Comparative *in vitro* Activities of Seven New β-Lactams, Alone and in Combination with β-Lactamase Inhibitors, Against Clinical Isolates Resistant to Third Generation Cephalosporins

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We examined the drug susceptibility pattern of Gram–negative bacilli to seven new β-lactams. A total of 277 non-duplicate gramnegative bacilli strains belonging to the Enterobacteriaceae family, *Pseudomonas* and *Acinetobacter* species, isolated from various clinical samples were tested for susceptibility to imipenem, piperacillin/tazobactam, cefoperazone/sulbactam, ticarcillin/clavulanate, cefdinir, cefepime and cefpirome with the disk diffusion technique. The percentage resistance was low for imipenem (7.2%), piperacillin/tazobactam (2.8%), cefoperazone/sulbactam (5.4%). However, a high frequency of resistance was observed to ticarcillin/clavulanate (83.9%), cefdinir (70.6%), cefepime (45.5%) and cefpirome (84.4%). We conclude that imipenem, piperacillin/tazobactam and cefoperazone/sulbactam are effective antibiotics in our environment, whereas ticarcillin/clavulanate, cefdinir, cefepime and cefpirome are relatively uneffective.

Key Words: β-lactam, β-lactam/β-lactamase inhibitor, Gram-negative bacilli.

In any community or nosocomial setting, widespread antibiotic usage influences the prevalence and distribution of antibiotic resistance in common pathogens. Antimicrobial usage is the only form of medical treatment where the choice of therapy for one patient can affect diseases suffered in the future by another, through the selection of resistant organisms followed by cross-infection to the new host.

Multiple drug resistance (MDR) mediated through R plasmids among Gram-negative bacteria has become a major nosocomial problem worldwide [1]. Due to multiple drug resistance to β-lactams, aminoglycosides and quinolones, antimicrobial treatment of nosocomial infections caused by these bacteria is compromised [2]. Among the β-lactams, third generation cephalosporins, such as ceftazidime, cefotaxime, and ceftriaxone are routinely used in our clinical setting, and resistance to these drugs, due to β-lactamase production, is rampant [3]. Broad-spectrum β-lactams, such as imipenem, cefdinir, cefepime and cefpirome, and β-lactamase inhibitor combinations, such as piperacillin/tazobactam, cefoperazone/sulbactam and ticarcillin/clavulanate, have been introduced in the market to overcome this resistance.

Imipenem, a broad spectrum β-lactam antibiotic and the first carbapenem to be available for clinical use, is an important drug for the treatment of serious Gram-negative bacterial infections. It offers the advantage of being more stable to most β-lactamases than third generation cephalosporins [4]. Piperacillin is a potent broad–spectrum ureidopenicillin, and tazobactam has potential for enhancing the clinical efficacy of piperacillin [5]. The combination of cefoperazone/sulbactam shows a marked degree of synergy against organisms that are resistant to cefoperazone alone, including *Acinetobacter* species and *Enterobacter* species [6]. Ticarcillin/clavulanate can be recommended for treatment of serious infections due to the synergistic effect between ticarcillin and clavulanate against *Enterobacteriaceae* and *Pseudomonas* [7]. Cefdinir, an advanced third-generation broad-spectrum oral cephalosporin, has good activity against many β-lactamase-producing organisms [8]. Cefepime and cefpirome, fourth generation cephalosporins that are resistant to many β-lactamases, are available for treatment of resistant nosocomial pathogens [9].

We measured the degree of *in vitro* activity of these new β-lactam drugs against clinical isolates belonging to the family *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* species, whichwere resistant to routinely-used third-generation cephalosporins, such as ceftazidime, cefotaxime and ceftriaxone.

Material and Methods

Test organisms. A total of 277 non-duplicate strains of Gram-negative bacteria isolated from in-patients of the Government Chandigarh Medical College and hospital, during the period of November 2002 to October 2003, were included in the study. The isolates were from wounds, sputum, tracheal secretions, bronchoalveolar lavage and various body fluids. Their pathogenic role was assigned only when isolated in pure culture from sites that are normally sterile (pus, body fluids) or when grown predominantly on repeated culture from sites with commensal flora (throat swab, sputum). The strains were identified and characterized by the following tests: gram stain, oxidase test, catalase test, motility by both hanging drop and semi-solid agar methods, Hugh & Leifson O/F test, citrate utilization, urease production, nitrate reduction, indole production, phenylpyruvic acid production, pigment

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production, lysine & ornithine decarboxylation, arginine dehydrolase test, and oxidation of 1% glucose, lactose, sucrose and mannitol [10].

Antibiotic sensitivity. Sensitivity to antibiotics was determined by the disk diffusion method of Stokes on Mueller-Hinton agar, as recommended by NCCLS [11]. All 277 strains were resistant to the third generation cephalosporins, ceftazidime, cefotaxime and ceftriaxone (30µg), and they were further tested against imipenem (10 µg), piperacillin/tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg), ticarcillin/clavulanate (75/10 µg), cefdinir (5 µg), cefpirome (30 µg), and cefepime (30 µg). All the above-mentioned antimicrobial discs were obtained from Hi-media, Mumbai (India), except cefoperazone/sulbactam, which was obtained from Pfizer (India). The control strains used were *E. coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662.

Results

The 277 strains isolated included *Pseudomonas* species (113), *Acinetobacte*r species (63), *E. coli* (42), *Klebsiella pneumoniae* (23), *Klebsiella oxytoca* (3), *Proteus vulgaris* (6), *Proteus mirabilis* (6), *Enterobacter* species (18) and *Citrobacter* species (3).

Overall only 7.2% of the 237 strains tested against imipenem showed resistance; the remaining 92.8% were sensitive (Table 1). Among these resistant strains, *Pseudomonas* species comprised 5.9%, *E coli* accounted for 0.8% and *Klebsiella pneumoniae*, 0.4%. None of the *Acinetobacter* species, as well as *Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris* and *Enterobacter* species were resistant to imipenem (Table 2).

Only 2.8% out of the143 isolates were resistant to piperacillin/tazobactam. One (0.8%) strain of *Pseudomonas* species and 3 (2%) strains of *Acinetobacter* species showed resistance. The rest of the139 strains (97.2%) were sensitive (Table 1).

Similarly, among the 150 isolates tested against cefoperazone/sulbactam, only 5.4 % were resistant. These included 4% *Pseudomonas* species and 0.7% each of *Acinetobacter* and *Enterobacter* species. No resistance was seen in *E. coli, Klebsiella oxytoca, Proteus mirabilis* and *Proteus vulgaris*.

Ticarcillin/clavulanate was tested against 31 isolates. Of these, 84% were resistant, including 29% *Pseudomonas* species, 35% *Acinetobacter* species, 10% *E. coli*, 6.4% *Klebsiella pneumoniae* and 3.5% *Enterobacter* (Table1).

Just over 70% of the 75 strains tested with cefdinir showed resistance. Among these, 6.8% were *Pseudomonas* species, 21.3% *Acinetobacter* species, 22.7% *E. coli*, 8% *Klebsiella pneumoniae*, 4% each of *Proteus vulgaris* and *Enterobacter* species, 2.6% *Proteus mirabilis* and 1.3% *Citrobacter* species.

Cefepime was moderately effective, with 45.5% of the 112 strains found resistant to it. Maximum resistance was seen with *Acinetobacter* species (13.3%), followed by *E. coli* (12.5%) and *Pseudomonas* species (11.6%). Cefpirome, another fourth generation cephalosporin, gave a very high resistance rate of 84% among the 45 strains tested against it. Among these resistant strains, there were 20% each of *Pseudomonas* species and *Acinetobacter* species, 33.4% *E. coli*, 4.4% each of *Klebsiella pneumoniae* and *Enterobacter* species and 2% *Citrobacte*r species (Table 2).

Table 1. Resistance and sensitivity percentages to new β-lactams

Antibiotics	% Resistance	% Sensitivity
Imipenem	7.2%	92.8%
Piperacillin/tazobactam	2.8%	97.2%
Cefoperazone/sulbactam	5.4%	94.6%
Ticarcillin/clavulanate	83.9%	16.1%
Cefdinir	70.6%	29.4%
Cefepime	45.5%	54.5%
Cefpirome	84.4%	15.6%

Discussion

Enterobacteriaceae and nonfermenting Gram-negative bacteria (nonfermenters) have emerged as important nosocomial pathogens, causing opportunistic infections in immunocompromised hosts [12]. The degree of resistance to antimicrobials has increased over the years. The use of broad-spectrum ß-lactams or a combination of ß-lactamase inhibitor with ß-lactams is currently the most successful strategy to combat resistance. Clinical experience confirms their effectiveness in the treatment of serious life-threatening and antibiotic-resistant infections [5].

In our study, resistance to imipenem was found to be low (7.2%), including 5.9% *Pseudomonas aeruginosa*, 0.8% *E. coli* and 0.4% *Klebsiella pneumoniae*. In an American study, resistance to imipenem by the E-test method was low, 4.2% [13]. Also meropenem, another carbapenem, was the most active drug (9.5% resistance) for *Pseudomonas aeruginosa* in a Belgian study [14]. However, Taneja et al. in India found a higher resistance rate of 36.4% in *Pseudomonas aeruginosa* and 42% in *Acinetobacter* species [12]. This difference can be attributed to the variation of resistance patterns to antimicrobials based on their usage; in our institute imipenem is still used as a reserve drug.

The resistance rate against piperacillin/tazobactam was lowest at 2.8%. An earlier study also suggested that piperacillin/tazobactam is a valuable approach for the treatment of infections caused by β-lactamase producing bacteria. In a study made in India, low resistance (16.3%) to piperacillin/tazobactam among Gram-negative bacilli was noted [15]. Similarly in America, Johnson et al. found a resistance rate of 5.8% only for piperacillin/tazobactam [13].

Table 2. Distribution of Drug Resistance Percentages Amongst the Isolated Gram-negative bacilli

Organisms isolated	Imipenem (n=237)	Piperacillin/ Tazobactam (n= 143)	Cefoperazone/ Sulbactam (n=150)	Ticarcillin/ clavulanate (n=31)	Cefdinir (n=75)	Cefepime (n=112)	Cefpirome (n=45)
Pseudomonas aeruginosa	5.9%	0.8%	4%	29%	68.5%	11.6%	20%
Acinetobacter species	0%	2%	0.7%	35%	21.3%	13.3%	20%
E. coli	0.8%	0%	0%	10%	22.7%	12.5%	33.4%
Klebsiella pneumoniae	0.4%	0%	0%	6.4%	8%	6.2%	4.4%
Klebsiella oxytoca	0%	-	-	-	-	-	-
Citrobacter species	-	0%	-	-	1.3%	-	2.2%
Enterobacter species	0%	0%	0.7%	3.5%	4%	0%	4.4%
Proteus vulgaris	0%	0%	0%	-	4%	0.9%	0%
Proteus mirabilis	0%	-	0%	-	2.6%	0.9%	-

Only 5.3% of the strains were resistant against cefoperazone/sulbactam. Various studies have shown that sulbactam increases the activity of cefoperazone against *Enterobacteriaceae* and nonfermenters [16]. Kucutkates et al. found cefoperazone/sulbactam to be very effective against Gram-negative bacilli in India [17].

Ticarcillin/clavulanate gave a very high resistance rate of 83.4%. Van Eldere et al. from Belgium found 37% of *Pseudomonas aeruginosa* strains to be resistant to ticarcillin/ clavulanate in a multicenter surveillance study [14]. Also Jones et al. from America showed ticarcillin/clavulanate to be the least active antimicrobial against *Enterobacteriaceae* and *Pseudomonas* species [18].

Cefdinir, a new third generation cephalosporin, gave a high resistance rate of 70.6%. Whereas Sader et al. found cefdinir to be a very effective antimicrobial in a study in America [19]. Cefepime and cefpirome, both fourth generation cephalosporins, gave high resistance rates of 45.5% and 84.4%, respectively. A study from Japan found similar high rates of resistance to cefepime (37.4%) and cefpirome (59.6%) [20]. In contrast, Johnson et al. found a low resistance rate against cefepime (4.5%) and cefpirome (5.0%) with the E-test strip method [13]. Also, the Malaysia/ Singapore antimicrobial resistance study group found only 7.7% resistance against cefepime and 8.9% against cefpirome [9]. The high rate of resistance for cefdinir, cefepime and cefpirome in India could be due to injudicious use of these new cephalosporins in our country.

The encouraging finding in our study was the low percentage resistance to imipenem, piperacillin/tazobactam and cefoperazone/sulbactam. However, caution is required; the use of these drugs must be restrictive and discriminative so as to prevent a rapid development of drug resistance. Our study highlights the need for antimicrobial susceptibility pattern determination from time to time so that proper guidelines for hospital antibiotics policies can be developed.

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