Original Research Article

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

Abstract

Introduction:

The anti-bacterial susceptibility pattern 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 including piperacillin/tazobactam, meropenem, cefoperazone+sulbactam and a novel antibiotic adjuvant entity, Ceftriaxone+Sulbactam+EDTA.

Methods:

Whole 362 clinical samples were collected from suspected patients at tertiary care transplant hospital, Mumbai (India) between June 2016 to November 2016 and subjected to bacterial identification. Susceptibility results were interpreted in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines except Ceftriaxone+Sulbactam+EDTA.

Results:

Of the total 203 samples which tested positive for gram-negative pathogens, the majority samples were of urine (44.3%) followed by sputum/endo-tracheal secretions (12.4%), 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% to the pathogens pool. 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 were 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 behaviour of piperacillin+tazobactam against all the pathogens were comparable to Cefoperazone+sulbactam. Pip/Taz displayed 67.3% and 46.0% and Cefoperazone+sulbactam displayed 69.3% and 53.9% sensitivity 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:

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 vary from hospital to hospital. The present study was undertaken to determine the susceptibility pattern of commonly used drugs cefoperazone+sulbactam, piperacillin+ tazobactam and meropenem and a novel antibiotic-adjuvant entity, Ceftriaxone+Sulbactam+EDTA 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 a common standard operating procedure (SOP).

Isolation and identification 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 Table1.

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. Organisms were identified on the basis of [9]

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), Piperacillin+tazobactam (110 μg) and Amoxicillin+clavulanate (30 μg), were procured from Microexpress, a division of Tulip Diagnostics Private Limited, Goa, India and used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB, Hi-Media, Mumbai, India) from 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 24 mm from center to center. The plates are then inverted and incubated for 16-18 hrs aerobically at 37° C within 15 minutes of disc application. Sensitivity of isolated organisms against antibiotics were reported as sensitive (S) or resistant (R) based on the breakpoints.

Results

A total of 362 different clinical samples collected from the patients were processed for the identification of pathogen isolates. Different types of clinical samples processed were urine, blood, sputum, endo-tracheal secretion, pus, fluid collections, tissues, body fluids. Out of the 362 samples analyzed, 56.1% (n=203) samples showed the growth of Gram-negative pathogen, 12.9% (n=47) tested positive for gram-positive 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, around 44.3% (90/203) samples were of urine followed by blood (12.3%), pus (9.3%), collection and sputum (7.3% each). The remaining samples such as endo-tracheal secretion, body fluid, tissue, drain fluid, necrotic tissue, ascitic fluid samples contributed <15.4% to the pool [Table 2].

Morphological and biochemical characterization of the samples (n=203) showing gram-negative isolates revealed presence of 9 different types. The detailed profile of various organisms collected 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* were found susceptible to meropenem whereas all the isolates of *E. aerogenes, E. cloacae,* and *P. mirabilis* were observed to be meropenem-susceptible [Fig-3 & 4]. As for the piperacillin+tazobactam, 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 behavior of Cefoperazone+sulbactam against all the isolates was comparable to piperacillin+tazobactam. Cefoperazone+sulbactam displayed 75%, 69.3%, 53.9% against *E. cloacae, E. coli* and *K. pneumoniae,* respectively. All the isolates of *C. freundii, E. aerogenes* and *P. mirabilis* were observed to be Cefoperazone+sulbactam susceptible whereas *S. maltophilia* exhibited complete resistance.

Discussion

In the face 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. The present study investigated the susceptibility profile of commonly used drugs including cefoperazone+sulbactam, piperacillin/tazobactam and meropenem and a novel antibiotic-adjuvant entity, Ceftriaxone+Sulbactam+EDTA in a tertiary care transplant hospital, Mumbai against Gram negative pathogens isolated from clinical samples. 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 causing bacterial infections [11].

Kumar *et al*., [12] has reported *E. coli* as the most predominant pathogen isolated from the 1180 clinical specimens suspected of bacterial infections. Antimicrobial resistance is an important concern for the public health authorities at global level. However, in developing countries like India, recent hospital and some community based data showed increase in burden of antimicrobial resistance. Research related to antimicrobial use, determinants and development of antimicrobial resistance, regional variation and interventional strategies according to the existing health care situation in each country is a big challenge. This paper discusses the situational analysis of antimicrobial resistance with respect to its problem, determinants and challenges ahead with strategies required in future to reduce the burden in India. Recent data from Google search, Medline and other sources were collected which was reviewed and analyzed by the authors. Hospital based studies showed higher and varied spectrum of resistance in different regions while there are limited number of community based studies at country level. There exists lacunae in the structure and functioning of public health care delivery system with regard to quantification of the problem and various determining factors related to antimicrobial resistance. There is an urgent need to develop and strengthen antimicrobial policy, standard treatment guidelines, national plan for containment

of AMR and research related to public health aspects of AMR at community and hospital level in India [8] [12]. Sachdeva [13] have also reported 51.7 % occurrence of *E. coli*. *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 sensitivity 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 Ceftriaxone+Sulbactam+EDTA 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].

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.*

196 *baumannii* and 9 isolates of *P. aeruginosa* were resistant to meropenem. A high rate of
197 meropenem resistance has been reported by other authors as well. Goyal et al. [17] have
198 shown that 6.4% and 6.3% of *A. baumannii* isolates were susceptible to doripenem and
199 meropenem in their study. Same study has reported that *P. aeruginosa* showed sensitivity of
200 60.3% for doripenem and 44.8% for meropenem. Similarly, Vraiya *et al.* [18] have reported
201 26% isolates as carbapenem resistant of the total 230 *P. aeruginosa* isolates tested for
202 susceptibility. Compared to our results, Arora et al. [19] have reported higher Meropenem
203 resistance of 73.1% in *Klebsiella spp.* and 23.8% in *E. coli*. Similar to our pattern, Wattal *et*
204 *al.* [20] have reported 31-51% Carbapenem-resistance in *Klebsiella spp.* and 2-13% in *E. coli*
205 in Delhi. A Carbapenem resistance of 14.6% in *E. coli* and 29.6% in *Klebsiella spp.* in
206 hospital isolates has been reported by Chauhan K *et al*. [20].

207 *E. coli and K. pneumoniae* exhibited 30-53% resistance rates against
208 Piperacillin+tazobactam and Cefoperazone+Sulbactam whereas the resistance rates by *P.*
209 *aeruginosa*, *A. baumannii, C. freundii* and *S. maltophilia* varied from 78% to 100%. High
210 resistance of Gram-negative pathogens to beta-lactam/BLIs has been consistently reported by
211 earlier studies and this could be possibly due to exponential rise in ESBL and MBL
212 producing strains globally [21] [22]. The AMR surveillance study conducted in India has
213 shown resistance against Pip-Taz has risen to 65-70%.

214 Results from the SENTRY Antimicrobial Surveillance Program, 2009–2012 has
215 shown that 69% of ESBL-producing *E. coli* isolates from patients with pneumonia were
216 found susceptible to Pip–Taz in vitro whereas only 26.9% of ESBL-producing *Klebsiella* spp.
217 isolates were susceptible to Pip–Taz [23]. Comparison of *in vitro* activities of ceftazidime,
218 piperacillin-tazobactam and cefoperazone-sulbactam in a retrospective study conducted at a
219 tertiary care cancer hospital in Mumbai has shown that for all bacterial isolates,
220 cefoperazone-sulbactam was sensitive against 58.3% isolates and piperacillin-tazobactam

against 48.1% [24]. The sensitivity pattern for the Enterobacteriacea group revealed that 67.9% of isolates were sensitive to Cefoperazone-Sulbactam and 45.4% to Piperacillin-Tazobactam [24]. Among the non-lactose fermenters, 52.5% isolates were sensitive to cefoperazone-sulbactam and 49.6% to piperacillin-tazobactam. For the *Pseudomonas* species, Piperacillin-tazobactam was sensitive against 58.4% and cefoperazone-sulbactam against 57.4% isolates.

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.

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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

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

318

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	Blood	62	25 (12.3)	37
3	Pus	22	19 (9.3)	3
4	Tissue	21	12 (5.9)	9
5	Collections	27	15 (7.3)	12
6	Sputum	23	15 (7.3)	8
7	ET Secretions	17	10 (5)	7
8	Body Fluids	21	13 (6.4)	8
9	Others	14	4 (1.9)	10
TOTAL (% of Total)		362	203 (56.1%)	159 (43.9%)

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

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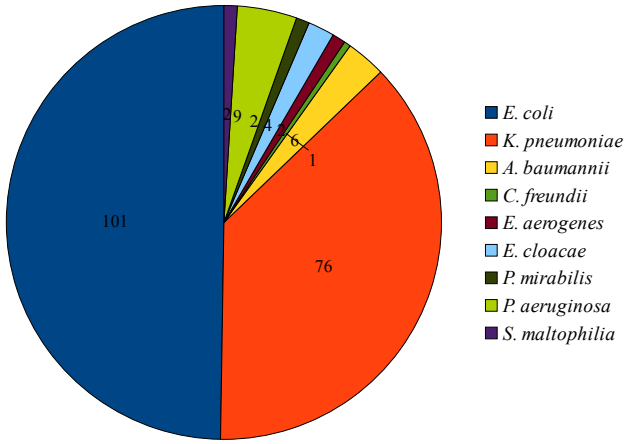
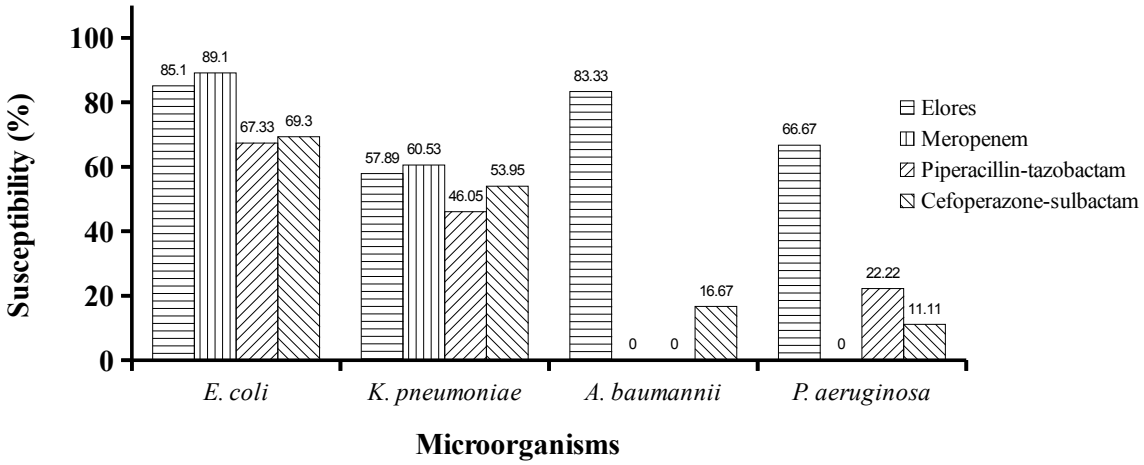


329 **Fig-2: Susceptibility profile of *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* to**

330 **Ceftriaxone+Sulbactam+EDTA.**

331



332

333 **[Fig-3]: Susceptibility profile of *C. freundii, E. aerogenes, E. cloacae, P. mirabilis and S.***

334 ***maltophilia* to Ceftriaxone+Sulbactam+EDTA.**

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