

Original Research Article

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A Single Center Observational Study to Evaluate Epidemiology and Susceptibility Patterns to Antimicrobial Agents at a Tertiary Care Hospital in India

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ABSTRACT

The continuous change in microbial sensitivity to antimicrobial agents necessitates to have knowhow of local susceptibility patterns so as to help clinicians choose the right pathogen specific antimicrobial therapies. This retrospective study assessed the phenotypic characteristics of pathogens isolated from different clinical specimens and their susceptibility to some of second line of antibiotics like Ceftriaxone-Sulbactam-EDTA (CSE-1034), Meropenem, Piperacillin-Tazobactam (Pip/Taz) and Cefaperazone-Sulbactam used in our hospital settings. A total of 241 Gram-negative isolates from 600 patients treated for various bacterial infections during May 2017 to December 2017 were included in the study. Four antimicrobial agents (Ceftriaxone-sulbactam-EDTA, Meropenem, Piperacillin-Tazobactam and Cefaperazone-Sulbactam) have been used and extended spectrum β -lactamases (ESBL) and metallo β -lactamases (MBL) production was confirmed by double-disk synergy test. Of the 241 Gram negative isolates obtained from 600 patients, 168 isolates were from Inpatient department (IPD) and 73 from Intensive care unit (ICU) patients. In IPD, the isolates were predominantly obtained from urine (52.4%) and wound (22.0%) whereas in ICU patients, the predominant specimens were respiratory (39.7%) and urine (36.9%). *E. coli* was detected in 41.5% of clinical specimens followed by *K. pneumoniae* (21.9%) and *P. aeruginosa* (15.4%). 65.6% of the isolates were reported beta-lactamase producers with 34.4% (83) as ESBL producers, 0.8% (2) as MBL producers and 30.3% (73) as ESBL+MBL producers. CSE-1034 had the greatest activity against both ESBL and ESBL+MBL producing isolates. ESBL isolates were sensitive to CSE-1034 (93.9%), Meropenem (83.1%), Cefaperazone-Sulbactam (61.4%) and Pip/Taz (51.8%). The susceptibility rates of ESBL/MBL producing isolates were CSE-1034 (75.2%), Cefaperazone-Sulbactam (8.2%), Meropenem (5.5%), and Pip-Taz (2.7%). This retrospective data suggests that CSE-1034 can be considered as an important therapeutic option for the treatment of infections caused by both ESBL and ESBL+MBL producing Gram-negative isolates

Keywords

Enterococcus faecium,
Staphylococcus aureus,
Klebsiella pneumoniae,
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Introduction

The unrestricted prescription of antibiotics by clinicians has led to an abrupt rise in anti-

microbial resistance, threatening the use of major available drugs for the treatment of various infections (Zaman *et al.*). Of particular concern in Asian sub-continent are

the Gram-negative infections particularly caused by ESKAPE pathogens which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* (Zellweger *et al.*, 2017). The primary mechanisms responsible for this resistance are the production of various classes of beta-lactamase enzymes including extended spectrum β -lactamases (ESBLs) and metallo β -lactamases (MBLs). ESBLs are β -lactamases capable of hydrolyzing various classes of antibiotics including penicillins, monobactams and other several groups of β -lactam antibiotics, notably third and fourth generation cephalosporins. MBLs are known as Carbapenem hydrolyzers (Rawat and Nair, 2010) (Munita and Arias, 2016). The majority of ESBL and MBL producing organisms produce more than one β -lactamase and thus show cross-resistance to other groups of antibiotics (Rawat and Nair, 2010). The prevalence of ESBL producers in India range from 28%-84% and MBL producers range from 7-71% (Singh *et al.*, 2015).

In addition to limiting the treatment options, the growing anti-microbial resistance is associated with a clinical and economic burden, including increased mortality, greater hospital and antibiotic costs, and longer stays in hospitals and intensive care units (Barriere, 2015). This is mainly attributed to improper empirical therapy in the beginning, causing delay in effective therapy and poor treatment outcomes.

For choosing the right kind of treatment for the management of infections, various factors are considered including individual risk factors, clinical severity and most importantly local epidemiology. The anti-microbial resistance pattern of pathogens varies in different geographical locales and the knowledge of local epidemiology is helpful to choose the effective antibiotic for treating

such pathogens. In the present study, we retrospectively analyzed the antibiotic susceptibility pattern of Gram-negative clinical isolates collected during the study period.

Materials and Methods

Source of isolates

This study was carried out in adult patients suffering from various bacterial infections and admitted to IPD or ICU for treatment from May 2017 to December 2017. The clinical samples used for pathogen isolation were wound, respiratory specimens, blood, fluids, tissue, bile, catheter tip, ear swabs and stools. The sample collection and processing were done as per standard microbiology laboratory operating procedures. Colony counts higher than or equal to 10^5 colony forming units (CFU)/mL were considered significant (Cheesbrough, 2005).

Pathogen Isolation and identification

The identification of clinical isolates was done on the basis of Gram-staining, colony morphology, motility and different biochemical reactions using standard techniques. Required clinical specimens were collected in sufficient amount aseptically which were then inoculated or streaked on different selective and non-selective culture media as per the standard microbiological procedures. Blood samples collected in brain heart infusion (BHI) broth were incubated aerobically overnight at 37°C followed by sub-culturing in the respective media (Cheesbrough, 2005).

Antibiotic susceptibility testing

In vitro susceptibility testing was done by Kirby-Bauer disk diffusion method for all the pathogen isolates as per Clinical Laboratory

Standard Institute (CLSI) guidelines. Discs of CSE-1034, Meropenem, Piperacillin/Tazobactam and Cefaperazone/ Sulbactam were used in the study.

The results were interpreted as per the interpretation criteria of the Clinical and Laboratory Standards Institute (CLSI) standards (Bauer and Kirby, 1966; CLSI, 2017).

CSE1034 is a novel antibiotic adjuvant entity of ceftriaxone and sulbactam (2:1 w/w) along with non-antibiotic adjuvant disodium EDTA (ethylene diamine tetra acetic acid), which displays a broad spectrum of activity against a range of infections caused by Gram negative organisms. This new drug has been approved by Drug Controller General of India (DCGI) (Nazish Fatima, Mohammed Shameem *et al.*, 2017).

CSE-1034, for which CLSI breakpoints are not available hence interpretative breakpoints provided by the manufacturer were used. Criteria for Enterobacteriaceae were >23mm - Sensitive, 20–22-Intermediate, and ≤19-Resistant and for other Gram-negative bacilli were >21 mm - Sensitive, 14–20-Intermediate, and ≤13- Resistant (Prabhu, Mohit Arora *et al.*, 2017). Data entered in excel sheet and analysed.

This study was approved by Bhatia hospital, Medical Research Society.

Results and Discussion

Sample collection and pathogen isolation

A total of 241 (40.2%) Gram-negative isolates obtained from 600 screened clinical specimens were included in this retrospective analysis. The major clinical samples processed for pathogen isolation were urine (47.7%), wound swabs and respiratory specimens (19.1% each)

whereas all other specimens contributed 14.1% to the total pool. The samples processed least were bile, catheter tip, ear swabs, and stools. For other details, refer to Table 1. Higher number of isolates (53.1%) was obtained from females.

Microbiological characteristics

The detailed profile of various organisms isolated is shown in Table 1. 41.5% of the isolates were *E. coli* followed by, *K. pneumoniae* (21.9%) and *P. aeruginosa* (15.4%).

E. coli was predominant in both ICU and IPD followed by *K. pneumoniae* (Table 1).

The susceptibility profile to different antibiotics based on family type is shown in Table 2. Overall, 83.4% (201) were reported to be sensitive to CSE-1034, 63.9% (154) to Meropenem, 49.4% (119) to Pip-Taz and 57.3% (128) to Cefaperazone-Sulbactam.

Overall, the susceptibility rates to all the antibiotics were higher in Enterobacteriaceae compared to Non-Enterobacteriaceae family.

The highest susceptibility among Enterobacteriaceae was shown to CSE-1034 (96.2%) and least was shown towards Pip/Taz (55.4%).

Among non-Enterobacteriaceae isolates also, the highest susceptibility was observed to Meropenem (45.6%) whereas lowest was reported to Pip/Taz (29.8%) (Table 2).

Susceptibility results of Enterobacteriaceae based on phenotypic characterization

As shown in Table 3 only two isolates of Enterobacteriaceae were reported as MBL producers. Both the isolates were reported CSE-1034-susceptible, one was Meropenem-

susceptible and none was reported susceptible to Pip/Taz and Cefaperazone-Sulbactam.

It was also seen that the susceptible percentage of ESBL producing Enterobacteriaceae to CSE-1034 was 97.3% and Meropenem (86.5%) which is significantly high compared to Pip-Taz (54.1%) and Cefaperazone-Sulbactam (64.9%).

44/184 (23.9%) of Enterobacteriaceae isolates were ESBL+MBL producers.

The susceptible percentage of ESBL+MBL producing Enterobacteriaceae isolates to CSE-1034 was 93.2%. Only 4.5% isolates each were susceptible to Meropenem and Pip/Taz and 11.4% were susceptible to Cefaperazone/Sulbactam.

Susceptibility results of Non-Enterobacteriaceae based on phenotypic characterization

As shown in Table 3 none of the isolates from non-Enterobacteriaceae family were reported as only MBL producers.

15.8% (9/57) of Non-Enterobacteriaceae isolates were identified as ESBL producers and 50.9% (29/57) were ESBL+MBL producers. The susceptibility rates of ESBL producing Enterobacteriaceae were CSE-1034 (66.7%), Meropenem (55.6%), Pip-Taz (33.3%) and Cefaperazone-Sulbactam (33.3%). The susceptibility rate of ESBL+MBL producing Enterobacteriaceae isolates to CSE-1034 was 48.3%. Only 6.9% isolates were susceptible to Meropenem and none to Pip-Taz and 3.4% isolates was susceptible to Cefaperazone-Sulbactam.

Table.1 Demographic and baseline characteristics of all study subjects

Characteristics		Total (n=241)	IPD (n=168)	ICU (n=73)
Gender	Male, n (%)	113 (46.9)	71	42
	Female, n (%)	128 (53.1)	97	31
Age (year)	Mean±SD		54.5±20.57	52.5±39.66
Clinical sample	Urine (%)	115 (47.7)	88 (52.4)	27 (36.9)
	Wound (%)	46 (19.1)	37 (22.0)	9 (12.3)
	Respiratory specimens (%)	46 (19.1)	17 (10.1)	29 (39.7)
	Blood (%)	12 (4.9)	9 (5.4)	3 (4.1)
	Fluids (%)	6 (2.5)	4 (2.4)	2 (2.7)
	Tissue samples (%)	6 (2.5)	5 (2.9)	1 (1.4)
	*Others (%)	10 (4.1)	8 (4.8)	2 (2.7)
Pathogen (n=241)				
Enterobacteriaceae (n=184)				
	<i>E. coli</i> (%)	100 (41.5)	72 (42.9)	28 (38.3)
	<i>K. pneumonia</i> (%)	53 (21.9)	33 (19.6)	20 (27.4)
	<i>E. cloacacae</i> (%)	10 (4.1)	8 (4.8)	2 (2.7)
	**Others (%)	21 (8.7)	18 (10.7)	3 (4.1)
Non-Enterobacteriaceae (n=57)				
	<i>P. aeruginosa</i> (%)	37 (15.4)	26 (15.5)	11 (15)
	<i>Acinetobacter spp.</i> (%)	16 (6.6)	9 (5.4)	7 (9.6)
	***Others (%)	4 (1.7)	2 (1.2)	2 (2.7)

*Others include bile, catheter tip, ear swabs, stool samples

**Others include *C. koseri*, *E. aerogenes*, *S. typhii*, *M. morgannii*, *Proteus spp.*, *Providencia spp.*, *S. marcescens*

***Others include *A. hydrophila*, *B. cepaceae*, *Streptomonas spp.*

Table.2 In vitro Susceptibility profile of various antibiotics tested

Antibiotic	Enterobacteriaceae (n=184)		Non-Enterobacteriaceae (n=57)		Overall susceptibility (n=241)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
CSE-1034	177 (96.2%)	7 (3.8%)	24 (42.1%)	33 (57.9%)	201 (83.4%)	40 (16.6%)
Meropenem	128 (69.6%)	54 (29.3%)	26 (45.6%)	30 (52.6%)	154 (63.9%)	87 (36.1%)
Pip/Taz	102 (55.4%)	82 (44.6%)	17 (29.8%)	40 (70.2%)	119 (49.4%)	122 (50.6%)
Cefaperazone-Sulbactam	117 (63.6%)	66 (35.9%)	21 (36.8%)	36 (63.2%)	138 (57.3%)	103 (42.7%)

Note: Isolates with Intermediate susceptibility have been considered as resistant

Table.3 In vitro Susceptibility profile of various antibiotics tested based on different phenotypes

Phenotypic characterization	Location	Family	CSE-1034 N (%)	Meropenem N (%)	Pip/Taz N (%)	Cefaperazone-Sulbactam N (%)
MBLs (n=2) (0.8%)	IPD/ICU (2/0)	Enterobacteriaceae (n=2)	2 (100)	1 (50)	0	0
	IPD/ICU (0/0)	Non-Enterobacteriaceae (n=0)	0	0	0	0
		Total (n=2)	2 (100)	1 (50)	0	0
ESBLs (n=83) (34.4%)	IPD/ICU (56/18)	Enterobacteriaceae (n=74)	72 (97.3)	64 (86.5)	40 (54.1)	48 (64.9)
	IPD/ICU (6/3)	Non-Enterobacteriaceae (n=9)	6 (66.7)	5 (55.6)	3 (33.3)	3 (33.3)
		Total (n=83)	78 (93.9)	69 (83.1)	43 (51.8)	51 (61.4)
ESBLs+ MBLs (n=73) (30.3%)	IPD/ICU (25/19)	Enterobacteriaceae (n=44)	41 (93.2)	2 (4.5)	2 (4.5)	5 (11.4)
	IPD/ICU (18/11)	Non-Enterobacteriaceae (n=29)	14 (48.3)	2 (6.9)	0	1 (3.4)
		Total (n=73)	55 (75.2%)	4 (5.5)	2 (2.7)	6 (8.2)

Multidrug-resistance (MDR) has been identified as an increasing health problem and poses a major challenge for patient management and public health around the globe. Though, various guidelines are available for the management of bacterial infections, adoption of these guidelines at the hospital level is challenged by the varied MDR resistance trends in different geographical locales. Thus, anti-microbial surveillance studies to monitor the MDR are

important to help clinicians choose a right empirical therapy. The current study provides insights in the antibiogram of commonly used drugs in a tertiary care hospital in India.

In this study, overall half of isolates were reported from urine samples followed by respiratory and wound specimens. Further data analysis based on specimens from IPD/ICU has shown that isolates were predominantly obtained from urine in IPD

followed by wound whereas in ICU patients, the predominant specimens were urine and respiratory. The distribution pattern of pathogens was almost similar in IPD and in ICU, with *K. pneumoniae* and *Acinetobacter spp.* numerically on the higher side in ICU patients. Mechanical ventilation and the common use of catheters in ICU patients could be one of the reasons for this uneven distribution of clinical specimens in the two populations. Our study results are concordant to other studies. Venkataraman *et al.*, (Venkataraman *et al.*, 2018) in their multicenter study on ICU patients ≥ 48 h, reported Ventilator Associated Pneumonia (VAP) followed by Catheter Associated Urinary Tract Infection (CAUTI) as the most common infections with *Acinetobacter* being the most common isolate in VAP patients and *Klebsiella* in CAUTI. Similarly, Rosenthal *et al.*, (Rosenthal *et al.*, 2006) have reported that ventilator-associated pneumonia posed the greatest risk (41% of all device-associated infections) followed by bloodstream infections (BSIs) (30%) and catheter-associated urinary tract infections (29%) in a study based on device-associated nosocomial infections in 55 ICUs of 8 developing countries, including India. In another study based on ICUs as part of the international infection control consortium from seven Indian cities, it was reported that the overall infection rates were 10.46/1000 ventilator days for VAP, 7.92/1000 catheter days for CLABSI, and 1.41/1000 catheter days for CAUTI (Mehta *et al.*, 2007)

In our study 65.6% of the isolates were reported to be beta-lactamase producers with 34.4% as ESBL producers, 0.8% as MBL producers and 30.3% as ESBL+MBL producers. This could be attributed to the fact that patients included in our study must have already been exposed to hospital environment and received some empiric antibiotics at the time of enrollment making them a higher risk

for developing multidrug resistance. Compared to IPD (62.5%), the beta-lactamase producers were more in ICU patients (69.9%). Moreover, 41.1% were MBL+ESBL producers in ICU patients compared to 25.6% among IPD patients, whereas ESBL producers were more in IPD patients (28.7% vs. 36.9%) (Table 3).

In our hospital, the highest susceptibility of ESBL isolates was reported towards CSE-1034 (93.9%) followed by Meropenem (83.1%). Pip-Taz susceptibility rate was 51.8% and Cefaperazone-Sulbactam was 61.4%. The AMR surveillance study conducted in India has shown resistance against Pip-Taz has risen to 65-70% (Kumar *et al.*, 2013).

Meropenem is being used for severe nosocomial infections, often in hospital units. Their value lies in their broad spectrum of action and in overcoming resistance in Gram-negative bacilli. Exposure and use of carbapenems for the treatment of diverse infectious diseases has appeared to be a main reason for the development of resistance (Kumar *et al.*, 2013). The emergence of carbapenem resistance among clinical isolates has raised fears that effective antimicrobial treatment options for these isolates may soon be severely limited (Ogutlu *et al.*, 2014). The low rate of carbapenem susceptibility reported among ESBLs in this surveillance study needs to be addressed. This pattern of resistance clearly indicates that other mechanisms of resistance to Carbapenems are increasing among ESBLs including efflux pump, membrane impermeability, etc. The emergence of ESBL/MBL producing isolates which is 30% in this study is also a matter of big concern as carbapenems are considered as the last resort drugs for serious bacterial infections. A significantly higher incidence of carbapenem-resistant Gram-negative bacteria has also been reported by Ghosh *et*

al., (Ghosh *et al.*, 2012) from AIIMS, Delhi. Similarly, Singh *et al.*, (Singh *et al.*, 2015) have reported that 15-22% of the gram-negative isolates were MBLs in their study.

Interestingly, a significant number of both ESBL and MBL-ESBL isolates were sensitive to CSE-1034. The high sensitivity of gram-negative pathogens to CSE-1034 has been reported in several other studies too (Chaudhary and Payasi, 2013a) (Chaudhary *et al.*, 2018) (Chaudhary *et al.*, 2017). In an antimicrobial susceptibility pattern study conducted by Sahu *et al.*, 100%, 64% and 63% of ESBL producing *A. baumannii*, *K. pneumoniae* and *E. coli* were reported to be susceptible to CSE-1034 [19]. In the same study, 89%, 60%, 42% and 41% of MBL producing isolates of *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, respectively were susceptible to CSE-1034 (Sahu *et al.*, 2014). In another anti-microbial susceptibility pattern study, 67–81% of ESBL producing *K. pneumoniae* isolates were reported to be highly susceptible to CSE-1034. Similarly, a study on antibiotic susceptibility pattern of various gram-negative pathogens isolated from ICU patients had reported that CSE-1034 has higher clinical efficacy compared to carbapenems (Chaudhary and Payasi, 2013b). These observations assume a huge importance in the backdrop of prescribing empirical therapy for MDR bacterial infections particularly caused by MBL producing isolates. As CSE-1034, a beta-lactam/beta-lactamase combination along with “EDTA” has a good susceptibility profile against all the Gram-negative pathogens tested, it can serve as an effective alternative to reduce the selection pressure on carbapenem-resistant strains through carbapenem over-use.

In conclusion, our analysis suggests that pathogens isolated from various clinical sources demonstrated resistance to a variety

of antimicrobials in our hospital. The high rate of antimicrobial resistance could be possibly explained by antibiotic misuse by public, inappropriate prescription by unskilled practitioners and laypersons, insufficient surveillance studies, poor drug quality and unhygienic conditions accounting for the spread of resistant bacteria. Moreover, CSE-1034 remains the most effective drug against both ESBL and MBL producing gram-negative isolates. The high resistance observed in this study warrants the need for surveillance of resistance pattern of antimicrobial agents administered to patients undergoing treatment for better patient management. A careful monitoring of antimicrobial use, in hospital, is required to identify the situations in which prescription patterns are contributing to the development of resistance.

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