Original Research Article

PREVALENCE AND SUSCEPTIBILITY ANALYSIS OF GRAM NEGATIVE PATHOGENS IN TERTIARY CARE TRANSPLANT HOSPITAL, MUMBAI

Abstract

Introduction:

The susceptibility pattern of antibiotics varies in different geographical regions and needs to be updated regularly to guide clinicians in choosing appropriate empirical therapies. This study was aimed to evaluate the susceptibility pattern of Gram negative clinical isolates towards commonly used antibiotics and a novel antibiotic adjuvant entity, CSE-1034 (Ceftriaxone+Sulbactam+EDTA).

Methods:

A retrospective observational analysis of antibiogram was performed to characterize the susceptibility pattern of different pathogen isolates from various clinical sources. A total of 203 Gram negative isolates identified from the period June 2015 to June 2016 were included in the study.

Results:

Of the total 203 gram-negative isolates, the majority were obtained from urine (44.3%) followed by respiratory specimens (12.3%), blood (12.3%), pus (9.3%) and collection/fluids (7.3%). The most predominant isolates were *Escherichia coli* (49.8 %) and *Klebsiella pneumoniae* (37.4%) whereas other pathogens contributed <5%. CSE-1034 and Meropenem were almost equally active against *E. coli* (85.1%: 89.1%) and *K. pneumoniae* (57.8%: 60.5%). The susceptibility of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to CSE-1034 was 83.3% and 66.6% whereas none of the isolates was reported Meropenem-susceptible. All the isolates of *Enterobacter aerogenes*, *Enterobacter cloacae,* and *Proteus*

mirabilis were reported 100% susceptible towards both CSE-1034 and Meropenem.

The susceptibility towards Piperacillin-Tazobactam (Pip-Taz) was comparable to cefoperazone-Sulbactam. Pip-Taz displayed 67.3% and 46.0% and Cefoperazone-Sulbactam displayed 69.3% and 53.9% susceptibility against *E. coli* and *K. pneumoniae*. All the isolates of *E. cloacae* and *P. mirabilis* were susceptible to both Cefoperazone-Sulbactam and Pip-Taz whereas the susceptibility of other isolates varied for the two antibiotics.

Conclusion:

The present study suggests that CSE-1034 may be considered as an important therapeutic option for Gram negative bacteria as monotherapy or as a part of combination therapy. It may also be considered as useful option to spare carbapenems.

Keywords: Antibiotic, Clinical isolates, CSE-1034, Prevalence, Susceptibility, Resistance

Introduction

Infections due to multi-drug resistant (MDR) pathogens are one of the leading causes of death and morbidity among hospitalized patients throughout the world [1]. Gram negative bacteria, especially members of *Enterobacteriaceae*, *Pseudomonadaceae* and *Moraxellaceae* are among the most important human pathogens and constitute the majority of bacteria isolated from clinical specimens [2]. These bacterial species form the main cause of sepsis, pneumonia, urinary tract infections, intra-abdominal infections and post surgical infections in intensive care units. In the past two decades, a worldwide increase in the number of infections caused by Gram-negative bacteria has been reported. In a study of 1265 intensive care units in 75 countries, 62% of infections were caused by Gram-negative bacteria [2]. Penicillins such as amoxicillin, cephalosporins such as Cefepime, Ceftazidime and Ceftriaxone, and carbapenems such as Imipenem, and Meropenem are commonly used antibiotics to treat the Gram negative bacterial infections [3]. However, over the span of last twenty years, a gradual rise in anti-microbial resistance to all the commonly prescribed antibiotics has been witnessed especially among *Klebsiella spp*., *Enterobacter spp*., *Pseudomonas spp*. and *Acinetobacter spp.* considered as the most deadly pathogens [4].

These enzymes are mainly encoded either by chromosomal genes or by genes located on movable genetic elements such as plasmids and transposons. Production of Extended-spectrum β- lactamase (ESBL) enzymes, is the predominant resistance mechanism adopted by Gram negative pathogens to counter β-lactam antibiotics [5]. Different research groups from India have reported the prevalence of ESBL producers between 28% to 84% [8,13,14] and the prevalence of MBLs range from 7–71% [6] [7] [8]. All these studies clearly point to the alarming situation of rising anti-microbial resistance globally as well as in India. In India, very limited number of microbial surveillance studies among hospitals are conducted. These

kind of studies are very helpful to the clinicians for choosing appropriate antibiotic therapies as resistance pattern varies from hospital to hospital. The present study was undertaken to determine the susceptibility pattern of commonly used drugs Cefoperazone-Sulbactam, Pip-Taz and Meropenem and a novel antibiotic-adjuvant entity, CSE-1034 in a tertiary care transplant hospital in Mumbai.

Material and methods

Sample collection

A total of three hundred sixty two different clinical specimens of urine, blood, sputum, endo-tracheal secretion, pus, fluid collections, tissues, body fluids were collected from patients suspected of infection during the period of June 2016 to November 2016. The collection and processing of the samples were done as per common standard operating procedures.

Sample collection and Isolation of pathogens

All the samples were collected and transported aseptically in sterile containers. Urine samples collected in sterile universal container were directly inoculated to the respective selective media. Other liquid specimens such as pus, sputum, and ET secretion collected in sufficient amount were inoculated on the different selective and non-selective culture media as per the standard microbiological techniques. Details of the culture media used for the isolation of pathogens from various clinical samples are given in Table 1.

Blood samples collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then sub-cultured on to the selective and non-selective media and incubated aerobically overnight at 37°C.

Pathogen Identification

Organisms were identified on the basis of colony morphology, Gram staining, motility, and biochemical reactions. Biochemical reactions were performed as described earlier [9] [15].

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [10]. Meropenem disc (10 µg), CSE-1034 disc (45 µg), Cefoperazone-Sulbactam (105 µg), and Pip-Taz (110 µg) were used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB, Hi-Media, Mumbai, India) from the isolated colony of pathogens selected from 18–24h 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° over the agar surface. After 3-5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24mm from center to center. The plates are then inverted and incubated for 16-18h aerobically at 37° C within 15 minutes of disc application. Sensitivity of isolated organisms against antibiotics was reported as sensitive (S) or resistant (R) based on the breakpoints.

Results

Out of the 362 samples analyzed, Gram-negative isolates were obtained from 56.1% (n=203) samples, Gram-positive isolates from 12.9% (n=47) samples while remaining 30.9% (n=112) samples displayed no growth [Table 2]. Among the samples (n=203) which showed the presence of Gram-negative isolates, 44.3% samples were of urine followed by respiratory specimens and blood (12.3% each), pus (9.3%), collection/fluids (7.3% each). [Table 2]. Morphological and biochemical characterization of Gram-negative isolates revealed presence of 9 different types of species. The detailed profile of isolates obtained from various clinical

samples is shown in Fig 2. The identified bacteria include *E. coli*, *K. pneumoniae*, *A. baumannii*, *C. freundii*, *E. aerogenes*, *E. cloacae*, *P. mirabilis*, *P. aeruginosa* and *S. maltophilia*. Among the identified isolates, the most predominant pathogens isolated were *E. coli* (49.8%, n=101/203) followed by *K. pneumoniae* accounting for 37.4% (76/203). Other pathogens isolated were *P. aeruginosa* (4.4%; 9/203), *A. baumannii* (2.9%; 6/203), *E. cloacae* (1.9%; 4/203), *P. mirabilis* (0.9%; 2/203), *E. aerogenes* (0.9%; 2/203), *C. freundii* (0.5%; 1/203) and *S. maltophilia* (0.5%; 1/203) [Fig-1]. *E. coli* was the major pathogen isolated from urine, blood, pus, fluid and collection samples whereas culture results of respiratory samples showed *K. pneumoniae* as the predominant pathogen. Antibiotic susceptibility profile for all the pathogens isolates is presented in Figure 2 and Figure 3. The susceptibility of the four most predominant pathogens *E. coli*, *K. pneumoniae, A. baumannii* and *P. aeruginosa* towards CSE-1034 was 85.2%, 57.9%, 83.3% and 66.7%, respectively [Fig. 2]. Susceptibility of other pathogens including *E. aerogenes, E. cloacae, and P. mirabilis* towards CSE-1034 was 100% [Fig-3].

Our data showed that the susceptibility of *E. coli* and *K. pneumoniae* towards Meropenem was 89.1% and 60.5%. Surprisingly, none of the isolates of *A. baumannii, P. aeruginosa* and *C. freundii* was found susceptible to Meropenem whereas all the isolates of *E. aerogenes, E. cloacae,* and *P. mirabilis* were Meropenem-susceptible [Fig- 3]. As for the Pip-Taz, the susceptibility rates exhibited were *E. coli* (67.3%) *K. pneumoniae* (46.1%), *P. aeruginosa* (22.2%). Similar to Meropenem, all the isolates of *E. aerogenes*, *E. cloacae* and *P. mirabilis* were Pip-Taz susceptible whereas no isolate of *A. baumannii, C. freundii* and *S. maltophilia* were observed to be Pip-Taz susceptible. The susceptibility of all the isolates to Cefoperazone-Sulbactam was comparable to Pip-Taz. *E. cloacae, E. coli* and *K. pneumoniae* displayed 75%, 69.3%, 53.9% susceptibility to Cefoperazone-Sulbactam respectively. All the isolates of *C. freundii, E. aerogenes* and *P. mirabilis* were Cefoperazone-Sulbactam

susceptible whereas *S. maltophilia* exhibited complete resistance.

Discussion

In the light of increasing antimicrobial resistance, it is important to have a knowhow of the susceptibility patterns of different hospitals so that clinicians would be able to provide befitting treatment against deadly microorganisms. Our data suggested, *E. coli* (49.8%) as the most prevalent pathogen among the identified isolates. Consistent with our results, various studies in the past have demonstrated that *E. coli* dominates the Gram-negative bacterial infections [11]. Kumar *et al*. [12] have reported *E. coli* as the most predominant pathogen isolated from the 1180 clinical specimens suspected of bacterial infections. Sachdeva *et al.* [13] have also reported 51.7% prevalence of *E. coli* infections. *K. pneumoniae* (37.4%) was observed as the second common pathogen after *E. coli*. which is also in accordance with results of other studies. Other isolates such as *P. aeruginosa* (4.4%), *A. baumannii* (2.9%), *E. cloacae* (1.9%), *P. mirabilis* (0.9%), *E. aerogenes* (0.9%), *C. freundii* (0.5%) and *S. maltophilia* (0.5%) also contributed to the pool of clinical isolates.

The antibiogram profile of four most prevalent pathogens including *E. coli*, *K. pneumoniae, A. baumannii* and *P. aeruginosa* towards Ceftriaxone+Sulbactam+EDTA has revealed 57-85% susceptibility whereas least prevalent pathogens including *E. aerogenes, E. cloacae, and P. mirabilis* exhibited 100% susceptibility. Similar kind of susceptibility pattern to CSE-1034 has been reported by several other studies also. Sahu *et al.* [13] have reported the susceptibility rates of 100%, 64% and 63% of ESBL producing *A. baumannii*, *K. pneumoniae* and *E. coli* to CSE-1034 respectively. Same study has reported 89%, 60%, 42% and 41% of MBL producing isolates of *A. baumannii*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* susceptible to CSE-1034. Similarly, in another antimicrobial susceptibility study on 515 MBL and ESBL+MBL producing isolates of *P. aeruginosa*, a susceptibility rate of 97.3% and 95.1% to CSE-1034 has been reported [14]. Greater susceptibility to CSE-1034

could be possible achieved via the multiple mechanisms through which CSE-1034 functions including enhanced antibiotic penetration into cell membrane, decreased expression of efflux pumps, inactivation of Carbapenemases and conjugation process by chelating various metal ions [15] [16] [17].

Our data has demonstrated varying susceptibility rates of different type of species towards Meropenem ranging from 100% by *E. aerogenes, P. mirabilis* and *E. cloacae,* 60-89% by *E. coli* and *K. pneumoniae* whereas *A. baumannii, P. aeruginosa, S. maltophilia* and *C. freundii* displayed zero susceptibility to Meropenem. All the 6 isolates of *A. baumannii* and 9 isolates of *P. aeruginosa* were resistant to Meropenem. A high rate of Meropenem resistance has been reported by other authors as well. Goyal *et al.* [18] have shown that 6.4% and 6.3% of *A. baumannii* isolates were susceptible to Doripenem and Meropenem in their study. Same study has reported that *P. aeruginosa* showed sensitivity of 60.3% for Doripenem and 44.8% for Meropenem. Similarly, Vraiya *et al.* [19] have reported 26% isolates as carbapenem resistant of the total 230 *P. aeruginosa* isolates tested for susceptibility. Compared to our results, Arora *et al.* [20] have reported higher Meropenem resistance of 73.1% in *Klebsiella spp.* and 23.8% in *E. coli*. Similar to our pattern, Wattal *et al.* [20] have reported 31-51% Carbapenem-resistance in *Klebsiella spp.* and 2-13% in *E. coli* in Delhi. A Carbapenem resistance of 14.6% in *E. coli* and 29.6% in *Klebsiella spp.* in hospital isolates has been reported by Chauhan K *et al*. [21].

E. coli and K. pneumoniae exhibited 30-53% resistance rates against Pip-Taz and Cefoperazone-Sulbactam whereas the resistance rates by *P. aeruginosa*, *A. baumannii, C. freundii* and *S. maltophilia* varied from 78% to 100%. High resistance of Gram-negative pathogens to BL/BLIs has been consistently reported by earlier studies and this could be possibly due to exponential rise in ESBL and MBL producing strains globally [22] [23]. The AMR surveillance study conducted in India has shown resistance against Pip-Taz has risen to

65-70%. Results from the SENTRY Antimicrobial Surveillance Program, 2009–2012 has shown that 69% of ESBL-producing *E. coli* isolates from patients with pneumonia were found susceptible to Pip-Taz in vitro whereas only 26.9% of ESBL-producing *Klebsiella spp.* isolates were susceptible to Pip-Taz [24]. Comparison of *in vitro* activities of Ceftazidime, Pip-Taz and Cefoperazone-Sulbactam in a retrospective study conducted at a tertiary care cancer hospital in Mumbai has shown that for all bacterial isolates, Cefoperazone-Sulbactam was sensitive against 58.3% isolates and Pip-Taz against 48.1% [25]. The sensitivity pattern for the Enterobacteriacea group revealed that 67.9% of isolates were sensitive to Cefoperazone-Sulbactam and 45.4% to Pip-Taz [25]. Among the non-lactose fermenters, 52.5% isolates were sensitive to Cefoperazone-Sulbactam and 49.6% to Pip-Taz. For the *Pseudomonas* species, Pip-Taz was sensitive against 58.4% and Cefoperazone-Sulbactam against 57.4% isolates.

Conclusion

The bacterial susceptibility and resistance profile of all isolates in this study have shown that CSE-1034 and Meropenem remain the most effective drugs against Gram negative pathogens, suggesting that use of CSE-1034 may be considered as an important therapeutic option for Gram negative bacteria as monotherapy or as a part of combination therapy even in multiple drug resistant bugs. It may also be considered as useful option to spare carbapenems. In addition, regular antimicrobial susceptibility surveillance is essential.

Ethical Disclaimer:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent : NA

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Table-1: Selective culture medium used for isolation of different pathogens.

Pathogen	Selective media
E. coli	MacConkey agar medium
A. baumannii	Leeds acinetobacter agar base medium
K. pneumoniae	Hicrome Klebsiella selective agar base medium
Proteus spp.	Eosin methylene blue agar medium (EMB) and MacConkey's agar medium
C. freundii	Chromogenic selective medium
Enterobacter species	EMB agar medium
S. maltophilia	VIA medium
P. aeruginosa	Cetrimide agar medium

Table 2: A profile of clinical samples used as a source of the pathogenic isolates.

Sr. No.	Clinical Specimen	Total No.	Gram-negative pathogen isolates N (%age)	Gram-positive isolate or No Growth
1	Urine	155	90 (44.3)	65
2	Respiratory specimens	40	25 (12.3)	15
3	Blood	62	25 (12.3)	37
4	Pus	22	19 (9.3)	3
5	Tissue	21	12 (5.9)	9
6	Collections	27	15 (7.3)	12
7	Body Fluids	21	13 (6.4)	8
8	Others	14	4 (1.9)	10
TOTAL		362	203 (56.1%)	159 (43.9%)

[Fig-1]: Prevalence of clinical isolates in different samples.

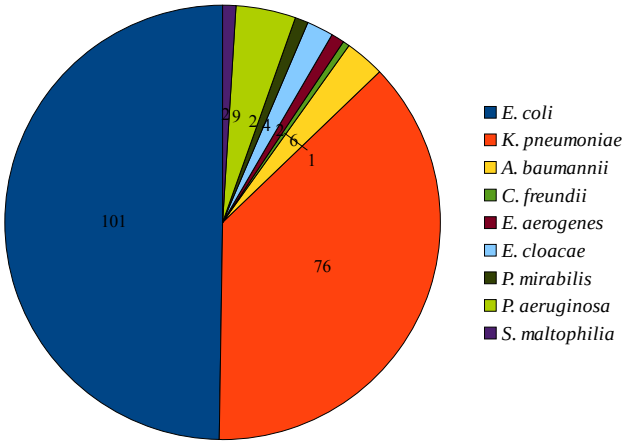
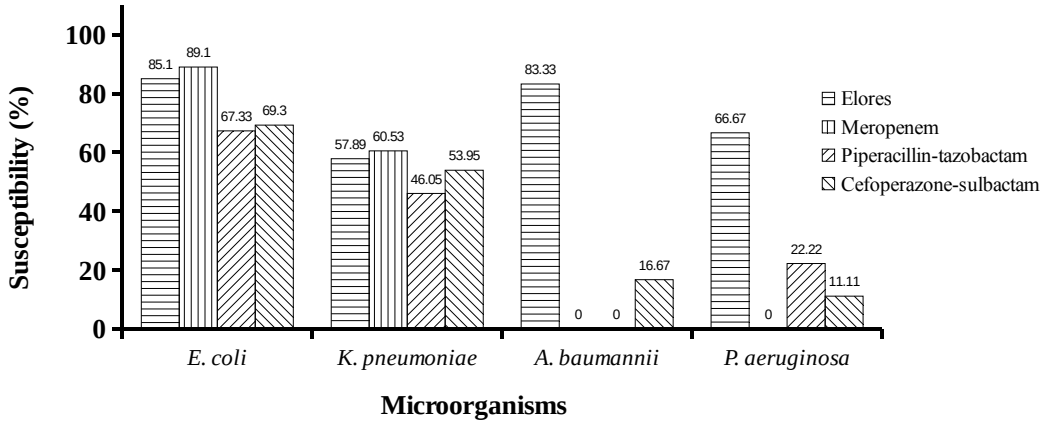


Fig-2: Susceptibility profile of *E. coli, K. pneumoniae, A. baumannii* and *P. aeruginosa* to Ceftriaxone+Sulbactam+EDTA.



[Fig-3]: Susceptibility profile of *C. freundii, E. aerogenes, E. cloacae, P. mirabilis and S. maltophilia* to Ceftriaxone+Sulbactam+EDTA.