ORIGINAL ARTICLE

Comparison of *in-vitro* Antibiotic Susceptibility of Ciprofloxacin, Cefotaxime, Ceftazidime and Cefepime against Gram Negative Bacilli Infections - A Study from Tertiary Care Centre

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ABSTRACT

Introduction: Infections from gram negative bacilli is a challenge for clinicians and laboratory personnel. Treatment of these infections remained as an area of concern. Both fluroquinolones and cephalosporins are most common choice of antibiotics. Despite Cephalosporins, being drug of choice they are expensive also showed many adverse reactions. This study, compares and reevaluates the susceptibility of gram negative bacteria to fluroquinolones (ciprofloxacin) compared to cephalosporins. **Method:** Various samples(pus, sputum, urine, blood and bodyfluids) were processed according to standard protocols. Antibiotic done susceptibility by using Kirby-baur disc diffusion method. ESBL and Amp C producers were identified using CLSI guidelines. **Result:** Among 400 isolates, majority were from pus followed by urine, sputum. The most common organism isolated was *Klebsiella spp*, (33.25%) *Escherichia coli* (29.5%), *Pseudomonas spp* (27.25%), *Enterobacter spp* (6.25%), *Citrobacter* 5 (1.25%), and *Acinetobacter spp* (2.5%). Isolates showed 20-80% susceptibility to ciprofloxacin, 30-60% to third and fourth generation cephalosporins. Klebsiella and Pseudomonas showed 64% and 31% susceptibility to ciprofloxacin. *Acinetobacter spp* showed 30% susceptibility to cefipime and 20% to ciprofloxacin. 34 isolates were ESBL 18 were AmpC producers, of which 15(44%) ESBL and 7(38%) of AmpC producers were ciprofloxacin susceptible. **Conclusion:** Ciprofloxacin was found to be more effective than the fourth generation cephalosporin (cefepime) against gram negative bacilli. Ciprofloxacin can be considered for treatment as it is more active and cost effective when compared to cephalosporins.

KEY WORDS: Fluroquinolones, Cephalosporins, Multidrug resistant, ESBL, Amp C.

Introduction

Gram-negative infections pose great threat and accounts for significant amount of public health problems. The rise of gram negative infections can be attributed to the ability of organisms to acquire resistance to the most upcoming antibiotics.[1] Among gram negative bacteria particularly members of family Enterobacteriaceae and non-fermenting gram negative bacilli gain our attention because of their ability to outgrow and develop into multidrug (MDR) and pan drug resistant bacteria(PANDR).[2] Among various drug resistance mechanisms described, ESBL, MBL, Amp C, Carbapenemase producing bacterial infections are responsible of higher mortality and morbidity among hospitalized patients there by increasing the cost and length of stay in hospitals.[3,4] Such infections always remained as a challenge for treating physicians

Access this article online

Quick Response Code:

Website: www.jmsh.ac.in

Doi: 10.46347/jmsh.v9i1.22.369

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and also led higher rates of treatment failure. Laboratory Identification of gram negative bacilli with detailed evaluation of antibiotic sensitivity testing plays a significant role in the treatment of such infections among various antibiotic classes, Cephalosporins and fluroquinolones (ciprofloxacin) are commonly used in the treatment of these infections. However, strains of *E. coli* and *Klebsiella spp* expressing extended-spectrum β lactamases that can hydrolyze most cephalosporins are a growing clinical concern. Beside the use of Cephalosporins can cause hypersensitivity reactions similar penicillins and results in greater collateral damage than the usage.[5] On the contrary, Fluoroquinolones, show an excellent activity against gram negative bacteria when compared to gram positive bacteria. Further, because they are cost effective half-life of 3- 5 hours and bioavailability of 70% makes fluoroquinolones a better choice of drug compared to cephalosporins. Also the drug is widely distributed in body fluids and tissues and well tolerated compared to cephalosporins.[6] With the emergence of drug resistance mechanisms, the gram negative bacteria that were earlier sensitive to cephalosporins now develop resistance to cephalosporins in the oxyimino group (cefotaxime, ceftazidime, ceftriaxone), 7-α methoxycephalosporins (cefoxitin or cefotetan) however not affected by available β-lactamase inhibitors (clavulanate, sulbactam, tazobactam)[7,8]. This leaves us with limited treatment options.

Development of resistance to extended-spectrum cephalosporins among Gram negative bacilli particularly among in *E. coli* and *K. Pneumoniae* has become a worldwide problem[9]. Besides increase in of ESBL-producing *Enterobacteriaceae* in the hospital settings, their dissemination and increased prevalence in the community poses a major threat, since they may turn into powerful reservoir for the continued influx of resistant strains into hospitals[10,11]. With the emergence of drug resistance to Fluoroquinolones and cephalosorins, there is a need to reevaluate the drug susceptibility and opt for a better choice of drug which is both effective and that has less adverse effects comparatively. Thus this study is done to compare the susceptibility of gram negative bacilli clinical isolates to ciprofloxacin with that of second, third and fourth generation of cephalosorins and to determine efficacy of fluroquinolones against ESBL and Amp C producing Gram negative bacilli. Hence the present study is taken to test and compare the susceptibility of gram negative bacilli clinical isolates to cephalosporins and ciprofloxacin, to test for susceptibility of ESBL and AmpC producers to ciprofloxacin

Materials and Methods

This is a prospective observational study done over a period of one year from 2018 March to 2019 April. The study was conducted at the department of Microbiology, Rajarajeswari medical college.

Sample collection

Various samples (pus, urine, sputum, vaginal swabs, ear swabs, pleural fluid, blood) were included in the study. All the samples were processed following the standard procedure.

Identification of bacteria

Gram negative bacterial isolates were identified by their colony characteristics and subjected to various biochemical reactions. The isolates were identified based on Gram stain, catalase test, oxidase test, nitrate test, Triple sugar iron test, urease test, indole test, citrate test and also various sugar fermentation and amino acid utilization tests.[12]

Antibiotic susceptibility testing

Identified gram negative bacilli were subjected to for Antibiotic susceptibility testing by Kirby Bauer disc diffusion method using cefuroxime, cefotaxime, ceftazidime, ceftazidime with clavulanic acid, cefoxitin, cefepime and ciprofloxacin as per CLSI guidelines.[13]

ESBL detection

ESBL producers were detected by disc diffusion method using ceftazidime and ceftazidime/clavulanic acid disc as per CLSI guidelines.Ceftazidime-clavulanic acid disc was placed toward the center of the plate, a ceftazidime disc 30 mg) was placed 15 mm out from the edge of ceftazidime-clavulanic acid disc at 90° angles, so that it's inner edge was 15 mm from it. Plates were incubated at 35°C, aerobically for 18-24hrs. Organism was detected as ESBL by >7mm zone with ceftazidime clavulanic acid than Ceftazidime alone. AmpC producers were detected by disc diffusion method using cefoxitin and cefepime discs. Cefoxitin zone of <18 mm was taken as cefoxitin resistant. Isolates resistant to cefoxitin and sensitive to cefepime was taken as AmpC producers.[13]

Results

A total of 400 Gram negative bacilli isolated from various clinical specimens were included. Among them 134 isolates were from pus samples, 16 from Ear swab,10 from vaginal swab, 02 from pleural fluid,92from sputum, 26 from blood and from 120 urine samples. Distribution of various clinical specimens are as shown in (Table 1)

Table 1: Distribution of various clinical specimens

Clinical specimen	Number (n)
Pus	136
Ear swab	36
Vaginal swab	12
Pleural fluid	02
Sputum	97
Blood	26
Urine	91
Total samples	400

The organisms isolated are *Klebsiella spp.* 133 (33.25%), *Escherichia coli* 118 (29.5%), *Pseudomonas spp* 109(27.25%), *Enterobacter spp* 25 (6.25%), *Citrobacter spp* 5 (1.25%), and *Acinetobacter spp* 10 (2.5%). Isolates from various clinical specimens are as shown in (Table 2)

Table 2: Isolates from various clinical specimens

Name of the isolates	Number (n)
Klebsiella spp	133
Escherichia coli	118
Pseudomonas spp	109
Enterobacter spp	25
Citrobacter spp	05
Acinetobacter spp	10
Total samples	400

Pseudomonas was isolated frequently from pus samples of burn wounds, ear swabs, Enterobacter from blood while *Klebsiella spp* from pus and respiratory tract, *Escherichia coli* from urine specimens. Distribution of various isolates in different clinical samples are as shown in (Table 3)

The susceptibility pattern of various isolates are shown in Table 4. All isolates showed good susceptibility to ciprofloxacin except *Acinetobacter spp* which was sensitive to cefepime.

Among 400 isolates, 34 isolates were ESBL producers and 18 isolates were AmpC producers out of which 15(44%) ESBL and 7(38%) of AmpC producers were ciprofloxacin susceptible.

Discussion

Resistance to third and fourth generation cephalosporins along against nosocomial gram negative bacteria poses a great threat to clinical outcome of the patients.[14] This could be because of unwarrented use of such antimicrobial agents both in the hospital settings also by itself prescribing practices of patients. Thus, changes in antimicrobial drug–prescribing patterns through formulary modification and continuous education of prescribers along with good infection control practices helps to combat this resistance.[15] Such resistance patterns always pushes a need for reevaluation of the susceptibility testing.

In a study done by Archibald L, et al in united states, Enterobacter cloacae showed 40% resistance to ceftazidime. In our study all *Entrobacter spp* (100) were resistant to ceftazidime (Table 4). This is probably related to production of stably derepressed chromosomal class-1 β-lactamase, which hydrolyzes β-lactam antibiotics other than carbapenems. Studies done by Verbist L.et al an JarlierV showed that lowest resistance to ciprofloxacin. Similarly In our study 64% of *Enterobacter spp*. was sensitive to ciprofloxacin.[16,17] In our study Klebsiellaspp showed 22% sensitivity to ceftazidime. Similar results were seen in studies done by Livermore DM et al and Philippon A et al, where *Klebsiella pneumoniae* showed 36% and 26% of susceptibility to ceftazidime respectivily. This could be because of the production of extended-spectrum β-lactamases.[18,19] Reports show that there is a substantial increase in resistance from 3.6% in 1990 to 14.4% in 1993 to ceftazidime among *K pneumoniae* in ICU isolates increased.[20]

In our study Gram negative bacilli susceptibility ranged from 25-60% to cefepime and 29-80% for ciprofloxacin. Thus ciprofloxacin was more effective compared to cefepime. A worrisome trend during the last two decades has been the development of resistance to extended-spectrum cephalosporins, e.g., cefotaxime, ceftazidime, and ceftriaxone. Such resistance is most often due to the breakdown of the extended-spectrum cephalosporin by extended-

Table 3: Distribution of various isolates in different clinical specimens

Gram negative bacilli	Pus	Ear swab	Vaginal swab	Pleural fluid	Sputum	Blood	urine
Klebsiella spp	40	3	4	1	72	5	8
E coli spp	7	11	8	-	7	3	82
Pseudomonas spp	81	20	-	-	8	-	-
Enterobacter spp	2	2			6	15	-
Citrobacter spp	3	-			-	1	1
Acinetobacter spp	3	-		1	4	2	-

Table 4: Susceptibility pattern ofGram negative bacterial isolates

Organism	Cefotaxime	Cefuroxime	Ceftazidime	Cefepime	Ciprofloxacin
Klebsiella	20(15)	20(15)	30(22)	35(26)	41(31)
E Coli	22(19)	22(19)	28(24)	70(60)	85(72)
Pseudomonas	9(8.3)	9(8.3)	26(25)	66(60)	70(64)
Enterobacter	-	-	-	11(44)	16(64)
Citrobacter	1(20)	2(40)	2(40)	2(40)	4(80)
Acinetobacter	-	-	-	3(30)	2(20)

spectrum β-lactamases (ESBLs), but it may also be due to plasmid-mediated or chromosomally hyperproduced Amp C.[21] Thus detection of ESβL- and AmpC beta lactum producing strains plays a pivotal role to prevent uncontrolled spread and also therapeutic failures. Early detection of the resistance patterns helps to formulate appropriate usage of antibiotics and helps in the effective implementation of containment measures. In our study 44% isolates were ESBL producers and 38% were AmpC producers. Though molecular methods are the gold standard for the detection of ESBL nad Amp C producers, because of unavailability of facilities at all centres in developing countries,various phenotypic methods are recommended for routine use to detect ESβL production in Gram-negative bacilli.[22]

In our study 20-80% of the isolates were susceptible to ciprofloxacin when compared to other class of antibiotics, especially third and fourth generation cephalosporins. Even among ESBL and Amp C produces high susceptibility was seen to ciprofloxacin. As there is growing resistance to third and fourth generation cephalosporins, it is a prerequisite to reevaluate the susceptibility of these isolates.Kaye et al.reported a protective effect of fluoroquinolone use against the emergence of resistance to third-generation cephalosporins in nosocomial isolates of *Enterobacter*.[23]Though our study does not generalize the use of fluroquinolones over cephalosporins, similar to Kaye et al. study, our study suggests that substitution of fluroquinolones for certain types of β-lactam antimicrobial drugs could be considered. The potential advantages of adding fluoroquinolones over third-generation cephalosporin resistance are: they can be administered orally; they are relatively nontoxic and inexpensive.[24]

Conclusion

In summary, there is a decrease in the percentage of antibiotic susceptibility across all isolates to cefuroxime, cefotaxime and ceftazidime. The most of the isolates were susceptible to cefepime and ciprofloxacin. Among them ciprofloxacin was more effective than cefepime among all tested organisms except the *Acinetobacter spp* in which cefepime was more effective. So considering the cost and adverse effects of cephalosporins, we suggest the use of ciprofloxacin, as first line of drug.

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How to cite this article: Vishwajith , Rao KA, Lakshminarayana SA. Comparison of *in-vitro* Antibiotic Susceptibility of Ciprofloxacin, Cefotaxime, Ceftazidime and Cefepime against Gram Negative Bacilli Infections - A Study from Tertiary Care Centre. J Med Sci Health 2023; 9(1):64-69

Date of submission: 12.08.2022
Date of review: 23.12.2022
Date of acceptance: 09.02.2023
Date of publication: 10.04.2023