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Comparison of Susceptibility of Clinical Isolates of Gram Negative Bacilli to Cephalosporins and Fluroquinolone

***Dr.Vishwajith[1], Dr.Anuradha.K[2], Dr.Venkatesha.D[3]**

[1,2,3]Department of Microbiology,
Mysore Medical College and Research Institute, Mysore – 570 001. INDIA.

Abstract:

BACKGROUND : Cephalosporins and ciprofloxacin are commonly used in the treatment of gram negative bacilli infections. Third generation cephalosporins are first choice but expensive than ciprofloxacin. So there is a need to compare their susceptibility.

OBJECTIVE: To compare the susceptibility of gram negative bacilli clinical isolates to cephalosporins and ciprofloxacin. Susceptibility of ESBL and AmpC producers to ciprofloxacin.

METHODOLOGY: Disk diffusion method performed on100 Gram negative bacilli isolates. Susceptibilityto cefuroxime, cefotaxime, ceftazidime, ceftazidime with clavulanic acid, cefoxitin, cefepime and ciprofloxacin were tested.

ESBL detected by>7mm zone with ceftazidime clavulanic acid than ceftazidime alone. AmpC detected by cefoxitin resistant and cefepime sensitive.

RESULTS: Pseudomonas isolates exudate7(29%),burns13(54%), bood4(17%).Susceptibility to ciprofloxacin, cefepime in exudate 4(57%),3(43%),burns 5(38%),4(31%) respectively. Blood isolates susceptible to cefepime3(75%)other cephalosporins and ciprofloxacin2(50%). Escherichia coli isolates 7(28%)exudates,3(12%)blood,7(28%)sputum and 9(36%)urine. Blood isolates100% susceptibility to ciprofloxacin, cefepime Exudate 2(28%),4(57%). Equal susceptibility to both 4(57%)sputum. Urine 6(66%),7(78%) respectively. Klebsiella 10(32%) exudates and burns,10(32%)sputum,2(6%)blood. Their susceptibility to ciprofloxacin 2(22%),1(11%),5(50%) and cefepime2(22%),2(22%),1(50%),5(50%) respectively. Enterobacter blood 6(50%), exudate, burns sputum2(17%)each. Susceptibility to ciprofloxacin2(100%) exudates and burns, 4(67%)blood. Resistance to cefepime seen in all except blood isolates4(67%). Citrobacter isolates from exudates3(60%), from blood and urine1(20%) each.Exudate1(33%) sensitive to all cephalosporins and ciprofloxacin. Blood,urine1(100%) sensitive to ciprofloxacin. Acinetobacter 3(100%) exudates.1(33%) sensitive only to cefepime Ciprofloxacin susceptibility seen in Pseudomonas54%,E.coli72%,klebsiella29%,Enterobacter66%,Citrobacter80% and acinetobacter resistant and cefepime susceptibility 21%,60%,25%,41%,40% and 33% respectively. Among 8 ESBL, 5AmpC producers 4(50%) and 2(40%) sensitive to ciprofloxacin respectively.

CONCLUSION: Ciprofloxacin was found to be more active than the fourth generation cephalosporin cefepime against gram negative bacilli. Since ciprofloxacin are cheaper it reduces the treatment cost.

Keywords: cephalosporins, ciprofloxacin, gram negative bacilli, antibiotic susceptibility test.

Introduction

Gram-negative bacteria are common causes of intra-abdominal infections (IAIs), urinary tract infections (UTIs), nosocomial pneumonia, and bacteremia.[1]The most common organisms responsible for these infections are multidrug resistant gram negative bacilli particularly members of the family *Enterobacteriaceae* and nonfermenting gram negative rods[2].

Antimicrobial resistance among GNB is increasing worldwide. This is a major public health problem and a cause for both substantial morbidity and mortality among hospitalized patients. A direct correlation has been shown between resistance of GNB and patient mortality, cost of patient care and length of stay in the hospital[3].

Cephalosporins and ciprofloxacin are commonly used in the treatment of these infections. Cephalosporins are similar to penicillins, but more stable to many bacterial β lactamases and therefore have a broader spectrum of activity. However, strains of *E coli* and *Klebsiella* sp expressing extended-spectrum β lactamases that can hydrolyze most cephalosporins are a growing clinical concern. Beside this Cephalosporins can cause hypersensitivity reactions that are identical to those of penicillins[4].

Fluoroquinolones were originally developed because of their excellent activity against gram-negative aerobic bacteria they had limited activity against gram-positive organisms.Further ciprofloxacin are cheap, and its half-life is 3- 5 hours with a bioavailability of 70% it can be orally administered twice daily. It is widely distributed in body fluids and tissues and well tolerated compared to cephalosporins[5].

In the Ambler structural classification of β-lactamases, AmpC enzymes belong to Class C, while in the functional classification scheme of Bush these are assigned to group 3[6][7] . These enzymes can be chromosomal or plasmid

encoded. AmpC β-lactamases are clinically significant,[8] since these confer resistance to cephalosporins in the oxyimino group (cefotaxime, ceftazidime, ceftriaxone), 7-α methoxy cephalosporins (cefoxitin or cefotetan) and are not affected by available β-lactamase inhibitors (clavulanate, sulbactam, tazobactam)[9]. Plasmid mediated AmpC β-lactamases differ from chromosomal AmpCs in being uninducible and are typically associated with broad multidrug resistance[10]. Plasmid mediated AmpC β-lactamases are present in isolates of *Klebsiella pneumoniae, K. oxytoca,Salmonella*spp., *Proteus mirabilis, Escherichia coli, Citrobacter freundii* and *Enterobacter aerogenes*[11]. In *E. coli*,high level production of chromosomally mediated AmpC β-lactamases is also present[12]. The treatment options for infections caused by organisms expressing AmpC β-lactamases are limited.

Resistance to extended-spectrum cephalosporins in *E. coli* and, in particular, *K. pneumoniae* has become a worldwide problem[13]. Dissemination of ESBL-producing *Enterobacteriaceae* in the community poses a new threat, since this may become a powerful reservoir for the continued influx of resistant strains into hospitals[14][15].

Thus there is a need for detecting AmpC β-lactamases so as to avoid therapeutic failures. The reports of AmpC β-lactamases from India are still limited. Therefore, there is a need to study for both extended spectrum β-lactamase (ESBL) and AmpC β-lactamases.

Resistance to Fluoroquinolones and cephalosorinsare increasing and there is a need to evaluate their resistance pattern. Keeping this in mind we decided to compare the susceptibility of gram negative bacilli clinical isolates to ciprofloxacin with that of second, third and fourth generation of cephalosorins.

Objectives

To compare the susceptibility of gram negative bacilli clinical isolates to cephalosporins and ciprofloxacin.

Susceptibility of ESBL and AmpC producers to ciprofloxacin

Materials and Methods

Gram negative bacterial isolates from samples of exudate, sputum, blood, urine and burn patients were identified using the conventional methods and tested for Antibiotic susceptibility testing by Kirby Bauer method using cefuroxime,cefotaxime,ceftazidime,ceftazidimewithclavulanic acid, cefoxitin, cefepime and ciprofloxacin as per CLSI guidelines[16].

ESBL producers were detected by disc diffusion method using ceftazidime and ceftazidime/clavulanic acid disc as per CLSI guidelines[16].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 18-24 h.Organism was detected as ESBL by >7mm zone with ceftazidimeclavulanic 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.

Results

A total of 100 Gram negative bacilli isolated from various clinical specimens were included. Among them 31 isolates were from exudates samples, 24 from burns, 19 from sputum, 16 from blood and 10 from urine samples. (Table 1)

TABLE 1: Distribution of various clinical specimens

EXUDATE	31
BURNS	24
SPUTUM	19
BLOOD	16
URINE	10
TOTAL SAMPLES	100

The organisms isolated are *Klebsiella spp*.31(31%), *Escherichia coli*25 (25%), *Pseudomonas spp*24 (24%), *Enterobacter spp*12 (12%), *Citrobacter spp* 5 (5%), and *Acinetobacter* spp3(3%).(Table 2)

Table 2: Isolates from various clinical specimens

KLEBSIELLA SPP	31
ESCHERICHIA COLI	25
PSEUDOMONAS SPP	24
ENTEROBACTER SPP	12
CITROBACTER SPP	5
ACINETOBACTER SPP	3
TOTAL SAMPLES	100

Pseudomonas was isolated frequently from burn wounds, *Enterobacter* from blood while *Klebsiella spp*from exudate and respiratory tract, *Escherichia coli* from urine specimens.(Table3)

Table 3: Distribution of various isolates in different clinical specimens

	Exudates	*Burns*	*Sputum*	*Blood*	*Urine*
PSEUDOMONAS	7	13	-	4	-
E COLI	7	-	7	3	9
KLEBSIELLA	10	9	10	2	-
ENTEROBACTER	2	2	2	6	-
CITROBACTER	3	-	-	1	1
ACINETOBACTER	3	-	-	-	-

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

Table 4: Susceptibility pattern of Gram negative bacterial isolates

Organism	*Cefotaxime*	*Cefuroxime*	*Ceftazidime*	*Cefepime*	*Ciprofloxacin*
Pseudomonas	2(8.3)	2(8.3)	5(21)	13(54)	15(62.5)
E Coli	3(12)	3(12)	3(12)	15(60)	18(72)
Klebsiella	2(6)	2(6)	2(6)	8(25)	9(29)
Enterobacter	-	-	-	5(41.6)	8(66)
Citrobacter	1(20)	2(40)	2(40)	2(40)	4(80)
Acinetobacter	-	-	-	1(33)	-

Among 100 isolates, 8 isolates were ESBL producers and 5 isolates were AmpC producers out of which 4(50%) isolates of ESBL and 2 isolates of AmpC producers were ciprofloxacin susceptible.

Klebsiella species isolates from exudates ,burns and sputum showed susceptibility to ciprofloxacin 2(22%),1(11%),5(50%) and cefepime2(22%), 1(50%), 5(50%) respectively.

Escherichia coli susceptibility to ciprofloxacin and cefepime, blood isolates showed 100% susceptibility whereas isolates from exudate showed 2(28%),4(57%), from urine 6(66%),7(78%) respectively. Equal susceptibility to both 4(57%) was observed in sputum isolates.

Pseudomonas species isolated from various specimens showed susceptibility to ciprofloxacin and cefepime from exudate 4(57%),3(43%),burns 5(38%),4(31%) respectively. Blood isolates were susceptible to cefepime3(75%)other cephalosporins and ciprofloxacin2(50%).

Enterobacter species isolated from exudate and burns showed 100% susceptibility tociprofloxacin, 4(67%) of blood isolates were susceptible. Only 4(67%) blood isolates were cefepime susceptible.

Citrobacter species isolated from blood, urine were 100% sensitive to ciprofloxacin. Exudate isolate1(33%) wassensitive to all cephalosporins and ciprofloxacin.

Acinetobacter species isolated from exudate alone. Out of which only 1(33%) was sensitive only to cefepime. All other antibiotics were resistant.

Ciprofloxacin susceptibility seen in *Pseudomonas* 54%, *E.coli*72%, *Klebsiella* 29%, *Enterobacter* 66%, *Citrobacter* 80% and *Acinetobacter* resistant .

Discussion

Bacterial resistance to ceftazidime is a serious problem. Approximately 40% of*Enterobacter cloacae* were resistant to ceftazidime, according to studies of ICU isolates in the United States between 1987 and 1991[17] and between 1994 and 1995[18]. In our study all *Entrobacter*spp 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. Previous use of third-generation cephalosporins is more likely to cause the selection of resistance to β-lactams in blood isolates of *Enterobacter* species, which is associated with higher mortality[19].

An increased ciprofloxacin resistance among *Enterobacter* species was seen in Belgium and France compared with previous studies performed in 1990[20] and 1991[21].In our study 66% of *Enterobacter* spp. was sensitive to ciprofloxacin.

In Portugal and France, 36% and 26%, respectively, of *Klebsiella pneumoniae* showed decreased susceptibility to ceftazidime. This was probably caused by the production of extended-spectrum β-lactamases[22][23]and is concordant with a study by Livermore and Yuan[24]. According to Jarlier et al,[21]decreased susceptibility to ceftazidime among *K pneumoniae* in French ICUs was 36% in 1991. In the United States, resistance to ceftazidime among *K pneumoniae* in ICU isolates increased from 3.6% in 1990 to 14.4% in 1993[25]. In our study *Klebsiella*spp showed 6% sensitivity to ceftazidime.

The potency of cefepime, a parenteral aminothiazoly lmethoxyimino cephalosporin, was assessed against 256 ceftazidime-resistant Gram-negative bacilli from five medical centers in the United States. In addition, cefepime activity was compared with that of ciprofloxacin and imipenem against 506 ceftazidime-resistant Gram-negative bacilli collected during an 11-medical-center international study. All US clinical isolates were susceptible (< or = 8 micrograms/ml) to cefepime except *Enterobacter cloacae* (94% susceptible) and *Pseudomonas aeruginosa* (19% susceptible). Enterobacteriaceae isolates from the 11-nation sample were > 80% cefepime susceptible with the exception of those from Brazil (48% susceptible) and Italy (55% susceptible[26].

These international, enteric isolates were also very susceptible to ciprofloxacin (55%-100% susceptible). Nonenteric organisms (Pseudomonas, Xanthomonas, and Acinetobacter) from the same international locations had overall rates of susceptibility of 47% for ciprofloxacin, and only 5% for cefepime.[26]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-spectrum β-lactamases (ESBLs), but it may also be due to plasmid-mediated or chromosomally hyperproduced AmpC[27].

Failure to detect ESβL- and AmpC βL-producing strains has contributed to their uncontrolled spread and therapeutic failures. Hence, their appearance in a hospital setting should be identified quickly so that appropriate antibiotic usage and containment measures can be implemented. For detection of various types of ESβLs, molecular methods are the best but the facilities are not available especially in the developing countries. Therefore, various phenotypic methods are recommended for routine use to detect ESβL production in Gram-negative bacilli[28]. ESBL producers were 8(8%) and AmpC βL producers were 5(5%) in our study.

Resistance to fluoroquinolones is frequently observed among ESBL producers[27]. In our study among ESBL producers 4(50%) and among AmpC producers 2(40%) were sensitive to ciprofloxacin.

Conclusion

In summary, we found that the percentage of decreased antibiotic susceptibility across all species to drugs cefuroxime, cefotaxime and ceftazidime.The most active agents were cefepime and ciprofloxacin. Among them ciprofloxacin was more active than cefepime among all tested organisms except the Acinetobacterspp in which cefepime

was more active. So considering the cost and adverse effects of cefepime and ciprofloxacin, the later can be used as first line of drug.

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