

Full Length Research Paper

Patterns of resistance to β -lactams and β -lactamase inhibitors in uropathogenic *Escherichia coli* strains isolated from animals in Portugal

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FMV 1953 uropathogenic *Escherichia coli* isolate was extremely resistant to amoxicillin, co-amoxiclav, ticarcillin, mecillinam, cefoxitime, cefixime, cefuroxime, cefotaxime, ceftazidime, ceftriaxone and aztreonam. From the resistance patterns observed we deduce the phenotype as producing a TEM-1 β -lactamase, a hyperproduced Amp-C β -lactamase and an OXA-3 β -lactamase or a PBP-2 mutation isolate.

Key words: Antibiotic resistance, uropathogenic *Escherichia coli*, phenotype characterization.

INTRODUCTION

Since the description of the first TEM-derived β -lactamases conferring resistance to clavulanate, a number of these enzymes have emerged in various parts of the world, as profiled by Jacoby and Bush (1997). There is little information available on resistance to β -lactam- β -lactamase inhibitor combinations in members of the family Enterobacteriaceae in Portugal (Caniça et al., 2000; Féria et al., 2002).

In this study, we describe the susceptibility of uropathogenic *E. coli* isolated from animals in Portugal to β -lactams and β -lactamase-inhibitor combinations and the relationship between the predominant mechanisms of resistance and the production of β -lactamases.

MATERIALS AND METHODS

Forty-five *E. coli* isolates from canines (28), felines (6), suines (7), equines (1) and humans (3) with significant bacteriuria and clinical symptoms of urinary tract infection (UTI) were collected from urine specimens at the Veterinary Medical School. Isolates were identified with the API 20E system (bioMérieux, Marcy l'Etoile, France). *E. coli* ATCC 25922, *E. coli* R111 (TEM-1), *E. coli* Sal (IRT-1), *E. coli* Guer (IRT-2), *E. coli* P37 (IRT-14) were used as a reference control for microdilution testing. The minimum inhibitory

concentration (MIC) microdilution assay was based on standard procedures (Medeiros, 1997). An inoculum of 5×10^5 cfu/mL was used. Susceptibility patterns were interpreted according to the recommendations of the Antibiogram Committee of the American Society for Microbiology (1992). Antibiotic and β -lactamase-inhibitor powders for MIC assays were provided by the following manufacturers: clavulanic acid and ticarcillin (GlaxoSmithKline, Oeiras, Portugal), mecillinam (Leo Pharmaceutical Products, Lisbon, Portugal), cefoxitime and cefixime (Lilly Farma, Oeiras, Portugal), ceftazidime (GlaxoWellcome, Oeiras, Portugal), ceftriaxone (Roche Pharmaceuticals, Amadora, Portugal), cefotaxime (Hoechst Marion Roussel, Sintra, Portugal), aztreonam (Bristol-Myers Squibb, Oeiras, Portugal). Amoxicillin was purchased from Sigma (Lisbon, Portugal). Clavulanate was used at a fixed concentration of 2 mg/L with serial two-fold dilutions of amoxicillin.

RESULTS AND DISCUSSION

The resistance patterns of 45 uropathogenic *E. coli* isolates to 10 β -lactams and one β -lactam- β -lactamase-inhibitor combination are shown in Table 1. 57.8% of these isolates were amoxicillin resistant, but only 28.6% were resistant to the combination of *amoxicillin* plus 2 mg/L clavulanate. We found that 53.3 and 24.4% of isolates were resistant to *ticarcillin* and *mecillinam*, respectively. Ceftriaxone, cefotaxime and ceftazidime showed a very similar resistance pattern, with 2.2, 2.2 and 4.5% of resistant isolates. Just 8.9% of isolates were resistant to cefoxitin and 4.4% to

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Table 1. *In vitro* activities of eleven antimicrobial agents against uropathogenic *E. coli* isolates ($n = 45$).

Antimicrobial ^a	Cumulative percentages of isolates inhibited at a concentration of (mg/L)																					
	0.0015	0.0015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096	
AML							0	11	33	38	42	42	44	56	58	60	75	90	100			
AMC							0	21	35	48	65	73	73	79	83	85	90	92	100			
TIC					0	4	4	6	6	21	31	40	46	46	48	52	65	67	75	83	100	
MEC		0	6	21	44	48	52	58	73	81	90	92	94	96	98	100						
CAZ	2	4	11	15	26	49	83	87	94	94	96	96	96	96	96	100						
CRO	2	4	15	52	77	85	88	96	96	96	96	96	96	98	100							
CTX	2	8	10	29	67	77	77	79	88	90	94	96	98	100								
CXM					0	2	2	5	12	24	77	93	93	93	100							
CFM		0	5	5	7	19	66	89	89	89	89	91	93	96	96	100						
FOX			0	2	9	9	11	15	32	65	89	91	91	91	95	100						
ATM		0	2	12	25	65	82	84	84	86	92	97	100									

^aAML, amoxicillin; AMC, co-amoxiclav; TIC, ticarcillin; MEC, mecillinam; CXM, cefuroxime; CAZ, ceftazidime; CRO, ceftriaxone; CTX, cefotaxime; ATM, aztreonam; FOX, ceftaxime; CFM, cefixime.

Table 2. MICs (mg/L) of the most resistant *E. coli* isolates ($n = 7$).

Isolate/control	MIC's (mg/L)										
	AML	AMC	TIC	MEC	FOX	CFM	CXM	CTX	CAZ	CRO	ATM
180	256	256	256	0.5	2	32	8	1	8	0.5	16
199	128	128	16	0.125	128	64	128	8	2	0.5	0.25
339	2048	64	2048	8	4	0.5	16	0.125	0.5	0.06	0.25
1074	4096	64	>4096	1	8	1	8	0.125	0.5	0.06	1
1187	4096	64	>4096	8	4	<0.03	8	2	0.5	0.03	0.25
1953	4096	512	>4096	4096	256	256	128	128	256	128	64
E939	4096	16	>4096	1024	4	0.5	8	0.125	0.03	0.125	0.25
ATCC	4	4	4	0.06	1	0.5	4	0.25	0.25	0.03	0.25
TEM-1	2048	128	>2048	4	ND	ND	ND	2	0.5	0.06	0.25
IRT-1	128	4	128	<0.125	0.5	ND	ND	0.015	0.25	0.015	0.25
IRT-2	1024	512	1024	8	2	0.25	ND	0.015	0.25	0.03	0.25
IRT-14	2048	1024	2048	0.25	4	ND	ND	0.015	0.25	0.06	0.06

ND – not determined.

cefuroxime. 9.1% of the isolates were resistant to cefixime. Only one of the isolates was resistant to aztreonam (Table 1). The FMV 1953 isolate showed resistance towards all tested antimicrobials (Table 2), suggesting the production of a TEM-1 β -lactamase, a hyper-expressed Amp-C β -lactamase and an OXA-3 β -lactamase or a PBP-2 mutations. Although *E. coli* is recognized as an important pathogen in UTIs, little is known about antimicrobial resistance mechanisms in such isolates. Our results demonstrate that canine uropathogenic *E. coli* isolates have a high level of *in vitro* resistance to amoxicillin (57.8%), mecillinam (24.4%),

ticarcillin (53.3%) and co-amoxiclav (28.6%), but that they are generally susceptible to other β -lactams. Similar data was found in other studies of uropathogenic *E. coli* in dogs in Portugal and the UK (Féria et al., 2000, 2002).

We found an important pattern of resistance to amoxicillin and co-amoxiclav, which are first-line and overused drugs for UTI therapy in dogs in Portugal. The production of TEM-1, AmpC and OXA-3 enzymes in uropathogenic canine *E. coli* is of concern. This study confirms that, even in animals, β -lactamase detection is important.

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REFERENCES

- American Society for Microbiology (1992). Section 5.2—Broth microdilution MIC testing. In *Clinical Microbiol. Procedures Handbook*, 1st edn, (Isenberg, H D, Ed.). American Soc.for Microbiol, Washington, DC.
- Bush K, Jacoby G, (1997). Nomenclature of TEM β -lactamases. *Antimicrob. J. Chemother.* 39: 1-3.
- Caniça M, Ferreira M, Vaz-Pato V, Ferreira E (2000). Grupo de Estudo Multicêntrico de Vigilância da Susceptibilidade aos Antibióticos, Mecanismos de resistência aos β -lactâmicos em estirpes de *Escherichia coli* de origem clínica. *Arq. Med.* 14: 71.
- Féria C, E Ferreira, JD Correia, J Gonçalves, M Caniça (2002). Patterns and mechanisms of resistance to β -lactams, β -lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *J. Antimicrob. Chemother.* 49: 77–85.
- Féria C, Correia JD, Machado J, Vidal R, Gonçalves J (2000). Urinary tract infection in dogs, analysis of 419 urocultures carried out in Portugal. In *Genes and Proteins Underlying Microbial.Urinary Tract Virulence*, (Emödy L, Pál T, Blurn-Oehler G Hacker IH, Eds). Kluwer Acad/Plenum Pub New York USA. pp. 301–4.
- Medeiros AA (1997). Evolution and dissemination of β -lactamases accelerated by generations of β -lactam antibiot. *Clin. Infect. Dis.* 24: suppl. 1 S19-S45.