In vitro Susceptibility Pattern of Major Gram Negative Isolates to Selected Antimicrobial Agents

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Authors' contributions

This work was carried out in collaboration between both the authors. Author SG designed the study, wrote the protocol and performed the experiments. Author PM wrote the first draft of the manuscript and managed the literature searches. Both authors managed the analysis of the study, read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

Background: The choice of choosing right anti-microbial therapy in hospitals depends on the knowledge of local anti-microbial susceptibility profile. This retrospective study was conducted to assess the in vitro susceptibility pattern of different pathogen isolates to various antibiotics including Cefepime-Amikacin-Antibiotic resistant breakers (ARBs)* in various hospitals across the Jaipur City.
Methods: To characterize the antimicrobial susceptibility pattern of different isolates from various hospitals across the Jaipur City, a retrospective, observational analysis was done for antibiogram data. A total of 1201 Gram negative isolates collected during the period from January 2017 to December 2017 were included in the study. Antibiotic sensitivity testing was done in accordance with the recommendations of Clinical Laboratory Standard Institute (CLSI) guidelines.
Results: Of the total 1201 Gram negative isolates included in this study, 51.6% were from wounds and pus specimens, 40.1% were from respiratory and 8.2% from blood. *P. aeruginosa* (49.7%) was the most frequently isolated pathogen distantly followed by *A. baumannii* (21.6%), *K. pneumoniae* (16.6%) and *E. coli* (12.1%). The highest susceptibility was reported to polymyxins (100%)

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including Colistin and Polymyxin B, among all the tested bacteria's and system wise. Among all the antibiotic tested, (Cefepime-Amikacin-ARBs*) sensitivity ranged for 87.9% to 52% on pathogens (*E. coli, K. pneumonia, P. aeruginosa*) tested from samples of skin and soft tissue, respiratory tract, blood stream, followed by Meropenem ranged for 78.4% to 55% on pathogens (*E. coli, K. pneumonia, P. aeruginosa*), followed by ceftazidime-tazobactam ranged for 82.7% to 58% on pathogens (*E. coli, K. pneumonia, P. aeruginosa*) and 22.7% sensitive for *A. baumannii* to Cefoperazone sulbactam. Based on pathogen type, *E. coli* exhibited highest overall susceptibility and the lowest was reported by *A. baumannii.* The susceptibility of *A. baumannii* ranged from 1-26% to all the tested antibiotics except polymyxins with 100% susceptibility.

Conclusions: This *in vitro* susceptibility data suggests that Cefepime-Amikacin-ARBs* can serve as important therapeutic option for the treatment of various resistant Gram-negative bacterial infections to relieve the excess pressure on last resort antibiotics, carbapenems and other drugs including Colistin and polymyxin B. Cefepime-Amikacin-ARBs*on the basis of antimicrobial susceptibility data can be considered as an effective therapeutic option for carbapenems in treating gram negative bacterial infections, and could be considered as a broad spectrum antibiotic sparer's like carbapenem, colistin and Polymyxin B.

1. INTRODUCTION

Other than limiting treatment choices, the rising anti-microbial resistance is associated with increased mortality and morbidity rates, and higher hospital and antibiotic costs [1,2]. One of the main factors that complicate the treatment of drug resistant infections and result in poor treatment outcomes is delayed effective empiric therapy [3,4]. Empiric use of combination antibiotic therapy in drug resistant serious infections has been shown to significantly reduce the associated morbidity and mortality, particularly in Gram-negative infections [5,6]. Compared to monotherapy, the potential benefits of combination therapy include synergistic mechanism of action, wider anti-bacterial spectrum and minimized risk of emerging resistance during therapy [7,8].

In the light of limited therapeutic options for drug resistant infections, combination therapies of one or more antibiotics along with Antibiotic resistant breakers are being increasingly employed for enhanced anti-bacterial effects against multidrug-resistant strains [5,6]. The use of broad-spectrum beta-lactam antibiotic in combination with aminoglycosides is in use for many years as empirical therapy for multi-drug resistant (MDR) nosocomial infections, because of the spectrum of activity against vast array of pathogens. Cefepime, a fourth-generation cephalosporin antibiotic, has lately replaced Ceftazidime as it has similar in vitro activity to that of Ceftazidime against *P. aeruginosa*, and a better activity than Ceftazidime against Gram-positive cocci and Enterobacteriaceae. Hence, Cefepime offers an alternative therapeutic option for empirical treatment, as a component of combination therapy. Cefepime-Amikacin-ARBs* (* stands for L-arginine as an antibiotic resistance breaker), a combination of Cefepime, Amikacin, and antibiotic adjuvant entities, L-arginine and chelating agent and antioxidants serves as an effective therapeutic strategy as Cefepime increases the effectiveness of Amikacin by weakening cell envelope and increasing cellular permeability. L-arginine through its anti-oxidant capacity, decreases the aminoglycoside induced nephrotoxicity and hepatotoxicity and enhances antibiotic efficacy under anaerobic conditions [9,10]. The chelating component interferes with the functioning of efflux pump, spread of resistance genes through conjugation and formation of extra-polymeric substance (EPS) layer of biofilms. The non-antibiotic adjuvant has an added advantage of providing boost to antioxidant defense of the host and thus preventing aminoglycoside induced tissue related injuries including nephrotoxicity and hepatotoxicity. In this study, the susceptibility profile of Gram-negative clinical isolates to different antibiotics including Cefepime-Amikacin-ARBs* were analyzed.

2. MATERIALS AND METHODS

2.1 Sample Collection

This study was carried on the susceptibility record of bacterial isolates from clinical specimens of patients suffering from respiratory tract infections, surgical site infections and blood stream Infections from Jan. 2017 to Dec. 2017.

Various samples included were blood, sputum, endo-tracheal secretions, tracheal secretions, BAL; pus and wound swabs from patients with bedsores, diabetic foot ulcer, traumatic and bed ridden patients. The sample collection and processing procedures were as per standard microbiology laboratory operations.

2.2 Pathogen Isolation and Identification

Colony morphology, motility, Gram-staining and different biochemical reactions were used to identify the pathogen isolates using standard techniques. The inoculums of required clinical specimens collected in sufficient amount were inoculated or streaked on desired culture media as per the standard microbiological procedures.

The clinical isolates antimicrobial susceptibility study was performed on theses isolates by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines. In brief, inoculum of 0.5 McFarland standards turbidity was prepared in saline from isolated colony of pathogens selected from 18-24 hour agar plates. A sterile cotton swab was dipped into the inoculum and streaked three times on the dried surface of a Mueller-Hinton agar (MHA) plate. After 5 minutes, antibiotic discs were applied and pressed down to check absolute contact with agar surface. The discs were apportioned in a minimum distance of 24 mm from the centre. The plates were then incubated for 16-18 hrs aerobically at 37°C. Sensitivity of isolated organisms against antibiotics was reported as sensitive (S) or resistant (R) based on the breakpoints.

2.3 Antibiotic Susceptibility Testing

Disc diffusion method was used to carry out the anti-microbial susceptibility testing. Discs of Amikacin, Cefepime, Cefepime-Amikacin-ARBs*, Cefoperazone-Sulbactam, Ceftazidime-Tazobactam, Piperacillin- Tazobactam, Tigecycline, Imipenem, Colistin, Polymyxin B and Meropenem discs were used to test the susceptibility of these antibiotics. The Zones of inhibition were measured the next day and were correlated with CLSI interpretive breakpoints to characterize them as sensitive, intermediate, and resistant.

3. RESULTS

3.1 Sample Collection, Pathogen Isolation and Microbiological Distribution

Of the total 1250 Gram-negative isolates analyzed, 1201 isolates were of the majorly identified species including *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* and 49 isolates of the other species. These Gram-negative isolates were obtained from 620 patients suffering from skin and soft tissue infections (SSTIs), 482 from patients suffering from respiratory infections, and 99 were from patients suffering from blood stream infections.

The percentage of major identified bacteria included *P. aeruginosa* (49.7%), *A. baumannii* (21.6%), *K. pneumoniae* (16.6%) and *E. coli* (12.1%).

3.2 Microbiology Test Results

The susceptibility profile of all Gram-negative isolates is shown in Table 1. The highest susceptibility of 100% irrespective of clinical specimen type was reported to polymyxins including Colistin and Polymyxin B, and lowest to Ceftazidime-Tazobactam. Next to polymyxins, the highest susceptibility was reported to Cefepime-Amikacin-ARBs*, (61.3%). The overall susceptibility to Cefoperazone-Sulbactam (22.6%) was almost similar to Cefepime (24.8%), and Piperacillin-Tazobactam (40.5%) was comparatively higher than Amikacin (33.4%). Among carbapenems, the susceptibility to Imipenem (48.2%) was slightly higher than Meropenem (45.0%).

Based on the type of clinical specimen, the pattern exhibited was little different from the aggregate pattern. The susceptibility of the isolates from blood were lowest to all the drugs except Colistin (100%), Polymyxin B (100%) and Cefepime-Amikacin-ARBs* (74.7%) compared to isolates from other specimens. The susceptibility of blood isolates to all other antibiotics ranged between lowest of 5% to Cefepime and highest of 30% to Meropenem. The susceptibility pattern among the isolates from respiratory specimens were Colistin (100%), Polymyxin B (100%), Cefepime-Amikacin-ARBs (50%). The susceptibility to BL-BLIs (Piperacillin-Tazobactam and Cefoperazone-Sulbactam) was 33-34% and carbapenems was 38-41%. Among isolates from SSTI specimens also, other than

Table 1. Pathogen wise *in vitro* susceptibility profile of various antibiotics tested

	Antibiotic (Susceptibility)	*E. coli* (n=145)		*K. pneumoniae* (n=199)			*P. aeruginosa* (n=597)		*A. baumannii* (n=260)	
		SSTIs (145)	RTI	SSTIs (99)	RTI (79)	BSIs (21)	SSTIs (350)	RTI (247)	SSTIs (104)	RTI (156)
Aminoglycosides	Amikacin (S)	102 (70.6%)	-	15 (15%)	27 (34%)	10 (47.8%)	125 (35.8%)	114 (46.0%)	5 (4.8%)	3 (2.0%)
Cephalosporins	Cefepime (S)	30 (20.8%)	-	5 (5%)	6 (7.6%)	4 (19.3%)	142 (40.7%)	109 (44.0%)	-	2 (1.0%)
AAE	Cefepime-Amikacin-ARBs* (S)	128 (87.9%)	-	74 (75%)	41 (52%)	13 (62.5%)	264 (75.4%)	170 (69.0%)	16 (15.8%)	30 (19.0%)
BL-BLIs	Ceftazidime-Tazobactam (S)	120 (82.7%)	-	-	21 (26%)	-	-	143 (58.0%)	-	2 (1.0%)
	Cefoperazone-Sulbactam	102 (70.6%)	-	15 (15%)	-	8 (40.4%)	122 (34.9%)	-	24 (22.7%)	-
	Pip-Taz (S)	102 (70.6)	-	20 (20%)	22 (28%)	9 (42.1%)	185 (52.9%)	136 (55.0%)	10 (9.1%)	2 (1.0%)
Carbapenems	Imipenem (S)	112 (77.5%)	-	25 (25%)	29 (37%)	11 (52.6%)	219 (62.6%)	153 (62.0%)	14 (13.6%)	16 (10.0%)
	Meropenem (S)	114 (78.4%)	-	30 (30%)	28 (36%)	11 (54.4%)	182 (52.0%)	136 (55.0%)	19 (18.2%)	20 (13.0%)
Polymyxins	Colistin (S)	145 (100%)	-	99 (100%)	79 (100%)	21 (100%)	350 (100%)	247 (100%)	104 (100%)	156 (100%)
	Polymyxin B (S)	145 (100%)	-	99 (100%)	79 (100%)	21 (100%)	350 (100%)	247 (100%)	104 (100%)	156 (100%)

Polymyxins, a very good susceptibility was reported to Cefepime-Amikacin-ARBs* (67.9%) followed by carbapenems (Imipenem: 57.4% and Meropenem: 52.7%).

Further based on pathogen type, *E. coli* exhibited highest overall susceptibility to all clinical pathogens and the lowest was reported against *A. baumannii. Escherichia. coli* exhibited lowest susceptibility to Cefepime (20.8%). The susceptibility rates to Amikacin, Cefoperazone-sulbactam and Piperacillin-Tazobactam were similar and equivalent to 70%. 77-79% of isolates were susceptible to carbapenems and 88% were susceptible to Cefepime-Amikacin-ARBs*. The susceptibility pattern of *P. aeruginosa* was in conformity to the overall pattern of all the isolates with highest susceptibility to Polymyxins (100%) followed by Cefepime-Amikacin-ARBs (72.7%) and Carbapenems (53.3-62.3%). The susceptibility pattern of *K. pneumoniae* isolates was also similar with 100% susceptibility to polymyxins followed by second highest susceptibility of 64.3% to Cefepime-Amikacin-ARBs. The susceptibility to Cefepime, BL-BLIs and carbapenems ranged from 7.5% to 35%. The susceptibility of *A. baumannii* was lowest among all the isolates ranging from 1-26% to all the tested antibiotics except polymyxins which showed 100% susceptibility.

4. DISCUSSION

Bacterial infections are caused by different organisms and the infecting organisms vary depending on the healthcare settings, geographical locales, the patient populations involved, and the antimicrobials received. The commonly reported gram-negative organisms include *K. pneumoniae, P. aeruginosa, A. baumannii,* and *E. coli* [11,12]. In concordance, we also reported the similar kind of pattern with most predominant pathogen as *P. aeruginosa* (49.7%), *A. baumannii* (21.6%), *K. pneumoniae* (16.6%) and *E. coli* (12.1%). Overall, it was observed that our incidence pattern was little different from the pattern reported all over country as SSTI's were most prevalent among all specimens accounting for 51.6% followed by respiratory specimens (40.1%) and blood specimens (8.24%). Mythri et al. [13] have reported urinary tract infections (34.8%) as the most frequent infection followed by pneumonia (21.7%), skin and sub-cutaneous infections (SSIs) (17.4%) and blood stream infections (13.0%). As Cefepime-Amikacin-ARBs* is mainly prescribed in bone and

joints infections, SSTIs, accidental cases, hospitalized patients with Ventilator-associated pneumonia (VAP), Hospital-acquired pneumonia (HAP), sepsis and intra-abdominal infections, thus this deviation in our pattern could be because of the inclusion criteria of patients tested for Cefepime-Amikacin-ARBs* in our study.

The anti-microbial susceptibility for the tested pathogens has shown a worrisome scenario for most antibiotics tested. The indiscriminate prescription of BL-BLIs as the 1st line of treatment in hospitals across India could be one of the reasons for the high anti-microbial resistance (AMR) reported towards them [14]. In support of our observations, the AMR surveillance study conducted in India has shown 65-70% resistance against Piperacillin-Tazobactam [15]. The most worrisome observation in this study is the growing carbapenem resistance. Almost half of the isolates were reported as carbapenem resistant. Carbapenem resistance rate ranging from 2% to 80% in *E. coli*, *Klebsiella* spp., Pseudomonas spp. and Acinetobacter spp. has been reported by Wattal et al. [16] in a tertiary care hospital in Delhi. A varying resistance rate of 17 to 22% among Enterobacteriaceae has been reported by Gupta et al. [17] whereas Datta et al. [18] have reported Carbapenem resistance of 7.87% among Enterobacteriaceae strains. Similar to our observations, a higher incidence of carbapenem-resistant Gram-negative bacteria has also been reported by Ghosh et al. [19] from AIIMS, Delhi. Comparatively, Singh et al. [20] have also reported 15-22% of Gram-negative isolates as MBLs in their study. Interestingly, the number of isolates sensitive to Cefepime-Amikacin-ARBs* were more than Meropenem. In a previous study, the susceptibility rate of Cefepime-Amikacin-ARBs* has been reported as 89.9%, 86.6%, 88% and 89% to *E. coli*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* respectively, which was high compared to other tested drugs [21]. Time kill curve analysis has shown that the MIC of Cefepime-Amikacin-ARBs* was 1.0 mg/l, 0.5 mg/l, 0.5 mg/l and 4.0 mg/l which was very low compared to 4.0 mg/l, 2.0 mg/l, 2.0 mg/l and 16.0 mg/l for Cefepime and 2.0 mg/l, 8.0 mg/l, 2.0 mg/l and 32.0 mg/l for Amikacin against *S. aureus*, *P. aeruginosa*, *E. cloacae* and *K. Pneumoniae* (Sanjay Mohan Srivastava, 2008). Similar, a study by Vijay and Manisha Kulkarni, on 320 isolates obtained from respiratory and urine samples, had highest isolation of *E. coli* (48.8%) followed by *K. pneumoniae* (22.7%), *P.*

aeruginosa (10.2%) and *A. baumannii* (10.2%) respectively and reported Cefepime-Amikacin-ARBs* susceptibility (87.5%) to be the highest as compared to Cefepime, Cefoperazone-sulbactam, Pip-taz, Meropenem, Tigecycline and Amikacin. Cefepime-Amikacin-ARBs* showed better susceptibility even in cefepime and amikacin resistant isolates [22].

Though almost 100% susceptibility was reported towards Colistin, however polymyxins are not the first line drug of choice among clinicians due to associated adverse effects. Neurotoxicity and nephrotoxicity are the two main adverse effects associated with colistin, and though the incidence of these adverse events does appear to be lower with moderate preparations, it is still substantial [23]. Moreover, the dosing issues in relation to renal clearance and in patients on renal replacement therapy are not completely clarified either. Moreover, in recent years there have been reports of outbreaks of Colistin-resistant infections worldwide [24,25]. As resistance to carbapenem is increasing, and because of lack of new class of antibiotic, it becomes imperative to explore the potential of combination therapy to enhance the anti-bacterial effect.

5. CONCLUSION

Overall, this *in vitro* surveillance data suggests that Cefepime-Amikacin-ARBs*, can be considered as an important therapeutic option for the treatment of various Gram-negative bacterial infections. Moreover, Cefepime-Amikacin-ARBs* can be considered as a Carbapenem sparing agent as it has shown better sensitivity as compared to other commonly used antibiotics including Carbapenems in resistant Gram-negative pathogens. The high rate of Carbapenem resistance observed is a topic of global concern and needs to be addressed at priority by stopping the irrational prescription of carbapenems and promoting various anti-microbial stewardship programs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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