Iranian Journal of Clinical Infectious Diseases
2009;4(1):13-18

ORIGINAL ARTICLE

Emergence of cefepime resistance in gram-negative induced nosocomial infections

Farzin Khorvash[1*], Kamyar Mostafavizadeh[1], Sina Mobasherizadeh[1], Mohaddeseh Behjati[1], Mansoor Salehi[2]

[1] *Department of Infectious Diseases and Tropical Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

[2] *Department of Molecular Genetics, Isfahan University of Medical Sciences, Isfahan, Iran*

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≤8µg/ml were sensitive to cefepime, ceftriaxone and ceftazidime, respectively. High level resistance with MIC≥256µg/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≥256µg/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

Received: 6 April 2008 *Accepted*: 14 July 2008
Reprint or Correspondence: Farzin Khorvash, MD. Department of Infectious Diseases and Tropical Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: Khorvash@med.mui.ac.ir

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. pneumonia, 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 (±SD) of 57±8 and 52±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 (20cases), 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 ≤8µg/ml and resistant if the MIC was ≥32µg/ml. For ceftriaxone all strains were considered susceptible if the MIC was ≤8µg/ml and resistant if the MIC was ≥64µg/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 ≤8µg/ml were sensitive to cefepime, ceftriaxone and ceftazidime, respectively. MIC of three agents required to inhibit 90% of isolates (MIC 90) were 256µg/ml and MIC of cefepime to inhibit 50% of organisms (MIC 50) was 96µg/ml, however, MIC 50 of the other two antibiotics were 256µg/ml (table 1).

Table 1. Antibiotic susceptibility pattern of all microorganisms

Drug Name	Break-points*	%R	%I	%S	MIC50	MIC90	MIC Range
Cefepime	S≤8 R≥32	65.4	5.2	29.4	96	256	0.047-256
Ceftazidime	S≤8 R≥32	72.5	4.2	23.3	256	256	0.047-256
Ceftriaxone	S≤8 R≥64	74	6.5	19.5	256	256	0.032-256

* µg/ml, R: resistant, S: sensitive, I: intermediate, MIC: minimal inhibitory concentration

Our data revealed a high level of resistance with MIC ≥256µg/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. pneumonia. 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
Pseudomonas Aeroginosa						
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
Citro-acterfreundii						
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
Acinetobacter						
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
Proteus Spp.						
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
E. coli						
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
Klebsiella Pneumonia						
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≤8µg/ml and R≥32µg/ml, however, for ceftriaxone is S≤8µg/ml and R≥64µg/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 cefpirome, 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. aeroginosa 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. aeroginosa 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. pneumonia. Regarding E. coli isolates, James reported ≥97% susceptibility rate to cefepime and ceftriaxone, but 3.8% were non-susceptible to ceftazidime. He also demonstrated ≥90% susceptibility rate to ceftriaxone for K. pneumonia (26). Sader showed high rates of resistance for cefoxitine (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 β-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≥256 μg/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.

ACKNOWLEDGEMENT

The authors wish to thank the research chancellor of Isfahan University of Medical Sciences for funding and the Infectious Diseases Research Center staff for their kind help.

REFERENCES

1. Diekema DJ, Pfaller MA, Jones RN, Doern GV, Winokur PL, Gales AC, et al. Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY antimicrobial surveillance program. Clin Infect Dis 1999;29:595–607.

2. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest 2000;118:146–55.

3. Chong Y, Lee K, Kwon OH. In vitro activities of Cefepime against Enterobacter cloacae, Serratia marcescens , Pseudomonas aeroginosa and other aerobic Gram-negative bacilli. J Antimicrob Chemother 1993; 32:21–29.

4. Jones RN, Biedenbach DJ, Gales AC. Sustained activity and spectrum of selected extended- spectrum betta lactams (carbapenems and cefepime) against Enterobacter spp and ESBL-producing Klebsiella spp: Report from the SENTRY antimicrobial surveillance program (USA, 1997–2000). Int J Antimicrob Agents 2003;21:1–7.

5. Bekir SK. Antimicrobial resistance surveillance of gram-negative bacteria isolated from intensive care units of four different hospitals in Turkey. Chemotherapy 2001;47:396–408.

6. Bryan CS, Reynolds KL, Brenner ER. Analysis of 1,186 episodes of gram-negative bacteremia in non-university hospitals: the effects of antimicrobial therapy. Rev Infect Dis 1983;5:629–38.

7. Geerdes HF, Ziegler D, Lode H. Septicemia in 980 patients at a university hospital in Berlin: prospective studies during 4 selected years between 1979 and 1989. Clin Infect Dis 1992;15:991–1002.

8. Leibovici L, Samra Z, Konigsbereger H, Drucker M, Ashkenazi S, Pitlik SD. Long-term survival following bacteremia or fungemia. JAMA 1995;274:807–12.

9. Perl TM, Dvorak M, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. JAMA 1995;274:338–45.

10. Carlos B, Marcela DC, Federico N, Silvia R, Jorgelina S. Comparative in vitro bactericidal activity between Cefepime and ceftazidime, alone and associated with amikacin, against carbapenem-resistant Pseudomonas aeroginosa strains. Diagn Microbiol Infect Dis 2000;37:41–44.

11. Domenech-Sanchez A, Pascual A, Suarez AI, Alvarez D, Benedi VJ, Martinez-Martinez L. Activity of nine antimicrobial agents against clinical isolates of Klebsiella pneumoniae producing extended-spectrum betta lactamases and deficient or not in porins. J Antimicrob Chemother 2000;46:847–63.

12. Husson MO, Quelquejay J, Fruchart A, Izard D. Comparative antibacterial activity of cefotaxime, ceftazidime and Cefepime with regard to different strains of Enterobacter aerogenes selected for their resistance to third generation cephalosporins. Pathol Biol 2000;48:933–39.

13. Jones RN. Impact of changing pathogens and antimicrobial susceptibility patterns in the treatment of serious infections in hospitalized patients. Am J Med 1996;100:3-12.

14. Washington JA, Jones RN, Gerlach EH, Murray PR, Allen SD, Knapp CC. Multicenter comparison of in vitro activities of FK-037, cefepime, ceftriaxone, ceftazidime, and cefuroxime. Antimicrob Agents Chemother 1993;37:1696-700.

15. Thornsberry C, Yee YC. Comparative activity of eight antimicrobial agents against clinical bacterial isolates from the United States, measured by two methods. Am J Med 1996;100:26-38.

16. Kollef MH, Vlasnik J, Sharpless L. Scheduled change of antibiotic classes: A strategy to decrease the incidence of ventilator-associated pneumonia. Am J Respir Crit Care Med 1997;156:1040–48.

17. Gruson D, Hilbert G, Vargas F. Strategy of antibiotic rotation: Long-term effect on incidence and susceptibilities of gram-negative bacilli responsible for ventilator-associated pneumonia. Crit Care Med 2003; 31:1908–14.

18. Gruson D, Hilbert G, Vargas F. Rotation and restricted use of antibiotics in a medical intensive care unit. Impact on the incidence of ventilator-associated pneumonia caused by antibiotic-resistant Gram-negative bacteria. Am J Respir Crit Care Med 2000;162:837–43.

19. Wayne PA. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M 100-S16 January 2006; volume 26 No. 3.

20. Marshall WF, Blair JE. The cephalosporins: Symposium on Antimicrobial Agents. Mayo Clin Proc 1999;74:187–95.

21. Fung-Tomc JC. Fourth-generation cephalosporins. Clin Microbiol Newsl 1997;19:129–31.

22. Joukhadar C, Klein N, Mayer BX, Kreischitz N, Delle-Karth G, Palkovits P, et al. Plasma and tissue pharmacokinetics of cefpirome in patient with sepsis. Crit Care Med 2002;30:1478–82.

23. Serap G, Oznur Ak, Nur B, Ayşe B, Serdar O. Susceptibility patterns and cross resistances of antibiotics against Pseudomonas aeruginosa in a teaching hospital of Turkey2002. Ann Clin Microbiol Antimicrob 2002;1:2.

24. Aksaray S, Dokuzouz B, Güvener E, Yücesoy M, Yulu N, Kocagöz S, et al. Surveillance of antimicrobial resistance among Gram-negative isolates from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother 2000;45:695-99.

25. Sader HS, Jones RN, Gales AC, Winokur P, Kugler KC, Pfaller MA, et al. Antimicrobial susceptibility patterns for pathogens isolated from patients in Latin American medical centers with a diagnosis of pneumonia: analysis of results from the SENTRY Antimicrobial Surveillance Program. Diagn Microbiol Infect Dis 1998;32:289-301.

26. James A, Mark E, Deborah C, Clyde T, Daniel F, Gregory A. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbiol Antimicrob 2004;3:7-11.

27. Rhomberg PR, Fritsche TR, Sader HS, Jones RN. Comparative antimicrobial potency of meropenem tested against Gram-negative bacilli: report from the MYSTIC surveillance program in the United States. J Chemother 2005; 17:459-69.

28. Aubert D, Poirel L, Chevalier J, Leotard S, Pages J, Nordmann P. Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in Pseudomonas aerugino. Antimicrob Agents Chemother 2001;45:1615–20.

29. Jacobson KL, Cohen SH, Inciardi JF, King JH, Lippert WE, Iglesias T, et al. The relationship between antecedent antibiotic use and resistance to extended spectrum cephalosporins in group 1 β-lactamase producing organisms. Clin Infect Dis 1995;21:1107–13.

30. Mebis J, Goossens H, Bruyneel P, Sion JP, Meeus I, Van Droogenbroeck J, et al. Decreasing antibiotic resistance of Enterobacteriaceae by introducing a new antibiotic combination therapy for neutropenic fever patients. Leukemia 1998;12:1627–29.