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In vitro activity of β-lactam and non–β-lactam antibiotics in extended-spectrum β-lactamase–producing clinical isolates of *Escherichia coli*

Antonio Sorlózano Puerto[a], José Gutiérrez Fernández[a,*], Juan de Dios Luna del Castillo[b], María José Soto Pino[a], Gonzalo Piédrola Angulo[a]

[a]*Department of Microbiology, School of Medicine, University of Granada, Granada 18012, Spain*
[b]*Department of Biostatistics, School of Medicine, University of Granada, Granada 18012, Spain*

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Abstract

The activity of different β-lactam and non–β-lactam antibiotics was assessed against extended-spectrum β-lactamase (ESBL)–producing and non-ESBL–producing clinical isolates of *Escherichia coli*. A phenotypic study to discover the presence of ESBLs in 399 clinical isolates of *E. coli* was made by the disk diffusion method following the Clinical and Laboratory Standards Institute (formely NCCLS, 2004) guidelines. The activity of different antibiotics was subsequently studied using the automated VITEK 2 system (bioMérieux, Marcy l'Étoile, France). One hundred fifteen isolates proved to be ESBL-producing and 284 non-ESBL–producing. Among the former, percentage susceptibilities to the antibiotics assayed were meropenem and amikacin, 100%; piperacillin/tazobactam, 97.4%; cefepime, 94.8%; amoxicillin/clavulanic acid, 84.3%; tobramycin, 84.3%; gentamicin, 83.5%; cefoxitin, 83.5%; nitrofurantoin, 71.3%; cotrimoxazole, 46.1%; norfloxacin, 29.6%; ciprofloxacin, 27%; and ofloxacin, 26.1%.

Keywords: *Escherichia coli*; ESBLs; Antibiotics; Susceptibility

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes produced by Gram-negative bacilli and confer resistance against all penicillins, cephalosporins (except cephamycins), and monobactams. The only β-lactams to retain their activity, apart from the cephamycins, are β-lactamase inhibitors and the carbapenems (Bradford, 2001). Although few studies have been made into the clinical use of cephamycins, some authors have described the appearance of mutants showing resistance to these antibiotics after their use due to a reduction in their permeability (Martinez-Martinez et al., 1996). Treatment with cefepime tends to be equally unsuccessful, even when used against isolates that appear to be susceptible in vitro (Paterson et al., 2001). Another therapeutic possibility is that of using penicillins in combination with β-lactamase inhibitors, but the clinical response to these depends upon the MIC shown by the microorganism in question. Carbapenems are the β-lactam antibiotics that show greatest activity against ESBL-producing Gram-negative bacilli (Johnson et al., 2002). Even so, they should be used with caution because they have been related to an increase in infection by *Acinetobacter baumanni* and *Pseudomonas aeruginosa* (Lee et al., 2004), which are resistant to them. Moreover, carbapenems are not a suitable choice for the treatment of community-acquired infections.

When ESBL-producing microorganisms are susceptible to non–β-lactam antibiotics such as fluoroquinolones, these may be useful in their treatment (Karas et al., 1996). Nevertheless, a frequent connection has been seen between the presence of ESBLs and a resistance to fluoroquinolones despite the fact that their genetic resistance is encoded at different sites (Paterson et al., 2000). Thus, it has been reported that up to 55% of ESBL-producing isolates are

* Corresponding author. Departamento de Microbiología, Facultad de Medicina, E-18012 Granada, Spain.
E-mail address: josegf@ugr.es (J. Gutiérrez Fernández).

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resistant to fluoroquinolones (Lautenbach et al., 2001b), and the transference of resistance to these antibiotics via plasmids has been clearly demonstrated in isolates of *Klebsiella pneumoniae* (Martinez-Martinez et al., 1998). Finally, coresistance has also been found to aminoglucosides, cotrimoxazole, and tetracyclins via plasmids, transposons, and integrons (Lautenbach et al., 2001a).

The international study SENTRY (Winokur et al., 2001) and the Spanish Group for Nosocomial Infections (Hernandez et al., 2003) have, among others, published results concerning the susceptibility of various ESBL-producing isolates to antibiotics. Both of these studies report high percentages of resistance to aminoglucosides, fluoroquinolones, and tetracyclines. Finally, we must emphasize the high frequency of ESBLs found in our study of isolates of *E. coli* (Sorlozano et al., 2004a), which has led us to investigate their susceptibility to different antibiotics.

Our aim has been to determine the activity of β-lactam and non–β-lactam antibiotics in clinical isolates of ESBL-producing strains of *E. coli* using an automated assay and to determine the levels of coresistance that exist in our environment and the therapeutic options open to us.

2. Materials and methods

We studied 399 clinical isolates of *E. coli* taken from patients attended to at the San Cecilio University Hospital in Granada (Spain) for a variety of infectious complaints. We identified them by biochemical tests using the Api20E gallery (bioMérieux, Marcy l'Etoile, France). The isolates were mainly taken from urine samples (92%), from female patients (71.2%) and from outpatients (76.4%). The samples were kept at −40 °C until use. After thawing, the purity of the samples was checked and suspensions were prepared to a 0.5 McFarland turbidity standard. They were then inoculated onto plates of Mueller-Hinton agar (bioMérieux) to carry out phenotypic analyses for the presence of ESBLs and susceptibility to cefoxitin and cefepime using a disk diffusion method and a susceptibility study made with the automatic VITEK 2 system (bioMérieux). *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were always used as reference strains.

In all the isolates, ESBL production was measured using the disk diffusion method (Oxoid, England) as recommended by the Clinical and Laboratory Standards Institute (formerly NCCLS) (NCCLS, 2004) and described elsewhere (Sorlozano et al., 2004b).

Although they do not figure within the criteria of the NCCLS for the detection of ESBLs, we assayed cefepime and cefoxitin by disk diffusion to determine the possible coexistence of type *AmpC* β-lactamases and reduced impermeability. To this end, we used disks of cefoxitin (30 μg) and cefepime (30 μg) (Oxoid) at a distance of 25 mm from a disk of amoxicillin plus clavulanic acid (20/10 μg) (Becton Dickinson, Franklin Lakes, NJ) to study the synergy of cefepime plus clavulanic acid.

The isolates were studied using the VITEK 2 system (Livermore et al., 2002). Following the manufacturer's instructions, they were inoculated onto AST-N020 cards, on which different concentrations of antibiotics were assayed. This system has proved useful in calculating MIC values (Ling et al., 2001).

We used Fisher exact test for tables $r \times s$ to compare the group of ESBL-producing isolates with the nonproducers with regard to the distribution of the clinical categories for each antibiotic obtained using the VITEK 2 system. The presence of a difference between the 2 groups with regard to the variables was the alternative hypothesis (H_1).

3. Results

The phenotypic reference method identified 115 isolates (28.8%) as ESBL producers. Among these, tests with

Table 1
Relationship between the ESBL-producing and non–ESBL-producing isolates against the antibiotics assayed using the VITEK 2 system

Antibiotic	Breakpoints (NCCLS, 2004)	ESBL producers (%)	Non-ESBL producers (%)	*P* value
Amoxicillin/ clavulanic acid	≤8/4	84.3	73.3	0.019
	16/8	13.9	15.4	
	≥32/16	1.8	11.3	
Piperacillin-tazobactam	≤16/4	97.4	97.2	1.000
	32/4–64/4	0.9	0	
	≥128/4	1.7	2.8	
Cefoxitin	≤8	83.5	69	0.004
	16	11.3	11.3	
	≥32	5.2	19.7	
Cefepime	≤8	94.8	100	0.001
	16	0.9	0	
	≥32	4.3	0	
Meropenem	≤4	100	100	1.000
	8	0	0	
	≥16	0	0	
Amikacin	≤16	100	100	1.000
	32	0	0	
	≥64	0	0	
Gentamicin	≤4	83.5	80.3	0.484
	8	2.6	0	
	≥16	13.9	19.7	
Tobramycin	≤4	84.3	84.5	1.000
	8	7	8.5	
	≥16	8.7	7	
Ciprofloxacin	≤1	27	50.7	<0.001
	2	3.4	0	
	≥4	69.6	49.3	
Norfloxacin	≤4	29.6	50.7	<0.001
	8	5.2	2.8	
	≥16	65.2	46.5	
Ofloxacin	≤2	26.1	50.7	<0.001
	4	3.5	0	
	≥8	70.4	49.3	
Nitrofurantoin	≤32	71.3	87.4	<0.001
	64	20	4.2	
	≥128	8.7	8.4	
Cotrimoxazole	≤2/38	46.1	50.7	0.440
	≥4/76	53.9	49.3	

cefepime and cefoxitin disks revealed that 95 isolates were susceptible to both antibiotics and that 13 were resistant to cefepime (with synergy with clavulanic acid) but were susceptible to cefoxitin and thus we considered that they all expressed ESBLs and showed neither alterations to their permeability nor *AmpC* β-lactamases. Four isolates were resistant to cefepime (with synergy with clavulanic acid) and to cefoxitin, and 3 isolates were susceptible to cefepime and resistant to cefoxitin. We considered that these 7 isolates, apart from producing ESBLs, may also show alteration to their permeability or the production of an *AmpC* β-lactamase. Among the 284 nonproducers of ESBLs, 228 were susceptible to cefepime and to cefoxitin, and 56 were susceptible only to cefepime. There was no synergy with clavulanic acid with any of these isolates.

A comparison of the activities of the different antibiotics between the ESBL-producing and non–ESBL-producing isolates, as determined by the VITEK 2 system, is set out in Table 1, before the correction made by the Advanced Expert System (AES) of VITEK 2.

The 115 ESBL-producing isolates were resistant in vitro to amoxicillin and cephalothin. They registered as being resistant to piperacillin, cefuroxime, cefotaxime, cefpodoxime, ceftazidime, and cefepime after the corrections made by the AES system for the isolates that presented MICs in vitro within the susceptibility range. There was no modification to the results in vitro for amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, or meropenem.

4. Discussion

In a previous study we found that the VITEK 2 system detected ESBLs to an acceptable extent in isolates of *E. coli* (Sorlozano et al., 2005). A study by the Spanish GEIH (Hernandez et al., 2005) records the susceptibility of ESBL-producing *E. coli* strains to carbapenems (100%), amikacin (93.5%), piperacillin/tazobactam (85%), cefoxitin (76.5%), gentamicin (66%), amoxicillin/clavulanic acid (69%), cotrimoxazole (25%), and ciprofloxacin (37.5%). Our results tended to agree with these except for the greater activity of piperacillin/tazobactam, cefoxitin, gentamicin, and above all, amoxicillin/clavulanic acid (84.3% versus 69% in the GEIH study). These results for piperacillin/tazobactam, cefoxitin, and amoxicillin/clavulanic were similar to Spanu et al. (2002) (91%, 78%, and 85%, respectively). As far as the clinical use of amoxicillin/clavulanic acid is concerned, it is important to determine first of all what the MIC for this antibiotic is, because in the treatment of a simple urinary tract infection in an outpatient, it could be a good therapeutic option whenever the MIC is low and within the range of susceptibility (Spanu et al., 2002). This is due to the fact that a coexistence of hyperproduction of an *AmpC* or SHV-1 enzyme and the ESBL will impede the activity of the amoxicillin/clavulanic acid combination (Navarro et al., 2002). Piperacillin/tazobactam showed activity in both groups, as did amoxicillin/clavulanic acid. According to some authors (cf. Spanu et al., 2002), the former is a reliable therapeutic choice for hospital patients with systemic infections caused by ESBL-producing microorganisms when they present MICs within the susceptibility range; other authors, on the other hand, do not advise its use because of reports of therapeutic failure (cf. Paterson et al., 2001). Whatever the case, its clinical inefficacy against resistant isolates in vitro (Rice et al., 1996) has been demonstrated, and its use could cause problems with the appearance of inhibitor-resistant TEM strains resistant to these combinations of antibiotics (Amyes and Miles, 1998).

Cefoxitin is active against ESBLs in vitro except when faced with alterations in permeability or the hyperproduction of cefaminases or *AmpC* β-lactamases coexisting with the ESBL (Navarro et al., 2002), in which case, the isolate proves itself resistant to this antibiotic. When the MIC for cefoxitin was ≤8 μg/mL against the ESBL-producing isolates, the VITEK 2 system only indicated the possible presence of ESBLs, but when it was ≥16 μg/mL, the system recognized the presence of ESBLs together with a probable association of impermeability. Cefoxitin proved itself to be most active against the ESBL-producers, possibly due to the existence of impermeability mechanisms or *AmpC* among the non–ESBL producers.

Cefepime was active against both ESBL-producing and non–ESBL-producing isolates, although the activity in the latter group was somewhat lower. Although the hydrolytic capacity of the ESBL enzymes against this antibiotic is variable (Navarro et al., 2002), its use is not recommendable because of failures described in the past. The AES system also registered resistance to cefepime in all the ESBL-bearing isolates.

Meropenem proved to be active against all the isolates, both ESBL producers and nonproducers. According to these results, meropenem, and consequently the carbapenems in general, would seem in principle to be the best therapeutical choice among the β-lactams for hospital use in combating infections caused by ESBL-producing microorganisms. In this regard, Thomson and Moland (2001) evaluated the activity of meropenem, cefepime, and piperacillin/tazobactam in vitro and found that, at least in those clinical cases where there was a high bacterial inoculate such as endocarditis, meningitis, septic arthritis, osteomyelitis, and abscesses, the best choice of treatment was meropenem.

Amikacin, gentamicin, and tobramycin were active against both ESBL-producing and non–ESBL-producing isolates. Fernandez-Rodriguez et al. (1992) found that 80% of ESBL-producing isolates were resistant to gentamicin, the most frequently associated mechanism being the presence of AAC(3)V, APH(3′), and APH(3′)l enzymes. On the other hand, Hadziyannis et al. (2000) found no connection between resistance to gentamicin and the presence of ESBLs.

The fluoroquinolones and nitrofurantoin assayed showed less activity against ESBL-producing isolates, probably because an increase in the consumption of fluoroquinolones

in the community as a whole has caused an increase in resistance to them in recent years (Oteo and Campos, 2004). The connection between the production of ESBLs and resistance against these antibiotics has been described before (Lautenbach et al., 2001b) and may derive partially from the joint transference of both mechanisms via plasmids (Martinez-Martinez et al., 1998). Nevertheless, this process might only explain a small part of the coresistance encountered. A possible increase in the use of fluoroquinolones against infections caused by ESBL-producing microorganisms that do not respond to treatment with β-lactams may well help to explain why coresistance has increased toward them.

As far as susceptibility to cotrimoxazole is concerned, the isolates were divided evenly, implying that this antibiotic is not really useful in these cases unless an antibiogram is made. In the same way as before, Hadziyannis et al. (2000) found no connection between the presence of ESBLs and resistance to cotrimoxazole.

In short, carbapenems prove to be the most suitable antibiotics against infections by ESBL-producing microorganisms because ESBLs offer considerable resistance to other antibiotic groups. Thus, carbapenems are often one of the only options available in the hospital environment. In our assays, amikacin also proved to be a suitable alternative. As far as infections of the urinary tract caused by these microorganisms in outpatients is concerned, we believe that amoxicillin/clavulanic acid is a good alternative if its activity is high enough.

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