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Activity of nine antimicrobial agents against clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases and deficient or not in porins

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Sir,

The most relevant single mechanism of resistance of Enterobacteriaceae to cephalosporins is the production of β-lactamases.[1] For *Klebsiella pneumoniae*, these enzymes are usually plasmid-mediated extended-spectrum β-lactamases (ESBLs) or, less commonly, plasmid-mediated AmpC-type enzymes. *K. pneumoniae* produces two major porins, outer membrane proteins (Omps) K35 and K36, which are homologous to *Escherichia coli* porins OmpF and OmpC, respectively.[2] In klebsiellae, simultaneous loss of both OmpK35 and OmpK36 causes increased resistance to cephalosporins and other β-lactams when ESBL or AmpC-type β-lactamase is also produced.[3] ESBLs do not confer resistance to cephamycins and carbapenems.[1] Cephamycins, however, are able to select for mutants that are deficient in porins, resulting in subsequent resistance to these agents.[4] Cefepime and cefpirome are poorly hydrolysed by AmpC-type β-lactamases but have less activity against ESBL-producing strains, particularly in porin-deficient organisms.[3] ESBL-producing *K. pneumoniae* are more frequently resistant to aminoglycosides and fluoroquinolones than *K. pneumoniae* strains not producing these β-lactamases. The purpose of this study was to evaluate the activity of cefepime, cefpirome, third-generation cephalosporins, carbapenems, amikacin and ciprofloxacin against clinical isolates of ESBL-producing *K. pneumoniae* with different porin profiles. Sixty-five non-consecutive clinical isolates of ceftazidime-resistant *K. pneumoniae* isolated at the Microbiology Laboratory of the University Hospital V. Macarena (Seville, Spain) in the period 1994–1997 were studied. Strains were selected to include 15 isolates deficient in porins (see below). *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* LB4 (producing SHV-5)[4] were also included as controls for antimicrobial susceptibility testing. *K. pneumoniae* C3 (producing OmpK35 and OmpK36)[5] and *E. coli* K-12 mutants deficient in OmpA, OmpF or OmpC were used as reference strains in the determination of porin profiles.

The activities of cefepime, cefpirome, cefotaxime, ceftazidime, cefoxitin, imipenem, meropenem, ciprofloxacin and amikacin were evaluated by a microdilution assay according to NCCLS guidelines.[6] A ⩾8-fold decrease in the activities of either ceftazidime or cefotaxime with clavulanic acid (2 mg/L) compared with ceftazidime or cefotaxime alone was taken as an indication of ESBL production. Omps were obtained after sonication of bacteria and sarkosyl treatment of cell membranes. Omps were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) using 11% polyacrylamide in the running gel. Bacteria were categorized as porin producers (P^+) if at least one protein band corresponding to either OmpK35 or OmpK36 was detected, and as porin-deficient (P^-) if no major bands in the region of porins were observed.

Fifty of the 65 clinical isolates of ESBL-producing *K. pneumoniae* produced at least one porin (mostly OmpK36). The remaining 15 strains were deficient in both OmpK35 and OmpK36. The proportion of isolates expressing or not expressing porins does not reflect their actual frequency of isolation among clinical isolates, as *K. pneumoniae* strains deficient in both porins are much less common than would be predicted from these figures.

The activities of nine antimicrobial agents against P^+ and P^- strains are presented in the Table. The activity of cefepime and cefpirome against both P^+ and P^- strains is higher than that of cefotaxime and ceftazidime. Nevertheless, and according to the NCCLS recommendations, ESBL-producing *K. pneumoniae* are considered resistant to all cephalosporins, independently of the *in vitro* activity of the different compounds within this group. The MIC_{50}s and MIC_{90}s of cefoxitin against P^+ and P^- strains indicate that loss of porins is critical for resistance to this cephamycin in ESBL-producing *K. pneumoniae*. Porin loss contributes to increasing the level of resistance to the other cephalosporins in ESBL-producing strains.

Both imipenem and meropenem were the most active

Table. MICs (mg/L) of nine antimicrobial agents against ESBL-producing clinical strains of *K. pneumoniae* expressing porins (P^+) and deficient in porins (P^-)

	P^+ strains (n = 50)			P^- strains (n = 15)		
Antimicrobial	range	MIC_{50}	MIC_{90}	range	MIC_{50}	MIC_{90}
Cefepime	0.25–128	4	64	2–256	64	256
Cefpirome	0.25–128	4	64	2–256	64	256
Cefotaxime	0.5–>256	16	>256	4–>256	128	>256
Ceftazidime	4–>256	256	>256	16–>256	>256	>256
Cefoxitin	1–16	4	8	64–256	128	128
Imipenem	0.06–2	0.125	0.5	0.06–1	0.25	1
Meropenem	0.03–0.125	0.06	0.06	0.03–2	0.125	2
Amikacin	0.25–>256	16	64	0.25–>256	16	64
Ciprofloxacin	0.06–64	8	16	0.06–8	4	8

agents against both bacterial groups. Meropenem was more active than imipenem against all P^+ strains and most P^- strains, but MIC ranges of meropenem against P^- strains were broader than those of imipenem. These results agree with previous reports on laboratory[7] and clinical[4] strains of *K. pneumoniae* deficient in both OmpK35 and OmpK36, and indicate that a subset of ESBL-producing *K. pneumoniae* strains have mechanisms of resistance that affect meropenem more than imipenem. The results of this study indicate that in a subset of strains deficient in porins, the activity of meropenem is affected more than that of imipenem. Loss of porins seems not to affect the *in vitro* activity of amikacin in ESBL-producing strains. This should be expected from the ability of amikacin to use the porin-independent self-promoted pathway of penetration into the bacterial cell.

It has been observed that resistance to fluoroquinolones is higher in ESBL producers than in non-producers.[8] The exact reasons for this relationship are not known. Previous reports considering clinical isolates and laboratory strains indicate that loss of porins causes a moderate increase in fluoroquinolone resistance.[5] Surprisingly, MIC_{50}s and MIC_{90}s of ciprofloxacin in the strains herein evaluated were higher for P^+ strains than for P^- strains. This apparent paradox could be explained considering that target mutations contribute more significantly to fluoroquinolone resistance than loss of porins, and that the more numerous group of P^+ strains can be expected to contain more organisms with multiple mutations in *gyrA*/*parC* than the group of P^- strains. New studies are currently in progress to evaluate this phenomenon.

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