



## Introduction

Gram-negative bacteria are common causes of intra-abdominal infections (IAIs), urinary tract infections (UTIs), nosocomial pneumonia, and bacteremia (Sievert *et al.*, 2013). The most common organisms responsible for these infections are multidrug resistant gram negative bacilli particularly members of the family *Enterobacteriaceae* and non fermenting gram negative rods (Bhattacharjee *et al.*, 2008).

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 (Shawn *et al.*, 2007).

Cephalosporins and ciprofloxacin are commonly used in the treatment of these infections. Cephalosporins are similar to penicillins, but more stable to many bacterial  $\beta$  lactamases and therefore have a broader spectrum of activity. However, strains of *E coli* and *Klebsiella* sp expressing extended-spectrum  $\beta$  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 (Bertram).

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 (Bertram).

In the Ambler structural classification of  $\beta$ -lactamases, AmpC enzymes belong to Class C, while in the functional classification scheme of Bush these are assigned to group 3 (Ambler *et al.*, 1980; Bush *et al.*, 1995). These enzymes can be chromosomal or plasmid encoded. AmpC  $\beta$ -lactamases are clinically significant, (Cordron *et al.*, 2000) since these confer resistance to cephalosporins in the oxyimino group (cefotaxime, ceftazidime, ceftriaxone), 7- $\alpha$  methoxy cephalosporins (cefoxitin or cefotetan) and are not affected by available  $\beta$ -lactamase inhibitors (clavulanate, sulbactam, tazobactam) (Phillipon *et al.*, 2002). Plasmid mediated AmpC  $\beta$ -lactamases differ from chromosomal AmpCs in being uninducible and are typically associated with broad multidrug resistance (Barnaud *et al.*, 1998). Plasmid mediated AmpC  $\beta$ -lactamases are present in isolates of *Klebsiella pneumoniae*, *K. oxytoca*, *Salmonella* spp., *Proteus mirabilis*, *Escherichia coli*, *Citrobacter freundii* and *Enterobacter aerogenes* (Thomson *et al.*, 2001). In *E. coli*, high level production of chromosomally mediated AmpC  $\beta$ -lactamases is also present (Black *et al.*, 2005). The treatment options for infections caused by organisms expressing AmpC  $\beta$ -lactamases are limited.

Resistance to extended-spectrum cephalosporins in *E. coli* and, in particular, *K. pneumoniae* has become a worldwide problem (Paterson *et al.*, 2005). 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 (Ben-Ami *et al.*, 2006; Pitout *et al.*, 2005).

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

Resistance to Fluoroquinolones and cephalosporins are 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 cephalosporins.

The main objectives of this study 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, ceftazidime with clavulanic acid, ceftazidime, cefepime and ciprofloxacin as per CLSI guidelines.

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 its 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 ceftazidime clavulanic acid than ceftazidime alone.

AmpC producers were detected by disc diffusion method using ceftazidime and cefepime discs. Ceftazidime zone of <18 mm was taken as ceftazidime resistant. Isolates resistant to ceftazidime and sensitive to cefepime was taken as AmpC producers.

### Results and Discussion

A total of 200 Gram negative bacilli isolated from various clinical specimens were included. Among them 62 isolates were from exudates samples, 48 from burns, 38 from sputum, 32 from blood and 20 from urine samples. (Table 1)

The organisms isolated are *Klebsiella spp.*62(31%), *Escherichia coli*50 (25%), *Pseudomonas spp.*48 (24%), *Enterobacter spp.*24 (12%), *Citrobacter spp.* 10 (5%), and *Acinetobacter spp.*6(3%).(Table 2)

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

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.

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%), 2(11%), 10(50%) and cefepime 4(22%), 2(50%), 10(50%) respectively.

*Escherichia coli* susceptibility to ciprofloxacin and cefepime, blood isolates showed 100% susceptibility whereas isolates from exudate showed 4(28%),8(57%), from urine 12(66%),14(78%) respectively. Equal susceptibility to both 8(57%) was observed in sputum isolates.

*Pseudomonas* species isolated from various specimens showed susceptibility to ciprofloxacin and cefepime from exudate 8 (57%), 6(43%), burns 10(38%), 8(31%) respectively. Blood isolates were susceptible to cefepime 6 (75%) other cephalosporins and ciprofloxacin4(50%).

*Enterobacter* species isolated from exudate and burns showed 100% susceptibility to ciprofloxacin, 8(67%) of blood isolates were susceptible. Only 8(67%) blood isolates were cefepime susceptible.

*Citrobacter* species isolated from blood, urine were 100% sensitive to ciprofloxacin. Exudate isolate2 (33%) was sensitive to all cephalosporins and ciprofloxacin.

*Acinetobacter* species isolated from exudate alone. Out of which only 2(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 .

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 and between 1994 and 1995

(Archibald *et al.*, 1997). In our study all *Enterobacter*spp were resistant to ceftazidime (Table 4). This is probably related to production of stably derepressed chromosomal class-1  $\beta$ -lactamase, which hydrolyzes  $\beta$ -lactam antibiotics other than carbapenems. Previous use of third-generation cephalosporins is more likely to cause the selection of resistance to  $\beta$ -lactams in blood isolates of *Enterobacter* species, which is associated with higher mortality.

An increased ciprofloxacin resistance among *Enterobacter* species was seen in Belgium and France compared with previous studies performed in 1990 (Verbist, 1991) and 1991 (Jarlier *et al.*, 1996). 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  $\beta$ -lactamases (Livermore, 1995) and is concordant with a study by Livermore and Yuan. According to Jarlier *et al.*, 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. In our study *Klebsiella* spp showed 6% sensitivity to ceftazidime.

The potency of cefepime, a parenteral aminothiazolylmethoxyimino 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 (Jones *et al.*, 1994).

**Table.1** Distribution of various clinical specimens

Exudate	62
Burns	48
Sputum	38
Blood	32
Urine	20
Total samples	200

**Table.2** Isolates from various clinical specimens

<i>Klebsiella spp</i>	62
<i>Escherichia coli</i>	50
<i>Pseudomonas spp</i>	48
<i>Enterobacter spp</i>	24
<i>Citrobacter spp</i>	10
<i>Acinetobacter spp</i>	6
Total samples	200

**Table.3** Distribution of various isolates in different clinical specimens

	Exudates	Burns	Sputum	Blood	Urine
<i>Pseudomonas</i>	14	26	-	8	-
<i>E coli</i>	14	-	14	6	18
<i>Klebsiella</i>	20	18	20	20	-
<i>Enterobacter</i>	4	4	4	12	-
<i>Citrobacter</i>	6	-	-	2	2
<i>Acinetobacter</i>	6	-	-	-	-

**Table.4** Susceptibility pattern of Gram negative bacterial isolates

Organism	Cefotaxime	Cefuroxime	Ceftazidime	Cefepime	Ciprofloxacin
<i>Pseudomonas</i>	4(8.3)	4(8.3)	10(21)	26(54)	30(62.5)
<i>E Coli</i>	6(12)	6(12)	6(12)	30(60)	36(72)
<i>Klebsiella</i>	4(6)	4(6)	4(6)	16(25)	18(29)
<i>Enterobacter</i>	-	-	-	10(41.6)	16(66)
<i>Citrobacter</i>	2(20)	4(40)	4(40)	4(40)	8(80)
<i>Acinetobacter</i>	-	-	-	2(33)	-

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. 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  $\beta$ -lactamases (ESBLs), but it may also be due to plasmid-mediated or chromosomally hyperproduced AmpC (Christian *et al.*, 2008).

Failure to detect ES $\beta$ L- and AmpC  $\beta$ L-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 ES $\beta$ Ls, 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 ES $\beta$ L production in Gram-negative bacilli (Sinha *et al.*, 2008). ESBL producers were 8(8%) and AmpC  $\beta$ L producers were 5(5%) in our study.

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

In 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 *Acinetobacter*spp 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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