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β-lactam susceptibility of *Escherichia coli* isolates from urinary tract infections exhibiting different resistance phenotypes

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Susceptibility to β-lactams was determined in 203 recent Spanish *E. coli* isolates from urinary tract infections exhibiting different resistance phenotypes: *a)* susceptible (n = 60); *b)* quinolone-resistant (n = 45); *c)* penicillinase (n = 64); *d)* hyperproduction of penicillinase (n = 8); *e)* inhibitor resistant TEM (IRT) (n = 18), and *f)* extended spectrum betalactamase (ESBL) (n = 8). Minimum inhibitory concentration (MIC) determination by agar dilution and susceptibility tests for ESBL detection by macrodilution were performed following CLSI recommendations. All the β-lactams tested showed high activity against susceptible and penicillinase phenotypes, with close to 100 % susceptibility. Hyperproduction of penicillinase increased MIC_{90} values for all antibiotics except for meropenem, with 100% resistance to cefuroxime and amoxicillin/clavulanic acid, and 100 % susceptibility to cefotaxime, piperacillin/tazobactam and meropenem. All the antibiotics, except for amoxicillin/clavulanic acid, exhibited high activity against IRT. Meropenem, cefminox and piperacillin/tazobactam exhibited the highest activity against ESBL, followed by amoxicillin/clavulanic acid. The most active compound among the parenteral antibiotics was meropenem, regardless of the resistance phenotype. Among the oral antibiotics, the most active compound was cefditoren with the exception of ESBL where amoxicillin/clavulanic acid where the MIC_{90} value was one dilution lower.

Key words:
E. coli. β-lactamase. Cefditoren.

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Sensibilidad a betalactámicos de aislados clínicos de *Escherichia coli* con distintos fenotipos de resistencia procedentes de infecciones urinarias

Se determinó la susceptibilidad a betalactámicos de 203 aislados recientes de *E. coli* procedentes de infecciones del tracto urinario en España y que presentaban distintos fenotipos de resistencia: *a)* susceptible (n = 60); *b)* resistente a quinolonas (n = 45); *c)* productor de penicilinasa (n = 64); *d)* hiperproductor de penicilinasa (n = 8); *e)* resistente a inhibidores de TEM (IRT) (n = 18), y *f)* productor de betalactamasas de espectro extendido (BLEE) (n = 8). La determinación de la concentración mínima inhibitoria (CMI) por dilución en agar y los tests de susceptibilidad para la detección de BLEE se realizaron siguiendo las recomendaciones del Clinical and Laboratory Standards Institute (CLSI). Frente a los fenotipos susceptible y productor de penicilinasa, todos los betalactámicos ensayados exhibieron gran actividad, con una sensibilidad cercana al 100% de los aislados. La hiperproducción de penicilinasa incrementó los valores de CMI_{90} de todos los antibióticos, excepto de meropenem, con un 100% de resistencia a cefuroxima y amoxicilina/clavulánico y un 100% de sensibilidad a cefotaxime, piperacilina/tazobactam y meropenem. Todos los antibióticos, excepto amoxicilina/clavulánico, presentaron gran actividad frente a las cepas IRT. Meropenem, cefminox y piperacilina/tazobactam presentaron la mayor actividad frente a BLEE, seguidas de amoxicilina/clavulánico. Entre los antibióticos parenterales, el compuesto más activo fue meropenem, con independencia del fenotipo de resistencia. Entre los antibióticos orales el compuesto más activo fue cefditoren, excepto frente a las cepas BLEE, donde amoxicilina/clavulánico presentó en un valor de CMI_{90} una dilución menor.

Palabras clave:
E. coli. Betalactamasa. Cefditoren.

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INTRODUCTION

Escherichia coli is the main etiological agent of community-acquired urinary tract infections. Amoxicillin, first generation cephalosporins and co-trimoxazole have been mainstays of oral therapy of *E. coli* infections, but nowadays they cannot be considered as useful agents due to high resistant rates, driving to recommendations to use amoxicillin/clavulanic acid, second generation cephalosporins as cefuroxime or quinolones. If parenteral therapy is needed, as in severe community-acquired pyelonephritis, the use of a quinolone, a combination of aminopenicillin/β-lactamase inhibitor, piperacillin/tazobactam and third generation cephalosporins has been advocated.

Resistance patterns suffer variations among the different geographical areas, depending on the antibiotic consumption pattern[1]. In Spain, despite that the highly susceptible wild-type *E. coli* phenotype represents nearly 50% of urinary isolates from the community[2], resistance rates to co-trimoxazole and nalidixic acid are >25%[3], and high resistance rates to quinolones (14.7%)[3] (related to quinolone consumption[1]) are found. Quinolone consumption is not only a risk factor for quinolone resistance, but also for infections due to isolates exhibiting specific β-lactamase production phenotypes in *E. coli* isolated from the community[4]. Production and hyperproduction of penicillinase are the most frequent resistant phenotypes (approx. 20% and 10%, respectively), with small isolation rates of Inhibitor Resistant TEM (IRT) and Extended Spectrum β-lactamase (ESBL) phenotypes (0.9% and 0.7%, respectively)[2]. Due to the high prevalence in our country of quinolone resistance, the endemicity of some β-lactamase production phenotypes, and the emergence of new β-lactamase production phenotypes among community *E. coli* isolates, surveillances and susceptibility studies with old and new, parenteral and oral, β-lactams are needed as a basic step to combat resistance.

The purpose of this study was to determine the susceptibility to different β-lactams of 203 Spanish *Escherichia coli* strains isolated along 2005 from urinary tract infections, exhibiting different resistance phenotypes.

MATERIAL AND METHODS

Strains

A total of 203 Spanish *Escherichia coli* strains isolated along 2005 from urinary tract infections in different Spanish hospitals and sent to the Instituto Valenciano de Microbiología (Valencia, Spain) for susceptibility testing were tested. Strains exhibited different resistance phenotypes: *a)* susceptible (controls) (n=60); *b)* quinolone-resistant (n=45) defined as non- β-lactamase production ciprofloxacin-resistant isolates; *c)* penicillinase (n=64); *d)* hyperproduction of penicillinase (n=8); *e)* IRT (n=18), and *f)* ESBL (n=8).

Antimicrobials

Antibiotics tested were one parenteral cephamycin (cefminox), one oral 2nd generation cephalosporin (cefuroxime), two 3rd generation cephalosporins (one oral, cefditoren, and one parenteral, cefotaxime), two penicillin/β-lactamase inhibitor combinations (amoxicillin/clavulanic acid, and one parenteral, piperacillin/tazobactam), and one carbapenem (meropenem). In addition a quinolone, ciprofloxacin, was also tested due to the high resistance prevalence in Spain.

Methods

MIC determination was performed by agar dilution following CLSI recommendations[5], with a final inocula of 1×10^4 cfu/spot in Mueller-Hinton agar (Difco laboratories, Detroit, Mi), and 35 ºC incubation for 20-24 h. Susceptibility tests for ESBL detection were performed by macrodilution following CLSI recommendations[5]. Antimicrobial concentrations ranged from 0.07 to 128 µg/ml. *E. coli* ATCC 25922, *E. coli* ATCC 35218 (for β-lactam/β-lactamase inhibitor combinations), and *Klebsiella pneumoniae* ATCC 700603 (ESBL) were used as control strains. Breakpoints used were those defined by CLSI[6].

RESULTS AND DISCUSSION

Twenty to thirty percent of adult women between 20 to 40 years of age has at least one episode of urinary tract infection[7], 90% of them are mild, candidates to oral treatment, being *E. coli* by far the most frequent isolate in these community infections. The existence of different β-lactamase resistance phenotypes should be kept in mind in an environment, as in Spain, where the use of quinolones is not advocated[8], because even the less prevalent phenotypes may cause infection in a significant number of patients due to the high incidence and prevalence of this disease. This warrants susceptibility studies including a significant number of isolates even of the less prevalent phenotypes.

The Table shows MIC50, MIC_{90} (µg/ml), range (µg/ml) and percentage of susceptible (S), intermediate (I) and resistant (R) strains. Among parenteral antibiotics (cefminox, cefotaxime, amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem and ciprofloxacin), the most active compound was meropenem, regardless the resistance phenotype. Among oral antibiotics (cefuroxime, cefditoren, amoxicillin/clavulanic acid and ciprofloxacin), the most active compound was cefditoren with the exception of the ESBL phenotype where amoxicillin/clavulanic acid exhibited one-dilution lower MIC_{90} value.

No susceptibility problems with β-lactams can be seen in the susceptible, quinolone-resistant phenotype and penicillinase phenotypes, where nearly 100% isolates were susceptible to all β-lactams according to CLSI breakpoints. No CLSI breakpoints are available for cefminox or cefditoren, but

Tabla 1 **MIC_{50}, MIC_{90} (µg/ml) and susceptibility to study drugs of strains from the different phenotypes**

Phenotype	Antibiotic	MIC_{50}	MIC_{90}	Range	%S	%I	%R
Susceptible (n=60)	Cefminox	0,25	0,5	0,06-0,5	NA	NA	NA
	Cefuroxime	1	2	0,125-4	100	—	—
	Cefotaxime	0,015	0,03	≤0,007-0,125	100	—	—
	Cefditoren	0,06	0,125	0,015-0,25	NA	NA	NA
	Amoxicillin/clavulanate	1	1	0,25-4	100	—	—
	Piperacillin/tazobactam	0,5	2	0,06-4	100	—	—
	Meropenem	≤0,007	≤0,007	≤0,007-0,015	100	—	—
	Ciprofloxacin	≤0,007	0,125	≤0,007-1	100	—	—
Quinolone-resistant (n=45)	Cefminox	0,5	0,5	0,125-16	NA	NA	NA
	Cefuroxime	1	4	0,5-16	93,4	6,6	—
	Cefotaxime	0,03	0,125	≤0,007-1	100	—	—
	Cefditoren	0,125	0,25	0,03-2	NA	NA	NA
	Amoxicillin/clavulanate	4	8	0,5-32	95,6	2,2	2,2
	Piperacillin/tazobactam	2	8	0,5-8	100	—	—
	Meropenem	≤0,007	0,015	≤0,007-0,015	100	—	—
	Ciprofloxacin	32	64	4-128	—	—	100
Penicillinase (n=64)	Cefminox	0,25	0,5	0,03-2	NA	NA	NA
	Cefuroxime	1	2	0,125-4	100	—	—
	Cefotaxime	0,015	0,03	≤0,007-1	100	—	—
	Cefditoren	0,06	0,125	0,015-0,5	NA	NA	NA
	Amoxicillin/clavulanate	4	8	1-16	96,9	3,1	—
	Piperacillin/tazobactam	1	4	0,06-4	100	—	—
	Meropenem	≤0,007	0,015	≤0,007-0,03	100	—	—
	Ciprofloxacin	≤0,007	0,25	≤0,007-16	90,6	—	9,4
Penicillinase hyperproduction (n=8)	Cefminox	16	64	8-64	NA	NA	NA
	Cefuroxime	32	64	16-64	—	—	100
	Cefotaxime	4	8	1-8	100	—	—
	Cefditoren	4	16	2-16	NA	NA	NA
	Amoxicillin/clavulanate	32	128	32-128	—	—	100
	Piperacillin/tazobactam	8	16	8-16	100	—	—
	Meropenem	≤0,007	0,03	≤0,007-0,03	100	—	—
	Ciprofloxacin	8	32	8-32	—	—	100
IRT (n=18)	Cefminox	0,25	0,5	0,125-16	NA	NA	NA
	Cefuroxime	1	4	0,5-8	94,5	5,5	—
	Cefotaxime	0,03	0,125	≤0,007-0,5	100	—	—
	Cefditoren	0,06	0,5	0,06-2	NA	NA	NA
	Amoxicillin/clavulanate	32	32	32-32	—	—	100
	Piperacillin/tazobactam	4	8	1-16	100	—	—
	Meropenem	≤0,007	0,015	≤0,007-0,015	100	—	—
	Ciprofloxacin	≤0,007	64	≤0,007-64	83,4	—	16,6
ESBL (n=8)	Cefminox	0,5	0,5	0,5-0,5	NA	NA	NA
	Cefuroxime	≥128	≥128	128-≥128	—	—	100
	Cefotaxime	16	64	8-64	12,5	62,5	35,0
	Cefditoren	8	32	4-32	NA	NA	NA
	Amoxicillin/clavulanate	8	16	8-16	71,0	29,0	—
	Piperacillin/tazobactam	2	2	0,5-2	100	—	—
	Meropenem	0,015	0,015	≤0,007-0,015	100	—	—
	Ciprofloxacin	8	64	0,125-64	12,5	—	87,5

MIC_{90} values against the strains tested from these phenotypes were ≤0.5 µg/ml, a concentration susceptible to the very high urine concentrations obtained after standard doses of these antibiotics (www.agemed.es)[9].

Penicillinase hyperproduction decreased the activity of penicillins, cephalosporins and the cephamycin tested in comparison to the penicillinase phenotype, by increasing MIC_{90} values obtained against the penicillinase phenotype for all antibiotics tested except for meropenem. This hyperproduction drived to 100% resistance to amoxicillin/clavulanic acid and cefuroxime, not affecting 100% susceptibility rates to parenteral antibiotics as cefotaxime, piperacillin/tazobactam and meropenem.

All antibiotics, except amoxicillin/clavulanic acid, exhibited high activity against IRT, suggesting that caution should be taken in the detection of this phenotype.

Lastly with the ESBL phenotype, as expected[10], activity of cephalosporins (cefuroxime, cefotaxime and cefditoren) was poor, while cefminox, piperacillin/tazobactam and meropenem maintained the highest activity. Amoxicillin/clavulanic acid was the oral compound with the highest intrinsic activity.

The emergence of *E. coli* β-lactamase resistant phenotypes in community urinary tract infections, currently showing very low prevalence but with associated resistance problems (as global decrease in susceptibility to oral β-lactams in the IRT phenotype or cephalosporin-resistance with the ESBL phenotype) increases the importance of susceptibility testing of *E. coli* isolates from community urinary tract infections, more even in severe cases implying parenteral therapy.

REFERENCES

1. Kahlmeter G. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECO.SENS study. Int J Antimicrob Agents 2003;22(Suppl. 2):49-52.
2. Alonso Sanz M, Abad Becquer MI. Fenotipos de resistencia en aislamientos urinarios de Escherichia coli en la comunidad: implicaciones terapéuticas. Med Clin (Barc) 2003;120:361-4.
3. Kahlmeter G, ECO.SENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrob Chemother 2003;51:69-76.
4. Rodríguez-Baño J, Navarro MD, Romero L y cols. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. J Clin Microbiol 2004;42:1089-94.
5. Clinical and Laboratory Standards Institute/NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that growth aerobically; Approved standard M7-A6. CLSI, Wayne, PA, USA, 2003.
6. Clinical and Laboratory Standards Institute/NCCLS. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement. CLSI/NCCLS document M100-S15. CLSI, Wayne, PA, USA, 2005.
7. Sanford JP. Urinary Tract Symptoms and Infections. Ann Rev Med 1975;26:485-98.
8. Naber KG. Treatment options for acute uncomplicated cystitis in adults. J Antimicrob Chemother 2000;46(Suppl. 1):23-7.
9. Sadaba B, Azanza JR, Quetglas EG, et al. Pharmacokinetic/pharmacodynamic serum and urine profile of cefditoren following single-dose and multiple twice- and thrice-daily regimens in healthy volunteers: a phase I study. Rev Esp Quimioter 2007;20:51-60.
10. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.