Research Paper

Activity *in vitro* of twelve antibiotics against clinical isolates of extended-spectrum beta-lactamase producing *Escherichia coli*

Antonio Sorlózano[1], José Gutiérrez[1], José María Romero[2], Juan de Dios Luna[3], Miguel Damas[4] and Gonzalo Piédrola[1]

[1] Department of Microbiology, School of Medicine, University of Granada, Spain
[2] Department of Immunology, School of Medicine, University of Granada, Spain
[3] Department of Biostatistics, School of Medicine, University of Granada, Spain
[4] Department of Pharmacy, San Cecilio University Hospital, Granada, Spain

Twelve beta-lactam and non-beta-lactam antibiotics were evaluated against 115 clinical isolates of extended-spectrum beta-lactamase-producing (ESBLs) *Escherichia coli* using a broth microdilution test in accordance with the CLSI guidelines. Susceptibility was 100% with imipenem, ertapenem and amikacin, 95.7% with piperacillin-tazobactam, 91.3% with cefoxitin, 87% with tobramycin, 81.7% with amoxicillin-clavulanate, 80% with cefepime, 67.8% with ceftazidime, 27.8% with ciprofloxacin, 27% with levofloxacin and 13% with ceftriaxone. Ertapenem was the antibiotic with the lowest minimum inhibitory concentrations (MICs) for all isolates. There were no clinically relevant differences in the activity of the antibiotics in the presence of CTX-M-9 or SHV enzymes.

Keywords: *Escherichia coli* / ESBLs / Microdilution / Susceptibility

Received: January 16, 2007; returned for modification: February 06, 2007; accepted: March 03, 2007

DOI 10.1002/jobm.200710318

Introduction

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria that confer resistance to all penicillins, cephalosporins (with the exception of cephamycins), and monobactams. Apart from cephamycins, the only beta-lactams to maintain activity are the beta-lactam combinations with beta-lactamase inhibitors and carbapenem antibiotics (Bradford 2001). The *in vivo* use of some of these antibiotics has led to therapeutic failure. For example, when using cephamycins, mutants have been generated which are resistant by virtue of a reduction in permeability (Martinez-Martinez *et al*. 1996). These antibiotics are therefore not useful in practice. Furthermore, the use of combinations of beta-lactams with beta-lactamase inhibitors is controversial (Johnson *et al*. 2002, Spanu *et al.* 2002). In any case, it is not advisable to use these antibiotics until the results of the antibiogram are known, and their use should be reserved for non-serious infections with demonstrated *in vitro* susceptibility.

Carbapenems have been reported to be the most active beta-lactam antibiotics against ESBL-producing Gram-negative bacilli (Johnson *et al*. 2002). These are not, however, an alternative in the management of community-acquired infections.

When ESBL-producing organisms are sensitive to other antibiotic groups these are useful as treatment. Nevertheless, it is important to investigate the activity of these other antibiotics, against which co-resistances can be found through plasmids, transposons or integrons (Lautenbach *et al*. 2001).

Since these enzymes frequently show a multiresistance pattern, treatment options are limited for infections caused by ESBL-producing organisms. Therefore they constitute a health-care challenge of great clinical importance.

With this in mind we designed the present study to determine the activity of different beta-lactam and non-beta-lactam antibiotics in clinical isolates of ESBL-producing strains of *E. coli* using a broth microdilution test.

Correspondence: Dr. José Gutiérrez, Departamento de Microbiología, Facultad de Medicina, Avda. de Madrid 11, E-18012 – Granada, Spain
E-mail: josegf@ugr.es

Materials and methods

Bacterial isolates

We studied 115 different clinical isolates of ESBL-producing *E. coli* identified in the Laboratory of Clinical Microbiology at the San Cecilio University Teaching Hospital in Granada (Spain) using the WIDER system (Francisco Soria Melguizo S.A., Spain) (Canton *et al.* 2000), in which we confirmed the presence or otherwise of ESBLs by the diffusion technique with discs of cefotaxime (30 μg), cefotaxime/clavulanate (30/10 μg), ceftazidime (30 μg) and ceftazidime/clavulanate (30/10 μg), in adherence to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2006a). Following phenotypic confirmation, determination of beta-lactamase and clonality was carried out by means of biochemical (determination of the isoelectric point) and molecular studies (PCR), following the procedures described elsewhere by our group (Sorlozano *et al.* 2007).

Sixty-seven isolates produced CTX-M-9 enzymes and 48 produced SHV enzymes. Of the total, 86.1% were from urine samples (58.6% producing CTX-M-9) whilst 77.4% were of community origin (55% producing CTX-M-9). Of the hospital samples, 69.2% produced CTX-M-9. A total of 41.7% of the isolates were CTX-M-9 producers originating from community and urine samples. In the case of the SHV producers, the figure was 31.3%.

Susceptibility determination

Microdilution was carried out in Mueller-Hinton broth, adjusted for Ca^{++} and Mg^{++}, in accordance with CLSI guidelines (CLSI 2006b). Each antibiotic was dissolved according to the manufacturers' recommendations. The following concentrations (in μg/ml) were tested in the microdilution procedure: amoxicillin-clavulanate 0.125/0.06 to 128/64, piperacillin-tazobactam 0.25 to 256, with a fixed concentration of tazobactam of 4 mg/l, ceftriaxone 0.5 to 512, cefoxitin 0.25 to 256, ceftazidime 0.25 to 256, cefepime 0.25 to 256, imipenem 0.008 to 8, ertapenem 0.008 to 8, amikacin 0.25 to 256, tobramycin 0.125 to 128, ciprofloxacin 0.125 to 128, and levofloxacin 0.125 to 128. The minimum inhibitory concentration (MIC) is defined as the lowest antibiotic concentration to completely inhibit bacterial growth. The isolates were considered to be susceptible, intermediate or resistant according to the recommendations of the CLSI.

Quality controls

Following the CLSI guidelines (CLSI 2006a), we used the following strains as quality control in all procedures: *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922.

Statistical analysis

The Fisher exact test for $r \times s$ tables was used to compare the clinical categories and MIC distributions between the two groups of ESBL isolates (CTX-M-9 and SHV) for each antibiotic tested. As an alternative hypothesis (H_1) we considered the presence of a difference between the groups compared for both variables.

Results

Table 1 shows the values (in μg/ml) of the MIC ranges, the MIC_{50} and MIC_{90} values, and percentage susceptibility to the 12 antibiotics tested of the 115 ESBL-producing isolates (67 and 48 producers of CTX-M-9 and SHV respectively). Of note is the fact that imipenem, ertapenem and amikacin were the only three antibiotics to show activity against 100% of the isolates.

The results of the Fisher exact test on comparing the CTX-M-9- and SHV-producing isolates *versus* the behavior of each antibiotic, from the perspective of MIC distribution and clinical category, were the following (p value for MIC distribution; p value for clinical category): amoxicillin-clavulanate (0.065; 0.342); piperacillin-tazobactam (0.810; 1.000); ceftriaxone (<0.001; <0.001); cefoxitin (0.828; 1.000); ceftazidime (<0.001; <0.001); cefepime (0.003; 1.000): imipenem (0.289; 1.000); ertapenem (0.178; 1.000); amikacin (0.102; 1.000); tobramycin (0.086; 0.049); ciprofloxacin (0.609; 0.674); levofloxacin (0.128; 0.523).

Ceftriaxone showed greater activity against SHV-producing isolates, whilst ceftazidime was more active against CTX-M-9-producing isolates. There were also significant differences for cefepime as regards MIC distribution, these concentrations being lower among the SHV producers, and for tobramycin in the distribution by clinical categories (increased percentage of resistance among SHV producers).

Discussion

In this study the ESBL-producing isolates proved themselves in general to be susceptible to a combination of amoxicillin and clavulanic acid, with no significant difference between the CTX-M-9- and SHV-producing strains. Other authors have obtained similar susceptibility percentages for ESBL-producing *E. coli*, [cf. for example the study conducted by the Spanish Group for Nosocomial Infections (GEIH) (Hernandez *et al.* 2005), who arrived at a figure of 69% for isolates susceptible to amoxicillin-clavulanic acid]. Some other authors

Table 1. *In vitro* activity of the 12 antibiotics tested against clinical isolates of extended-spectrum beta-lactamase producing *E. coli*.

	ESBL-producers *n* = 115				CTX-M-9-producers *n* = 67				SHV-producers *n* = 48			
	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Susceptible (%)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Susceptible (%)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Susceptible (%)
Amoxicillin-clavulanate	2/1–32/16	8/4	32/16	81.7	2/1–32/16	8/4	16/8	85.1	2/1–32/16	8/4	32/16	77.1
Piperacillin-tazobactam	≤0.25/4–128/4	2/4	8/4	95.7	≤0.25/4–128/4	2/4	8/4	95.5	≤0.25/4–64/4	2/4	16/4	95.8
Ceftriaxone	2–>512	16	512	13	16–>512	64	512	0	2–>512	16	256	31.3
Cefoxitin	0.5–32	4	8	91.3	0.5–32	4	8	91	1–32	4	8	91.7
Ceftazidime	≤0.25–>256	2	64	67.8	≤0.25–16	1	4	95.5	≤0.25–>256	32	128	29.2
Cefepime	≤0.25–128	4	32	80	≤0.25–128	4	16	80.6	≤0.25–128	2	32	80
Imipenem	0.03–2	0.125	0.25	100	0.03–0.25	0.125	0.25	100	0.03–2	0.125	0.25	100
Ertapenem	≤0.008–0.5	0.03	0.125	100	≤0.008–0.125	0.03	0.06	100	≤0.008–0.5	0.016	0.125	100
Amikacin	≤0.25–8	1	4	100	0.5–8	1	4	100	≤0.25–8	1	8	100
Tobramycin	≤0.125–128	0.5	8	87	≤0.125–128	0.5	4	92.5	≤0.125–32	0.5	8	79.2
Ciprofloxacin	≤0.125–128	16	64	27.8	≤0.125–128	16	64	29.9	≤0.125–128	16	64	25
Levofloxacin	≤0.125–>128	8	16	27	≤0.125–64	8	16	29.9	≤0.125–>128	8	16	22.9

(Hoban *et al*. 2005), however, have obtained lower values (12.5%).

Piperacillin-tazobactam was found to be very active against ESBL isolates, as has also been observed by Hoban *et al*. (2005), Casellas *et al*. (2003) and Sader *et al*. (2005), who found percentages of susceptibility to this antibiotic of 79.2%, 75.6% and 86.2%, respectively, whilst showing no significant differences between the CTX-M-9- and SHV-producers.

Ceftriaxone presented higher MICs because of the hydrolysis of this drug by ESBLs. Moreover, CTX-M-type ESBL-producing organisms exhibit high resistance to this antibiotic (Bradford 2001). This would explain why the MIC_{50} and MIC_{90} values are higher in the CTX-M-9-producing group than in the SHV producers – resulting from a significantly different distribution of the MICs between the two groups.

The susceptibility of ESBL isolates to cefoxitin and cefepime seems to be variable and depends on the study in question. Sader *et al*. (2005) found that 91.3% of their isolates were susceptible to cefoxitin. With cefepime the values were 24.3%, 50% and 93.8% in studies by Casellas *et al*. (2003), Hoban *et al*. (2005) and Sader *et al*. (2005) respectively. We found both cefoxitin and cefepime to be active against ESBL-producing organisms, though their clinical use is not advisable (Martinez-Martinez *et al*. 1996, Paterson *et al*. 2001). No significant differences between the two groups of isolates were observed in the activity of cefoxitin. The CTX-M-9 producers did however show significantly higher MICs for cefepime since they hydrolyze this antibiotic more efficiently than do the SHV producers (Yu *et al*. 2002).

Because of the reduced capacity of CTX-M-9 enzymes to hydrolyze ceftazidime (Bradford 2001) the MICs obtained in this group are significantly lower and fall within the susceptibility range found for the SHV-producing isolates against this antibiotic. Casellas *et al*. (2003) reported a susceptibility of 7.4%, far lower than our own value, since they investigated CTX-M2-producing *E. coli*.

Imipenem and ertapenem were the only beta-lactam antibiotics to show activity against 100% of the isolates, with no significant differences between the two enzymes. In this context, ertapenem was the antibiotic with the lowest MICs for each isolate. Thus this drug constitutes the best *in vitro* option of all the antibiotics tested. Although ertapenem and imipenem are both active against *E. coli*, the greater intrinsic activity of ertapenem seems to be due to its greater affinity for PBP-3 compared with imipenem (Kohler *et al*. 1999).

In the same way as in our study, where ertapenem exhibited the lowest MIC_{90} (0.125 μg/ml), other authors have also found the most active carbapenem antibiotic *in vitro* to be ertapenem, with a MIC_{90} of 0.06 μg/ml as opposed to 0.5 μg/ml for imipenem, 16 μg/ml for cefepime, or more than 128 μg/ml in the case of piperacillin-tazobactam when tested against ESBL-producing isolates of *K. pneumoniae* in a study published by Livermore *et al*. (2001). Alhambra *et al*. (2004) found an MIC_{90} of 0.03 μg/ml for ertapenem when testing against ESBL-producing *E. coli* isolates.

Amikacin was found to be active against 100% of the ESBL-producing isolates, with no significant differences found between the two groups of enzymes. Tobramycin was also seen to be very active against these

isolates, particularly against the CTX-M-9 producing organisms.

The quinolones showed lower active, with no significant differences between the CTX-M-9- and SHV-producing isolates. The association between the production of ESBLs and resistance to these antibiotics has been well established (Valverde *et al.*, 2004), though such resistance is not observed in all cases (Sader *et al.* 2005).

The carbapenems are undoubtedly very active *in vitro* against ESBL-producing isolates and, in view of the presence of resistances associated to other antibiotic groups, often constitute one of the few treatment options available for such organisms.

References

Alhambra, A., Cuadros, J.A., Cacho, J., Gomez-Garces, J.L. and Alos, J.I., 2004. *In vitro* susceptibility of recent antibiotic-resistant urinary pathogens to ertapenem and 12 other antibiotics. J. Antimicrob. Chemother., **53**, 1090–1094.

Bradford, P.A., 2001. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev., **14**, 933–951.

Canton, R., Perez-Vazquez, M., Oliver, A., Sanchez del Saz, B., Gutierrez, M.O., Martinez-Ferrer, M. and Baquero, F., 2000. Evaluation of the Wider system, a new computer-assisted image-processing device for bacterial identification and susceptibility testing. J. Clin. Microbiol., **38**, 1339–1346.

Casellas, J.M., Tome, G., Bantar, C., Bertolini, P., Blazquez, N., Borda, N., Couto, E., Cudmani, N., Guerrera, J., Juarez, M.J., Lopez, T., Littvik, A., Mendez, E., Notario, R., Ponce, G., Quinteros, M., Salamone, F., Sparo, M., Sutich, E., Vaylet, S. and Wolff, L., 2003. Argentinean collaborative multicenter study on the in vitro comparative activity of piperacillin-tazobactam against selected bacterial isolates recovered from hospitalized patients. Diagn. Microbiol. Infect. Dis., **47**, 527–537.

Clinical and Laboratory Standards Institute, 2006a. Performance Standards for Antimicrobial Susceptibility Testing. Sixteenth Informational Supplement. M100–S16. Clinical and Laboratory Standards Institute, Wayne, Pa.

Clinical and Laboratory Standards Institute, 2006b. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically (Seventh Edition). Approved Standard. M7–A7. Clinical and Laboratory Standards Institute, Wayne, Pa.

Hernandez, J.R., Martinez-Martinez, L., Canton, R., Coque, T.M., Pascual, A. and Spanish Group for Nosocomial Infections (GEIH), 2005. Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. Antimicrob. Agents Chemother., **49**, 2122–2125.

Hoban, D.J., Bouchillon, S.K., Johnson, B.M., Johnson, J.L., Dowzicky, M.J. and Tigecycline Evaluation and Surveillance Trial (TEST program) Group, 2005. *In vitro* activity of tigecycline against 6792 Gram-negative and Gram-positive clinical isolates from the global tigecycline evaluation and surveillance trial (TEST Program, 2004). Diagn. Microbiol. Infect. Dis., **52**, 215–227.

Johnson, D.M., Biedenbach, D.J. and Jones, R.N., 2002. Potency and antimicrobial spectrum update for piperacillin-tazobactam 2000: Emphasis on its activity against resistant organisms populations and generally untested species causing community-acquired respiratory tract infections. Diagn. Microbiol. Infect. Dis., **43**, 49–60.

Kohler, J., Dorso, K.L., Young, K., Hammond, G.G., Rosen, H., Kropp, H. and Silver, L.L., 1999. *In vitro* activities of the potent, broad-spectrum carbapenem MK-0826 (L-749,345) against broad-spectrum β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates. Antimicrob. Agents Chemother., **43**, 1170–1176.

Livermore, D.M., Oakton, K.J., Carter, M.W. and Warner, M., 2001. Activity of Ertapenem (MK-0826) versus *Enterobacteriaceae* with potent β-lactamases. Antimicrob. Agents Chemother., **45**, 2831–2837.

Lautenbach, E., Patel, J.B., Bilker, W.B., Edelstein, P.H. and Fishman, N.O., 2001. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis., **32**, 1162–1171.

Martínez-Martínez, L., Hernandez-Alles, S., Alberti, S., Tomas, J.M., Benedi, V.J. and Jacoby, G.A., 1996. In-vivo selection of porin deficient mutants of *Klebsiella pneumoniae* with increased resistance to cefoxitin and expanded-spectrum cephalosporins. Antimicrob. Agents Chemother., **40**, 342–348.

Paterson, D.L., Ko, W.C., Gottberg, A., Casellas, J.M., Mulazimoglu, L., Klugman, K.P., Bonomo, R.A., Rice, L.B., McCormack, J.G. and Yu, V.L., 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol., **39**, 2206–2212.

Sader, H.S., Fritsche, T.R. and Jones, R.N., 2005. Potency and spectrum trends for cefepime tested against 65746 clinical bacterial isolates collected in North American medical centers: Results from the SENTRY Antimicrobial Surveillance Program (1998–2003). Diagn. Microbiol. Infect. Dis., **52**, 265–273.

Sorlozano, A., Gutierrez, J., Luna, J.D., Oteo, J., Liebana, J., Soto, M.J. and Piedrola, G., 2007. High presence of extended-spectrum β-lactamases and resistance to quinolones in clinical isolates of *Escherichia coli*. Microbiol. Res.

Spanu, T., Luzzaro, F., Perilli, M., Amicosante, G., Toniolo, A., Fadda, G. and Italian ESBL Study Group, 2002. Occurrence to extended-espectrum β-lactamases in members of the family *Enterobacteriaceae* in Italy: Implications for resistance to β-lactams and other antimicrobial drugs. Antimicrob. Agents Chemother., **46**, 196–202.

Valverde, A., Coque, T.M., Sanchez-Moreno, M.P., Rollan, A., Baquero, F. and Canton, R., 2004. Dramatic increase in prevalence of fecal carriage of extended-spectrum β-lactamase-producing *Enterobacteriaceae* during nonoutbreak situations in Spain. J. Clin. Microbiol., **42**, 4769–4775.

Yu, W.L., Pfaller, M.A., Winokur, P.L. and Jones, R.N., 2002. Cefepime MIC as a predictor of the extended-spectrum beta-lactamase type in *Klebsiella pneumoniae*, Taiwan. Emerg. Infect. Dis., **8**, 522–524.