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Original

High rates of antimicrobial co-resistance among *Enterobacteriaceae*: comparative analysis between clinical isolates resistant and susceptible to third-generation cephalosporins

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SUMMARY

We compared the antimicrobial co-resistance of 3,402 clinical isolates of Enterobacteriaceae *resistant to third-generation cephalosporins (2,569 ESBL-producing and 833 AmpC overproducing) with that of 16,220 susceptible isolates, in order to determine the impact of resistance to third-generation cephalosporins on the likelihood of resistance to other antimicrobial classes.* Enterobacteriaceae *resistant to third-generation cephalosporins, independently of their mechanism of resistance, were significantly more resistant to other classes of antimicrobials than susceptible isolates (*p *<0.001). Percentages of co-resistance to ciprofloxacin, gentamicin, tobramycin and trimethoprim-sulfamethoxazole of resistant isolates were: 51%, 39%, 53% and 51%, respectively. However, among the susceptible isolates, percentages were 17%, 7%, 6% and 19%, respectively. Fosfomycin exhibited excellent* in vitro *activity against urinary isolates (92%), mainly against ESBL-producing organisms (90%), and is a good alternative treatment of infections caused by multidrug resistant* Enterobacteriaceae. *Amikacin and imipenem were the most active antimicrobials against all species tested.*

Key words: Co-resistance - Beta-lactamases - *Enterobacteriaceae* resistance - Third-generation cephalosporins

Altos porcentajes de corresistencia antimicrobiana entre Enterobacteriaceae*: análisis comparativo entre aislamientos clínicos resistentes y sensibles a las cefalosporinas de tercera generación*

RESUMEN

En este estudio se ha comparado la corresistencia antimicrobiana de 3402 aislamientos clínicos de Enterobacteriaceae *resistentes a las cefalosporinas de tercera generación (2569 productores de BLEE y 833 hiperproductores de AmpC) y 16.220 sensibles, con el fin de determinar la repercusión de la resistencia a las cefalosporinas de tercera generación asociada a resistencia a otra clase de antimicrobianos. Las enterobacterias resistentes a las cefalosporinas de tercera generación, independientemente de su mecanismo de resistencia, fueron significativamente más resistentes que las sensibles (*p *<0.001). Los porcentajes de corresistencia a ciprofloxacino, gentamicina, tobramicina y cotrimoxazol de los aislamientos resistentes fueron 51%, 39%, 53% y 51%, respectivamente; sin embargo, tales porcentajes en los aislamientos sen-*

sibles fueron 17%, 7%, 6% y 19%, respectivamente. La fosfomicina presentó una excelente actividad in vitro *frente a los aislamientos urinarios (92%), principalmente frente a los microorganismos productores de BLEE (90%), y parece ser una buena opción para el tratamiento de las infecciones causadas por enterobacterias multirresistentes. Amikacina e imipenem fueron los antimicrobianos más activos frente a todas las especies probadas.*

Palabras clave: Corresistencia - Betalactamasas - Resistencia *Enterobacteriaceae* - Resistencia cefalosporinas

Resistance to third-generation cephalosporins among *Enterobacteriaceae* is a cause of increasing concern worldwide (1-5). The production of plasmid-mediated, extended-spectrum beta-lactamases (ESBLs) and the derepressed production of the chromosomal AmpC beta-lactamase are the main mechanisms of resistance to beta-lactams among members of the *Enterobacteriaceae* family. The ESBLs are enzymes capable of hydrolyzing penicillins, broad-spectrum cephalosporins, including third and fourth-generation, and aztreonam. They are not active against cephamycins and carbapenems, and are inhibited by beta-lactamase inhibitors such as clavulanic acid. The AmpC-type beta-lactamases hydrolyze third-generation cephalosporins but are not inhibited by clavulanic acid (4). Clinical failures have been reported when third-generation cephalosporins are used to treat infections caused by pathogens with these mechanisms of resistance. In the case of ESBL producers, failures have been reported even when the microorganism showed *in vitro* susceptibility to the cephalosporin used for the treatment (6). Clinical failures have been described as well when third-generation cephalosporins were used to treat *Enterobacter* spp. and other AmpC producing *Enterobacteriaceae* due to the selection of stably derepressed mutants that hyperproduce the AmpC-type beta-lactamases (7-10).

The increased prevalence of ESBLs in certain microorganisms and their spread into new hosts, as well as the dissemination of AmpC-overproducing strains, is modifying the therapeutic choices, which are becoming seriously reduced (11). Furthermore, the plasmids bearing genes encoding ESBLs also frequently carry genes responsible for the resistance to other families of antimicrobial agents, such as aminoglycosides and cotrimoxazole (12), and a marked association between resistance to fluoroquinolones and ESBL-positive *Enterobacteriaceae* has also been described (13-17). Most studies describing the co-resistance of *Enterobacteriaceae* to different antimicrobial classes rely on the description of outbreaks, or refer to particular types of patients or clinical syndromes, or to the situation in a specific ward, and thus provide biased information (18-21). Data regarding antimicrobial co-resistance without a previous selection is scarce, and reports including all potentially useful non-beta-lactam antimicrobials for *Enterobacteriaceae* resistant to third-generation cephalosporins are lacking.

The aim of this study was to compare, in a non-selected sample, the antimicrobial co-resistance of clinical isolates of *Enterobacteriaceae* resistant to third-generation cephalosporins (ESBL-positive and AmpC overproducers) with that of susceptible isolates, in order to determine the impact of resistance to third-generation cephalosporins on the likelihood of resistance to other antimicrobial classes.

MATERIALS AND METHODS

Our institution is a 1800-bed tertiary university hospital serving a population of 750,000 people in Madrid. For the purpose of this study, all clinical isolates of *Enterobacteriaceae* collected in our microbiology laboratory during 2004 and 2006 were analyzed retrospectively.

Identification and antimicrobial susceptibility testing of the isolates were performed by an automated microdilution system using MicroScan Combo Neg 1S and Combo Urine 1S panels (Dade Behring, Sacramento, Calif.) following the manufacturer's guidelines. Antimicrobials tested were ampicillin, ticarcillin, piperacillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefazolin, cefuroxime, cefoxitin, cefotaxime, ciprofloxacin, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, imipenem, meropenem, and fosfomycin (only for urinary isolates). Breakpoints were applied following Clinical and Laboratory Standards Institution (CLSI) recommendations (22). Although CLSI breakpoints for fosfomycin are only defined for *Escherichia coli*, we used the same breakpoints for other *Enterobacteriaceae* (23). Confirmatory testing for the presence of ESBLs was performed by the double-disk diffusion test (24, 25). Susceptibility data were compared by using a chi-square test (qualitative variables). A p value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 19,622 clinical isolates of *Enterobacteriaceae* were studied and included *E. coli* (11,775 isolates),

Table 1. Comparative analysis of co-resistance between *Enterobacteriaceae* resistant and susceptible to third-generation cephalosporins.

Antimicrobial agents analyzed[a]	Resistant		Susceptible		
	%	(No. isolates)	%	(No. isolates)	p
Ciprofloxacin	51	1,734	17	2,792	<0.001
Gentamicin	39	1,323	7	1,152	<0.001
Tobramycin	53	1,802	6	1,050	<0.001
Amikacin	6	209	1	92	<0.001
Trimethoprim-sulfamethoxazole	51	1,735	19	3,097	<0.001
Imipenem	1	26	0	2	<0.001

Klebsiella pneumoniae (2345), *Klebsiella oxytoca* (818), *Proteus mirabilis* (1831), *Enterobacter cloacae* (1231), *Enterobacter aerogenes* (268), *Citrobacter freundii* (224), *Morganella morganii* (645) and *Serratia marcescens* (485). Other enterobacteria species isolated in our laboratory over the study period (a total of 1173, including 797 *Salmonella* isolates) were not included in the analysis due to the low numbers of the particular species). The origin of the isolates was blood (2430 isolates, 12%), urine (13,637 isolates, 69%) and other origins (3555 isolates, 19%). Among these, 16,220 isolates (83%) were susceptible to third-generation cephalosporins, and 3402 (17%) were resistant and comprised 2569 (76%) ESBL-producing strains, and 833 (24%) AmpC overproducers.

Enterobacteriaceae resistant to third-generation cephalosporins, independently of their mechanism of resistance, were significantly more resistant to other classes of antimicrobials than susceptible isolates ($p < 0.001$) (Tables 1 and 2). About half of the ESBL-producing strains and one-third of the AmpC-overproducing isolates were resistant to ciprofloxacin, gentamicin, tobramycin and trimethoprim-sulfamethoxazole (Table 2). Blood isolates resistant to third-generation cephalosporins were significantly more resistant to other antimicrobial classes than susceptible isolates ($p < 0.001$). Among the resistant isolates, 55%, 48%, 56% and 55% were also resistant to ciprofloxacin, gentamicin, tobramycin and trimethoprim-sulfamethoxazole, respectively. However, among the susceptible isolates, these percentages decreased to 18%, 7%, 6% and 29%, respectively ($p < 0.001$). Urinary isolates resistant to third-generation cephalosporins were significantly more resistant to ciprofloxacin (65% *vs.* 44%) and trimethoprim-sulfamethoxazole (60% *vs.* 46%) than isolates from non-urinary samples ($p < 0.001$). Fosfomycin exhibited good *in vitro* activity against urinary isolates. It was active against 80% and 94% of isolates resistant and susceptible to third-generation cephalosporins, respectively. In addition, the activity of fosfomycin was superior against ESBL-producing strains (90%) as compared to AmpC-overproducing strains (51%).

Table 3 shows the percentages of isolates susceptible to the antimicrobial agents analyzed. Amikacin and imipenem were the most active antimicrobials against all species tested.

DISCUSSION

This study demonstrates that in a non-selected sample of *Enterobacteriaceae* recovered over a period of two years in a general hospital, the isolates resistant to third generation cephalosporins are significantly more resistant to other classes of antimicrobials. Over the past 20 years a high increase in the frequency of ESBL-producing *Enterobacteriaceae* as well as AmpC overproducers has been observed (1, 3, 4). Their presence, associated with concomitant resistance to other antibiotics, is a serious therapeutic problem (1, 26) that requires alternative therapies. For a number of years we have observed in our institution a high prevalence of *Enterobacteriaceae* resistant to third-generation cephalosporins which prompted us to look for thera-

Table 2. Co-resistance of *Enterobacteriaceae* resistant to third-generation cephalosporins: ESBL-producing strains and AmpC overproducers.

Antimicrobial agents analyzed	% of isolates resistant to:	
	ESBL-producing strains	AmpC overproducers
Ciprofloxacin	55	40
Gentamicin	38	42
Tobramycin	58	39
Amikacin	7	2
Trimethoprim-sulfamethoxazole	55	39
Imipenem	0	3

Table 3. Proportions of isolates susceptible to the antimicrobial agents analyzeda*.

Microorganism and group	No. isolates	% of isolates susceptible to:					
		GEN	TOB	AMIK	CIP	SEXT	IPM
E. coli							
CTX-R**	1687	67	53	94	28	34	100
CTX-S***	10,088	92	92	99	77	80	100
K. pneumoniae							
CTX-R**	719	54	17	89	82	71	100
CTX-S***	1,626	97	97	99	95	91	100
K. oxytoca							
CTX-R**	147	38	40	95	69	49	100
CTX-S***	671	98	99	100	95	93	100
P. mirabilis							
CTX-R**	16	62	62	100	69	31	100
CTX-S***	1815	90	94	99	88	61	100
E. cloacae							
CTX-R**	410	57	54	98	61	64	97
CTX-S***	821	99	99	100	99	97	100
E. aerogenes							
CTX-R**	101	62	58	95	86	93	94
CTX-S***	167	97	97	100	100	99	100
C. freundii							
CTX-R**	39	72	82	100	69	87	100
CTX-S***	185	94	96	99	94	89	100
M. morgannii							
CTX-R**	234	54	73	99	37	29	100
CTX-S***	411	82	93	100	84	68	100
S. marcescens							
CTX-R**	49	71	60	98	92	96	100
CTX-S***	436	99	89	99	92	98	100

*GEN: gentamicin; TOB: tobramycin, AMK: amikacin; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; IPM: imipenem.
**CTX-R: indicates resistance to third-generation cephalosporins, independent of the resistance mechanism.
***CTX-S: indicates susceptibility to third-generation cephalosporins.

peutic alternatives. The results of this study confirmed high rates of co-resistance with fluoroquinolones, trimethoprim-sulfamethoxazole and aminoglycosides (with the exception of amikacin), which have important clinical implications since only amikacin and imipenem seem to be an appropriate empirical alternative when a severe infection due to a third-generation cephalosporin-resistant microorganism is suspected. Several studies have reported that inappropriate initial antimicrobial therapy for bacteremia caused by ESBL-producing *E. coli* or *K. pneumoniae* is associated with a significant higher mortality rate than when initial therapy involves an active agent (27-30), and treatment of *Enterobacter* bacteremia with third-generation cephalosporins may select for mutant strains that overproduce the AmpC beta-lactamase which is also associated with higher mortality rates (7).

Numerous studies have identified risk factors for nosocomial colonization or infection by multiresistant pathogens that include advanced age, underlying diseases and severity of illness, inter-institutional transfer of the patient, especially from a nursing home, prolonged hospitalization, gastrointestinal surgery or transplantation, exposure to invasive devices of all types, and exposure to antimicrobial drugs, especially cephalosporins and fluoroquinolones (31, 32). Thus, in areas with a high incidence of *Enterobacteriaceae* resistant to third-generation cephalosporins, it is necessary to identify the patients with severe infections who should receive empirical treatment with an agent active

against these organisms, and it would be prudent to consider a carbapenem as the initial therapy. Concerning the treatment of urinary tract infections, our study shows high rates of resistance to fluoroquinolones and to trimethoprim-sulfamethoxazole. Fosfomycin exhibits excellent *in vitro* activity against *Enterobacteriaceae* resistant to third-generation cephalosporins, mainly against ESBL-producing organisms, and is a good option to consider for the treatment of infections caused by multidrug-resistant *Enterobacteriaceae*. After many years of fosfomycin use, it continues to be active against the most common uropathogens (23, 33, 34).

In summary, the present high incidence of *Enterobacteriaceae* resistant to third-generation cephalosporins associated with high rates of resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole limits the alternatives for the empirical treatment of infections caused by these microorganisms, and suggests that it is necessary to implement infection control measures in order to limit the spread of ESBLs and other forms of resistance in *Enterobacteriaceae*.

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