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In vitro activity of cefepime and cefpirome compared to other third-generation cephem antibiotics against gram-negative nosocomial pathogens

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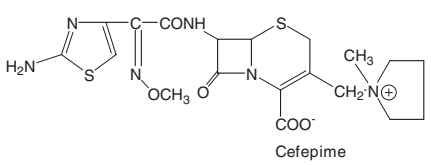
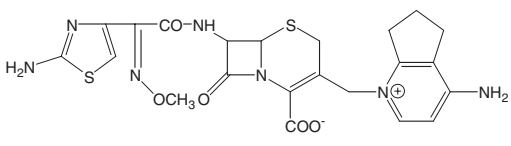
The *in vitro* activities of new expanded spectrum of fourth-generation cephalosporins, cefepime and cefpirome, were compared with those of three third-generation cephalosporins, cefoperazone, ceftazidime, and ceftriaxone, that are commonly used in the treatment of serious infections caused by aerobic gram-negative bacteria. The agar dilution method described by the US National Committee for Clinical Laboratory Standards was used to determine the minimum inhibitory concentrations of antibiotics tested. 302 clinical isolates, representing a cross-section of *Klebsiella* and *Enterobacter* species and *Pseudomonas aeruginosa* were tested. Cefepime was considerably more active than other antibiotics tested, against *Klebsiella* species and *Enterobacter* species, and demonstrated activity similar to ceftazidime against *Pseudomonas aeruginosa*. Ceftazidime was active against *Pseudomonas aeruginosa* but was less potent against *Enterobacter* species. Cefoperazone and ceftriaxone were less active than ceftazidime against *Pseudomonas aeruginosa.* Cefepime had slightly greater activity than cefpirome against the gram-negative bacteria tested. However, cefepime and cefpirome were found to be highly active against many resistant organisms that traditionally have been difficult to treat.

1. Introduction

Cephalosporins have been in extensive use since the 1960s because of their broad spectrum of antibacterial activity and favorable safety record (Fung-Tomc 1997; Marshall and Blair 1999; Robert et al. 1996). Over the past several decades major improvements in cephalosporins were achieved by modifying the basic structure of moieties attached to the cephem nucleus. Structural modifications of the cephem nucleus have produced a variety of antibacterial agents with improved intrinsic activity as well as improved β-lactamase stability. Cefepime and cefpirome are new quaternary ammonium cephalosporins that have been introduced into clinical practice. The considerable increase in potency resulting from the insertion of a quaternary ammonium group at the C-3′ position has led to these compounds being termed "fourth-generation" cephalosporins. These compounds have a more balanced antimicrobial spectrum of activity against gram-positive and gram-negative organisms compared to third-generation cephalosporins (Giamarellou 1999; Hancock and Bellido 1996; Kessler 2001; Pechere and Wilson 1995; 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-lactamase 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). This benefit is also clinically relevant (Giamarellou 1993; Mouton et al. 1993).



Cefepime



Cefpirome

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). These three genera account for 15–35% of all serious nosocomial infections (Schaberg et al. 1991)). *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 (Carlos et al. 2000; Domenech-Sanchez et al. 2000; Husson et al. 2000; Ronald et al. 2003). The severity of the infections caused by these three genera of bacteria in hospitalized patients puts a premium on developing new drugs with high *in vitro* activity, clinical efficacy, low toxicity and potential for resistance.

In this study, we compared the *in vitro* activity of 5 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. 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.

2. Investigations and results

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 percent susceptible at breakpoint for three genera to each of the antimicrobial agents are given in Table 1.

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 (Table 2).

Enterobacter was generally less susceptible to the third-generation cephalosporins than was *Klebsiella*. Ceftazidime, ceftriaxone, and cefoperazone each inhibited $\leq 75\%$ of the 93 isolates tested at the susceptibility breakpoint. Of the 26 strains resistant to cefoperazone, ceftazidime, or ceftriaxone (29% of strains tested), 96% were susceptible to cefepime, and 88% were susceptible to cefpirome (Table 2).

Pseudomonas aeruginosa isolates had the greatest proportion of resistant strains. Although cefepime and cefpirome 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, 72% were susceptible to cefoperazone, and all isolates were resistant to ceftriaxone (Table 2). The rank order of activity of the cephalosporins against *Pseudomonas aeruginosa* was cefepime (86% susceptible) > ceftazidime (84% susceptible) > cefpirome (81% susceptible) > cefoperazone (75% susceptible) > ceftriaxone (11% susceptible) (Table 1).

As a majority of the published cefepime clinical trials have used ceftazidime as the comparator drug, the relative

Table 1: Comparison of the *in vitro* antibacterial activity of 5 antimicrobial agents against 302 strains of nosocomial bacteria

Organism (no. tested) and antimicrobial agent	MIC (μg/ml)			% Susceptible
	Range	MIC_{50}	MIC_{90}	
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
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
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

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

Organism	n	Cefepime	Cefpirome	Ceftazidime	Cefoperazone	Ceftriaxone
Klebsiella species	11	2	5	10	9	8
Enterobacter species	26	1	3	24	15	25
Pseudomonas aeruginosa	89	14	19	16	25	89
Total	126	17 (13.5%)	27 (21.4%)	50 (39.7%)	49 (38.9%)	122 (96.8%)

* 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.

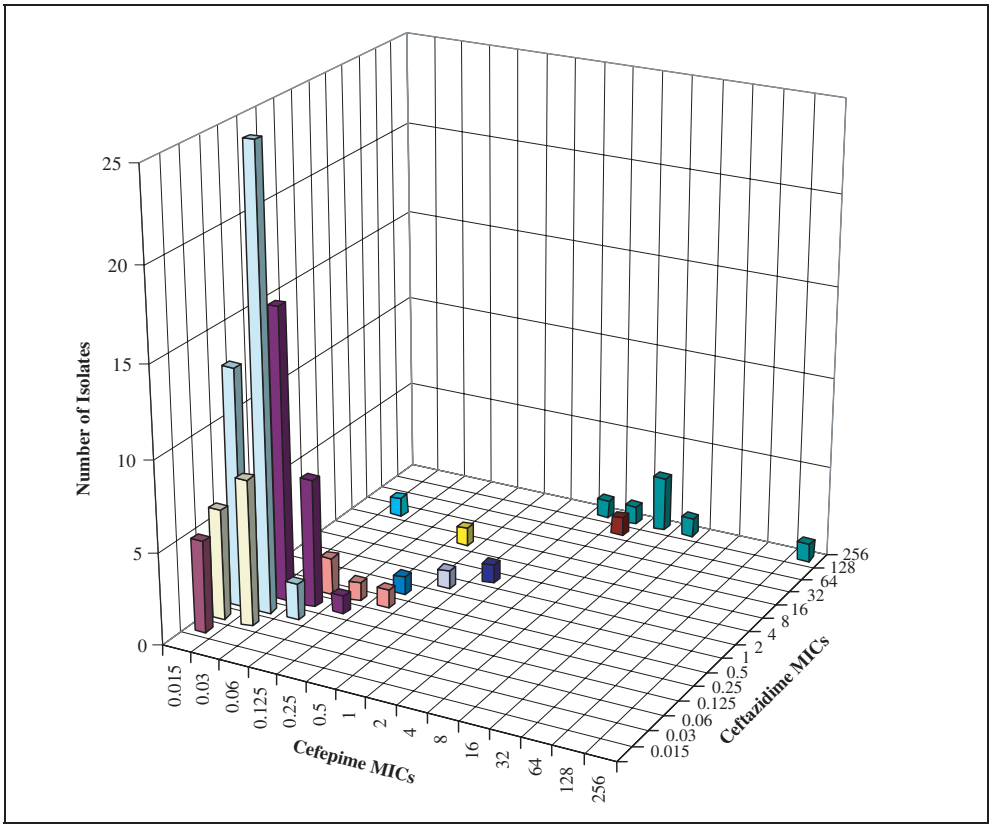


Fig. 1:
Comparison of *Klebsiella* spp. MICs (μg/mL) for cefepime and ceftazidime (n = 109). The height of the columns indicates the number of isolates with a given relative combination of susceptibilities. The NCCLS breakpoint for susceptible organisms is 8 μg/mL for both agents

MIC values of these two agents were plotted for each of the three genera (Fig. 1–3). Breakpoints for resistance are shown on these charts. These data indicate that cefepime was considerably more active against *Klebsiella* species and *Enterobacter* species, and demonstrated similar activity to ceftazidime against *Pseudomonas aeruginosa.*

3. Discussion

In the current study, the activities of fourth-generation cephalosporins, cefepime and cefpirome were compared with those of three parenteral third-generation cephalosporins, commonly used to treat serious infections caused by gram-negative bacilli.

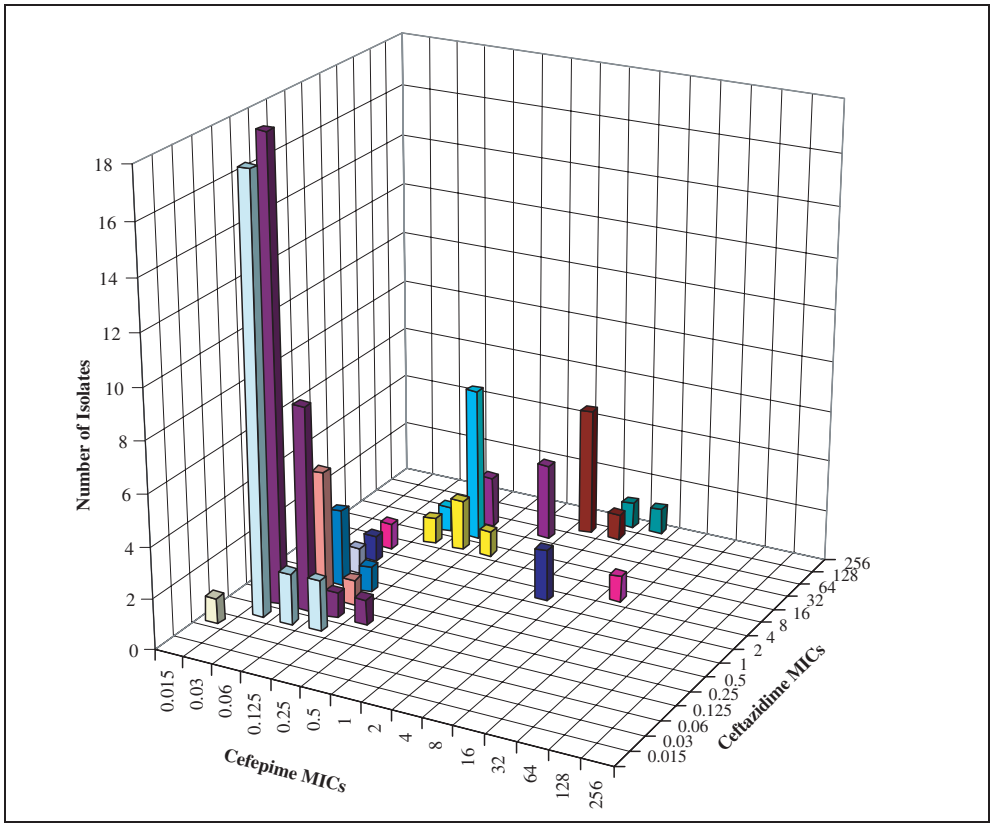


Fig. 2:
Comparison of *Enterobacter* spp. MICs (μg/mL) for cefepime and ceftazidime (n = 93). The height of the columns indicates the number of isolates with a given relative combination of susceptibilities. The NCCLS breakpoint for susceptible organisms is 8 μg/mL for both agents

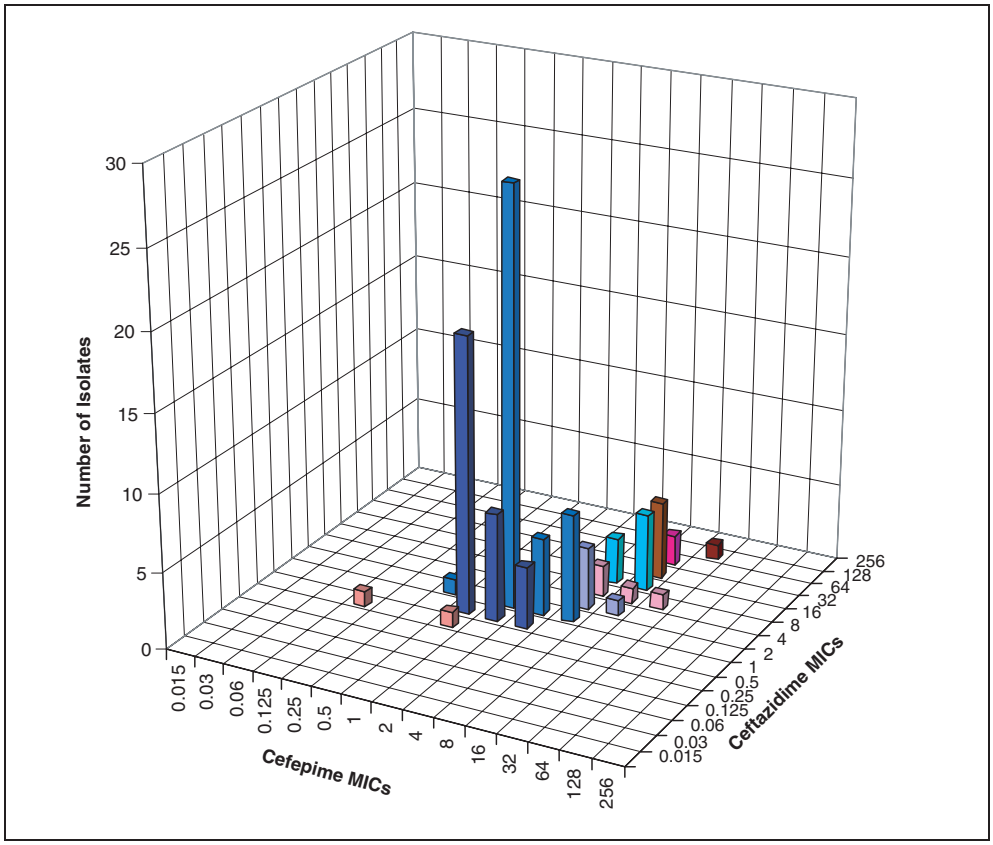


Fig. 3:
Comparison of *Pseudomonas aeruginosa* MICs (μg/mL) for cefepime and ceftazidime (n = 100). The height of the columns indicates the number of isolates with a given relative combination of susceptibilities. The NCCLS breakpoint for susceptible organisms is 8 μg/mL for both agents

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 antimicrobials are constantly being sought to overcome emerging bacterial resistance. The fourth-generation cephalosporins, cefepime and cefpirome, were found to be slightly more potent than the third-generation cephalosporins 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).

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 appeared to be equal to or greater than their susceptibility to the other cephalosporins. Cefepime had slightly greater activity than cefpirome against gram-negative bacilli tested (Jan et al. 2001). Both antibiotics, cefepime and cefpirome demonstrated excellent *in vitro* activity against multiple isolates of *Klebsiella*, *Enterobacter*, and *Pseudomonas aeruginosa* from a hospital setting, including many resistant to other antimicrobials. However, the clinical role of cefepime and cefpirome will largely depend on their efficacy in clinical studies of patients with infections caused by bacteria that are difficult to treat. Preliminary clinical trials indicate that cefepime and cefpirome may be valuable in the treatment of serious bacterial infections caused by *Enterobacter* species, *Pseudomonas aeruginosa*, and other gram-negative or gram-positive pathogens (Giamarellou 1999; Joukhadar et al. 2002; Kessler 2001; Lewis et al. 1999).

4. Experimental

4.1. Materials

The antimicrobial agents were obtained as follows: cefepime (Bristol-Myers Squibb Co., Princeton, New Jersey); cefpirome (Hoechst Marion Roussel, Bridgewater, New Jersey); ceftazidime (Glaxo, Inc., Research Triangle Park, North Carolina); ceftriaxone (Hoffmann LaRoche, Nutley, New Jersey); cefoperazone (Pfizer-Roerig Pharmaceuticals, West Haven, Connecticut). 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.

4.2. 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 pneumonie*, 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) document (NCCLS 2000).

Antibiotics were dissolved in the appropriate diluent, 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 5×10^4 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 h 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. Breakpoints for susceptibility were taken from the NCCLS; the proposed breakpoint of 8 μg/ml was used for cefepime.

References

Barradell LB, Bryson HM (1994) Cefepime, a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 47: 471–505.

Bell JM, Turnidge JD, 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 DC, Federico N, Silvia R, Jorgelina S (2000) Comparative *in vitro* bactericidal activity between cefepime and ceftazidime, alone and associated with amikacin, against carbapenem-resistant *Pseudomonas aeruginosa* strains. Diag Microbiol Infect Dis 37: 41–44.

Chong Y, Lee K, Kwon OH (1993) *In-vitro* activities of cefepime against *Enterobacter cloacae*, *Serratia marcescens*, *Pseudomonas aeruginosa* and other aerobic gram-negative bacilli. J Antimicrob Chemother 32: 21–9.

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 AI, Alvarez D, Benedi VJ, Martinez-Martinez L (2000) Activity of nine antimicrobial agents against clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases and deficient or not in porins. J Antimicrob Chemother 46: 847–863.

Fung-Tomc J, Dougherty TJ, Simich-Jacobson V, Kessler RE (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 JC (1997) Fourth-generation cephalosporins. Clin Microbiol Newsl 19: 129–31.

Giamarellou H (1993) Low-dosage cefepime as treatment for serious bacterial infections. J Antimicrob Chemother 32: 123–132.

Giamarellou H (1999) Fourth generation cephalosporins in the antimicrobial chemotherapy of surgical infections. J Chemother 11: 486–93.

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

Hancock RE, Bellido F (1996) Antibacterial *in vitro* activity of fourth generation cephalosporins. J Chemother 2: 31–6.

Husson MO, Quelquejay J, Fruchart A, 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: 933–9.

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 BX, Kreischitz N, Delle-Karth G, Palkovits P, Heinz G, Muller M (2002) Plasma and tissue pharmacokinetics of cefpirome in patient with sepsis. Crit Care Med 30: 1478–82.

Kessler RE (2001) Cefepime microbiologic profile and update. Pediatr Infect Dis J 20: 331–336.

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

Lewis MT, Biedenbach DJ, Jones RN (1999) *In vitro* evaluation of cefepime and other broad-spectrum beta-lactams against bacteria from Indonesian medical centers. The Indonesia antimicrobial resistance study group. Diagn Microbiol Infect Dis 35: 285–90.

Marshall WF, Blair JE (1999) The cephalosporins: Symposium on antimicrobial Agents. Mayo Clin Proc 74: 187–95.

Mouton Y, Chidiac C, Humbert G, et al. (1993) A non-comparative, multicentre study of cefepime in the treatment of serious bacterial infections. J Antimicrob Chemother 32: 133–140.

National Committee for Clinical Laboratory Standards, NCCLS (2000) Methods for antimicrobial susceptibility testing of aerobic bacteria. Approved standard. Villanova, Pa, M7-A5.

Pechere JC, Wilson W, Neu H (1995) Laboratory assessment of antibacterial activity of zwitterionic 7-methoxyimino cephalosporins. J Antimicrob Chemother 36: 757–71.

Ramphal R, Hoban DJ, Pfaller MA, Jones RN (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: 125–9.

Robert E, Kessler, Fung-Tome J, Wallingford, Connecticut (1996) Susceptibility of bacterial isolates to β-lactam antibiotics from U.S. clinical trials over a 5-years period. Am J Med 100: 6A–19S.

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.

Schaberg DR, Culver DH, Gaynes RP (1991) Major trends in the microbial etiology of nosocomial infections. Am J Med 3b: 72S–75S.

Sofianou D, Tsoulfa S, Kontodimou L, Polydorou F, Malaka E (1997) Comparative *in vitro* activity of cefepime against nosocomial isolates. J Chemother 5: 341–6.

Thornsberry C, Brown SD, Yee YC, Bouchillon SK, Marler JK, Rich T (1993) *In-vitro* activity of cefepime and other antimicrobials: survey of European isolates. J Antimicrob Chemother 32: 31–53.

Watanabe NA (1996) Newer antipseudomonal cephalosporins. J Chemother 2: 48–56.

Wynd MA, Paladino JA (1996) Cefepime: a fourth-generation parenteral cephalosporin. Ann Pharmacother 30: 1414–1424.