidemic and plug gaps in knowledge regarding the activity of Ceftazidime-avibactam against CRE causing bloodstream infections.

According to the results of ATLAS (Antimicrobial Testing Leadership and Surveillance) surveillance 2018-2019, conducted in nine centres across India, 24% carbapenem-resistant (CR) *E.coli* and 51% carbapenem-resistant *K.pneumoniae* were found to be susceptible to CZA.[8] In the current study, 37% CR *E coli* and 31% CR *K. pneumoniae* were found susceptible to CZA. Another study from Uttar Pradesh revealed 75% CRE to be susceptible.[16] These differences highlight the need for continuous surveillance to note subtle shifts and to develop site-specific empirical therapy guidelines.

CZA has a broad spectrum of activity, being highly active against Ambler class A, class C and some class D beta-lactamases. However, it exhibits limited activity against class B metallo-beta-lactamases. Aztreonam, belonging to the monobactam group, remains stable against MBLs. Thus, combining CZA with aztreonam is a workable therapeutic option for MBL-producing CREs.[2, 4,17,18] There is ample evidence regarding the predominance of NDM and OXA-48-like enzymes, alone or in combination, in CRE isolates in India.[8,10,19,20] The recent Indian Council of Medical Research (ICMR) guideline recommends the use of prolonged infusion of ceftazidime-avibactam and aztreonam for carbapenem-resistant Enterobacterales.[4]

In the current study, only 31% and 4% of CRE were susceptible to CZA and AT alone, respectively, whereas

97% of isolates showed synergy with the combination of the two. Taha R et al and Falcone M et al have also reported similar optimistic results regarding the synergy, christening this combination the "glimmer of hope."[16-17] The combination of CZA and ATM has shown a synergistic effect in vitro and in vivo and is supported by clinical trials worldwide. Falcone M et al conducted an observational study on 102 adults with bloodstream infections caused by metallo-β-lactamase-producing Enterobacterales. They compared the outcomes of patients receiving ceftazidime-avibactam in combination with aztreonam versus those receiving a combination of other agents. Thirty-day mortality was 19% and 44% for the CZA/AT and alternate groups, respectively, highlighting the potential clinical benefit of the former.[17] Similar results have been presented by other authors too.[2,10,16]

In the current study, a high degree of resistance was observed to several other antibiotics tested as well. Overall, amikacin showed the highest susceptibility rate of 61% (63/104), followed by minocycline 30% (31/104). 11% of isolates exhibited susceptibility to cotrimoxazole (CoT), and 31% of CRE isolates were susceptible to tigecycline (Tgc). This is in sharp contrast to Kachari et al and Khare et al., in which 91.6% and 86.1% isolates were found to be susceptible.[21-22] This difference can probably be explained by differences in the way this drug in used.

Colistin resistance was observed in 12% CRE isolates, comprising 12(15%) and 1 (5.3%) strains of *K.pneumoniae* and *E.coli* respectively. The difference between *E. coli* and *K. pneumoniae* in colistin resistance was not found to be statistically significant (p = 0.25). This finding is consistent with results reported by El-mahallawy et al[23] and slightly lower than that documented by R Bir[24] et al in 2022. Taha R et al detected colistin resistance in 15% of CR- *E. coli* and 23.75% of CR *K. pneumoniae* isolates.[16] Interestingly, all the 13 colistin-resistant isolates in the current study exhibited susceptibility to the to CZA and AT combination. 2 strains that were resistant to the CZA + AT combination showed intermediate susceptibility to colistin.

Contemporary data demonstrate the use of polymyxins and tigecycline as first-line therapy for treating CRE. However, the large volume of distribution of tigecycline, and the unreliable pharmacodynamics, and pharmacokinetics as well as the emergence of resistance during therapy associated with polymyxins, limit their clinical utility.[2,9] A favourable susceptibility profile associated with the use of CZA and aztreonam supports their use as first-line therapy against CREs in our setup.[11,2,16] Its role as a colistin-sparing agent can be quite pivotal. The finding of two strains that did not exhibit synergy to this combination implies that, if not used prudently, we may lose this final shot at CRE. Veeraraghavan B et al and Bhattacharya S have also expressed concerns regarding an "early demise" of CZA.[25,26]

This study has a few limitations. This is a single-centre study; therefore it may not provide a comprehensive picture of CRE across the entire area. Nonetheless, it is important to note that this is a tertiary-level care teaching super-speciality hospital serving a wide catchment area in and around Jaipur, thereby providing a fair insight into the issue. Secondly, due to infrastructural constraints, genotypic characterization could not be done.

Despite this fact, to the best of our knowledge, this is the first study from Western India to focus on CRE causing bloodstream infections, especially with respect to susceptibility to the CZA. It also explores the role of the CZA and AT combination and it's the excellent activity against CRE blood isolates. By filling this knowledge gap, the study contributes meaningfully to the development of empirical therapy guidelines in such a scenario, ultimately leading to improved clinical outcomes.

Conclusion

This study aims to sensitize clinicians to the activity of CZA on CRE isolates in the critically ill patients. The need of the hour is that therapy for CRE infections should be individualized based on disease severity, patient characteristics and antibiotic susceptibility results. While CZA alone was effective against only 31%, its combination with aztreonam has demonstrated excellent in vitro activity against CRE isolated from blood cultures. This combination can thus, be considered as a suitable therapeutic option in sepsis patients. Its use as a polymyxin-sparing regimen cannot be overemphasized.

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Authors' Contribution
Conceptualization: Ekadashi Rajni.
Data curation: Ekadashi Rajni, Himanshi Galav, Kanika Bairwa.
Formal analysis: Ekadashi Rajni.
Methodology: Ekadashi Rajni.
Supervision: Ekadashi Rajni.
Validation: Ekadashi Rajni.
Visualization: Ekadashi Rajni.
Writing–original draft: Himanshi Galav, Kanika Bairwa.
Writing–review & editing: Ekadashi Rajni, Himanshi Galav, Kanika Bairwa.

Competing Interests
The authors declare that there is no competing interest.

Ethical Approval
Approval of the Institutional Ethics Committee was obtained prior to the commencement of this study (MGMC&H/IEC/JPR/2022/697). Dated: 14 May 2022. Since the patients' information was used in a completely confidential manner, no informed consent was obtained from the participants.

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