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Original Research Article

Antimicrobial susceptibility profiling of carbapenem resistant gram-negative bacilli with special reference to ceftazidime-avibactam and ceftriaxone-sulbactam with EDTA

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ABSTRACT

Background: Increased morbidity and mortality rates attributable to multidrug resistant organisms (MDROs) such as carbapenem resistant organisms (CROs) are being increasingly reported ultimately leading to limited therapeutic options. Several potential strategies are being explored to develop new antibiotics for Carbapenem-resistant organisms.
Methods: This was a retrospective study where Antimicrobial susceptibility testing for CZA and CSDE was done using the Kirby-Bauer disc diffusion method. Patient's clinical conditions were assessed by the clinician and demographic details were recorded in the hospital EMR system. The outcome was decided based on the therapeutic effect of the antibiotics on the patients via primary and secondary endpoints.
Results: A total of 150 patients were included in the study, 41 (27.3%) were susceptible to CSDE and turned culture negative. The antibiotic susceptibility patterns of different antibiotics were compared against each other, especially with CZA and CSDE.
Conclusions: Colistin is still the drug with maximum susceptibility against the gram negative bacterial infections although CSDE and CZA are the novel antibiotics and show promising results and may be used on a case to case basis depending on the pharmacokinetics and pharmacodynamics which could be the limiting factor at times restricting use of Colistin.

Keywords: Antimicrobial resistance, Carbapenem resistant *Enterobacteriaceae*, Ceftazidime-avibactam, Ceftriaxone-sulbactam EDTA

INTRODUCTION

Antimicrobial resistance has been an area of concern for a long time and has shown rapid rise in recent times.[1] Carbapenem resistant organisms (CROs) are showing an increase in trend leading to difficulty in therapy. Increased morbidity and mortality rates attributable to multidrug resistant organisms (MDROs) such as CROs are being increasingly reported ultimately leading to limited therapeutic options.[2,3] WHO has categorised and prioritised carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant *Enterobacterales* (CRE)

and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) as the most alarming danger amongst the gram negative MDROs.[4] The prevalence of carbapenem resistant *Enterobacterales* is 77.8% in oncology patients.[5]

Overdependence on carbapenem for the therapy of cefoperazone-sulbactam and piperacillin–tazobactam resistant *Enterobacterales* has led to rise of carbapenem resistant *Enterobacterales*. Hence there is an urgent need of developing new therapeutic options in beta-lactam group.[6] Colistin resistant MDROs are being increasingly reported amongst which *Klebsiella* species are also showing colistin resistance.[7]

Ceftazidime-avibactam (CZA) has been approved for the antimicrobial therapy of Hospital acquired pneumonia, ventilator associated pneumonia, intra-abdominal infection, urinary tract infections, and also for the treatment of the infections caused by aerobic gram negative rods specially in multi drug resistant cases.[8]

A multidrug therapeutic antibiotic formulation of ceftriaxone sulbactam with ethylenediamine tetra-acetic acid (CSDE) has been developed for the multidrug Gram-negative rods specially MDR pathogens producing ESBL and MBL.[9] The scientific rationality of this combination is explained by different mechanisms of antibacterial actions of sulbactam and EDTA with ceftriaxone. EDTA has anti biofilm and metal chelation properties which attributes to its antibiotic effect. Beta lactamase producing microbes can be countered by sulbactum, a beta lactamase inhibitor. Membrane porosity of the drug combination is facilitated by EDTA due to which MIC value of drug is also reduced.[10]

The current scenario of multi and pan drug gram negative rods have left us with no option but to look for alternative or novel therapeutic options. This would allay the development of resistance and simultaneously spare the remaining antibiotics by reducing selection pressure.[10,11]

The aim of the study was to meet the current challenges faced during treatment of multidrug gram-negative organisms and to provide an alternate solution to this currently emerging resistance in gram negative rods while we wait for a definitive solution.

Objective of the study

The objective of the study was to compare susceptibility of other antimicrobials with ceftazidime-avibactam and ceftriaxone-sulbactam with EDTA and the clinical efficacy of ceftriaxone-sulbactam with EDTA in treatment of infected patients.

METHODS

This was a retrospective study of specimens sent for bacteriology culture over a period of 6 months (1st December 2020 to 30th May 2021) at a tertiary care oncology centre. A total of 150 patients were included in the study. Approval was obtained on 28 August 2021 from the Institutional Ethics Committee of the institution with approval number OIEC/11000515/2021/00003. As it was a retrospective study, the ethics committee granted a waiver of the need to obtain individual patient consent. The study was conducted according to the ethical guidelines established by the Declaration of Helsinki, Good Clinical Practice guidelines, and those established by the Indian Council of Medical Research. The study received no funding and was not registered in any clinical trial registry because it was a retrospective analysis.

Inclusion criteria

All registered patients whose specimen were sent for bacteriology culture and showing growth of pathogen resistant to first line drugs were included in the study.

Exclusion criteria

All specimens showing no growth/contaminants/colonizer in culture were excluded.

Study methodology

Specimens were processed by standard culture, identification and susceptibility techniques. Antimicrobial susceptibility testing for CZA and CSDE was done using the Kirby-Bauer disc diffusion method. Patient's clinical conditions were assessed by the clinician and demographic details were recorded in the Hospital EMR system. The clinical response of patients who were given CSDE for treatment of infection was evaluated with help of the clinician. The outcome was decided based on the therapeutic effect of the antibiotics on the patients via primary and secondary endpoints. The primary endpoint was taken as survival in reference to sepsis and secondary endpoints were procalcitonin correlation in response to treatment (increase/decrease), length of stay in reference to sepsis.

This was retrospective analysis study where data was collected of 6 months' duration from 1st December 2020 to 30th May 2021, formal sample size calculation was not required. Data was presented as absolute numbers, mean, and standard deviation, or percentages. Continuous variables were expressed as mean±standard deviation or median±IQR. Normality of the data of continuous variables were checked using Shapiro-Wilks test. Categorical variables were expressed as raw numbers and percentages. Patient characteristics were analysed using descriptive statistics. The comparison of antimicrobial susceptibility testing of different antimicrobials breakpoints was performed using Fisher exact test, and was represented using odds ratio (95% CI) and p values. A p value less than or equal to 0.05 was considered statistically significant. All analysis was performed using SPSS version 22 software.

RESULTS

A total of 150 patients were included in the study, among which 51 (34%) were females and 99 (66%) were males. There were 46 (30.7%) patients below the age 20 years and 32 (21.3%) patients were above 60 years. Of 150 patients CSDE was tested in all the patients however CZA was tested only in 110 patients. Among all the 150 patients, 41 (27.33%) were susceptible to CSDE and turned culture negative. The antibiotic susceptibility patterns of different antibiotics were compared against each other, especially with CZA and CSDE. A Chi-Square test was applied for comparison between all the antibiotics, if cell counts were >5, otherwise Fisher exact test was performed. The data also contained missing information for the antibiotics, which were ignored for analysis.

Table 1: Contingency table for comparing the antibiotic susceptibility between ceftazidime-avibactam and ceftriaxone-sulbactam with EDTA using Fisher exact test.

Antimicrobials		Ceftriaxone-Sulbactam EDTA		P value	Odds ratio	95% CI
		Resistant	Susceptible			
Ceftazidime avibactam	Resistant	54	1	4.348e-13	90.40	(13.43,3814.53)

Table 2: Contingency table for comparing the antibiotic susceptibility between ceftazidime-avibactam and colistin using Fisher exact test.

Antimicrobials		Colistin		P value	Odds ratio	95% CI
		Resistant	Susceptible			
Ceftazidime avibactam	Resistant	1	79	0.5988	0.43	(0.007, 8.48)
	Susceptible	2	68			

Table 3: Contingency table for comparing the antibiotic susceptibility between Ceftriaxone-Sulbactam with EDTA and Colistin using Fisher Exact Test.

Antimicrobials		Colistin		P value	Odds ratio	95% CI
		Resistant	Susceptible			
Ceftriaxone-sulbactam EDTA	Resistant	1	73	0.2491	0.24	(0.004, 4.68)
	Susceptible	2	34			

Table 4: Susceptibility pattern of Different antibiotics.

Antimicrobials	CZA	CSDE	PTZ	AMC	CFS	IMI	MERO	ETP	COLI
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Resistant	80 (53.3)	74 (49.3)	96 (64)	119 (79.3)	96 (64)	89 (59.3)	92 (61.3)	93 (62)	03 (2)
Sensitive	70 (46.7)	36 (24)	54 (36)	25 (16.7)	54 (36)	61 (40.7)	58 (38.7)	52 (34.7)	147 (98)

CZA-Ceftazidime-Avibactam; CSDE-Ceftriaxone-Sulbactam with EDTA; PTZ-Piperacillin-Tazobactam; CFS-Cefoperazone-Sulbactam; AMC-Amoxicillin-Clavulanate; IMI-Imipenem; MERO- Meropenem; ETP-Ertapenem; COLI-Colistin.

Table 5: Comparison of death and discharge between susceptibility and resistant for ceftriaxone-sulbactam with EDTA.

Antimicrobials	Ceftriaxone-Sulbactam with EDTA		χ^2 Value	P value
	Susceptible	Resistant		
Death	0	16	7.45	0.006
Discharge	36	58		

Table 6: Comparison of death and discharge between resistant and susceptibility for ceftazidime-avibactam.

Antimicrobials	Ceftazidime-avibactam		P value	Odds ratio	95% CI
	Susceptible	Resistant			
Death	04	19	0.003	0.20	(0.05, 0.64)
Discharge	66	61			

Table 1 shows that p value was obtained to be 4.348e-13 (<0.05), this implies that the antibiotic effect of CZA and CSDE are statistically significant of each other and there is presence of a relationship between these antibiotics. The sample Odds Ratio obtained was 90.40. It was found that only 1 out of 110 (0.9%) bacterial strains was resistant for CZA while it was susceptible for CSDE, which was less than 35 out of 110 (31.8%) bacterial strains which were susceptible to both CZA and CSDE.

Table 2 shows that p value was obtained to be 0.598 (>0.05), this implies that CZA and colistin are not statistically significant of each other and there was no relationship between these antibiotics. The sample odds ratio obtained was 0.43. It was found that 79 out of 150 (52.7 %) bacterial strains which were resistant for CZA were susceptible for colistin, which were more than 68 out of 150 (45.3%) bacterial strains which were susceptible to both CZA and colistin.

Table 3 shows that p value was obtained to be 0.249 (>0.05), this implies that CSDE and colistin were not statistically significant of each other and there is no relationship between these antibiotics. The sample odds ratio obtained was 0.24. It was found that 73 out of 110 (66.4%) bacterial strains which were resistant for CSDE were susceptible for colistin, which was more than 34 out of 110 (30.9%) bacterial strains which were susceptible to both CSDE and colistin. Susceptibility pattern of various antimicrobials is shown in Table 4.

There were 42 cases where the bacterial culture turned to no growth subsequent to antibiotic therapy. Among them, there were 23 bacterial strains out of 42 (54.8%) which were susceptible to CZA antibiotic, 9 bacterial strains out of 42 (21.4%) which were susceptible to CSDE, and 42 bacterial strains out of 42 (100%) which were susceptible to colistin.

The length of stay of 122 patients had a mean of approximately 30 days. The minimum number of days that any patient had stayed was 2 days and maximum was 110 days. The length of stay for the patients who were discharged was greater than those in whom death had occurred.

A total of 39 patients were assessed for Procalcitonin levels, among which all the 6 patients who succumbed to death had raised procalcitonin levels while among the 39 patients who were discharged increased procalcitonin levels were observed in 10 patients and majority i.e., 23 patients were found to have decreased procalcitonin levels.

A total of 125 (84.7%) patients were discharged, which indicates positive outcome. On the other hand, death occurred in only 23 (15.3%) patients.

The comparison of death and discharge between susceptible and resistant bacteria for CSDE is shown in Table 5. The p value obtained was 0.006 (<0.05), this implies that the effect from CSDE on death/discharge is statistically significant and there is presence of a relationship between them. It was found that 36 out of 110 (32.7%) patients who were infected with bacterial strains susceptible to CSDE, were treated and no mortality occurred in these patients.

The p value (table 6) was obtained to be 0.003 (<0.05), this implies that the effects from CZA on death/discharge is statistically significant and there is presence of a relationship between them. The odds ratio was 0.20. It was found that there were 4 out of 150 (2.7%) patients who were infected with bacterial strains susceptible from CZA and had death, while there were 66 out of 150 (44%) patients who were infected with bacterial strains susceptible from CZA, were treated and discharged.

Similarly, for colistin comparison was done and the p value was obtained to be 0.39 (>0.05), this implies that the effect from colistin on death/discharge was not statistically significant and there is absence of a relationship between them. The odds ratio was 0.35. It was found that there were 22 out of 150 (14.7%) patients who were infected with bacterial strains susceptible from colistin and had death, while there were 125 out of 150 (83.3%) patients who were infected with bacterial strains susceptible from colistin, were treated and discharged.

DISCUSSION

Majority of the patients were male i.e., 66% and the age group with the maximum number of patients was 0-20 years.

Among 150 bacterial strains, 54 were resistant and 35 were susceptible to both CSDE and CZA, which may imply that they had a similar pattern of antibiotic susceptibility. There exists a statistical relationship between the antibiotic susceptibility test results of these antibiotics.

Most of the bacterial strains (68) which were susceptible to CZA were also susceptible to colistin. There were only 2 bacterial strains which were resistant to colistin and susceptible to CZA while there were 79 bacterial strains which were resistant to CZA but susceptible to colistin. This implies that Colistin has higher antibiotic susceptibility rates than CZA.

Zalas-Wiecek et al reported similar results i.e., 98% susceptibility to CZA for *Enterobacterales* strains.[13] Most of the bacterial strains (34) which were susceptible to CSDE were also susceptible to colistin. There were only 2 bacterial strains which were resistant to colistin and susceptible to CSDE while there were 73 bacterial strains which were resistant to CSDE but susceptible to colistin. This suggests that Colistin has higher antibiotic susceptibility rates than CSDE.

However, a study by Singh et al showed that CSDE had higher susceptibility (95%) for *Enterobacteriaceae* as compared to colistin (89%).[14]

Gupta et al suggested CSDE as an effective agent for ESBL producing MDROs.[9]

Colistin exhibited maximum susceptibility of 98%, while CZA and CSDE showed susceptibilities of 46% and 24% respectively.

A study by Wenzler et al showed 78% susceptibility to CZA15. A study by Wang et al showed susceptibility of 97.14% for CZA in *K. pneumoniae* isolate.[16]

However, a study by Duin et al showed that CRE isolates were more susceptible to CZA than colistin and concluded CZA as an alternative to colistin.[17]

Hakeam et al reported that CZA had better performance in clinical outcomes vs. colistin (46.8% versus 20.4%, respectively; p=0.047).[18] Among the 42 cases where the bacterial culture turned to no growth subsequent to antibiotic therapy; 54.8% bacterial strains were susceptible to CZA antibiotic, 39.1% bacterial strains were susceptible to CSDE, and all the 42 bacterial strains were susceptible to colistin.

The length of stay of 122 patients had a mean of approximately 30 days. The minimum number of days that any patient had stayed was 2 days and the maximum was 110 days. Procalcitonin levels increased in 10.7% of cases while it decreased in 15.3% of cases. Mortality occurred in 6 cases, all of which showed an increasing trend of procalcitonin, implying that these cases could be suffering with severe sepsis which may be the cause of their death, while on the other hand, 23 patients who were discharged had decreased procalcitonin. Study by Xu et al also showed that procalcitonin levels remain increased for longer time in patients with severe bacterial sepsis.[19]

The majority (84.7%) of the patients admitted for different bacterial infections including sepsis were treated and discharged while death occurred only in 15.3% of the patients, which may be attributable to non-infectious aetiologies such as the primary disease too. Study by Gudiol et al showed that cancer and neutropenia are major risk factors for severe sepsis and mortality.[20] The patients who had lesser length of stay had more chances of death, implying that they may be suffering with severe sepsis at the time of admission leading to shorter span of stay in hospital and earlier death.

There were no patients who were infected with bacterial strains which were susceptible from CSDE and had died; while there were 36 out of 110 (32.7%) patients who were infected with bacterial strains susceptible from CSDE, were treated and discharged. There were 4 out of 150 (2.7%) patients who were infected with bacterial strains susceptible from CZA and had death, while there were 66 out of 150 (44%) patients who were infected with bacterial strains susceptible from CZA, were treated and discharged. There were fewer deaths in the patients who had infections with the bacterial strains susceptible to CSDE and CZA. This finding suggests that these antibiotics may stand a chance in severe bacterial infection or sepsis therapy. Treating MDR gram negative infections with CZA have resulted in better clinical outcomes.[21] Study by Rathish et al also showed 79% susceptibility to CZA and its role in decreasing mortality with CRE infections to 27%.[22] There were 22 out of 150 (14.7%) patients who were infected with bacterial strains susceptible from colistin and had succumbed to death, while there were 125 out of 150 (83.3%) patients who were infected with bacterial strains susceptible from colistin, were treated and discharged. A study by Hakeam et al also showed good clinical outcome with use of CZA as compared to colistin 46.8% versus 20.4%, respectively; (p=0.047).[18] Death occurred in a total of 23 patients, out of which 13 deaths were attributable to sepsis, non-sepsis related deaths were 6 and no reason for death could be found in 4 cases.

Limitation of study

This was a retrospective study where both the antimicrobials CZA and CSDE were tested for limited number of samples. A larger number of sample size would have added more power to the study. More clinical data of patients would have given a better idea about the in vivo usage of these antimicrobial agents. Further prospective studies with larger sample sizes can significantly enhance the value.

CONCLUSION

Colistin is still the drug with maximum susceptibility against the gram negative bacterial infections although CSDE and CZA are the novel antibiotics and show promising results and may be used on a case to case basis depending on the pharmacokinetics and pharmacodynamics which could be the limiting factor at times restricting the use of colistin. Procalcitonin test is a good investigation to detect present or impending bloodstream bacterial infection. It may be used optimally for early diagnosis of occult cases of systemic bacterial infection, especially in early phase of sepsis. Negative culture reports may be helpful to correlate successful antibiotic therapy.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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