Original Article

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Evaluation of Ceftriaxone-Sulbactam-EDTA Adjuvant Combination against Multidrug Resistant Bacteria in Tertiary Care Hospital, Guntur, Andhra Pradesh, India

Microbiology Section

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ABSTRACT

Introduction: In India, multiple antibiotic resistance are rapidly growing in the bacterial population with a rising threat to public health. To overcome the effect of extended-spectrum beta-lactamases (ESBL), Metallo beta-lactamases (MBL) and carbapenemase-producing organisms, very few antibiotics are effective, the need of the hour is a new antibiotic or drug combination. Various studies suggested that antibiotic adjuvant therapies can be an alternate approach to curb the rate of drug resistance in microorganisms. A new antibiotic combination of ceftriaxone+sulbactam+Ethylene Diamine Tetra Acetic Acid (EDTA) (CSE) has recently been proposed to tackle Multidrug Resistant (MDR) organisms.

Aim: To evaluate the in-vitro efficacy of a new antibiotic adjuvant entity CSE, among the Vitek-2 (Biomerieux, France) confirmed MDR strains isolated from specimens of Intensive Care Unit (ICU) patients.

Materials and Methods: A cross-sectional descriptive study was conducted on a total of 100 consecutive MDR organisms isolated from specimens of ICU admitted patients in NRI general hospital and super specialty care centre in Guntur district, Andhra Pradesh, India from January 2021-May 2021. Bacterial growth on the medium was identified using an automated Vitek-2 system using Gram negative and Gram positive identification card and Antimicrobial Susceptibility Testing (AST) cards. The isolates were identified as ESBL, MBL, AmpC-beta-lactamase, and carbapenemases producing organisms and tested for sensitivity to CSE drug-using Epsilometer (E)-test strips with Minimum Inhibitory Concentrations (MIC) gradient of 0.016-256 μg/mL, and interpreted as sensitive, and resistant based on breakpoints. A descriptive statistical analysis was done by calculating the frequencies of the variables.

Results: The more prevalent MDR pathogens were *Escherichia coli* (36%), *Pseudomonas aeruginosa* (18%), *Klebsiella pneumoniae* (15%), and *Proteus* species (10%). Among *Escherichia coli* MDR isolates, 16.7% were ESBL+MBL producing and 83.3% were carbapenemase-producing with good susceptibility to CSE 86.7% and 83.3%, respectively. Among *Pseudomonas aeruginosa* 83.3% of ESBL+MBL and carbapenemase-producing organisms were susceptible to CSE. Overall, 88% of MDR strains were sensitive and 12% were resistant to novel CSE combinations among 100 MDR isolates.

Conclusion: The novel antibiotic-adjuvant combination CSE is highly effective (88% susceptibility) against ESBL/MBL, AmpC, and carbapenemase-producing MDR bacteria. The enhanced susceptibility may be due to the synergistic effect of all three molecules in the combination.

Keywords: Antibiotic-adjuvant combination, Epsilometer-test, Ethylene diamine tetra acetic acid, Multidrug resistant bacteria, Vitek-2

INTRODUCTION

The indiscriminate use of antibiotics has raised a global concern due to the emergence of MDR organisms, especially in Southeast Asia [1,2]. In India, multiple antibiotic resistance are rapidly growing in the bacterial population with a rising threat to public health. Among all bacterial species, Gram negative bacterial resistance has been considered the major menace as these organisms (ESBL, MBL, AmpC and carbapenemase-producing organisms) have encoded genetic information for MDR mechanisms. Carbapenems are structurally stable against most MDR pathogens and thus have a frontline role in the treatment regimen [3]. However, in the last few years, resistance to carbapenems has also raised an urgent need to develop newer alternatives to cope with increasing MDR pathogens [4].

The antibiotic-resistant ESKAPE pathogens like *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species are capable of escaping the biocidal effects of antimicrobial agents. The resistance mechanisms include enzymatic inactivation, modification of drug targets, changing cell permeability through porin loss or increase in expression of efflux pumps, and mechanical protection provided by biofilm formation, production of ESBL and carbapenemases in Gram negative pathogens, and due to mutant transpeptidase gene in Gram positive organisms that have low affinity for beta-lactams which are currently posing serious therapeutic dilemmas to medical practitioners [5-8].

The prevalence of ESBL, MBL, and AmpC beta lactamase producers among Gram negative organisms ranges were high in studies from Katmandu and Bhubaneswar [9,10]. In India, studies from Karad, Maharashtra, and Ludhiana, the prevalence of carbapenem resistance was high [11,12]. Beyond carbapenems, colistin and tigecycline are the last-line therapeutic drugs in MDR pathogens [13,14].

The newer alternatives include the use of newer antibiotic molecules or re-shuffling the role of existing molecules i.e., a combination of antibiotic treatment or adjunctive antibiotic therapies. This is a novel approach that uses an adjuvant molecule lacking intrinsic antibiotic activity along with antimicrobial to overcome microbial resistance [15]. The novel antibiotic adjuvant combination, CSE is one such combination of ceftriaxone (3rd generation beta-lactam cephalosporin), sulbactam (beta-lactamase inhibitor), and disodium EDTA (class-1 antibiotic resistance breaker). The in-vitro activity of ceftriaxone can be restored by the synergistic effect of this combination and

become effective against MDR pathogens producing ESBLs/MBLs, and carbapenemases [16,17]. The efficacy of the new antibiotic adjuvant entity of ceftriaxone+sulbactam+adjuvant EDTA was proven in a wide range of infections [18]. As most of the bacterial isolates are carbapenemase, ESBL, MBL, and AmpC beta-lactamase producing sparing only colistin for the treatment in ICU patients. Based on these sensitivity patterns there is an urgent need to find alternative antibiotic adjuvant entities. Keeping in view the above background, the present study sought to evaluate the in-vitro efficacy of a new antibiotic adjuvant entity (CSE), among the Vitek-2 confirmed ESBL, MBL, AmpC, and carbapenemase-producing strains isolated from different ICU specimens.

MATERIALS AND METHODS

A descriptive cross-sectional study was done for five months (January 2021-May 2021) in NRI General and super specialty Hospital in Chinakakani, Guntur, Andhra Pradesh, India. Institutional Ethical Clearance (IEC) was obtained (NRIAS/IEC/96/2014). A total of 865 ICU specimens like urine 242 (28%), pus swab 294 (34%), sputum 34 (3.9%), tracheal aspirate 58 (6.7%), blood 217 (25.1%), sterile body fluids 20 (2.3%), were received in the Microbiology laboratory during the study period.

Inclusion criteria: The study included all Vitek 2 confirmed ESBL, MBL, Carbapenemase, and AmpC beta lactamase-producing organisms.

Exclusion criteria: Organisms that were not flagged as any of the ESBL, MBL, Carbapenemase, or AmpC beta lactamases producing by Vitek 2 were excluded.

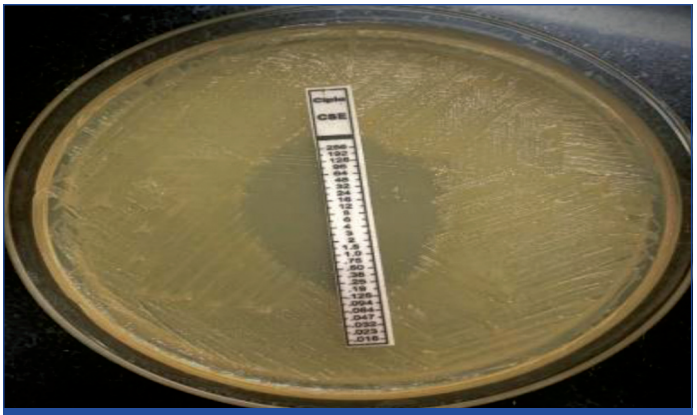
Study Procedure

Processing of specimens: Blood samples were collected in blood culture bottles and incubated in BacTAlert (Biomerieux, France) machine and samples that signaled positive were then subcultured. All other samples including positive signaled blood samples were inoculated on blood agar and MacConkey's agar, incubated for 18-24 hours at 37°C, and growth was examined. Each sample growth was identified using standard microbiological procedures [19].

Identification and characterisation of the isolates: After obtaining pure bacterial growth, further identification and antibiotic sensitivity were done using automated Vitek 2 compact (Biomerieux SA,) with identification cards and AST cards i.e. for Gram positive cocci N628 card, for fermenter bacilli N280 card and non fermenting bacilli N281card. Growth was assessed quantitatively using an optical reader [20].

Out of 865 specimens, 580 (67%) specimens were culture positive. Among them, the study included a total of 100 consecutive MDR isolates confirmed by the Vitek 2 compact system with phenotypes flagged as carbapenemase-producing, ESBL, MBL, and Amp beta-lactamase-producing organisms [21,22]. These MDR isolates were subcultured on nutrient agar. These isolates were further tested for antibiotic susceptibility of CSE E-strips with MIC gradient of 0.016-256 µg/mL using Epsilometer test on Muller Hinton agar medium according to CLSI standards [23].

Epsilometer (E) test procedure: The 0.5 McFarland standards nutrient broth inoculum was prepared from the isolated pathogen from nutrient agar plates. The inoculum was lawn cultured using a sterile cotton swab by rotating several times at 60° on the Mueller Hinton agar plate. After drying for 3-5 minutes, the CSE E- strip (Elores, Cipla Ltd) MIC scale facing upward was applied and then pressed to make sure complete contact with the MHA plate and the maximum concentration nearest the rim of the plate [13,16]. Following 16-18 hours of incubation at 37°C, an elliptical zone of inhibition that intercepts the strip was taken as a MIC value [Table/Fig-1]. The MIC value was read at complete inhibition of all growth. The sensitivity of the CSE E- strip MIC zone of inhibition was interpreted as sensitive (S), and resistant (R) based on breakpoints provided by the manufacturer [Table/Fig-2] [24,25].



[Table/Fig-1]: CSE E- Test on Mueller-Hinton agar plate.

Quality control strain	MIC (mg/mL)
Escherichia coli ATCC 25922	0.016-012
Pseudomonas aeruginosa ATCC 27853	8-32
Klebsiella pneumoniae NCTC 13439	1-4

[Table/Fig-2]: Quality control and expected MIC (µg/mL) [24,25].

STATISTICAL ANALYSIS

The descriptive statistical analysis was done by calculating the frequencies.

RESULTS

In the present study, out of 865 specimens, 100 MDR organisms confirmed on Vitek 2 compact ID and AST system, were isolated. Out of which majority of specimens (42%) were pus/wound followed by urine, blood, tracheal aspirate, sputum, and body fluids specimens [Table/Fig-3].

	Specimen					
Isolate-n	Pus	Urine	Blood	Tracheal aspirates	Sputum	Body fluids
E. coli (36)	14	15	4	1	1	1
P. aeroginosa (18)	12	4	0	0	2	0
Klebsiella pneumoniae (15)	4	2	4	5	0	0
Proteus spp. (10)	4	6	0	0	0	0
Staph. aureus (9)	4	0	5	0	0	0
Enterobacter cloacae (6)	3	2	0	0	0	1
Acinetobacter baumannii (5)	1	0	0	4	0	0
Stenotrophomonas (1)	0	0	1	0	0	0
Total	42	29	14	10	3	2

[Table/Fig-3]: Total number of MDR isolates from different specimens.

Analysing the sensitivity patterns: The sensitivity and resistance details of each isolated MDR strain are outlined in [Table/Fig-4]. Of these 100 isolates, a total of 63 were carbapenemase-producing followed by 28 were ESBL+MBL and nine (9) were AmpC beta-lactamase-producing organisms.

As shown in [Table/Fig-4], out of 100 MDR isolates 36 (36%) were *Escherichia coli*, the most prevalent MDR pathogens followed by *Pseudomonas aeruginosa* 18 (18%), *Klebsiella pneumoniae* 15 (15%), *Proteus* species 10 (10%). Among 36 (36%) *Escherichia coli*, 6 (16.7%) were ESBL+MBL, and 30 (83.3%) were carbapenemase-producing and showed good susceptibility to adjuvant CSE (Ceftriaxone+Sulbactam+EDTA) of 5 (83.3%) and 26 (86.7%) respectively. The second most prevalent 18 (18%) *Pseudomonas aeruginosa* isolates, 6 (33.3%) were ESBL+MBL, and 12 (66.7%) carbapenemase-producing organisms of which 5 (83.3%) and 10 (83.3%) showed susceptibility to CSE respectively. Among 15 (15%) *Klebsiella pneumoniae* isolates, 10 (66.7%) were

Isolate-n (%)	Characterisation of Isolate-n (%)	CSE sensitive n (%)	CSE resistant n (%)
E. coli (36)	Carbapenemases 30 (83.3%)	26 (86.7%)	4 (13.3%)
	ESBL+MBL 6 (16.7%)	5 (83.3%)	1 (16.7%)
P. aeruginosa (18)	Carbapenemases 12 (66.7%)	10 (83.3%)	2 (16.7%)
	ESBL+MBL 6 (33.3%)	5 (83.3%)	1 (16.7%)
K. pneumoniae (15)	Carbapenemases 10 (66.7%)	9 (90%)	1 (10%)
	ESBL+MBL 5 (33.3%)	5 (100%)	0
Proteus spp. (10)	Carbapenemases 7 (70%)	7 (100%)	0
	ESBL+MBL 3 (30%)	2 (66.7%)	1 (33.3%)
S. aureus (9)	AmpC 9 (100%)	8 (88.9%)	1 (11.1%)
Enterobacter cloacae (6)	Carbapenemases 4 (66.7%)	4 (100%)	0
	ESBL+MBL 2 (33.3%)	2 (100%)	0
Acinetobacter.baumannii (5)	ESBL+MBL 5 (100%)	4 (80%)	1 (20%)
Stenotrophomonas- (1)	ESBL+MBL 1 (100%)	1 (100%)	0
Total isolates (100)		88 (88%)	12 (12%)

[Table/Fig-4]: Antibiotic sensitivity of MDR isolates against ceftriaxone+sulbactam+EDTA drug.
ESBL: Extended-spectrum β-lactamases; MBL: Metallo-β-lactamases; AmpC: AmpC-β-lactamase

carbapenemase, and 5 (33.3%) were ESBL+MBL producers, of which 5 (100%) and 9 (90%) were susceptible to CSE respectively. Other less isolated organisms including *Proteus* species, *Staphylococcus aureus, Enterobacter cloacae*, *Acinetobacter baumannii* and *Stenotrophomonas maltophila* are shown in [Table/Fig-4].

Overall, 88% of isolates were sensitive and 12% were resistant to the novel CSE combination among all 100 MDR isolates obtained from ICU.

aeruginosa (18%), and *Klebsiella pneumoniae* (15%). In line with the current findings, several studies, have also reported a higher prevalence of *Escherichia coli*. A study by Al-Zahrani AJ and Akhtar N showed 48% *Escherichia coli* and 18% *Klebsiella pneumoniae* [27]. Oberoi L et al., reported MDR isolates of which 33.3% were *Escherichia coli* followed by *Pseudomonas aeruginosa* (25%) and *Klebsiella pneumoniae* 16.6% [28].

Among eight different MDR strains isolated in the present study, the prevalence of carbapenemase producers were 63 (63%), ESBL and MBL producers were 28 (28%), and AmpC producer pathogens were 9 (9%). Similar to present study Mathias A et al., reported 70% carbapenemase-producing [12]. Govindaswamy A et al., also reported 65% of carbapenemase-producers [22]. Pawar SK et al., reported a low percentage of 31.7% carbapenemase-producing isolates [11]. In other studies, Nepal K et al., reported ESBL and MBL as 34.5% and 21% [9]. Jena J et al., reported ESBL and MBL as 31.5% and 19.29% [10]. Singhal S et al., reported 8% AmpC beta lactamases which was similar to the present study [29]. As per World Health Organisation (WHO), in the South-East Asian Region, 16-68% of *Escherichia coli* and 34-81% of *K. pneumoniae* were resistant to 3rd generation cephalosporin [30].

Present in-vitro study, which evaluated the efficacy of CSE, an antibiotic-adjuvant combination, among the Vitek-2 confirmed MDR strains isolated from ICU patients reported 66.6-100% susceptibility, and 0-33.3% resistance to CSE. Overall sensitivity to CSE was 88 (88%) and resistance was 12 (12%) among the MDR strains. Similarly, in a study by Chaudhary M et al., they evaluated the effect of the addition of EDTA (a potent class β-Metallo β- lactamase inhibitor, 3 mg/mL) and its salts with ceftriaxone + sulbactam (2:1) combination (CSE1034), and stated that this combination lowers MIC to >8 fold and possess combined effect against most ESBL producing bacteria [15]. As tabulated in [Table/Fig-5], several other studies from India have confirmed the reduced sensitivity of various antibiotics to ESBL producing bacteria and higher susceptibility to CSE [16,18,31-33]. Furthermore, Arora S and Munshi N; Nema S et al., and Chakravorty S and Arun P also reported better susceptibility to CSE compared to other carbapenems (imipenem and meropenem) against Gram negative bacilli [32-34].

Isolate	Kumar M et al., [31] (2015) Uttar Pradesh	Arora S and Munshi N [32] (2015) Pune, Maharashtra	Chaudhary M et al., [18] (2017) 17 centres in India	Nema S et al., [33] (2017) Bhopal, Madhya Pradesh	Singh S et al., [16] (2020) Lucknow, Uttar Pradesh	Present study (2022) Guntur, Andhra Pradesh
E. coli	83.8%	81%	98%	93.3-96%	94%	83.3-86.6%
P. aeroginosa	80%	82%	90%	-	97%	83.3%
K. pneumoniae	81.9%	73%	93%	93.3-96%	94%	90-100%
Proteus spp.	90.2%	-	100%	93.3-96%	94%	66.6-100%
Acinetobacter baumannii	82%	82%	89%	93.3-96%	97%	80%
Enterobacter cloacae	-	-	98%	93.3-96%	94%	100%

[Table/Fig-5]: Ceftriaxone+sulbactam+EDTA Susceptibility patterns [16,18,31-33].

DISCUSSION

Infection with MDR strains of Gram negative bacterial pathogens can lead to severe morbidity and mortality [1]. Thus, the use of antibiotic adjuvants that lack intrinsic antibiotic activity along with antimicrobial (e.g., CSE) is a novel alternative to deal with MDR microorganisms (ESBL, MBL, AmpC, and carbapenemase-producing). It disturbs bacterial physiological function, inhibits antibiotic resistance elements, enhances uptake of the antibiotic through the bacterial membrane, blocks efflux pumps, changes the physiology of resistant cell's function i.e., alteration in biofilm formation, and prevents the transfer of resistant plasmid [6,17,26].

In the present study, *Escherichia coli* was found to be the most prevalent MDR pathogens (36%), followed by *Pseudomonas*

The present study suggested that this novel combination therapy (β-lactam antibiotic+β-lactamase inhibitor+EDTA) is highly effective against ESBL/MBL, AmpC, and carbapenemase-producing MDR bacteria. The enhanced susceptibility may be due to the synergistic effect of all three molecules in the combination. EDTA enhances the permeability of ceftriaxone and sulbactam in ESBL pathogens and can chelate the divalent ions required for MBL activity which increases its susceptibility against ESBL/MBL producing bacteria [35]. Hence, the carbapenem class of drugs can be reserved for specific high-risk patients.

Limitation(s)

The limitations of the present study include the absence of clinical efficacy details and a lack of comparison with other antibiotics.

Further studies are required in a large patient population and clinical correlation of the efficacy of CSE against MDR organisms. This study was an approach to discover the effectiveness of this novel therapeutic combination in hospital settings.

CONCLUSION(S)

The present study showed high (88%) in-vitro susceptibility of MDR isolates to a novel antibiotic-adjuvant combination CSE. This novel antibiotic-adjuvant combination is a promising approach to deal with MDR bacterial infections. However, a clinical study in a large patient population is required to validate these in-vitro study results.

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