

Trends in Antibiotic Resistance among Major Bacterial Pathogens Isolated from Different Specimens at a Tertiary Care Hospital in India

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Original Research Article

ABSTRACT

Introduction and Aim: The know-how of local antibiograms is very important in the light of continuous changes in resistance patterns in different geographical niches. This retrospective observational study assessed the in vitro susceptibility pattern of pathogen isolates from different clinical samples to commonly used antibiotics including Potentox (Amikacin+Cefepime+L-arginine) in our hospital settings.

Methodology: A total of 320 isolates obtained from 1372 respiratory and urine clinical specimens from patients treated for bacterial infections during Jan. 2017 to Dec. 2017 were included in the study.

Results: Of the total 320 isolates, 75% were from urine and 25% from blood. *E. coli* (48.8%) was the most frequently isolated pathogen followed by *K. pneumoniae* (22.7%), *P. aeruginosa* (10.2%) and *A. baumannii* (10.2%). Meropenem and Potentox were the most active agents against all the isolates exhibiting 86.9% and 87.5% susceptibility rates respectively. The susceptibility to Imipenem was similar to Tigecycline (81.9%) and Amikacin was similar to Cefoperazone-Sulbactam. The

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lowest susceptibility rate was reported to Cefepime (55.3%) followed by Pip-taz (62.5%). The susceptibility to Colistin was 94.0%. Based on pathogen type, the highest susceptibility was reported towards *E. coli* and lowest towards *A. baumannii*. Susceptibility pattern for Amikacin and Cefepime resistant isolates were; Colistin (75.6%), Potentox (51.25%), Meropenem (50%) and Tigecycline (39%), with less than 30% susceptibility to Imipenem, Pip-taz and Cefoperazone-Sulbactam.

Conclusions: This antimicrobial susceptibility data suggest that Potentox can be considered as an important therapeutic option for the treatment of various gram-negative bacterial infections and in cases which are both Amikacin and Cefepime resistant to avoid excess consumption of carbapenems and where Tigecycline is not feasible.

Keywords: Meropenem; potentox; polymyxins; susceptibility.

1. INTRODUCTION

Bacterial infections are one of the leading killers worldwide, and the rising microbial resistance continues to hinder the effective control of this pandemic health problem. Currently, most of the existing antibiotics are increasingly becoming redundant for an increasing number of bacterial species and the number of new antibiotics being developed is unable to keep up with the rate of resistance [1][2]. One of the effective therapeutic strategies to enhance the effectiveness of some antibiotics is the weakening of cell envelope and increasing cellular permeability[3][4]. This kind of mechanism is exploited by compounds that target the bacterial cell membrane or cell wall and has been used in the formulation of Potentox. Potentox is a combination of one of the aminoglycosides, Amikacin and a beta-lactam antibiotic, Cefepime and an antibiotic adjuvant entity, L-arginine. The beta-lactams inhibit cell wall synthesis which facilitates the passage of aminoglycosides into the periplasmic space of the microbes which freezes the 30S initiation complex (30S-mRNA-tRNA) by binding irreversibly to the 30S ribosome, and inhibiting the further process. L-arginine increases the antioxidant capacity thereby decreasing the aminoglycoside induced nephrotoxicity and hepatotoxicity and also enhances antibiotic susceptibility under anaerobic conditions, presumably by increasing the metabolic rate [5][6]. The pharmacokinetic interactions between beta-lactam and aminoglycoside antibiotics have been studied extensively. Documented studies report that concurrent administration of Cefepime and Amikacin does not alter the pharmacokinetics of either antibiotic [7][8]. The pharmacokinetic properties of Cefepime before, during, and Amikacin co-administration and those of Amikacin before, during, and after cefepime co-administration are reported to be in excellent agreement with the previously reported

pharmacokinetic properties of these drugs individually.

Combination antibiotic therapy, primarily used to broaden the antimicrobial spectrum is frequently used to treat severe Gram-negative infections. Potential benefits of combination therapy compared to monotherapy include synergistic mechanism of action, wider anti-bacterial spectrum and minimized risk of emerging resistance during therapy [3]. In the light of limited evidence-based treatment options, combination therapies are being increasingly employed for enhanced anti-bacterial effects against multidrug-resistant strains. In the present study, we evaluated the antibiotic susceptibility of Gram-negative clinical isolates to commonly used antibiotics with special emphasis on Potentox.

2. MATERIALS AND METHODS

2.1 Sample Collection

This retrospective study was carried on bacterial isolates from clinical samples of patients suspected of bacterial infections from Jan 2017 to Dec. 2018. The clinical samples used for pathogen isolation were urine and respiratory specimens. Respiratory samples included sputum, endo-tracheal secretions, TT secretions and BAL. The sample collection and processing were done as per standard microbiology laboratory operating procedures.

2.2 Pathogen Isolation and Identification

Identification of pathogen isolates was done based on colony morphology, motility, Gram-staining and different biochemical reactions using standard techniques. The required clinical samples were collected in sufficient amount in an aseptic manner in sterile containers. The inoculums of collected specimens were inoculated or streaked on different selective and

non-selective culture media as per the standard microbiological procedures.

2.3 Antibiotic Susceptibility Testing

Anti-microbial susceptibility was carried out using disc diffusion methods as per Clinical Laboratory Standard Institute (CLSI) guidelines. Amikacin, Cefepime, Potentox, Cefoperazone-Sulbactam, Piperacillin/tazobactam, Tigecycline, Imipenem and Meropenem discs were procured from Himedia Laboratories, Pvt. Ltd, Mumbai, India and used in the study. The sensitivity of Colistin was done using MIC method. Zones of inhibition were measured next day after 24 hours of incubation and were correlated with CLSI interpretive breakpoints to characterize them as sensitive, intermediate, and resistant.

3. RESULTS

3.1 Sample Collection and Pathogen Isolation

Out of the total 1372 samples, respiratory (579) and urine samples (793) were obtained from patients suffering from different infections and were processed for pathogen isolation. Of the total samples analyzed, the Gram-negative isolates were obtained from 25.7% (n=352) samples, Gram-positive from 8.9% (n=122) and 65.5% (n=898) displayed no growth at all. The demographic and baseline characteristics of all study subjects are presented in Table 1. For other details and demographic characteristics, refer to Table 1.

3.2 Microbiological Characteristics

The major identified bacteria include *E. coli* (48.8%), *K. pneumoniae* (22.7%), *P. aeruginosa*

(10.2%) and *A. baumannii* (10.2%). The other pathogens contributed just 9% to the total pool and were not included in the study. The distribution pattern of various organisms isolated in clinical samples is shown in Table 1.

Antibiogram profile for all Gram-negative isolates included in the study is shown in Table 2 and Fig. 1. The lowest sensitivity rate of 55.3% was reported to Cefepime followed by Pip-taz (62.5%) and the highest to Colistin (94.0%). 3/4th of the isolates were reported susceptible to Amikacin and Cefoperazone-Sulbactam. Among Carbapenems, the susceptibility rates were slightly higher for Meropenem (86.9%) compared to Imipenem (81.2%) and similar to Potentox (87.5%). The susceptibility to Tigecycline (81.9%) was similar to Imipenem.

Based on pathogen type, the highest susceptibility was reported towards *E. coli* and lowest towards *A. baumannii*. In conformity to the overall susceptibility pattern, *E. coli* exhibited lowest susceptibility to Cefepime (61.0%). 90% of *E. coli* isolates were reported susceptible to Tigecycline, Imipenem, Meropenem and Colistin. Among *K. pneumoniae* isolates, almost 60% of the isolates were reported susceptible towards Cefepime (58.8%) and Pip-Taz, whereas susceptibility reported towards Potentox, Tigecycline and Imipenem was almost similar and equivalent to 80%. Around 90-94% of *K. pneumoniae* isolates were susceptible to Meropenem and Colistin.

P. aeruginosa isolates were reported to have lowest susceptibility to Tigecycline (30.6%). Almost half of the isolates were susceptible to Amikacin and Cefepime. The susceptibility towards Cefoperazone-Sulbactam was similar to Imipenem (69.4%). The susceptibility towards Potentox was 88.9% and Colistin was 91.7%.

Table 1. Demographic and baseline characteristics of all study subjects (n=320)

Characteristics		Clinical sample		
		Total (n=320)	Urine (n=240)	Respiratory (n=80)
Gender	Male, n (%)	180 (56.3)	126	54
	Female, n (%)	140 (43.7)	114	26
Age (year)	Mean±SD		53.8±21.74	51.3±25.44
Causative pathogen				
	<i>E. coli</i> n (%)	172	150 (62.5)	22 (27.5)
	<i>K. pneumoniae</i> n (%)	80	64 (26.7)	16 (20.0)
	<i>P. aeruginosa</i> n (%)	36	20 (8.3)	16 (20.0)
	<i>Acinetobacter spp.</i> n (%)	32	6 (2.5)	26 (32.5)

Table 2. *In vitro* Susceptibility profile of various antibiotics tested

Antibiotic (Susceptibility)	<i>E. coli</i> (n=172) n (%)	<i>K. pneumoniae</i> (n=80) n (%)	<i>P. aeruginosa</i> (n=36) n (%)	<i>A. baumannii</i> (n=32) n (%)
Amikacin (S)	152 (88.4)	56 (70)	19 (52.8)	11 (34.4)
Cefepime (S)	105 (61.0)	47 (58.8)	20 (55.6)	5 (15.6)
Potentox (S)	159 (92.4)	66 (82.5)	32 (88.9)	23 (71.9)
Cefoperazone-Sulbactam (S)	142 (82.6)	52 (65)	25 (69.4)	22 (68.8)
Pip-Taz (S)	124 (72.1)	48 (60)	21 (58.3)	7 (21.9)
Tigecyclin (S)	163 (94.8)	62 (77.5)	11 (30.6)	26 (81.3)
Imipenem (S)	159 (92.4)	66 (82.5)	25 (69.4)	10 (31.3)
Meropenem (S)	163 (94.8)	72 (90)	29 (80.6)	14 (43.8)
Colistin (S)	164 (95.3)	75 (93.8)	33 (91.7)	29 (90.6)

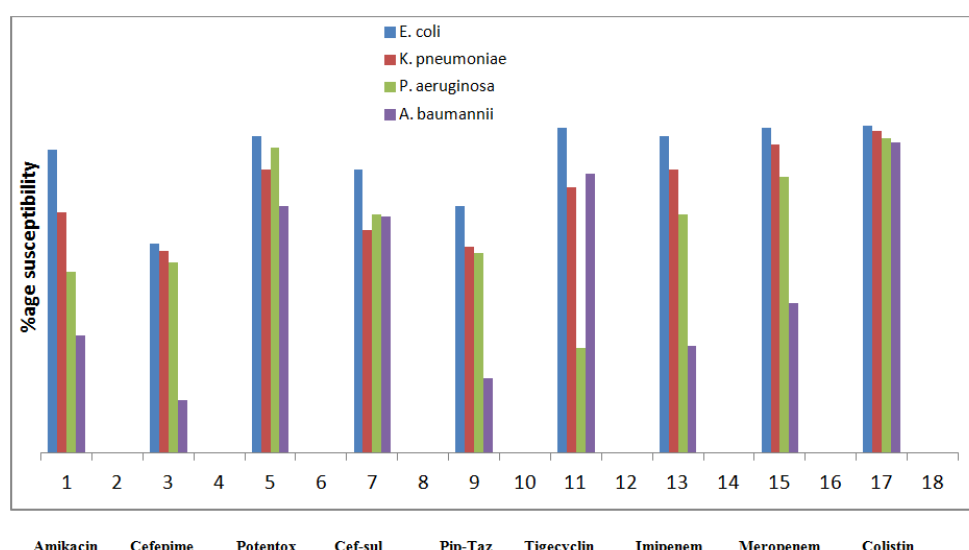


Fig. 1. Bar graph showing *In vitro* Susceptibility of various antibiotics tested

Table 3. *In vitro* Susceptibility profile of Amikacin-resistant, Cefepime-resistant and Amikacin+Cefepime resistant isolates to various antibiotics tested

Antibiotic (sensitivity)	Amikacin resistant (n=82) n (%)	Cefepime resistant (n=147) n (%)	Amikacin+Cefepime resistant (n=82) n (%)
Amikacin (S)	-	82 (55.7)	-
Cefepime (S)	-	-	-
Potentox (S)	42 (51.2)	107 (72.8)	42 (51.2)
Cefoperazone-Sulbactam (S)	17 (20.7)	67 (68.0)	17 (20.7)
Pip-Taz (S)	3 (3.7)	27 (18.4)	3 (3.7)
Tigecyclin (S)	32 (39.0)	94 (63.9)	32 (39.0)
Imipenem (S)	23 (28.0)	87 (59.2)	23 (28.0)
Meropenem (S)	41 (50.0)	105 (71.4)	41 (50.0)
Colistin (S)	62 (75.6)	42 (28.6)	64 (75.6)

Next to Cefepime (15.9%), the *A. baumannii* isolates reported lowest susceptibility towards Carbapenems (Imipenem; 31.3%, Meropenem;

43.8%) and Amikacin (34.4%). The susceptibility towards Potentox (71.9%) was almost similar to Cefoperazone-Sulbactam (68.8%). 81.3% of *A.*

baumannii were susceptible to Tigecycline and 90.6% to Colistin.

Further analysis of susceptibility pattern has shown that all 82 Amikacin resistant strains were Cefepime-resistant. However, out of 147 Cefepime resistant isolates, only 65 were resistant to Amikacin. The susceptibility of Amikacin resistant and Cefepime resistant isolates to different antibiotics in descending order were Colistin (75.6%), Potentox (51.25%), Meropenem (50%) and Tigecycline (39%). The susceptibility to Imipenem, Pip-Taz and Cefoperazone-Sulbactam was less than 30%.

4. DISCUSSION

Multidrug-resistant Gram-negative bacteria resistant to multiple antibiotics have been identified as a major challenge for patient management and public health. Though multiple guidelines are available for the management of bacterial infections, however microbial surveillance studies at small and large scale levels are important as the predominant pathogens and their sensitivity pattern varies in different geographical locales. Thus, publishing anti-microbial resistance (AMR) surveillance studies is very important from clinician's point of view as it can help to choose the right empiric therapy. The current study provided insights in the antimicrobial susceptibility pattern of commonly used drugs in a tertiary care hospital in India.

In this study, it was observed that urinary pathogens were the most prevalent isolates accounting for around 65.8% of the total number. In concordance with the pattern in Asian countries particularly India where more incidences of Gram-negative pathogens are reported, Gram-negative pathogens were isolated from maximum number of clinical samples processed. Multiple studies conducted all across India have reported Gram-negative pathogens as the majorly isolated pathogens. 76.4% isolates have been reported as Gram negative in a retrospective anti-microbial susceptibility study conducted at a tertiary care centre in Delhi, India [9]. Similarly, Karanwal *et al.* [10] and Prabhash *et al.* [11] have also reported 78% and 68.1% of the total isolates as Gram-negative isolates.

The anti-microbial susceptibility results have shown that the susceptibility to cefepime was lowest among all the drugs. Cefepime is a fourth

generation cephalosporin used highly for the treatment of Gram-negative infections. The rising rate of ESBL producing pathogens could be one of the reasons for low sensitivity to Cefepime. In concordance to our studies, various studies have reported the growing resistance of Cefepime all over India. Among BL/BLI combinations, a high rate of resistance was observed towards Pip-Taz. The indiscriminate consumption of Pip-Taz could be one of the vital reasons for the high AMR reported towards the normally recommended second line of treatment in our hospital. The AMR surveillance study conducted in India has shown resistance against Pip-Taz has risen to 65-70 %. Among Carbapenems, the susceptibility rates for Meropenem (86.9%) were higher compared to Imipenem (81.2%) and similar to Potentox (87.5%). The emergence of carbapenem-resistant strains, which ranges from 13-19% in this study is a matter of big concern as carbapenems are considered as the last resort drugs for ESBL strains. Similar to our observations, Singh *et al.* [9] have also reported 15-22% of gram-negative isolates as MBLs in their study. Comparatively, a higher incidence of carbapenem-resistant Gram-negative bacteria has also been reported by Ghosh *et al.* [12] from AIIMS, Delhi. As resistance to carbapenems is increasing, and because of a lack of new antibiotics, it becomes imperative to explore the potential of combination therapy to enhance the anti-bacterial effects of available drugs. Interestingly, the number of isolates sensitive to Potentox were equivalent to meropenem. Moreover, 50% of the isolates which were both Amikacin and Cefepime resistant were reported Meropenem and Potentox susceptible. The results from various published *in vitro* studies have shown that antibiotic combinations demonstrate synergistic or bactericidal effects against bacteria that are resistant to the individual drugs [13]. Combination therapy provides broad-spectrum coverage, bactericidal activity and potential synergic effects, and minimises the development of resistance during treatment. Though, the susceptibility reported to Tigecycline was comparatively good, it is usually not considered as a drug of choice by many physicians for treatment of bloodstream or urinary tract infections because of its inability to maintain the minimum Serum and urinary MIC levels required for its anti-bacterial activity. Moreover, a higher mortality rate in patients treated with Tigecycline than with comparator agents has been reported in meta-analyses of various clinical trials [3][14][15].

5. CONCLUSION

Overall, this antimicrobial susceptibility data suggest that Potentox can be considered as an important therapeutic option for the treatment of various Gram-negative bacterial infections to avoid excess consumption of carbapenems and in situations where Tigecycline is not feasible. Moreover, Potentox can serve as an effective alternative to Meropenem in cases which are both Amikacin and Cefepime resistant.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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