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Original

Activity of ertapenem and other antimicrobials against ESBL-producing enterobacteria isolated from urine in patients from Madrid

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SUMMARY

The objective of this study was to evaluate the activity of ertapenem and other antimicrobials against extended-spectrum β-lactamase (ESBL)-producing enterobacteria isolated from patients' urine samples at 4 community healthcare centers in the Madrid (Spain) area and to determine the prevalence of ESBL-producing enterobacteria in community-acquired urinary tract infections. The antibiotic susceptibility results were compared by patient age and sex. A total of 293 strains were studied. The minimum inhibitory concentration (MIC) for each antibiotic was determined using the agar dilution method. The tested carbapenems were the antibiotics with the greatest activity (ertapenem MIC_{90}=0.06 mg/l; imipenem MIC_{90}=0.5 mg/l), with no intermediate or resistant strains being observed. High rates of resistance to ciprofloxacin (80.9%) and cotrimoxazole were observed (62.1%). The global prevalence of ESBL-producing enterobacteria was 3.6% (293/8,139). Prevalence according to areas was 5.3% in Getafe, 3.45% in Argüelles, 3.02% in Alcalá de Henares and 3.56% in Móstoles. The global prevalence of ESBL-producing Escherichia coli *was 4.15% (279/6,721). The analysis of resistance according to patient sex (males versus females) showed no significant differences. The analysis of resistance according to patient age (<50 years* versus *≥50 years) showed statistically significant differences (more resistance among subjects ≥50 years old) for cotrimoxazole (OR=0.43, 95%CI: 0.20-0.93, p=0.018) and ciprofloxacin (OR=0.32, 95%CI: 0.14-0.74, p=0.0027). In view of the good activity shown by ertapenem, and the continuous increase in the prevalence of ESBL strains, this antibiotic and some of the others could be a good choice for the treatment of community-acquired urinary tract infections produced by such bacteria in Spain.*

Key words: Ertapenem - Antibiotics - Extended-spectrum β-lactamases - Urinary tract infections

Actividad de ertapenem y otros antimicrobianos frente a enterobacterias productoras de BLEE aisladas de orina en pacientes de Madrid

RESUMEN

El objetivo de nuestro estudio fue evaluar la actividad del ertapenem y de otros antimicrobianos frente a cepas de enterobacterias productoras de BLEE aisladas de orina de pacientes de la comunidad en cuatro áreas sanitarias de Madrid, así como establecer la prevalencia de enterobacterias productoras de BLEE en este tipo de infecciones. Los resultados de sensibilidad antibiótica se compararon por edad y sexo. Se estudiaron 293 cepas. La CMI de cada antibiótico se determinó por el método de dilu-

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ción en agar. Los carbapenemes probados fueron los antibióticos con mayor actividad (ertapanem CMI_{90} = 0,06 mg/l; imipenem CMI_{90} = 0,5 mg/l), sin que se observara ninguna cepa con sensibilidad intermedia o resistente. Se encontraron altas tasas de resistencia a ciprofloxacino (80,9%) y a cotrimoxazol (62,1%). La prevalencia global de enterobacterias productoras de BLEE fue del 3,6% (293/8139). La prevalencia según el área de salud fue del 5,3% en Getafe, del 3,45% en Argüelles, del 3,02% en Alcalá de Henares y del 3,56% en Móstoles. La prevalencia global de Escherichia coli *productoras de BLEE fue del 4,15% (279/6721). En el análisis de la resistencia por sexo (hombres frente a mujeres) no se encontraron diferencias significativas. En el análisis por edad (<50 años frente a ≥50 años) se encontraron diferencias estadísticamente significativas (más resistencia en ≥50 años) para el cotrimoxazol (OR = 0.43, IC95%: 0,20-0,93, p = 0.018) y el ciprofloxacino (OR = 0.32, IC95%: 0,14-0,74, p = 0.0027). Considerando la buena actividad mostrada por el ertapenem y el continuo aumento en la prevalencia de cepas productoras de BLEE, este antibiótico y algún otro podrían ser una buena elección para el tratamiento de las infecciones del tracto urinario adquiridas en la comunidad producidas por cepas productoras de BLEE en España.*

Palabras clave: Ertapenem - Antibióticos - Betalactamasas de espectro extendido - Infección urinaria

INTRODUCTION

Ertapenem is a parenteral, 1-β methyl carbapenem, a member of the β-lactam class of antibiotics. The drug possesses a broad spectrum of action against Gram-positive and Gram-negative bacteria (aerobes as well as anaerobes). In addition, it has a long half-life of about 4.5 hours, allowing for once-daily dosing via both the intramuscular and intravenous routes, and is eliminated in urine, where it reaches high concentrations. Following i.v. administration of 1.0 g, the drug reaches a serum peak concentration of 150 mg/l. After 12 and 24 hours, the levels are 4 mg/l and 1-2 mg/l, respectively (1).

Preliminary data suggest that ertapenem may be effective as monotherapy for community-acquired infections, including urinary tract infections produced by single or mixed bacterial isolates (2, 3). In addition, a number of studies have confirmed that ertapenem is resistant to hydrolysis mediated by many β-lactamases (both plasmidic and chromosomal), including extended-spectrum β-lactamases (ESBL) (4-7).

The present study was designed to evaluate the activity of ertapenem and other antimicrobial agents against extended-spectrum β-lactamase (ESBL)-producing enterobacteria isolated from patients' urine samples at four community healthcare centers in the Madrid (Spain) area. Another objective was to determine the prevalence of ESBL-producing enterobacteria in community-acquired urinary tract infections and to compare the antibiotic susceptibility results by patient age and sex in order to determine possible statistically significant differences.

MATERIALS AND METHODS

Bacterial isolates

A total of 293 urinary pathogens from the microbiology laboratories of Príncipe de Asturias University Hospital (Alcalá de Henares), Getafe University Hospital, the Primary Care Center of Argüelles and Móstoles Hospital –all located in the Community of Madrid (Spain)– were used in this study. The strains were collected from November 2005 to June 2006. The base population of this study consisted of outpatients from these healthcare centers. During this period, 8,139 enterobacteria isolates were screened for production of ESBL, of which 6,721 were *Escherichia coli*.

A total of 234 strains were isolated from females (79.5%) and 60 from males (20.5%). Each bacterium was identified by standard laboratory methods. The detection of ESBL production was based on the agar diffusion technique according to the standardized conditions of the Clinical and Laboratory Standards Institute guidelines (8), using *E-test*® strips of cefotaxime/cefotaxime clavulanate and ceftazidime/ceftazidime clavulanate, and also cefoxitin discs.

Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) for each antibiotic was determined using the agar dilution method (8). The following antimicrobial agents obtained from their respective manufacturers were studied: ampicillin, cefazolin, cefuroxime, cefotaxime, amoxicillin/clavulanate, piperacillin/tazobactam, imipenem, ertapenem, gentamicin, amikacin, fosfomycin, ciprofloxacin and cotrimoxazole. Mueller-Hinton agar was used as culture medium, in which a dilution of the bacterial suspension was inoculated at a McFarland turbidity equivalent of 0.5, representing approximately 10^4 colony-forming units (CFUs) per spot applied with the Steers replicator (Craft Machine Inc., Chester, PA, USA). The plates were incubated for 18-24 hours at 35 °C. The breakpoints considered were those stated in the Clinical and Laboratory Standards Institute 2005 manual (8).

The control strains were *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *E. coli* ATCC 35218, *Staphylococcus aureus.* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Statistical analysis

The χ^2 test and Fisher's exact test were used. A two-tailed p-value of ≤0.05 was considered significant.

RESULTS

The 293 strains studied were distributed by species as follows: 279 *E. coli*, 11 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca* and 1 *Citrobacter koserii*.

Table 1 reports the results corresponding to MIC range, MIC_{50} and MIC_{90}, along with the susceptibility and resistance data of the 279 strains of *E. coli*.

The tested carbapenems were the antibiotics with the greatest activity, with no strain exhibiting an MIC above the established cutoff value. Comparison of activity showed ertapenem to be the most active. The MIC_{90} of ertapenem (0.06 mg/l) was three dilutions below that of imipenem (0.5 mg/l).

High rates of resistance to ciprofloxacin (80.9%) and cotrimoxazole were observed (62.1%) among all strains studied.

The global prevalence of ESBL-producing enterobacteria was 3.6% (293/8,139). Prevalence rates according to healthcare centers were 5.3% in Getafe, 3.45% in Argüelles, 3.02% in Alcalá de Henares and 3.56% in Móstoles.

The global prevalence of ESBL-producing *E. coli* was 4.15% (279/6,721). Prevalence rates according to healthcare centers were 5.97% (56/938) in Getafe, 3.94% (75/1,903) in Argüelles, 3.6% (74/2,057) in Alcalá de Henares and 4.06% (74/1,823) in Móstoles.

The analysis of resistance according to patient sex (males versus females) showed no significant differences for any of the antibiotics studied. The analysis of resistance according to patient age (<50 years *versus* ≥50 years) showed statistically significant differences (more resistance among subjects ≥50 years old) for cotrimoxazole (OR=0.43, 95%CI: 0.20-0.93, p=0.018) and ciprofloxacin (OR=0.32, 95%CI: 0.14-0.74, p=0.0027).

DISCUSSION

Urinary tract infections (UTI) are among the most frequent human infections and cause considerable morbidity among the outpatient population.

While the etiology of UTI does not differ to any significant degree among zones, the microbial resistance rates have experienced great variations. As a result, the empirical treatment of such infections requires constant updating of the antibiotic susceptibility of the principal urinary tract pathogens found in each zone, country and institution involved.

Reports of isolation of ESBL-producing *E. coli* and *Klebsiella* spp. are increasingly frequent in Spain. This increase is observed not only in the hospital setting but also in community-based infections (9-12). In comparison to earlier data from the healthcare centers participating in this study (data not shown), and based on data in the literature referring to Spain (9-12), an increase in the prevalence of community-acquired urinary infections is observed (including those seen in hospital emergency services), produced by ESBL-producing strains of *E. coli*, particularly of

Table 1. *In vitro* susceptibility of 279 ESBL-producing *E. coli* strains to 13 antibiotics (MIC in mg/l).

Antibiotic	MIC range	MIC_{50}	MIC_{90}	%S	%I	%R
Ampicillin	>16	>16	>16	0	0	100
Cefazolin	>16	>16	>16	0	0	100
Cefuroxime	>16	>16	>16	0	0	100
Cefotaxime	1->16	>16	>16	–	–	100
Amoxicillin/clavulanate	4/2->32/16	8/4	32/16	56.3	29.7	14
Piperacillin/tazobactam	≤1/4->64/4	4/4	32/4	77.1	17.9	5.0
Imipenem	≤0.06-1	0.25	0.5	100	0	0
Ertapenem	≤0.008-0.12	0.03	0.06	100	0	0
Gentamicin	≤0.5->8	≤0.5	>8	81.3	2.2	16.5
Amikacin	≤1->16	2	8	99.3	0	0.7
Fosfomycin	≤1->64	2	16	93.6	0	6.4
Ciprofloxacin	≤0.06->4	>4	>4	15.5	2.5	82.0
Cotrimoxazole	≤0.5/9.5->2/38	>2/38	>2/38	37.3	–	62.7

the CTX-M type, conditioning resistance to third-generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefixime and others). These infections are fundamentally found in elderly people with concomitant disease processes and recent prior antibiotic treatments, although they can also be seen in patients without such risk factors. These results have also been described worldwide (13). On the other hand, ESBL-producing strains are usually also resistant to other groups of antibiotics such as cotrimoxazole, quinolones and/or aminoglycosides, which further complicates the treatment scenario (14). Knowledge of the prevalence statistics of ESBL-producing enterobacteria and of their susceptibility to antibiotics, together with the characteristics of the patients involved, may help select coherent and effective empirical treatments.

The strains selected for the present study, according to the previously described criteria, were all found to be susceptible only to ertapenem and imipenem. Ertapenem was the most active of the antibiotics studied, with an MIC_{90} of 0.06 mg/l. Other authors have shown the excellent activity of ertapenem against strains of ESBL-producing *E. coli* and *K. pneumoniae*, but with such strains collected between 1995 and 2001 (15). Our work provides similar results but with a very recent collection of ESBL-producing strains a few years after the introduction of ertapenem in the therapeutic armamentarium, perhaps because resistance to these drugs implies an excessive biological cost for bacteria. We documented high rates of resistance or intermediate resistance to antibiotics commonly used to treat urinary infections, such as amoxicillin/clavulanate, gentamycin, ciprofloxacin and cotrimoxazole. The greater resistance to the latter two antibiotics among patients over the age of 50 years could be attributed to increased consumption in this population group, as well as other risk factors such as increased frequency of complicated UTIs, the presence of urinary catheters, etc., thus further complicating the treatment of infections of this kind in this particular patient age group.

The resistance to other antibiotics very often seen in our ESBL-producing strains facilitates their persistence and spread, and thus complicates their control.

A fact that should be pointed out is the good *in vitro* activity of fosfomycin in strains of this kind, with low resistance rates (6.8% for the global strains studied). Further studies are needed to assess the drug's clinical efficacy against strains of this type.

Due to the growing prevalence of bacteria of this kind in Spain, future studies would be advisable to explore prevalence and antibiotic susceptibility in order define their evolution over time.

In conclusion, in view of the good activity shown by ertapenem against the strains studied here, its known lack of activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and the continuous increase in prevalence of ESBL-producing strains, this drug could be a good choice for treating community UTIs produced by ESBL-producing strains in Spain.

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