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LETTER TO THE EDITOR

Multiresistant extended-spectrum β-lactamase producing *Escherichia coli* in human urine samples in Portugal

Sir,

The introduction of antimicrobial drugs in clinical practice is usually followed by the emergence of resistant strains. The inadequate use, auto-medication, exclusive use of extended-spectrum drugs, or the premature break of antimicrobial treatments are factors to take in consideration.[1]

Multiresistant extended-spectrum β-lactamase (ESBL) producing-*Escherichia coli* are among the top six most worrying human pathogens.[2] This organism is a common inhabitant of the human intestinal tract but, in certain cases, it may cause infectious diseases, such as enteric, lung, nervous, and urinary tract infections. In recent years, *E. coli* strains have been developing resistance to some antimicrobial agents used to treat infections and are associated with therapeutic failure, causing great concern.[3]

Sixty-five ESBL-producing *E. coli* isolates, obtained from individual human urine samples, were selected for study. The isolates were obtained monthly from urine samples of patients at the Hospital Center of Trás-os-Montes and Alto Douro, from November 2009 to January 2011. Susceptibility to 16 antibiotics (ampicillin, amoxicillin + clavulanic acid, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole/trimethoprim, tetracycline and chloramphenicol) was tested by the disk-diffusion method in all recovered isolates and ESBL-phenotypic detection was confirmed out by double-disk test according to the CLSI criteria.[4]

The presence of resistance genes as well the classification in phylogenetic groups (A, B1, B2, or D) were performed by PCR (Table 1).

The highest levels of resistance were observed for cefotaxime, ciprofloxacin, and nalidixic acid. Other studies in urinary human *E. coli* isolates showed lower values.[5] We demonstrated that the CTX-M group of β-lactamases was the predominant ESBL type identified in our isolates, with the $bla_{CTX\text{-}M\text{-}15}$ being the most frequent *bla* gene detected. In Portugal, only dispersed cases of detection of $bla_{CTX\text{-}M\text{-}15}$ have been reported.[6] The bla_{TEM} gene was detected in 73.8% of the isolates, bla_{SHV} in 1.5%, bla_{OXA} in 16.9% and $bla_{CTX\text{-}M}$ in 89.2% of the 65 *E coli* isolates. The *aac*(3)-II gene was detected in 97.4% of the gentamycin-resistant isolates; 51.3% of the streptomycin-resistant isolates presented the *aad*A gene; the *tet*(A) and/or *tet*(B) genes were presented in 45.3% and 37.5% of the tetracyclin-resistant isolates, respectively; the *cml*A gene was detected in 54.5% of the chloranphenicol-resistant isolates; and *sul*1, *sul*2, and/or *sul*3 were detected in 40%, 37.5%, and 37.5% of the sulfamethoxazol/trimethoprim-resistant isolates, respectively.

It is important to note that the majority of isolates belonged to B2 (63.1%) and D (26.1%) phylogroups and almost all were multi-resistant. The expression of CTX-M-type β-lactamases may explain this fact.[6]

The class 1 integron was present in 35.3% of the sulfamethoxazol/trimetoprim-resistant isolates. The variable region of the class 1 integron was detected in 12 isolates, containing the *dfr*A15 and *aad*A1 genes (seven isolates) or the *dfr*A17, *dfr*A2+ and *aad*A5 genes (five isolates). These genes were previously detected in *E. coli* isolated from human urine samples, conferring resistance to sulfamethoxazol/trimethoprim and streptomycin, respectively.[7]

The *gyr*A gene presented two mutations causing amino acid changes (S83L and D87N). These mutations have

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Table 1 Phenotypic and genotypic profile of the 59 extended-spectrum β-lactamase producing *Escherichia coli* isolates recovered from human urine samples

Isolates	Phenotype of resistance to beta-lactam antibiotics	Phenotype of resistance to other antibiotics	Beta-lactamase genes detected	Other resistant genes	Amino acid changes		Genetic elements	Phylogenetic groups
					*gyr*A	*par*C		
RP1	AMC-ATM-CTX	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP2	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 3	ATM-CTX	AMP-CIP-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 4	AMC-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-S	bla_{TEM-1B}; $bla_{CTX-M-15}$	*tet*(B); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 5	AM-ATM-CTX-CAZ	AMP-TET-CIP-TOB-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 6	AMC-ATM-CTX-CAZ	AMP-TET-CIP-TOB-SXT-NAL-STR	$bla_{CTX-M-15}$	*aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 7	ATM-CTX	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 8	CTX	AMP-GEN-CIP-TOB-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1	B2
RP 9	ATM-CTX	AMP-TET-GEN-CIP-TOB-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1[*dfr*A1+*aad*A1]	B1
RP 10	AMC-ATM-CTX	AMP-TET-GEN-CIP-TOB-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aac*(3)-II; *aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 11	AMC-ATM-CTX-CAZ	AMP-TET-CIP-TOB-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; Int1; rvint1[*dfr*A15+*aad*A1]	B2
RP 12	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 13	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 14	ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 15	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B2
RP 16	CTX	AMP	bla_{TEM-1B}; $bla_{CTX-M-9}$				ISEcp1; *orf477*	B2
RP 17	CTX	FOX-AMP-TET-GEN-CIP-NAL	bla_{TEM-1B}; $bla_{CTX-M-9}$	*aac*(3)-II; *tet*(B); *gyr*A; *par*C; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*	B1

RP 18	CTX-CAZ	AMP-TET-GEN-CIP-TOB-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-9}$	*aac*(3)-II; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 19	ATM-CTX	AMP-GEN-CIP-TOB-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf*477	B2
RP 20	AMC-ATM-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(B); *sul*1; *sul*2; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 21	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-AK-TOB-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*tet*(A); *tet*(B); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 22	AMC-ATM-CTX-CAZ	AMT-TET-GEN-CIP-AK-TOB-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *sul*1; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 23	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 24	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 25	AMC-ATM-CTX-CAZ	CAZ-AMP-TET-CIP-TOB-NAL	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*tet*(A); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 26	AMC-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(B); *sul*1; *sul*2; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 27	AMC-ATM-CTX-CAZ	CAZ-AMP-TET-CIP-AK-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 28	ATM-CTX	AMP-CIP-AK-TOB-NAL	$bla_{CTX-M-15}$	*aad*A; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 29	AMC-ATM-CTX-CAZ	AMP-TET-CIP-AK-TB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aad*A; *tet*(B); *sul*1; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 30	ATM-CTX-CAZ	AMP-TET-GEN-CIP-A-TOB-SXT-NAL-STR	bla_{TEM-1B}; bla_{OXA}; $bla_{CTX-M-15}$	*aad*A; *tet*(A); *sul*1; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 31	AMC-ATM-CTX	AMP-TET-GEN-CIP-IMP-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-9}$	*tet*(A); *sul*1; *sul*2; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		D
RP 32	ATM-CTX	AMP-TET-CIP-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*sul*1; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B1
RP 33	ATM-CTX	AMP-TET-GEN-CIP-TOB-C-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aad*A; *tet*(B); *sul*1; *sul*2; *sul*3; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 34	ATM-CTX-CAZ	AMP-TET-CIP-NAL	$bla_{CTX-M-15}$	*gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 35	AMC-ATM-CTX	AMP-TET-CIP-TOB-C-SXT-NAL-STR	bla_{TEM-1B}; bla_{SHV-12}	*gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D

(*continued on next page*)

Table 1 (*continued*)

Isolates	Phenotype of resistance to beta-lactam antibiotics	Phenotype of resistance to other antibiotics	Beta-lactamase genes detected	Other resistant genes	Amino acid changes		Genetic elements	Phylogenetic groups
					*gyr*A	*par*C		
RP 36	AMC-ATM-CTX	AMP-GEN-CIP-TOB-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 37	AMC-ATM-CTX-CAZ	FOX-AMP-TET-GEN-CIP-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 38	ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*cml*A; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 39	AMC-ATM-CTX	AMP-TET-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*tet*(A); *tet*(B); *cml*A; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 40	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		D
RP 41	AMC-ATM-CTX-CAZ	FOX-AMP-TET-GEN-CIP-AK-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*tet*(B); *cml*A; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 42	ACM-ATM-CTX	AMP-TET-CIP-TOB-CHL-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cml*A; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ofr477	D
RP 43	AMC-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-S	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cml*A; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; Int1; rvint1[*dfr*A17+*dfr*A29+*aad*A5]	D
RP 44	AMC-ATM-CTX	AMP-TET-CIP-TOB-CHL-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*tet*(A); *tet*(B); *cml*A; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 45	AMC-ATM-CTX	AMP-TET-GEN-CIP-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*sul*1; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; Int1; rvint1[*dfr*A17 +*dfr*A29+*aad*A5]	B1
RP 46	AMC-ATM-CTX	TET-GEN-CIP-IMP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cml*A; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 47	AMC-CTX	AMP-TET-CIP-TOB-CHL-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477	D
RP 48	ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	$bla_{CTX-M-15}$	*cml*A; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; IS903	B2
RP 49	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cml*A; *sul*1; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	ISEcp1; *orf*477; IS903	B2
RP 50	AMC-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	$bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cml*A; *sul*1; *sul*2; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; IS903	B2
RP 51	AMC-CTX	AMP-TET-CIP-SXT-NAL-STR	$bla_{CTX-M-15}$	*tet*(B); *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; IS903; Int1	B2
RP 52	AMC-CTX	AMP-TET-GEN-CIP-SXT-NAL-STR	$bla_{CTX-M-15}$	*aac*(3)-II; *sul*1; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V		B2
RP 53	AMC-ATM-CTX-CAZ	AMP-CIP-SXT-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*sul*1; *gyr*A; *par*C	S83L+D87 N	S80I+E84 V	*orf*477; IS903; Int1; vint1 [*dfr*A17+*dfr*A29 +*aad*A5]	B2

RP 54	AMC-ATM-CTX-CAZ	AMP-TET-GEN-CIP-TOB-NAL	$bla_{CTX-M-15}$	*aac*(3)-II; *tet*(A); *gyrA*; *parC*	S83L+D87 N	S80I+E84 V	ISEcp1; *orf477*; IS903	B2
RP 55	CTX	AMP-TET-CIP-CHL-NAL	$bla_{CTX-M-15}$	*tet*(B); *gyrA*; *parC*	S83L+D87 N	S80I+E84 V	*orf477*; IS903	B2
RP 56	AMC-ATM-CTX-CAZ	AMP-GEN-NAL	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *gyrA*	S83L+D87 N		ISEcp1; *orf477*; IS903	B2
RP 57	AMC-CTX	AMP-TET-GEN-CIP-TOB-CHL-SXT-NAL-STR	bla_{TEM-1B}; $bla_{CTX-M-15}$	*aac*(3)-II; *tet*(B); *cmlA*; *sul1*; *sul2*; *gyrA*; *parC*	S83L+D87 N	S80I+E84 V	*orf477*; IS903; Int1; rvint1 [*dfrA17*+*dfrA29*+*aadA5*]	B2
RP 58	AMC-ATM-CTX-CAZ	AMP-TET-CIP-TOB-SXT-NAL-STR	$bla_{CTX-M-15}$	*sul1*; *gyrA*; *parC*	S83L+D87 N	S80I+E84 V	*orf477*; Int1; rvint1[*dfrA17*+*dfrA29*+*aadA5*]	B2
RP 59	AMC-ATM-CTX-CAZ	AMP-GEN-CIP-TOB-NAL	$bla_{CTX-M-15}$	*aac*(3)-II; *gyrA*; *parC*	S83L+D87 N	S80I+E84 V	*orf477*; IS903	B2

AK = amikacin; AMC = amoxicillin+clavulanic acid; AMP = ampicillin; ATM = aztreonam; CAZ = ceftazidime; CHL = chloranphenicol; CIP = ciprofloxacin; CTX = cefotaxime; FOX = cefoxitine; GEN = gentamicin; IMP = imipenem; NA = nalidixic acid; STR = streptomycin; SXT = sulfamethoxazole/trimethoprim; TET = tetracycline; TOB = tobramycin.

previously been reported as the most frequent on the *gyr*A gene, in *E. coli* isolates of human origin.[3] The *par*C gene was detected in the isolates that were simultaneously resistant to nalidixic acid and ciprofloxacin, and two non-silent mutations were detected (S80I and E84V).

In conclusion, our study shows a wide variety of resistance genes and inclusion of resistant genes in integrons, in multiresistant ESBL-producing *E. coli* isolates, obtained from urinary human samples, in a clinical setting. It is important for clinicians and infection-control practitioners to characterize the different types of resistance quickly to allow efficient reduction of the spread of these bacteria and help to select more appropriate antibiotics.

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