

# A Comparative *In Vitro* Sensitivity Study of “Ceftriaxone–Sulbactam–EDTA” and Various Antibiotics against Gram-negative Bacterial Isolates from Intensive Care Unit

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## ABSTRACT

**Introduction:** A rapid increase in multidrug-resistant (MDR) strains is being seen across the globe especially in the Southeast Asian region, including India. Carbapenems and colistin form the mainstay of treatment against gram-negative pathogens, especially extended-spectrum beta-lactamase (ESBL)- and metallo-beta-lactamase (MBL)-producing isolates. However, due to increased resistance to carbapenems and toxicity of colistin, especially in intensive care units (ICUs), carbapenem-sparing antibiotics like ceftriaxone–sulbactam–EDTA (CSE) combination needs to be evaluated.

**Materials and methods:** Bacterial isolates cultured from various clinical samples from all ICUs for a period of 9 months were evaluated. Bacterial identification was performed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and antibiotic susceptibility testing were performed by disk diffusion and E test method. Antibiogram of various antibiotics was noted. Extended-spectrum beta-lactamase- and MBL-producing bacteria were identified by phenotypic methods. Antibiotic sensitivity results of CSE were compared with the comparator drugs like colistin, carbapenems, and tigecycline in Enterobacteriaceae, *Acinetobacter* spp., and *Pseudomonas* spp. along with ESBL and MBL producers.

**Results:** A total of 2,760 samples of blood, cerebrospinal fluid (CSF), respiratory samples, tissue, and pus were collected from ICUs with maximum isolates from pus (37%) followed by respiratory samples (31%) and blood (27%). *Escherichia coli* and *Klebsiella pneumoniae* were the predominant gram-negative pathogens accounting for 56% of the isolates followed by *Acinetobacter* spp. (23%) and *Pseudomonas* spp. (15%). Extended-spectrum beta-lactamase screening was positive for 57% (1,069/1,877) isolates; whereas 43% (732/1,877) were MBL producers. According to the antibiotic susceptibility results, CSE was the most effective antibiotic showing 94% sensitivity for carbapenem-sensitive Enterobacteriaceae and 97% for carbapenem-resistant *Acinetobacter* and *Pseudomonas* spp. Among the other drugs, colistin was found to be the most effective showing almost 95% sensitivity in both the Enterobacteriaceae and non-Enterobacteriaceae group (both ESBL + OXA/NDM). Ceftriaxone–sulbactam–EDTA was also found much more effective (95%) as compared to Colistin (89%) toward ESBL- and MBL-producing strains of Enterobacteriaceae and non-Enterobacteriaceae group. Among the carbapenems, imipenem was the most effective drug against Enterobacteriaceae showing 34% sensitivity and ertapenem proved to be least effective.

**Conclusion:** In our present study, CSE emerged as a potent antibacterial agent against MDR gram-negative infections; both for ESBL as well as MBL producers. Hence, in light of present study, we strongly recommend inclusion of CSE in routine sensitivity panel and may be used as a carbapenem- and colistin-sparing drug and a promising option against ESBL and MBL producers especially in ICU.

**Keywords:** *Acinetobacter*, Antibiogram, Bacteria, Ceftriaxone–sulbactam–EDTA, New antibiotic, Resistance.

*Indian Journal of Critical Care Medicine* (2020); 10.5005/jp-journals-10071-23573

## INTRODUCTION

Emergence of multidrug-resistant (MDR) organisms globally is an increasing threat and public health hazard,<sup>1,2</sup> especially in the Southeast Asian region, including India.<sup>1,3</sup> Prevalence of extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) is rising in India and varies between 8% and 78%.<sup>4,5</sup> Carbapenems form the mainstay of treatment against these gram-negative pathogens.

Several reports suggest that B-lactam/B-lactamase inhibitors (BL/BLI) combinations like cefoperazone–sulbactam, piperacillin–tazobactam and ampicillin–sulbactam can be used as suitable options for treating less severe cases,<sup>6,7</sup> whereas carbapenems can be used as reserve drug for more severe cases.

Looking beyond carbapenems, therapeutic options are very narrow, with colistin and tigecycline in line.<sup>8,9</sup> These drugs are last resort for serious gram-negative pathogens. These are also reported to be failing in some cases due to rise in development of resistance. It can very soon land us with no choice of antibiotics for these MDR infections.<sup>10,11</sup> Thus, all these studies call for a research on

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**How to cite this article:** Singh S, Sahu C, Patel SS, Singh A, Yaduvanshi N. A Comparative *In Vitro* Sensitivity Study of “Ceftriaxone–Sulbactam–EDTA” and Various Antibiotics against Gram-negative Bacterial Isolates from Intensive Care Unit. *Indian J Crit Care Med* 2020;24(12): 1213–1217.

**Source of support:** Nil

**Conflict of interest:** None

carbapenem-sparing drugs to combat these multidrug infections. “Ceftriaxone–sulbactam–EDTA (CSE)” serves as one of the options.

Ceftriaxone–sulbactam–EDTA is a novel patented antibiotic adjuvant entity<sup>12</sup> with a combination of ceftriaxone (third-generation

beta-lactam cephalosporin), sulbactam (beta-lactamase inhibitor), and disodium EDTA (class 1 antibiotic resistance breaker). This combination restores the *in vitro* activity of ceftriaxone against ESBL/MBL-producing gram-negative bacteria, including enzyme families that belong to Ambler class A (TEM, SHV, CTX-M), class B (NDM, VIM, IMP), class C (some variants of AmpC), and class D (OXA ESBLs). The bactericidal activity of ceftriaxone results from inhibition of mucopeptide synthesis in the bacterial cell wall. EDTA results in reduction in efflux transporter expression, bacterial biofilm eradication (inhibition to form biofilm and making biofilms porous by divalent ion chelation when administered along with antibiotic compounds), and inhibition of curli formation. Sulbactam forms a protein complex with beta-lactamases and irreversibly blocks their destructive hydrolytic activity. Ceftriaxone–sulbactam–EDTA alters the membrane potential, resulting in disturbing ATP balance required for efflux pump activity and hence is effective in bacterial strains where mechanisms are overactive, such as *Pseudomonas aeruginosa* and *Escherichia coli*.<sup>12</sup>

The present study was designed to evaluate the efficacy of CSE a novel carbapenem-sparing drug in MDR gram-negative isolates especially for ESBL-producing bacteria by comparing the susceptibility patterns with other commonly used carbapenems (meropenem/ertapenem/imipenem). Ceftriaxone–sulbactam–EDTA efficacy was also compared with last resort drugs like colistin and tigecycline especially in MBL-producing bacteria.

## MATERIALS AND METHODS

### Place and Duration of Study

The study was conducted in Microbiology Department at our tertiary care hospital between September 2018 and May 2019.

### Sample Collection

A total of 1,877 gram-negative isolates were obtained from 2,760 clinical samples of blood, cerebrospinal fluid (CSF), respiratory samples, tissue, and pus collected from intensive care units (ICUs) patients for routine cultures.

### Characterization of Samples

The gram-negative isolates were further screened for ESBL and MBL production. Screening of isolates for ESBL production was performed as per the Clinical Laboratory Standards Institute (CLSI) guidelines.<sup>13</sup> Isolates exhibiting zone size  $\leq 25$  with ceftriaxone (30  $\mu$ g),  $\leq 22$  for ceftazidime (30  $\mu$ g), and  $\leq 27$  with cefotaxime (30  $\mu$ g) were considered as possible ESBL producer. Extended-spectrum beta-lactamase production was confirmed by disk potentiation test using ceftazidime (30  $\mu$ g) and cefotaxime (30  $\mu$ g) antibiotic disks with and without clavulanic acid (10  $\mu$ g) and by double disk susceptibility test (DDST).<sup>13</sup> Similarly, phenotypic detection of MBL among clinical isolates was carried out using imipenem (10  $\mu$ g) and imipenem (10  $\mu$ g) + EDTA (750  $\mu$ g) disks as described by Yong et al.<sup>14</sup>

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion method as recommended by the CLSI (2020).<sup>13</sup> The disk of meropenem (10  $\mu$ g), ertapenem (10  $\mu$ g), and imipenem (10  $\mu$ g) was obtained from Hi-Media (Mumbai, India). Antibiotic disks of “CSE” were procured from Abtek biological (Liverpool, United Kingdom). Colistin and tigecycline were applied as E-strips and MIC breakpoints were interpreted according to the CLSI guidelines.<sup>13</sup>

Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth from isolated colony of pathogens selected from 24 hours agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller–Hinton agar (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60°C over the agar surface. After 3–5 minutes, antibiotic disks and E-strips were applied and pressed down to ensure complete contact with agar surface. The disks were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates were then inverted and incubated for 16–18 hours aerobically at 37°C within 15 minutes of application. Sensitivity of isolated organisms against antibiotics was reported as sensitive (S), intermediate (I), or resistant (R) based on the breakpoints as per the CLSI guidelines.

## RESULTS

### Characterization of Isolates

A total of 2,760 samples of blood, CSF, respiratory samples, tissue, and pus were collected from ICUs during the study period of 9 months from September 2018 to May 2019. Among all the clinical isolates, 68% (1,877/2,760) were gram-negative pathogens. Maximum isolates were collected from pus (37%), respiratory samples (31%), and blood (27%) (Fig. 1). *Escherichia coli* and *Klebsiella pneumoniae* were the predominant gram-negative pathogens accounting for 56% of the isolates followed by *Acinetobacter* spp. (23%) and *Pseudomonas* spp. (15%). All the gram-negative isolates were then screened for ESBL and MBL production. Extended-spectrum beta-lactamase screening was positive for 57% (1,069/1,877) isolates, whereas 43% (732/1,877) were MBL producers.

### Antimicrobial Susceptibility Results

Antibiotic susceptibility results were interpreted as per the CLSI guidelines and CSE was compared with the other comparator drugs namely colistin, tigecycline, and carbapenems. These drugs were analyzed in different pathogen groups which were (i) carbapenem-sensitive Enterobacteriaceae, (ii) carbapenem-resistant *Acinetobacter*, (iii) carbapenem-resistant *Pseudomonas*, (iv) Enterobacteriaceae (both ESBL + OXA/NDM) (Fig. 2), (v) *Acinetobacter* spp. and *Pseudomonas* spp. (both ESBL + NDM/OXA)

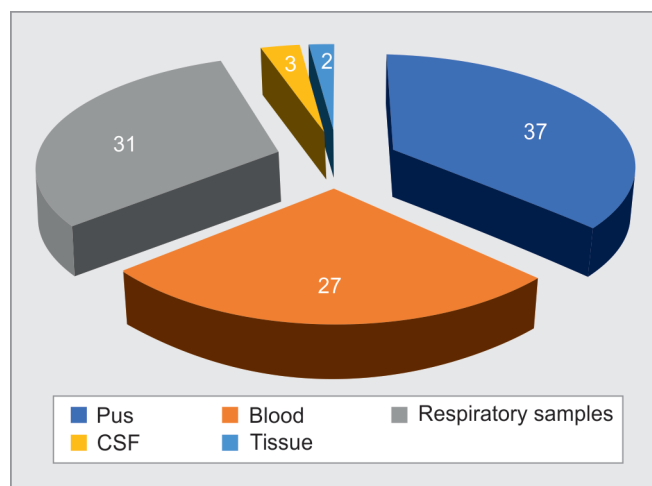
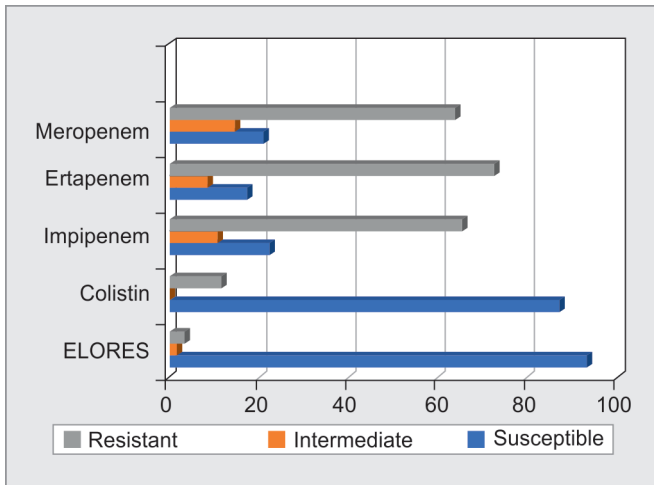
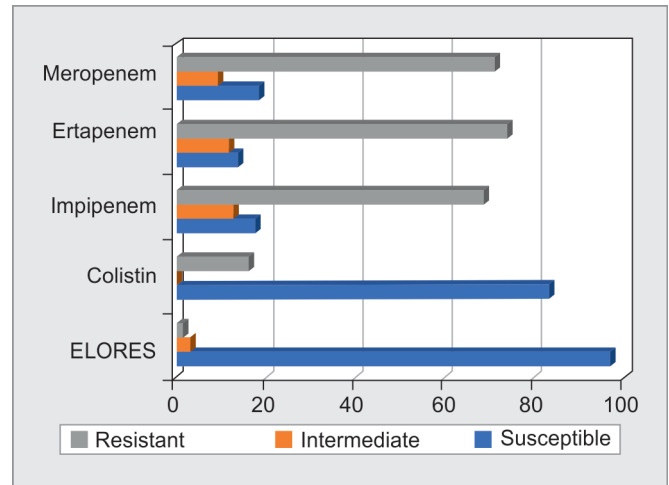


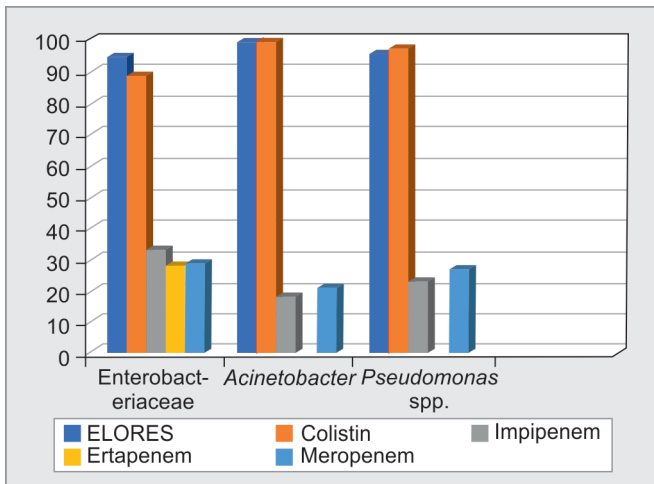
Fig. 1: Characterization of samples. CSF, cerebrospinal fluid



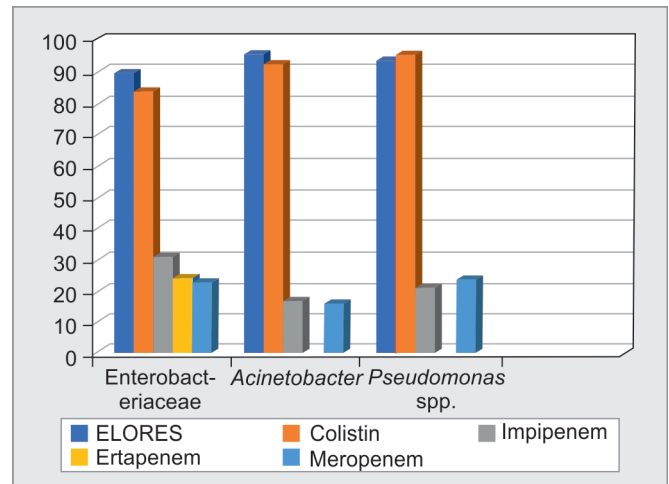
**Fig. 2:** Susceptibility pattern of Enterobacteriaceae (both ESBL + NDM/OXA) for different antimicrobial agents



**Fig. 3:** Susceptibility pattern of *Acinetobacter* and *Pseudomonas* spp. (both ESBL + NDM/OXA) for different antimicrobial agents



**Fig. 4:** Susceptibility pattern in percentage of different antimicrobials for ESBL producers



**Fig. 5:** Susceptibility pattern in percentage of different antimicrobials for MBL producers

(Fig. 3), (vi) ESBL producers alone, and (vii) MBL producers alone (Figs 4 and 5).

On analyzing the results of antibiotic sensitivity (Figs 2 and 3), CSE was the most effective antibiotic showing 94% sensitivity for carbapenem-sensitive Enterobacteriaceae and 97% for carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas*. Among the other drugs, colistin was found to be the most effective showing almost 95% sensitivity in both the Enterobacteriaceae and non-Enterobacteriaceae group. The carbapenem drugs, however, showed less sensitivity in both the groups (ESBL + OXA/NDM) Enterobacteriaceae vs non-Enterobacteriaceae: imipenem 23 vs 18%, ertapenem 18 vs 14%, and meropenem 21 vs 19%, respectively. None of the isolates of *A. baumannii* were resistant to “CSE”, whereas 6, 8, and 3% isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, respectively, were resistant to “CSE” (Figs 2 and 3).

“CSE” was also found effective toward ESBL- and MBL-producing strains of Enterobacteriaceae and non-Enterobacteriaceae group. Among *Acinetobacter* group, CSE and colistin were effective in 100% of the isolates. Ceftriaxone-sulbactam-EDTA also showed higher sensitivity toward Enterobacteriaceae (95%) as compared to colistin (89%). Among the carbapenems, imipenem was the most effective

drug against Enterobacteriaceae showing 34% sensitivity and ertapenem was the least effective. Meropenem showed moderate sensitivity toward ESBL producers (Fig. 4).

For the MBL producers, CSE showed 90% sensitivity toward Enterobacteriaceae, 96% toward *Acinetobacter* spp., and 94% toward *Pseudomonas* spp. and colistin showed intermediate sensitivity as 84, 93, and 96% to the above isolates, respectively. Overall, CSE was found to be more effective as compared to colistin. Among the carbapenems, imipenem was again the most effective drug against Enterobacteriaceae showing 31% sensitivity (Fig. 5).

Ertapenem and meropenem were less sensitive (24 and 23%, respectively) (Fig. 5).

## DISCUSSION

Extended-spectrum beta-lactamase and MBL producers have rendered most of the beta-lactam antibiotics ineffective in today’s scenario. Colistin and tigecycline are considered the last resort drugs. Several BL/BLI combinations are in the pipeline and some already have approval for use against these MDR pathogens. Some of these combination drugs are ceftolozane/tazobactam, ceftazidime-avibactam, ceftaroline-avibactam, imipenem/

MK-7655, plazomicin (ACHN-490), and eravacycline (TP-434).<sup>15</sup> So, we are pondering for new options to combat these threats. "CSE" serves the purpose in such MDR cases.

In a study from New Delhi, India, 79% of the *K. pneumoniae* isolates were MBL producers.<sup>4</sup> The prevalence of ESBL producers was 57% and MBL producers was 43% in our study. It is very much comparable to the studies where MBL producers were 42.1% in tertiary care hospital, Kolkata and 56% in MGM Medical College and Hospital, Kamothe, Navi Mumbai, India, respectively.<sup>5,16</sup>

In a study by Chaudhary et al., ceftriaxone + sulbactam in the ratio of 2:1 along with EDTA disodium (3 mg/mL) (CSE1034) lowered MIC to >8-fold and possessed synergy against the most ESBL-producing microorganisms.<sup>17</sup> CSE1034 is effective against MDR pathogen producing ESBLs, MBLs like NDM-01 and prevents "transfer of resistant plasmid" and hence the spread of resistance is controlled.<sup>17-23</sup>

In the present study, a total of 2,760 samples from blood, CSF, respiratory samples, tissue, and pus were collected and gram-negative bacteria were predominant pathogens (68%). Ceftriaxone-sulbactam-EDTA stood out among rest of the drugs by showing 94% sensitivity for Enterobacteriaceae and 97% for *Acinetobacter* and *Pseudomonas* spp., followed by colistin (95%). A similar study performed by Satras reported superior antibacterial activity of CSE (87.80%) as compared to carbapenem drugs (meropenem: 62.67% and imipenem: 60.95%) and was far better to piperacillin + tazobactam (48.42%) against 758 clinical pathogens.<sup>24</sup> Antibiogram profile depicted CSE as most susceptible drug toward both Enterobacteriaceae and non-Enterobacteriaceae isolates.<sup>24</sup> In our study, among, the "ESBL group", CSE and colistin showed 100% sensitivity toward *Acinetobacter*. "CSE" was much more effective toward Enterobacteriaceae (95%) as compared to colistin (89%). For the MBL producers too, CSE showed remarkable sensitivity toward Enterobacteriaceae, *Acinetobacter*, as well as *Pseudomonas* spp. A study by Prabhu et al., also concluded similar results with CSE proving to be a much better and effective alternative than ceftriaxone alone for combating ESBL and MBL producers.<sup>25</sup> Our findings were further supported by similar studies performed by Bagga<sup>26</sup> and Sahu et al.,<sup>27</sup> who demonstrated CSE as having maximum susceptibility profile against gram-negative bacteria compared to other carbapenems and piperacillin + tazobactam.

Some limitations of our study were in the terms of comparison with other novel drug options like ceftazidime-avibactam, ceftolozane-tazobactam, etc., and lack of clinical efficacy data. The effective dose of EDTA should also be explored clinically. However, our study was a planned in a large population group of ICU patients with a variety of samples and in difficult to treat MDR organisms. It was an approach to explore the potency of this novel therapeutic option, CSE in our clinical/hospital settings.

## CONCLUSION

In our present study, CSE emerged as a potent antibacterial agent against gram-negative infections. It was the most effective agent followed by colistin. Ceftriaxone-sulbactam-EDTA showed excellent activity in all the groups of Enterobacteriaceae and *Acinetobacter* as well as ESBL and MBL producers. It can very well act as a carbapenem sparer drug in carbapenem-sensitive organisms, and colistin sparer drug in carbapenem-resistant organisms. Hence, in light of the present study, we strongly recommend inclusion of "CSE" in routine sensitivity panel and it may prove to be a promising option against ESBL and MBL producers.

## ACKNOWLEDGMENTS

Authors like to thank Abtek Biological (Liverpool, United Kingdom) for providing "Elores" disks.

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