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Activities of Imipenem and Cephalosporins against Clonally Related Strains of *Escherichia coli* Hyperproducing Chromosomal β-Lactamase and Showing Altered Porin Profiles

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Forty clonally related clinical isolates of *Escherichia coli* from hospitalized patients were resistant to cefoxitin (MICs, >256 μg/ml) and ceftazidime (MICs, 32 to 256 μg/ml) and were intermediate or resistant to cefotaxime (MICs, 16 to 128 μg/ml) but susceptible to both cefepime (MICs, 0.5 to 2 μg/ml) and imipenem (MICs, 0.125 to 0.25 μg/ml). Resistance to β-lactams was related to high-level production of AmpC β-lactamase and loss of OmpF porin.

Most *Escherichia coli* strains do not produce clinically relevant levels of the chromosomally encoded AmpC β-lactamase (4). Gene amplification or mutations at either the promoter and/or the attenuator of the structural β-lactamase gene result in AmpC hyperproduction (2, 5, 11, 16, 20). This causes increased resistance to penicillins, cephalosporins, and β-lactam–β-lactamase inhibitor combinations. β-Lactams penetrate into gram-negative bacteria throughout nonspecific porins (17). Two major porins have been described in *E. coli*: OmpF and OmpC (24). Loss of nonspecific porins in *E. coli* and other *Enterobacteriaceae* are related to increased levels of resistance to β-lactams, particularly when combined with production of an efficient β-lactamase(s) (1, 3, 8–10, 13, 21). Zwitterionic cephalosporins (cefepime, cefpirome, etc.) are more active against organisms producing increased levels of inducible chromosomal AmpC β-lactamase than are older oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime, etc.) (7). We have, however, scarce information on the activity of zwitterionic cephalosporins against *E. coli* hyperproducing AmpC. In this study, we have evaluated the in vitro activities of imipenem and cephalosporins against clonally related clinical isolates of *E. coli* hyperproducing chromosomal β-lactamase and the pattern of porin expression in these isolates.

Forty-four cefoxitin-resistant (MICs, >32 μg/ml) isolates of *E. coli* obtained from clinical samples (January to December 1994) have been studied. Forty isolates were obtained from inpatients, and four were obtained from outpatients. Identification and preliminary susceptibility testing were performed using commercial panels (panels 6P; Pasco). All 40 isolates from inpatients showed similar patterns of resistance to β-lactams, fluoroquinolones, co-trimoxazole, and aminoglycosides. A variety of resistance patterns were observed in isolates from outpatients, none of them being identical to that of isolates from inpatients (data not shown). Genomic DNA from the 44 clinical isolates and from strain ATCC 25922 was separated after digestion with *Xba*I by pulsed-field gel electrophoresis (PFGE) as previously described (14). All 40 isolates from inpatients presented the same PFGE pattern, in contrast to the 4 isolates from outpatients and strain ATCC 25922, which showed patterns unrelated to each other and to that of isolates from inpatients (Fig. 1). The activities of cefoxitin (Sigma, Madrid, Spain), cefotaxime (Sigma), ceftriaxone (Sigma), ceftazidime (Glaxo, Madrid, Spain), cefepime (Bristol-Myers Squibb, Madrid, Spain), and imipenem (Merck Sharp and Dhome, Madrid, Spain) against all 44 isolates were determined by microdilution (15). MIC ranges and MICs at which 90% of isolates were inhibited, respectively, for the 40 isolates from inpatients were as follows (in micrograms per milliliter): >256 and >256 (cefoxitin); 0.5 to 2 and 2 (cefepime), 16 to 128 and 32 (both cefotaxime and ceftriaxone), 32 to 256 and 128 (ceftazidime), and 0.125 to 0.5 and 0.5 (imipenem). All strains were resistant to cefoxitin and ceftazidime, intermediate or resistant to cefotaxime and ceftriaxone, and susceptible to both cefepime and imipenem (15).

Eight isolates from hospitalized patients, including organisms covering the whole range of MICs of ceftazidime and cefotaxime for the 40 isolates from inpatients, were further studied. MICs of cephalosporins and imipenem were determined with Etest strips (AB Biodisk, Solna, Sweden) in the presence of clavulanic acid (2 μg/ml) or BRL 42715 (4 μg/ml). A clinical strain of *Klebsiella pneumoniae* (HUS57/94) producing SHV-5 β-lactamase (unpublished results) was used as a control in these studies. MICs of both cefoxitin and cefotaxime did not decrease in the presence of clavulanic acid. BRL 42715, however, significantly decreased the MICs of both cefoxitin and cefotaxime (Table 1). Both β-lactamase inhibitors decreased the MIC of cefotaxime, but not of cefoxitin, for *K. pneumoniae* HUS57/94 (data not shown).

Plasmid DNA was obtained by the alkaline-lysis method (12) and analyzed by electrophoresis in 0.7% agarose gels. All eight isolates contained a plasmid of 5.1 kb; isolate HUS23/94 additionally contained a plasmid of 8.0 kb. Attempts to transfer resistance to cephalosporins from isolates HUS31/94, HUS23/94, HUS36/94, and HUS47/94 to *E. coli* J53-2 (F^- *pro met* Rif^r) by conjugation, transformation (heat shock method), and electroporation, using ampicillin (50 μg/ml), cefoxitin (10 μg/ml), or cefotaxime (10 μg/ml) as selective agents (22), consistently failed. Although strains of *E. coli* producing AmpC-type β-lactamase from plasmid have been described (6), these results

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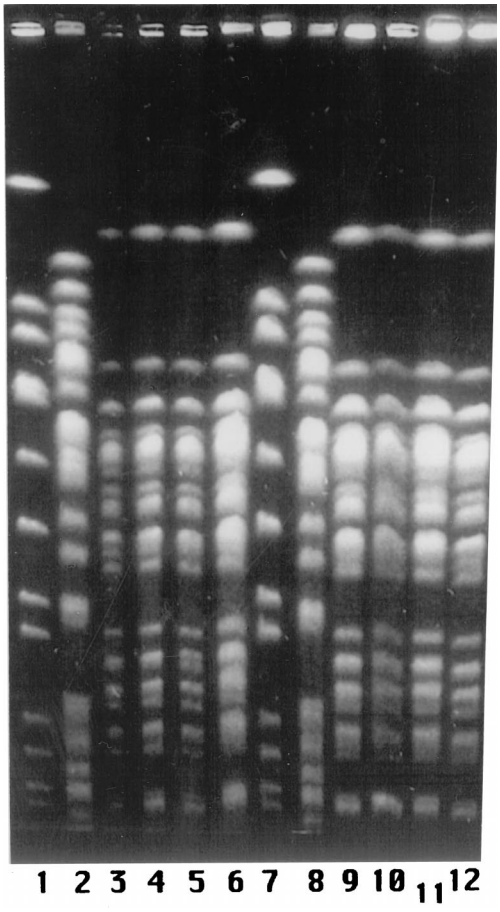

FIG. 1. PFGE profiles of *E. coli* clinical isolates hyperproducing chromosomal β-lactamase. Lanes 1, 2, 7, and 8: markers. Lanes 3, 4, 5, 6, 9, 10, 11, and 12: isolates HUS4/94, HUS7/94, HUS23/94, HUS31/94, HUS34/94, HUS36/94, HUS42/94, and HUS47/94, respectively.

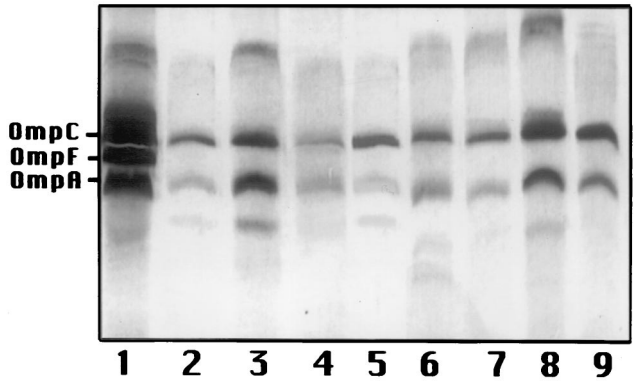

FIG. 2. Outer membrane protein profiles of *E. coli* clinical isolates HUS4/94 (lane 2), HUS7/94 (lane 3), HUS23/94 (lane 4), HUS31/94 (lane 5), HUS34/94 (lane 6), HUS36/94 (lane 7), HUS42/94 (lane 8), and HUS47/94 (lane 9). Lane 1: *E. coli* JF568 ($OmpA^+$ $OmpF^+$ $OmpC^+$).

suggest that the most probable cause of β-lactam resistance in our isolates is of chromosomal origin.

Isoelectric point (pI) determinations using crude supernatants were performed by isoelectric focusing using the Phast-System (gel pI range, 3.5 to 9; Pharmacia, Sant Cugat del Vallés, Spain). All eight isolates produced a β-lactamase with a pI of ≥9. Isolate HUS23/94 also produced a β-lactamase with a pI of 5.4, compatible with TEM-1. β-Lactamase activity was determined spectrophotometrically using crude supernatants from sonicated cells. One unit of activity was defined as the amount of enzyme that hydrolyzes 1 μmol of cephaloridine per min at 37°C. Inhibition of β-lactamase was determined after preincubation (10 min at 37°C) of supernatants with cloxacillin (250 μM) or clavulanic acid (2 μM). Cloxacillin and clavulanic acid inhibited 88.7 to 99.6% and 0.6 to 8.1% of the β-lactamase activity, except in isolate HUS23/94. Outer membrane protein profiles from bacteria grown to logarithmic phase in Mueller-Hinton broth or in nutrient broth were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% acrylamide and 6 M urea in the running gel). OmpF porin was absent in the eight isolates grown in either Mueller-Hinton broth or nutrient broth (Fig. 2).

Hyperproduction of AmpC causing resistance of clinical isolates of *E. coli* to oxyimino-cephalosporins has been previously documented (2, 4, 16), but the epidemiological relationship of the strains included in those studies has not been reported. In this study we have shown that 40 isolates from inpatients were

TABLE 1. MICs of cephalosporins and cephalosporins plus β-lactamase inhibitors[a]

E. coli isolate	MIC (μg/ml) of drug(s) by method								pI	β-Lactamase activity[b]	% β-lactamase inhibition by:	
	Microdilution			Etest								
	CAZ	FOX	CTX	CAZ	FOX	FOX-BRL	CTX	CTX-BRL			CLOX[c]	CA[d]
HUS34/94	32	>256	16	64	>256	24	12	0.25	≥9	470	99.6	1.9
HUS47/94	64	>256	16	96	>256	24	16	0.25	≥9	215	99.1	7.6
HUS36/94	64	>256	16	24	>256	12	8	0.25	≥9	218	99.0	6.3
HUS4/94	64	>256	16	64	>256	16	8	0.25	≥9	346	99.1	8.1
HUS23/94	64	>256	32	256[e]	>256	96	48	0.38	≥9 + 5.4[f]	2,057	32.5	31.4
HUS42/94	128	>256	32	64	>256	64	16	0.5	≥9	575	99.0	0.6
HUS31/94	128	>256	32	256[e]	>256	24	16	0.25	≥9	514	99.0	1.9
HUS7/94	256	>256	128	256[e]	>256	8	24	0.75	≥9	394	87.7	2.6

[a] Abbreviations for cephalosporins: CAZ, ceftzidime; FOX, cefoxitin; CTX, cefotaxime. Abbreviations for β-lactamase inhibitors: CA, clavulanic acid; BRL, BRL 42715. MICs were determined by microdilution or Etest for eight isolates of AmpC-hyperproducing *E. coli*

[b] Milliunits of enzyme/milligram of protein.

[c] Cloxacillin (250 μM).

[d] Clavulanic acid (2 μM).

[e] Difficult-to-read end-point.

[f] Two β-lactamase bands were detected.

clonally related but epidemiologically unrelated to other AmpC-hyperproducing strains (data not shown) cultured from outpatients in the same geographical area. These 40 isolates presumably represent a single strain causing a nosocomial outbreak. Data on patients from whom the isolates were obtained are not available for a complete analysis, thus precluding an adequate epidemiological study.

The isolates described in this report were resistant to oxyimino-cephalosporins but susceptible to both cefepime and imipenem, two compounds highly stable to AmpC β-lactamases from other enterobacteria (7). This study suggests that both cefepime and imipenem may represent a therapeutic alternative for infections caused by AmpC-hyperproducing *E. coli* strains, as suggested for other organisms (23). Loss of both OmpF and OmpC porins in laboratory mutants of *E. coli* determines an 8- to 16-fold increase in the MICs of cephalosporins (9). Loss of OmpF alone in *E. coli* mutants expressing increased amounts of AmpC determines a two- to fourfold increase in the MICs of cephalosporins (10). There is scarce information on the number and nature of porins expressed by clinical isolates of *E. coli*, and the relationship of porin expression, if any, with antimicrobial resistance. Reguera et al. (21) reported that seven out of eight clinical strains of *E. coli* resistant to amoxicillin-clavulanic acid expressed only OmpC or expressed OmpF with altered electrophoretic mobility. In order to evaluate the role of porins in antimicrobial resistance, it would be necessary to compare Omp profiles and susceptibility data of bacteria cultured in the same medium. OmpF, an important porin for β-lactam penetration (18, 19), is not expressed in high-osmolarity media, such as Mueller-Hinton broth, the most commonly used medium for susceptibility testing. In the isolates herein studied, we have observed that OmpF is not expressed in Mueller-Hinton broth (as would be expected); nor is it expressed in the low-osmolarity medium nutrient broth, suggesting that this porin is actually not expressed by these isolates. This also suggests that resistance due to cooperation between β-lactamase production and altered permeability demonstrated in laboratory mutants of *E. coli* can also be expressed by clinical isolates.

The level of resistance to cephalosporins varied in different (but clonally related) isolates. It does not seem probable that variations in β-lactamase activity accounts for the differences in susceptibility to cefotaxime and ceftazidime. This is suggested by both the poor correlation between enzyme activity and MICs (even if strain HUS23/94 is not considered) and by the differences of MICs of cefoxitin and cefotaxime in the presence of BRL 42715. It is possible that other mechanisms (penicillin-binding protein expression, active efflux, etc.) contribute to the observed phenotypes of resistance. New studies are in progress to evaluate these possibilities.

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