Susceptibility to Ceftriaxone-sulbactam-EDTA in gram negative MDR & non-MDR isolates at tertiary care centre JNMCH Aligarh, Uttar Pradesh

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ABSTRACT:

Introduction: A rapid increase in multidrug-resistant (MDR) is being reported across India. Ceftriaxone-sulbactam-EDTA (CSE) is a promising therapeutic option available in cases of infections caused by ESBL and MBL producing pathogens proving to be a carbapenem-sparing antibiotic. However, CSE effectiveness needs to be evaluated due to rapidly increasing antimicrobial resistance. **Objectives:** Following prospective observational study was directed to generate data on in-vitro susceptibility of MDR and NON-MDR to Ceftriaxone-sulbactam-EDTA (CSE). **Materials and Methods:** Gram negative bacterial isolates cultured from various non-repetitive clinical samples of indoor and outdoor patients of a tertiary care centre of UP for a period of 9 months (1 April 2022 to 31 December 2022) were included in the study. Antimicrobial susceptibility testing to antibiotics was done using Kirby-Bauer disk diffusion and VITEK methods. **Results:** During study period, 82.3% (229/278) and 17.6% (49/278) of MDR and Non-MDR gram negative isolates respectively. Among the isolates 51% of MDR and 95% of Non-MDR were found to be susceptible to CSE. **Conclusions:** Present study shows that in-vitro susceptibility to CSE varies from 51% to 95% depending on the organism. A trend of increasing resistance in multidrug-resistant (MDR) organisms is being reported across all isolates. Ceftriaxone-sulbactam-EDTA fixed dose combination is a promising therapeutic option in cases of infections caused by MDR (especially ESBL and MBL producing pathogens) acting as carbapenem-sparing antibiotics.

Keywords: Ceftriaxone-sulbactam-EDTA, susceptibility, multi-drug resistant.

Introduction: The rapid increase of antimicrobial resistance with emergence of multidrug resistant (MDR) organism producing extended spectrum beta lactamases (ESBL) and metallo-beta lactamases (MBL) conferring sufficient protection from antibiotics action, poses a therapeutic challenge to treating physicians. A vast majority of healthcare-associated infections in India are caused by MDR gram-negative bacteria [1]. Apart from being associated with increased morbidity and mortality among hospitalized patients, these infections are associated with increased health care costs burdening and an overall negative impact on the economy. [2,3]. Carbapenems and colistin form the mainstay of treatment against gram-negative pathogens, especially ESBL and MBL producing isolates. In view of the increasing failure rate of β-lactams [4] and increased resistance to carbapenems with toxicity of colistin, especially in intensive care units (ICUs), there is a need for a new antibiotic/combination of antibiotics which can work more efficiently against ESBLs and MBLs. [5,6,7}. A new approach to improve the existing antimicrobial agents is the use of Antibiotic Adjuvant Therapy (AAE). Prompt use of AAE (ceftriaxone, sulbactam with adjuvant EDTA) has been reported to have proven efficacy in a wide range of infections [8,9]. The in vitro, preclinical, microbiological and molecular studies have demonstrated it to be more effective than penicillins, cephalosporins, beta-lactam and beta-lactamase inhibitor combinations including piperacillin + tazobactam,cefoperazone + sulbactam, amoxicillin + clavulanate [6,7,8,9] proving to be a carbapenem-sparing antibiotic which requires further susceptibility testing and required evaluation. To the best of our knowledge, there is no data available from this tertiary care center on in-vitro susceptibility profile of clinical MDR and Non-MDR Gram-negative bacterial isolates to this AAE. Keeping this void in mind, the following study has been conducted with the aim of generating preliminary laboratory data on this subject.

Material and Methods: The following study was conducted at tertiary care Centre JNMCH, AMU in Department of Microbiology. Bacterial isolates of both in-patients and ICUs were studied for a period of nine months from April 2022-December 2022.

Sample collection: During the study period, a total of 278 Gram negative bacterial isolates were obtained from tracheal, urinary and blood samples collected from in-patients and ICUs.

Sample grouping: Gram negative bacterial isolates were further classified as MDR (being non-susceptible to at least one agent in three or more antimicrobial categories [10]) and non-MDR after screening them as per the Clinical Laboratory Standards Institute (CLSI) guidelines [11].

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed by subjecting the isolates to Kirby-Bauer disk diffusion and VITEK methods with antibiotic panel of Ceftriaxone-sulbactam-EDTA (CSE).

Results:

With a total of n=278 isolates, 74 were obtained from tracheal and the remaining 204 being blood and urine, collected from both inpatients and outpatients during the study period of 9 months from April 2022 to December 2022. Out of total clinical isolates, 82.3% (229/278) were gram-negative MDR, while remaining 17.6 % (49/278) were found to be non-MDR gram negative ones. A major contribution 57.55% (160/278) of MDR species came from blood and urinary samples. *Acinetobacter* spp. and *Escherichia coli* were the most predominant gram-negative pathogens comprising 43.2% and 74.4% of tracheal and blood urinary isolates respectively

CSE susceptibility results: Among tracheal isolates most of them were multidrug resistant (MDR) species, *Acinetobacter* 43.24 % (32/74) was most commonly detected, followed by *klebsiella* 29.74 % (22/74), *E. coli* 16.21% (12/74), *Pseudomonas* 5.40 (4/74) and others 5.40% (4/74).

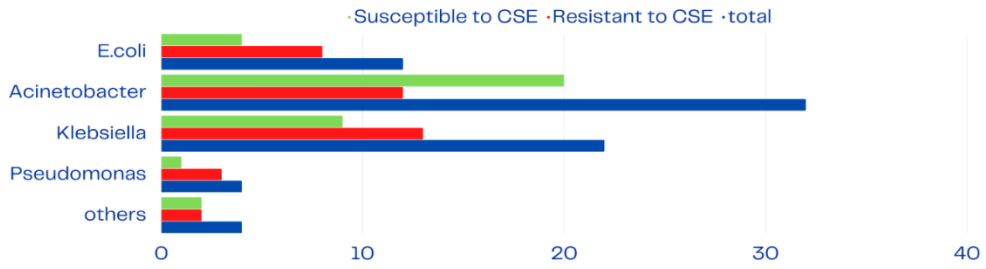


Fig.1 Tracheal isolates susceptiblity organism wise

Isolated bacteria (MDR & non-MDR)	Total isolates (tracheal)	Susceptible to CSE, n/N (%)
Acinetobacter spp.	32	20/32 (62)
Klebsiella spp.	22	9/22 (40)
E.coli	4	1/4 (25)

Pseudomonas spp.	4	1/4 (25)
Others	4	2/4 (50)

Table. 1 Susceptibility of Tracheal isolates to CSE

Only 52.6 % (38/74) isolates including both MDR and non-MDR were susceptible to CSE. However, there is a high degree of resistance in half of isolates.

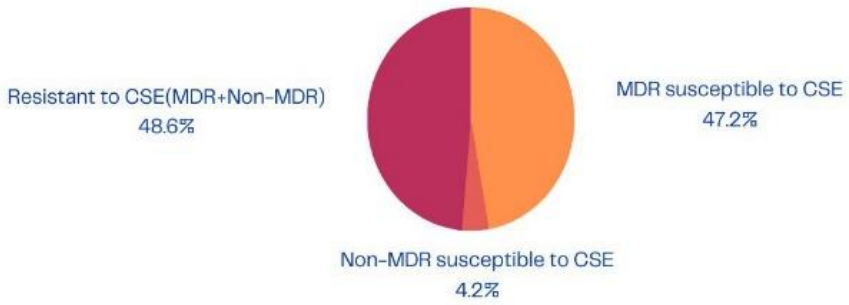


Fig.2 Tracheal isolates overall

E.Coli 78.43% (160/204) is the most common isolate among urinary samples, followed by *klebsiella* 17.15% (35/204) and *proteus* 3.43% (7/204). A total 78.43% (160/204) of isolates came out to be MDR with 67.5% (108/160) of them were susceptible to CSE.

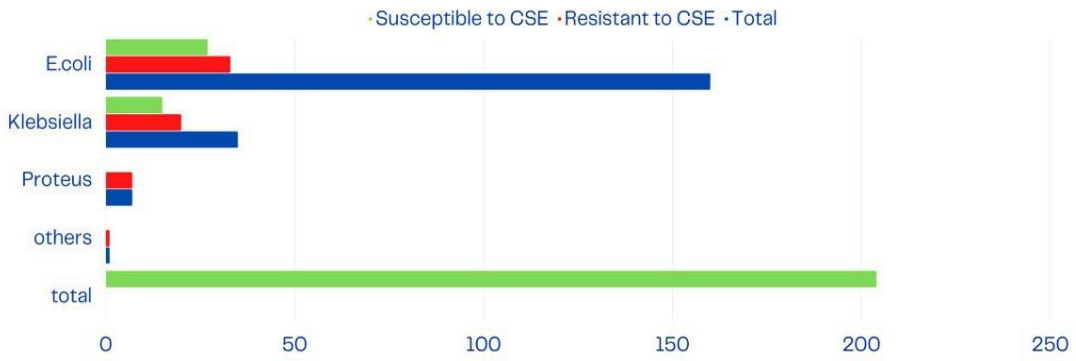


Fig.3 Blood urinary isolates susceptibility organism wise

Isolated bacteria (MDR & non-MDR)	Total isolates (Blood & Urine)	Susceptible to CSE, n/N (%)
E.coli	160	27/160 (16.8)
Klebsiella spp.	35	15/35 (42.8)
Proteus spp.	7	0/7 (0)

Table. 2 Susceptibility of blood and urinary isolates to CSE

A peculiar finding showed that all non-MDR 20% (44/204) isolated from blood and urinary samples were CSE susceptible.

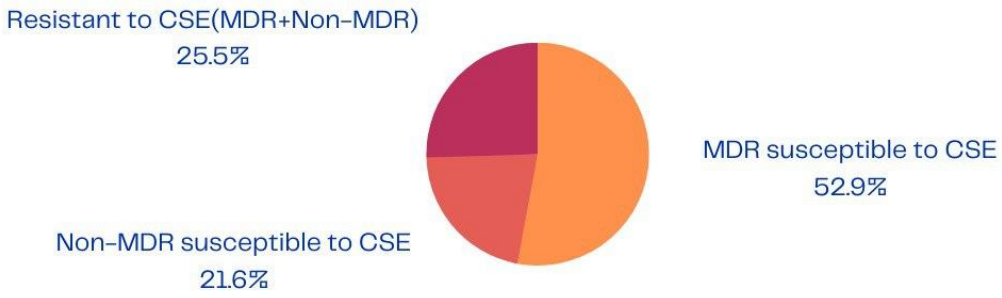


Fig.5 blood urinary isolates

During the study period, 82.3% (229/278) and 17.6% (49/278) of MDR and Non-MDR gram negative isolates were detected respectively.

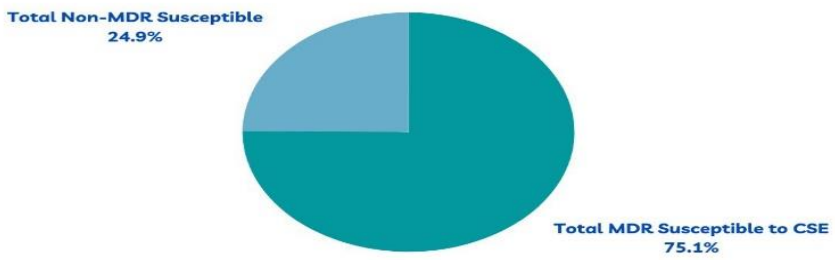
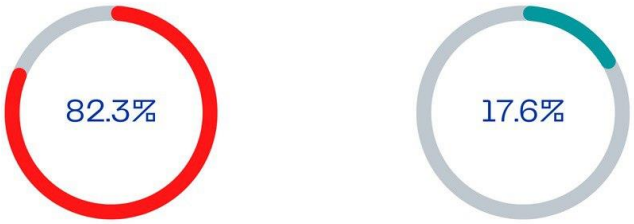


Fig.6 Total clinical isolates (both tracheal and urinary

Among the total isolates 51% of MDR and 95% of Non-MDR were found to be susceptible to Ceftriaxone-sulbactam-EDTA.



(a) MDR (b) non-MDR

Fig.7 Total bacterial isolates

Discussion:

Extended-spectrum beta-lactamase and MBL producers have rendered most of the beta-lactam antibiotics [12], which are the most widely prescribed ones in both community and nosocomial infections. Use of these agents for a long duration has however resulted in a dramatic increase in the rates of resistance that now threatens the utility of most of the large drug family [13]. Meropenem, Tigecycline and Colistin are the last remaining resorts and there are chances they may become ineffective in near time as increasing carbapenem resistance among Gram-negative bacteria has been documented greatly in recent year [14]. The need of hour is to combat increased antimicrobial resistance by using proper combination of including beta-lactam and beta lactamase inhibitors (BL+BLI). This study retrospectically documents clinical isolate's susceptiblity to AAE

of Ceftriaxone-sulbactum-EDTA which has given some promising results as depicted in study by Chaudhary et al., which shows 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. CSE is an effective against MDR pathogen producing ESBLs, MBLs like NDM-01 and prevents “transfer of resistant plasmid” and hence the spread of resistance is controlled [15]. Furthermore, studies have shown that ceftriaxone-sulbactam-EDTA combination is a promising therapeutic option as carbapenem sparer in cases of infections caused by ESBL and MBL producing pathogens, respectively [16,17,18,19].

Conclusion.

Present study shows that in-vitro susceptibility to CSE varies from 51% to 95% depending on the gram-negative organism. Preliminary data suggest MDR being more susceptible to CSE as compared to non-MDR. A trend of increasing resistance in multidrug-resistant (MDR) organisms is being reported across all isolates thus judicious use with antibiotic stewardship is required while prescribing CSE. Ceftriaxone-sulbactam-EDTA fixed dose combination may be a promising therapeutic option in cases of infections caused by MDR (especially ESBL and MBL producing pathogens) acting as carbapenem-sparing antibiotics Further evaluation and more study is required.

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