

## Emergence of cefepime resistance in gram-negative induced nosocomial infections

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### ABSTRACT

**Background:** The rapid emergence of antibiotic resistance, especially broad-spectrum antibiotics, resulted in the avid use of new potent antibiotics. Ceftriaxone and ceftazidime, two third-generation cephalosporin, are usually used to manage complicated and uncomplicated infections. The use of cefepime in resistant infections is increasing gradually, which put this potent antibiotic at risk of resistance.

**Patients and methods:** During an 18-month period, a total of 220 gram-negative bacteria including *Pseudomonas* spp, *Serratia* spp, *Acinetobacter* spp, *Proteus* spp, *E-coli* and *Klebsiella* spp. have been isolated by standard microbiological methods from nosocomial surgical site, abscess, blood stream and urinary tract infections. MIC of antibiotics on isolated bacteria was determined by gradient concentration method.

**Results:** Totally, 29.4%, 19.5% and 23.3% of isolated bacteria with MIC $\leq$ 8 $\mu$ g/ml were sensitive to cefepime, ceftriaxone and ceftazidime, respectively. High level resistance with MIC $\geq$ 256 $\mu$ g/ml to cefepime, ceftriaxone and ceftazidime was also observed in 47.1%, 70.8% and 62.5% of cases, respectively ( $p<0.05$ ). High level resistance to cefepime were more commonly observed for *pseudomonas* (73.1%) and *Klebsiella* spp. (73.5%), respectively ( $p<0.05$ ).

**Conclusion:** According to CLSI criteria, 47.1% of isolated bacteria in this study showed high level of resistance (MIC $\geq$ 256 $\mu$ g/ml) to cefepime. Therefore application of cefepime, as a drug of choice, for gram-negative organisms is not reasonable. Our result demonstrated that this potent antibiotic should not be used as a choice for empiric antibiotic therapy, in the cases of nosocomial infections caused by gram-negative organisms.

**Keywords:** Nosocomial infections, Gram-negative bacteria, MIC, Antibiotic resistance, Cephalosporins. (Iranian Journal of Clinical Infectious Diseases 2009;4(1):13-18).

### INTRODUCTION

Gram-negative bacteria remain important hospital pathogens, particularly for critically ill patients (1,2). *Klebsiella*, *Enterobacter* species, and *Pseudomonas aeruginosa* are among the most commonly isolated nosocomial pathogens (3-5). The mortality rate for patients infected with gram-

negative bacteria is 20 to 30% (6-9). Appropriate antimicrobial treatment is often critical to decrease morbidity and mortality among hospitalized patients with infections (1,2). More recently, gram-negative species have emerged as important pathogens capable of exhibiting resistance to third-generation cephalosporins and other broad-spectrum agents (10-12).

Expanded-spectrum cephalosporin such as ceftriaxone and ceftazidime are broad-spectrum

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agents that have been widely prescribed by physicians in Iran for hospitalized patients with variety of infections for almost 15 years. Cefepime, a “fourth-generation” cephalosporin recently introduced in Iran, has an extended spectrum of activity that encompasses both gram positive organisms, such as *S. aureus* and *S. pneumoniae*, and gram-negative pathogens, including *Pseudomonas aeruginosa*, *Enterobacter* spp, and other members of the Enterobacteriaceae family that are becoming increasingly resistant to expanded-spectrum cephalosporins (12-15).

Several previous studies have examined the impact of cycling of empirical antimicrobial therapy within individual hospitals (16,17). These studies reported favorable alterations in the antimicrobial susceptibility of clinical isolates of gram-negative bacteria (18).

Therefore, the aim of the present study was to evaluate the sensitivity-specificity pattern of gram-negative nosocomial pathogens to the third generation cephalosporins (ceftriaxone and ceftazidime) and fourth-generation cephalosporin (cefepime). These data will serve to establish baseline information on gram-negative bacilli and their in-vitro susceptibility to antimicrobial agents.

#### PATIENTS and METHODS

This cross sectional study was performed during 2005 and 2006, on nosocomial gram-negative bacteria isolated from patients hospitalized in Al-Zahra hospital (a 860-bed university teaching hospital) in Isfahan, Iran. The study population included 130 males and 90 females with the mean age ( $\pm$ SD) of  $57\pm 8$  and  $52\pm 6$  years, respectively.

All gram-negative bacteria isolated from patients with nosocomial infections were enrolled in the study. Demographics, past medical history, site of infection and type of gram-negative bacteria were collected. Specimens taken from surgical wound (45 cases), lower respiratory tract (20 cases), urinary tract (65 cases) and blood stream (90 cases)

were cultured. The method of urine sample collection was case dependent and included urine from midstream urine, catheter, or suprapubic aspiration. A wound infection was identified by the presence of purulent discharge from the incision with erythematous cellulitis, induration or pain, and demonstrable fluid collection noted on ultrasound after surgery. Aspirates were obtained by preparing the wound area with alcohol, inserting a sterile needle through the healing incision and aspirating fluid into a sterile syringe. For the patients with nosocomial pneumonia (fever, increase sputum production and infiltration in chest radiography), specimens from lower respiratory tract were obtained with bronchoalveolar lavage (BAL). Drawn blood samples from the patients were cultured on blood agar media and incubated at 35°C for 18–24 hours. Totally, 220 gram-negative bacteria have been isolated by standard microbiological methods from nosocomial surgical site, abscess, blood stream and urinary tract infections. Minimum inhibitory concentrations (MICs) of ceftriaxone, ceftazidime and cefepime on isolated bacteria were determined using the agar plate dilution method in accordance with the National Committee for Clinical Laboratory Standards. (E-Test, AB BIODISK Co. Sweden). Quality control was tested by *E. coli* ATCC25922.

The breakpoints indicated in the last edition of CLSI M100-S16 tables (19) were used to determine susceptibility and resistance. For cefepime and ceftazidime all strains were considered susceptible if the MIC was  $\leq 8\mu\text{g/ml}$  and resistant if the MIC was  $\geq 32\mu\text{g/ml}$ . For ceftriaxone all strains were considered susceptible if the MIC was  $\leq 8\mu\text{g/ml}$  and resistant if the MIC was  $\geq 64\mu\text{g/ml}$ . Finally, data were analyzed by Whonet 5 and SPSS software (version 13, SPSS Inc., USA).

#### RESULTS

Of 220 gram-negative bacteria isolated from nosocomial infections, 100 (45.4%) were *E. coli*,

47 (21.4%) *Klebsiella* Spp, 38 (17.3%) *Pseudomonas* Spp, 20 (9.1%) *Acinetobacter* Spp, 9 (4.1%) *Proteus* Spp and 6 (2.7%) were *Citrobacter* Spp.

According to the standard breakpoints, 29.4%, 19.5% and 23.3% of isolated bacteria with MIC  $\leq 8\mu\text{g/ml}$  were sensitive to cefepime, ceftriaxone and ceftazidime, respectively. MIC of three agents required to inhibit 90% of isolates (MIC 90) were  $256\mu\text{g/ml}$  and MIC of cefepime to inhibit 50% of organisms (MIC 50) was  $96\mu\text{g/ml}$ , however, MIC 50 of the other two antibiotics were  $256\mu\text{g/ml}$  (table 1).

**Table 1.** Antibiotic susceptibility pattern of all microorganisms

Drug Name	Break-points*	%R	%I	%S	MIC50	MIC90	MIC Range
<b>Cefepime</b>	S $\leq 8$ R $\geq 32$	65.4	5.2	29.4	96	256	0.047-256
<b>Ceftazidime</b>	S $\leq 8$ R $\geq 32$	72.5	4.2	23.3	256	256	0.047-256
<b>Ceftriaxone</b>	S $\leq 8$ R $\geq 64$	74	6.5	19.5	256	256	0.032-256

\*  $\mu\text{g/ml}$ , R: resistant, S: sensitive, I: intermediate, MIC: minimal inhibitory concentration

Our data revealed a high level of resistance with MIC  $\geq 256\mu\text{g/ml}$  to cefepime, ceftriaxone and ceftazidime in 47.1%, 70.8% and 62.5% of cases, respectively. Furthermore, we demonstrated that *Pseudomonas* spp. (73.1%) and *Klebsiella* spp. (73.5%) were the highest resistant organisms to cefepime ( $p < 0.05$ ).

Regarding *E. coli*, 36.5%, 32.5% and 28.6% of isolates were susceptible to cefepime, ceftazidime and ceftriaxone, respectively. However, these figures were 23.5%, 17.2%, and 6.9%, respectively, for *K. pneumoniae*. Among *P. aeruginosa*, cefepime had the highest rate of susceptibility (23.1%) when compared with ceftazidime (21.7%) and ceftriaxone (7.1%). *Acinetobacter* was generally less susceptible to cefepime (6.7%) and ceftriaxone (6.2%) than ceftazidime (14.3%). Against *Citrobacter Freundii*,

ceftriaxone (66.7%) was more effective than ceftazidime (50%) and cefepime (25%). When comparing different microorganisms, *Proteus* species were generally more susceptible to the tested antimicrobial agents, indeed, all *Proteus* isolates were sensitive to cefepime and ceftazidime, however, ceftriaxone was effective on 66.7% of isolates.

Table 2 represents the range of observed antimicrobial MIC values and the MIC required to inhibit 50% and 90% of the isolates (MIC 50 and MIC 90, respectively), as well as the percentage of susceptibility at breakpoint for six genera to each of the tested antimicrobial agents.

**Table 2.** Antibiotic susceptibility pattern of selective microorganisms\*

	%R	%I	%S	MIC50	MIC90	MIC-Range
<b><i>Pseudomonas Aeruginosa</i></b>						
Cefepime	73.1	3.8	23.1	32	256	0.75 - 256
Ceftazidime	69.6	8.7	21.7	256	256	0.75 - 256
Ceftriaxone	85.7	7.1	7.1	256	256	4 - 256
<b><i>Citro-acterfreundii</i></b>						
Cefepime	50	25	25	12	256	1.5 - 256
Ceftazidime	50	0	50	4	256	4 - 256
Ceftriaxone	33.3	0	66.7	4	256	4 - 256
<b><i>Acinetobacter</i></b>						
Cefepime	66.7	26.7	6.7	96	256	0.5 - 256
Ceftazidime	85.7	0	14.3	256	256	8 - 256
Ceftriaxone	87.5	6.2	6.2	256	256	6 - 256
<b><i>Proteus Spp.</i></b>						
Cefepime	0	0	100	.047	2	0.047 - 2
Ceftazidime	0	0	100	4	8	4 - 8
Ceftriaxone	33.3	0	66.7	1.5	256	0.5 - 256
<b><i>E. coli</i></b>						
Cefepime	61.5	1.5	36.9	128	256	0.047 - 256
Ceftazidime	65	2.5	32.5	256	256	0.094 - 256
Ceftriaxone	69.8	1.6	28.6	256	256	0.032 - 256
<b><i>Klebsiella Pneumonia</i></b>						
Cefepime	73.5	2.9	23.5	256	256	0.047 - 256
Ceftazidime	86.2	6.9	6.9	256	256	0.25 - 256
Ceftriaxone	69	13.8	17.2	256	256	0.094 - 256

\* Breakpoints for cefepime and ceftazidime are S $\leq 8\mu\text{g/ml}$  and R $\geq 32\mu\text{g/ml}$ , however, for ceftriaxone is S $\leq 8\mu\text{g/ml}$  and R $\geq 64\mu\text{g/ml}$   
R: resistant, S: sensitive, I: intermediate, MIC: minimal inhibitory concentration

## DISCUSSION

The accepted worldwide use of cephalosporins for the treatment of bacterial infections is due to their safety and pharmacokinetic features (20-22).

Despite the advent of new drugs against the emergent bacterial resistance, cefepime and ceftazidime, the fourth-generation cephalosporins, were found to be slightly more potent than the third-generation cephalosporins tested against *Klebsiella* species. In this study, the antimicrobial effect of cefepime was compared with commonly used third generation cephalosporins in life threatening gram-negative induced systemic infections.

Our results demonstrated that 73.1% and 69.6% of *P. aeruginosa* isolates were resistant to cefepime and ceftazidime, respectively. This degree of resistance is quite low when compared with prior studies. Gencer et al. found that 65% and 54% of *Pseudomonas* isolates were sensitive to ceftazidime and cefepime, respectively (23). In another study, resistance to cefepime was detected only in 30% of *P. aeruginosa* isolates (24).

Our data demonstrated 66.7% cefepime-resistance of *Acinetobacter*, however, cefepime-resistance of *Acinetobacter* was reported 6.7% in Sader (25) and 50% in Aksaray study (24).

Low susceptibility rate of *E. coli* isolates to cefepime and cephalosporins was also described in our study. This rate was 32.5%, 36.9%, and 28.6% for ceftazidime, cefepime and ceftriaxone, respectively. The associated figures were 23.5%, 6.9%, and 17.2%, respectively, for *K. pneumoniae*. Regarding *E. coli* isolates, James reported  $\geq 97\%$  susceptibility rate to cefepime and ceftriaxone, but 3.8% were non-susceptible to ceftazidime. He also demonstrated  $\geq 90\%$  susceptibility rate to ceftriaxone for *K. pneumoniae* (26). Sader showed high rates of resistance for cefoxitin (73.0%), ceftazidime (69.4%) and ceftriaxone (65.9%) among *Klebsiella* spp isolates.

Our data demonstrated that the ranking order of susceptibility rate for gram-negative nosocomial pathogens is as follow: 29.4% for cefepime, 23.3% for ceftazidime and 19.5% for ceftriaxone. Rhomberg showed this ranking as follow: 91.2% for cefepime, 89.0% for ceftazidime and 69.1% for ceftriaxone (27), while Aubert showed similar susceptibility rate to ceftazidime (78.8-81.9%) and cefepime (80-83.4%), respectively (28).

Resistance to the tested antibiotics is partly attributed to the pattern of antibiotic use, i.e., previous administration of broad-spectrum cephalosporin that is associated with the emergence of resistance to group 1  $\beta$ -lactamase-producing organisms (29). In these cases, when resistance to third or fourth generation cephalosporins occurred, carbapenems are the drug of choice.

Reduction in the incidence of ceftazidime resistance had been observed after restricted use (30). Thus, some reports of high susceptibility to ceftazidime and low susceptibility to cefepime may reflect the decreased ceftazidime use. However, ineffective hospital infection control and poor antibiotic policies may probably result in increasing rates of resistance to all antibiotics, including cephalosporins.

In conclusion, according to CLSI criteria, 47.1% of isolated bacteria in this study showed high level of resistance ( $MIC \geq 256 \mu\text{g/ml}$ ) to cefepime. Therefore application of cefepime as a drug of choice, for gram-negative organisms, is not reasonable. Our result demonstrated that this potent antibiotic should not be used as a drug of choice for empiric antibiotic therapy, if nosocomial infections caused by gram-negative organisms.

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