*Acta Pharmaceutica Turcica*
46: 67-72 (2004)

# In Vitro Study of Antibacterial Activity of Cefepime and Cefpirome against Gram-Negative Clinical Isolates, Compared with Those of Nine Broad-Spectrum Agents

**Haitham Tumah**

Department of Pharmaceutical Technology, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan

## Abstract

The in vitro activities of cefepime and cefpirome were compared with those of cefoperazone, ceftazidime, ceftriaxone, ciprofloxacin, aztreonam, gentamicin, pipercillin, tobramycin, and ticarcillin/clavulanic. 302 clinical isolates, representing a cross-section of *Klebsiella* and *Enterobacter* species and *Pseudomonas aeruginosa* were tested. Cefepime and cefpirome demonstrated excellent activity against the majority of strains in all three genera of bacteria tested, as did ciprofloxacin and tobramycin. Ceftazidime was active against *Pseudomonas aeruginosa* but was less potent against *Klebsiella* and *Enterobacter* species. Cefoperazone, and ceftriaxone were less active than ceftazidime against *Pseudomonas aeruginosa.*

**Key words**: Cefepime, Cefpirome, Antibacterial activity, Cross-resistance

## Introduction

Cefepime and cefpirome are new semi-synthetic cephalosporins which have been recently introduced into clinical practice. The remarkable increase in their activity resulting from the insertion of a quaternary ammonium group at the C-3' position of the cephem nucleus, has led to these compounds being termed "fourth-generation" cephalosporins (Pechere *et al.*, 1995.). These compounds have a more balanced antimicrobial spectrum of activity against gram-positive and gram-negative organisms compared to third-generation cephalosporins and other broad-spectrum agents (Giamarellou, 1999; Fung-Tomc, 1997; Hancock and Bellido, 1996; Marshall and Blair, 1999; Kessler, 2001; Wynd and Paladino, 1996). They exhibit poor affinity as substrates for Bush-Jacoby-Medeiros Class 1 chromosomally mediated β-lactamases and a high degree of resistance to enzymatic hydrolysis by these enzymes (Denis *et al.*, 1998; Fung-Tomc *et al.*, 1989; Jan *et al.*, 2001; Pechere *et al.*, 1995; Wynd and Paladino, 1996). These compounds also have been shown to be poor inducers of β-lactamases expression in *Enterobacter*, *Citrobacter*, *Serratia*, and *Pseudomonas* species (Barradell and Bryson, 1994; Giamarellou, 1999; Kessler, 2001; Thornsberry *et al.*, 1993; Watanabe, 1996). Because of their zwitterionic nature at physiologic pH, cefepime and cefpirome have been shown to penetrate the outer membrane porins of gram-negative bacteria faster than third-generation cephalosporins (Hancock and Bellido, 1992; kessler 2001; Pechere *et al.*, 1995). As a result of these properties, both antibiotics have shown to be highly active in vitro against a broad range of organisms frequently isolated from patients in tertiary care university hospitals (Kessler, 2001; Kuriyama *et al.*, 2002; Sofianou *et al.*, 1997). *Klebsiella* species, *Enterobacter* species, and *Pseudomonas aeruginosa* are among the most commonly isolated nosocomial pathogens in tertiary-care university hospitals (Chong *et al.*, 1993; Ronald *et al.*, 2003). *Pseudomonas aeruginosa* has long been recognized as a virulent pathogen with significant resistance to many

available antimicrobial agents. More recently, *Enterobacter* and *Klebsiella* species have emerged as important pathogens capable of exhibiting resistance to third-generation cephalosporins and other antibiotics (Carlos *et al.*, 2000; Domenech-Sanchez *et al.*, 2000; Husson *et al.*, 2000; Ronald *et al.*, 2003).

In this study, we compared the in vitro activity of 11 antimicrobial agents against 302 clinical isolates of *Klebsiella* species, *Enterobacter* species, and *Pseudomonas aeruginosa*. The agents tested were the fourth-generation cephalosporins cefepime and cefpirome; the third-generation cephalosporins, cefoperazone, ceftazidime, and ceftriaxone; the aminoglycosides, gentamicin and tobramycin; the penicillin, ticarcillin-clavulanic acid and piperacillin; the monobactam, aztreonam; and the fluoroquinolone, ciprofloxacin. Patterns of cross-resistance in strains resistant to one or more cephalosporins were examined, to see if other β-lactam agents might be useful, even when resistance to one of these agents had already been documented.

## Materials and Methods

The isolates were collected from 302 patients in tertiary care university hospitals, who had definite nosocomial infections due to *Klebsiella* species, *Enterobacter* species, or *Pseudomonas aeruginosa*. Breakdown by strains showed 103 isolates of *Klebsiella pneumoniae*, 6 of *Klebsiella oxytoca*, 61 of *Enterobacter cloacae*, 32 of *Enterobacter aerogenes*, and 100 of *Pseudomonas aeruginosa*.

The organisms were stored at -70°C in trypticase-soy broth with 20% glycerol (BBL Microbiology Systems, Cockeysville, Maryland) until ready for batch susceptibility testing. They were thawed and passed 3 times to assure purity and viability. Minimum inhibitory concentrations (MICs) were determined using the agar plate dilution method in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Antibiotics solutions were prepared on the day of use according to the manufactures recommendation, and serial 2-fold dilutions were added to molten BBL Mueller-Hinton Gold II agar (BBL Microbiology Systems, Cockeysville, Maryland). After slight cooling and drying of the plates, a Steers replicator was used to place aliquots containing approximately 5 x $10^4$ colony-forming units per drop for 28 test strains along with 4 quality control strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) per plate. The plates were incubated at 35°C and read 18 later. MIC was defined as the lowest concentration at which there was no growth, a faint haze or fewer than 3 discrete colonies. Plates were read in duplicate, and the higher MIC value was recorded.

Antibiotics were commercially obtained or kindly provided by manufacturing companies, including cefepime and aztreonam (Bristol-Myers Squibb Co., Princeton, NJ); cefpirome (Hoechst M. R, Bridgewater, NJ); ceftazidime (Glaxo; Research Triangle Park, NC), ceftriaxone (Hoffman LaRoche; Nutley, NJ), cefoperazone (Pfizer-Roering Pharmaceuticals; New York, NY), ciprofloxacin (Bayer Pharmaceutical Division; West Haven, CT), tobramycin (Eli Lilly; Indianapolis, IN), piperacillin (Lederle Laboratories; Wayne, NJ), ticarcillin-clavulanic acid (Smith-Kline Beecham; Philadelphia, PA), and gentamicin (Schering-Plough; Bloomfield, NJ). Quality control strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), were purchased from American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852, USA.

## Results

Table 1 shows the range of observed antimicrobial MIC values and the MIC required to inhibit 50% and 90% of isolates ($MIC_{50}$ and $MIC_{90}$, respectively), and percentage of isolates of the three genera susceptible at breakpoint to each of the antimicrobial agents.

Table 1. Comparison of in vitro antibacterial activity of 11 broad-spectrum agents against 302 strains of nosocomial bacteria

| Organism (no. tested) and antimicrobial agent | MIC (μg/ml) | | | % Susceptible |
|---|---|---|---|---|
| | Range | MIC50 | MIC90 | |
| *Klebsiella* species (109) | | | | |
| Cefepime | 0.015-128 | 0.03 | 0.25 | 98 |
| Cefpirome | 0.015-256 | 0.06 | 0.5 | 95 |
| Cefoperazone | 0.03-256 | 0.25 | 4 | 92 |
| Ceftazidime | 0.03-256 | 0.125 | 2 | 91 |
| Ceftriaxone | 0.015-256 | 0.03 | 0.5 | 93 |
| Ciprofloxacin | 0.008-16 | 0.06 | 0.25 | 94 |
| Aztreonam | 0.015-256 | 0.03 | 2 | 92 |
| Gentamicin | 0.125-64 | 0.5 | 2 | 90 |
| Piperacillin | 1-256 | 4 | 128 | 86 |
| Tobramycin | 0.5-16 | 2 | 2 | 91 |
| Ticarcillin/clavulanic* | 1-256 | 2 | 16 | 90 |
| *Enterobacter* species (93) | | | | |
| Cefepime | 0.015-16 | 0.06 | 2 | 99 |
| Cefpirome | 0.015-32 | 0.125 | 4 | 97 |
| Cefoperazone | 0.125-256 | 0.25 | 64 | 70 |
| Ceftazidime | 0.06-128 | 0.25 | 64 | 75 |
| Ceftriaxone | 0.03-256 | 0.125 | 32 | 73 |
| Ciprofloxacin | 0.008-8 | 0.03 | 0.06 | 97 |
| Aztreonam | 0.03-128 | 0.125 | 64 | 70 |
| Gentamicin | 0.25-64 | 0.5 | 1 | 95 |
| Piperacillin | 1-256 | 2 | 128 | 74 |
| Tobramycin | 0.25-64 | 0.5 | 1 | 95 |
| Ticarcillin/clavulanic acid* | 0.5-256 | 4 | 128 | 65 |
| *Pseudomonas aeruginosa* (100) | | | | |
| Cefepime | 0.125-32 | 2 | 16 | 86 |
| Cefpirome | 0.125-32 | 4 | 32 | 81 |
| Cefoperazone | 0.5-256 | 8 | 128 | 75 |
| Ceftazidime | 0.5-128 | 2 | 16 | 84 |
| Ceftriaxone | 1-256 | 16 | 256 | 11 |
| Ciprofloxacin | 0.06-16 | 0.25 | 2 | 88 |
| Aztreonam | 0. 25-128 | 4 | 32 | 79 |
| Gentamicin | 0.5-64 | 4 | 8 | 79 |
| Piperacillin | 0.5-256 | 4 | 128 | 89 |
| Tobramycin | 0.5-64 | 1 | 2 | 97 |
| Ticarcillin/clavulanic acid* | 1-256 | 32 | 128 | 81 |

* Ticarcillin combinations included a fixed concentration of clavulanic acid (2 μg/ml)

*Klebsiella* species were generally more susceptible to the antimicrobial agents tested than were *Enterobacter* or *Pseudomonas* species. All antimicrobial agents inhibited at least 90% of the isolates of *Klebsiella* at or below their breakpoint. Of the 11 *Klebsiella* strains that demonstrated resistance to ceftazidime, cefoperazone, or ceftriaxone (10% of total isolates

tested), 9 were susceptible to cefepime and 8 were susceptible to cefpirome, 4 were susceptible to ciprofloxacin (Table2).

Table 2. Comparison of cross-resistance among selected antimicrobial agents-number of resistant strains*

| Organism | n | Cefepime | Cefpirome | Ceftazidime | Cefoperazone | Ceftriaxone | Ciprofloxacin |
|---|---|---|---|---|---|---|---|
| *Klebsiella* spp. | 11 | 2 | 3 | 10 | 9 | 8 | 7 |
| *Enterobacter* spp. | 26 | 1 | 3 | 24 | 15 | 25 | 3 |
| *Ps. aeruginosa* | 89 | 14 | 19 | 16 | 25 | 89 | 12 |
| Total | 126 | 17 (13.5%) | 27(21.4%) | 50 (39.7%) | 49 (38.9%) | 122(96.8%) | 22 (17.5%) |

* 126 isolates were resistant to one or more of the following antibiotics: ceftazidime, cefoperazone, and ceftriaxone. Of these, 109 were susceptible to cefepime and 99 were susceptible to cefpirome.

*Enterobacter* species were less susceptible to the third-generation cephalosporins than were *Klebsiella* species. Ceftazidime, ceftriaxone, and cefoperazone each inhibited 75% or fewer of the 93 isolates tested at the susceptibility breakpoint. Of the 26 strains resistant to ceftazidime, cefoperazone, or ceftriaxone (28% of strains tested), 96% were susceptible to cefepime, and 88% were susceptible to cefpirome, 88% were susceptible to ciprofloxacin (Table 2). The combination of ticarcillin and clavulanic acid was no more active than was pipercillin alone.
*Pseudomonas aeruginosa* isolates had the greatest proportion of resistant strains. Although cefepime , cefpirome, ceftazidime, ciprofloxacin, piperacillin, and tobramycin each inhibited >85% of the isolates, 89% were resistant to one or more of the third-generation cephalosporins. Of these 89 resistant isolates, 84% were susceptible to cefepime, 79% were susceptible to cefpirome, 82% were susceptible to ceftazidime, 87% were susceptible to ciprofloxacin, 72% were susceptible to cefoperazone, and all isolates were resistant to ceftriaxone (Table2).
The data in table I indicate that cefepime was considerably more active against *Klebsiella* species and *Enterobacter* species, and demonstrated similar activity to ceftazidime against *Pseudomonas aeruginosa.*

## Discussion

In this study, the activities of fourth-generation cephalosporins, cefepime and cefpirome were compared with those of a large number of parenteral antimicrobial agents commonly used to treat serious infections caused by gram-negative bacillary.
The cephalosporins have been widely accepted in the treatment of bacterial infection because of their excellent clinical profile, including safety and pharmacokinetic features (Fung-Tomc, 1997; Joukhadar *et al.,* 2002; Marshall and Blair, 1999). However, newer antimicrobial agents are constantly being sought to overcome emerging bacterial resistance. The fourth-generation cephalosporin, cefepime and cefpirome, were found to be more potent than the third-generation cephalosporins and other agents tested against *Klebsiella* species. However, in the case of *Enterobacter* species, a 25-30% greater susceptibility rate was noted for cefepime and cefpirome (99% and 97%, respectively) than for the other cephalosporins. Compared to the third-generation cephalosporins, cefepime was clearly more active against *Pseudomonas aeruginosa* (86%) than ceftriaxone (11%) and cefoperazone (75%) but was similarly as active as ceftazidime (84%) (Barradell and Byson, 1994; Bell and Turnidge, 2001; Ramphal *et al.,* 2000; Sofianou *et al.,* 1997; Thornsberry *et al.,* 1993). Cefpirome (81%) was slightly less active than cefepime and ceftazidime but more active than ceftriaxone, and cefoperazone against *Pseudomonas aeruginosa.* Although this study did not specifically select for highly resistant strains, these were nevertheless present in the sample of isolates collected, and their

susceptibility to cefepime and cefpirome appeared to be equal to or greater than their susceptibility to the other cephalosporins. Cefepime had slightly greater activity than cefpirome against gram-negative bacillary tested (Jan *et al.*, 2001). Cefepime and cefpirome were similarly as active as ceftazidime, and ciprofloxacin against the large number of *Pseudomonas aeruginosa* isolates that were resistant to ceftriaxone (89%).

## References


Barradell, L.B. and Bryson, H.M. (1994). Cefepime, a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs* 47:471-505.

Bell, J.M., Turnidge, J.D. and Cefepime Study Group (2001). Multicentre study of the in vitro activity of cefepime, a broad-spectrum cephalosporin, compared to other broad-spectrum agents. *Pathology* 33:53-60.

Carlos, B., Marcela, D.C., Federico, N., Silvia, R. and Jorgelina, S. (2000). Comparative in vitro bactericidal activity between cefepime and ceftazidime, alone and associated with amikacin, against carbapenem-resistant Pseudomonas aeruginosa strains. *Diagnostic Microbiology and Infectious Disease* 37:41-44.

Chong, Y., Lee, K. and Kwon, O.H. (1993). In-vitro activities of cefepime against Enterobacter cloacae, Serratia marcescens, Pseudomonas aeruginosa and other aerobic gram-negative bacilli. *J. Antimicrob. Chemother.* 32:21-29.

Denis, P., Kistof, E., Sabine, L., and a Belgian Multicentre Study Group (1998). Comparative in-vitro activity of cefpirome against isolates from intensive care and hematology/oncology units. *J. Antimicrob. Chemother.* 41:443-450.

Domenech-Sanchez, A., Pascual, A., Suarez, A.I., Alvarez, D., Benedi, V.J., Martinez-Martinez, L. (2000). Activity of nine antimicrobial agents against clinical isolates of Klebsiella pneuminiae producing extended-spectrum β-lactamases and deficient or not in porins. *J. Antimicrob. Chemother.* 46:847-863.

Fung-Tomc, J., Dougherty, T.J., Simich-Jacobson, V. and Kessler, R.E. (1989). Activity of cefepime against ceftazidime- and cefotaxime-resistant gram-negative bacteria and its relationship to β-lactamase levels. *Antimicrob. Agents Chemother.* 33:498-502.

Fung-Tomc, J.C. (1997). Fourth-generation cephalosporins. *Clin. Microbiol. Newsl.* 19:129-131.

Giamarellou, H. (1999). Fourth-generation cephalosporins in the antimicrobial chemotherapy of surgical infections. *J. Chemother.* 11:486-493.

Hancock, R.E.W.and Bellido, F. (1992). Factors involved in the enhanced efficacy against gram-negative bacteria of fourth-generation cephalosporins. *J. Antimicrob Chemother.* 29:1-6.

Hancock, R.E. and Bellido, F. (1996). Antibacterial in vitro activity of fourth-generation cephalosporins. *J. Chemother.* 2:31-6.

Husson, M.O., Quelquejay, J., Fruchart, A. and Izard, D. (2000). 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.* 48:933-939.

Jan, M., Bell., John, D., Turnidge., and the Cefepime Study Groups (2001). Multicentre study of the in vitro activity of cefepime, a broad-spectrum cephalosporin, compared to other broad-spectrum agents. *Pathology* 33:53-60.

Joukhadar, C., Klein, N., Mayer, B.X., Kreischitz, N., Delle-Karth, G., Palkovits, P., Heinz, G. and Muller, M. (2002). Plasma and tissue pharmacokinetics of cefpirome in patient with sepsis. *Crit. Care. Med.* 30:1478-1481.

Kessler, R.E. (2001). Cefepime microbiologic profile and update. *Pediatr. Infect. Dis. J.* 20:331-336.

Kuriyama, T., Karasawa, T., Nakagawa, K., Nakamura, S. and Yamamoto, E. (2002). Antimicrobial susceptibility of major pathogens of orofacial odontogetic infections to 11 beta-lactam antibiotics. *Oral. Microbiol. Immunol.* 5:285-289.

Marshall, W.F., Blair, J.E. (1999). The cephalosporins: Symposium on Antimicrobial Agents. *Mayo Clin. Proc.* 74: 187-195.

National Committee for Clinical Laboratory Standards (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard-Fifth edition. *NCCLS* document M7-A5, Wayne, Pennsylvania 19087-1898, USA.

Pechere, J.C., Wilson, W. and Neu, H., (1995). Laboratory assessment of antibacterial activity of zwitterionic 7- methoxyimino cephalosporins. *J. Antimicrob. Chemother.* 36: 757-771.

Ramphal, R., Hoban, D.J., Pfaller, M.A. and Jones, R.N. (2000). Comparison of the activity of two broad-spectrum cephalosporins tested against 2,299 strains of Pseudomonas aeruginosa isolated at 38 North American medical centers participating in the SENTRY Antimicrobial Surveillance Program, 1997-1998. *Diagn. Microbiol. Infect. Dis.* 36:125-129.

Ronald, N., Jones., Douglas, J., Biedenbach., Ana, C., Gales (2003). Sustained activity and spectrum of selected extended-spectrum β-lactams (carbapenems and cefepime) against Enterobacter spp. and ESBL-producing Klebsiella spp.: report from the SENTRY antimicrobial surveillance program (USA, 1997-2000). *International Journal of Antimicrobial Agents* 21:1-7.

Sofianou, D., Tsoulfa, S., Kontodimou, L., Polydorou, F. and Malaka, E. (1997). Comparative in vitro activity of cefepime against nosocomial isolates. *J. Chemother.* 5:341-346.

Thornsberry, C., Brown, S.D., Yee, Y.C., Bouchillon, S.K., Marler, J.K.and Rich, T. (1993). In- vitro activity of cefepime and other antimicrobials: survey of European isolates. *J. Antimicrob. Chemother.* 32:31-53.

Watanabe, N.A. (1996). Newer antipseudomonal cephalosporins. J. Chemother. 2:48-56.

Wynd, M.A. and Paladino, J.A. (1996). Cefepime: a fourth-generation parenteral cephalosporins. *Ann. Pharmacother.* 30:1414-1424.

*Received:30.09.2003*
*Accepted:29.11.2003*