Ciprofloxacin-resistant, CTX-M-15-producing *Escherichia coli* ST131 clone in extraintestinal infections in Italy

M. Cerquetti, M. Giufrè, A. García-Fernández, M. Accogli, D. Fortini, I. Luzzi and A. Carattoli

Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Abstract

Quinolone and β-lactam resistance mechanisms and clonal relationships were characterized among *Escherichia coli* isolates resistant to ciprofloxacin and extended-spectrum cephalosporins associated with human extra-intestinal infections in Rome. The *E. coli.* ST131 clone was found to be prevalent. This clone invariably carried a specific pattern of substitutions in the topoisomerase genes and all isolates but one produced CTX-M-15. One ST131 isolate produced SHV-12. The new ST131 variant described here is of particular concern because it combines fluoroquinolone resistance and chromosomally encoded CTX-M-15.

Keywords: IncF plasmids, multidrug resistance, replicon typing, septicemia, ST131, urinary infections

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Corresponding author: A. Carattoli, Department of Infectious, Parasitic and Immuno-Mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome-Italy
E-mail: alecara@iss.it

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) isolates are involved in a wide spectrum of human diseases, including urinary-tract infections (UTIs), septicaemia and neonatal meningitis, and the management of such infections has become complicated as a result of combined fluoroquinolone and extended-spectrum cephalosporin resistance [1].

Sixty-four ciprofloxacin-resistant extra-intestinal isolates of *E. coli* isolates (MIC ≥4 mg/L), each obtained from different patients in Rome, Italy, between March and September 2006, at the San Camillo Forlanini Hospital and at the BIOS Diagnostic Laboratory, from blood (23 isolates) and urine (41 isolates) samples, respectively, were included in this study. During this period, 852 cases of human septicaemia occurred in the hospital, 68 of which were caused by *E. coli*, whereas, of the 1061 community-acquired UTIs, 323 were caused by *E. coli*.

MICs and confirmation of the presence of extended-spectrum β-lactamases (ESBLs) were performed by E-test (BioMérieux S.A., Marcy l'Etoile, France) and breakpoints were interpreted based on CLSI susceptibility criteria [2]. The vast majority of isolates were highly resistant to ciprofloxacin (MIC_{90} ≥ 32 mg/L), irrespective of the isolation site (blood or urine). The prevalence of ciprofloxacin resistance was higher among bloodstream isolates than among those from UTIs (23/68; 33.8% vs. 41/323; 12.7%). Fifteen out of 64 isolates (23%) were confirmed to be ESBL producers (Table 1).

The 64 isolates were screened for plasmid-mediated quinolone resistance (PMQR) genes [3–6]. Two isolates (3.1%) possessed both *qnrB1* and *aac(6')-Ib-cr* genes, whereas 12 (18.8%) carried the *aac(6')-Ib-cr* gene only, confirming that the *aac(6')-Ib-cr* gene appeared to be more prevalent than the *qnr* genes [7]. Identification of the β-lactamase-encoding genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM} and bla_{OXA-1}) was carried out in a subgroup of 18 isolates including all the ESBL-producing and the PMQR- positive isolates [8–10]. Of the 15 ESBL producers, 14 contained $bla_{CTX-M-15}$ and one harboured bla_{SHV-12}. The percentage of CTX-M-15-producing *E. coli* was significantly higher among blood isolates than among UTI isolates (10/23; 43.5% vs. 4/41; 9.8%; p 0.0017).

Plasmids were transferred by transformation (MAX Efficiency DH5α; Invitrogen, Milan, Italy) or conjugation (RifR *E. coli* CSH26) and assigned to plasmid families [11]. The ESBL and PMQR genes were all located on plasmids, except in strains IN6 and IN33 both from cases of septicaemia. Strain IN6 carried plasmids, although the $bla_{CTX-M-15}$ gene was identified within the chromosome by the PFGE-I-*CeuI* method [12], whereas, in strain IN33, this gene was located on both chromosome and IncF plasmid, suggesting the presence of more than one copy of the ESBL gene within the same cell. In all the remaining isolates, the $bla_{CTX-M-15}$ gene was identified on plasmids belonging to the IncF group (50–200 kb), carrying more than one repF replicon, as previously described (Fig. 1) [13]. Strains IN21 and IN44 both carried the *qnrB1* gene on IncHI2 plasmids.

The quinolone resistance determining region (QRDR) of the *gyrA*, *gyrB* and *parC* genes was examined for the ESBL producers and PMQR-positive isolates (Table 1) [14]. All the

TABLE 1. Genotypic and phenotypic characteristics of extended-spectrum β-lactamase-producing and/or *qnr* -and *aac(6')-Ib-cr*-positive isolates

Isolate	GyrA ParC Type	GyrA		ParC				β-lactamase genes				MIC (mg/L)			
		Ser83	Asp87	Ser80	Glu84	*qnr*	*aac(6')-Ib-cr*	bla_{TEM}	bla_{CTX-M}	bla_{SHV}	bla_{OXA-1}	CIP	LV	CRO	CTX
UR 3	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
UR 14	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
UR 40	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 30	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 31	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 34	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 40	A	Leu	Asn	Ile	Val	–	+	bla_{TEM-1}	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 16	A	Leu	Asn	Ile	Val	–	+	–	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 33	A	Leu	Asn	Ile	Val	–	+	–	$bla_{CTX-M-15}$[a]	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 6	A	Leu	Asn	Ile	Val	–	–	bla_{TEM-1}	$bla_{CTX-M-15}$[a]	–	–	≥32	≥32	≥64	≥64
IN 36	A	Leu	Asn	Ile	Val	–	–	–	$bla_{CTX-M-15}$	–	–	≥32	24	≥64	≥64
IN 22	A	Leu	Asn	Ile	Val	–	–	–	–	bla_{SHV-12}	–	≥32	32	32	4
UR 36	B	Leu	Asn	Ile	Glu	–	+	bla_{TEM-1}	–	–	–	≥32	≥32	0.064	0.125
UR 41	B	Leu	Asn	Ile	Glu	–	+	–	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	≥32	≥64	≥64
IN 12	B	Leu	Asn	Ile	Glu	–	–	bla_{TEM-1}	$bla_{CTX-M-15}$	–	–	≥32	≥32	≥64	≥64
IN 21	C	Leu	Tyr	Ile	Val	*qnrB1*	+	bla_{TEM-1}	–	–	bla_{OXA-1}	≥32	≥32	0.094	0.38
IN 43	D	Leu	Gly	Ser	Lys	–	+	–	$bla_{CTX-M-15}$	–	bla_{OXA-1}	≥32	8	≥64	≥64
IN 44	E	Leu	Tyr	Ile	Gly	*qnrB1*	+	bla_{TEM-1}	–	–	bla_{OXA-1}	≥32	≥32	0.125	0.25

CIP, ciprofloxacin; LV, levofloxacin; AM, ampicillin; CRO, ceftriaxone; CTX, cefotaxime.
UR, isolate from urine; IN, isolate from blood.
[a]Chromosomal integration of the $bla_{CTX-M-15}$ gene.

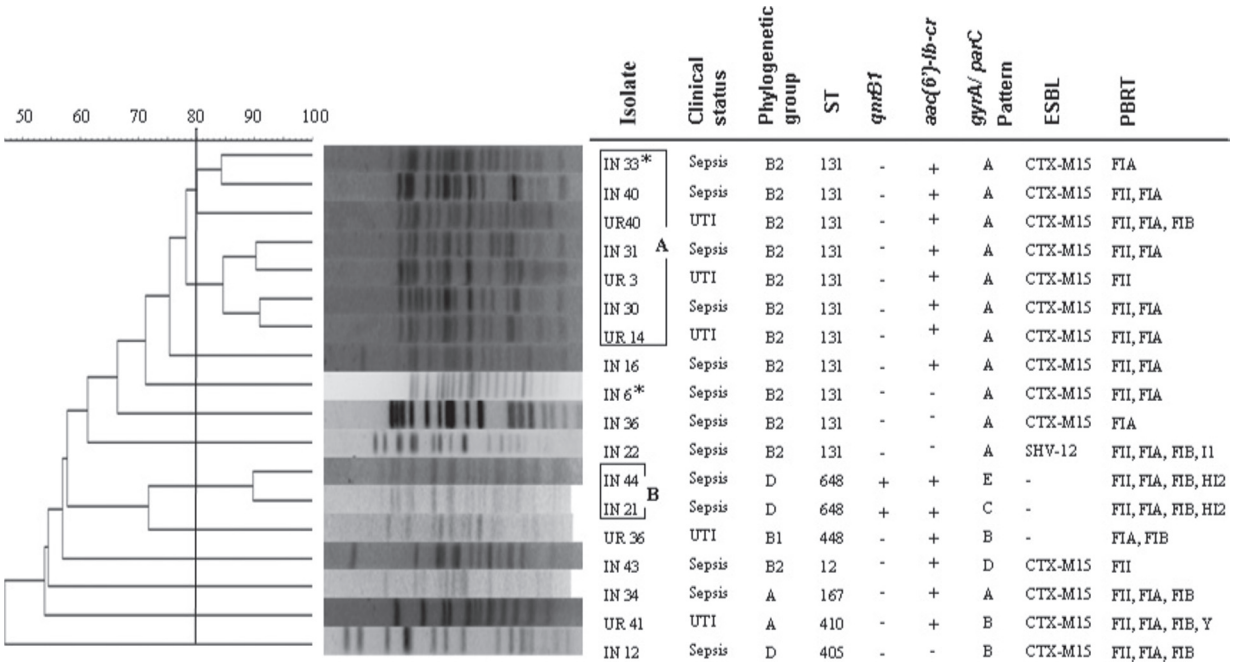

FIG. 1. Genetic relatedness among 18 extended-spectrum β-lactamase (ESBLs)-producing and/or *qnrB1*- and *aac(6')-Ib-cr*-positive *E. coli* isolates. Cluster analysis of the pulsed field gel electrophoresis (PFGE) patterns, phylogenetic groups and multilocus sequence typing genotyping, together with the main characteristics of the resistance genotypes are shown. DNA fragments were analysed with the Diversity Database Fingerprinting Software, version 2 (Bio-Rad Laboratories, Hercules, CA, USA). The band tolerance used was 1.0%. Similarity analysis of the PFGE patterns was performed with Dice's coefficient and clustering by the unweighted pair group method with arithmetic mean. Strains with a coefficient of similarity value ≥80% were considered to belong to the same PFGE clonal group (clusters A and B in the boxes). *$bla_{CTX-M-15}$ gene integrated in the chromosome. PBRT, polymerase chain reaction-based replicon typing.

isolates exhibited double mutations in GyrA at codons Ser83 and Asp87. The same Ser83 → Leu amino acid change was detected in each strain, whereas different mutations occurred at the Asp87 codon, generating the Asp87 → Asn or Asp87 → Gly or Asn87 → Tyr amino acid changes. Further single or double mutations were detected at the Ser80 and/

or Glu84 codons of ParC (Ser80 → Ile and Glu84 → Val or Glu84 → Gly). No change in QRDR of GyrB was observed. The different patterns of amino acid substitutions were designated as patterns A to E; the predominant was pattern A (12/18; 66.7%) (Table 1).

Genetic relatedness among 18 ESBL-producing and PMQR positive isolates was assessed by *Xba*I-pulsed field gel electrophoresis (PFGE) analysis (New England Biolabs, Inc., Ipswich, MA, USA), phylogenetic grouping and multilocus sequence typing (MLST) (Fig. 1) [15,16].

Most of the isolates (12/18; 66.7%) belonged to the phylogenetic group B2, and seven isolates constituted a clonal group named PFGE-cluster A (Fig. 1). MLST analysis identified seven distinct STs; the predominant was ST131, comprising 11 isolates (11/18; 61%), including the seven of PFGE-cluster A. ST648 was assigned to the two *qnrB1*-positive strains (PFGE-cluster B). Most of the ST131 isolates were CTX-M-15-producers (10/11) and *aac(6')-Ib-cr*-positive (8/11). Notably, all the ST131 isolates showed the characteristic GyrA/ParC pattern A, irrespective of the ESBL type or the presence or absence of PMQR genes.

Over the past year, several reports have described the emergence and dissemination across the globe of uropathogenic and invasive *E. coli* ST131 isolates producing CTX-M-15 and, in several cases, showing ciprofloxacin resistance by unidentified mechanisms [17]. Our results demonstrated that the *E. coli* ST131 clone had a high level of ciprofloxacin resistance associated with a specific QRDR mutation pattern. The same pattern was found in other European ST131 isolates, including a high level resistant one where it was associated with OMP loss, whereas a slightly different pattern occurred in ST131 isolates from Korea, supporting the hypothesis of a different origin for these microorganisms [18,19]. Of note, in our study, two ciprofloxacin-resistant ST131 isolates from cases of septicaemia further evolved by chromosomal integration of the $bla_{\text{CTX-M-15}}$ gene. Despite the chromosomal location of ESBL genes in *Enterobacteriaceae* being regarded as a very rare event, the integration of the $bla_{\text{CTX-M-15}}$ gene is apparently not so exceptional, having been reported in *E. coli* from four continents and in *Salmonella* from internationally adopted children [13,20]. The chromosomal integration of the $bla_{\text{CTX-M-15}}$ gene might contribute to the stable maintenance and diffusion of this resistance gene in particularly successful clones.

We also identified one ST131 strain that has acquired the $bla_{\text{SHV-12}}$ gene and displayed the same topoisomerase pattern of the other ST131 isolates. The acquisition of β-lactamases other than CTX-M-15 in ST131 has recently been reported in Japan and Norway. In Japan, ST131 produced CTX-M-2-group enzymes because this CTX-M group is predominant in Asia, whereas, in Norway, it produced CMY-2 [21,22]. These observations suggest that ST131 have had a worldwide dissemination even before the acquisition of ESBL genes, and this clonal strain can abruptly change the prevalence of infections with ESBL-producing *E. coli* within a country [21]. To conclude, the new variant of the ST131 clone described here is of particular concern because it combines both chromosomally encoded ESBLs and topoisomerase gene mutations, within the successful genetic context of the ST131 clone.

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Transparency Declaration

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Tn*5090*-like class 1 integron carrying bla_{VIM-2} in a *Pseudomonas putida* strain from Portugal

C. Santos[1], T. Caetano[1,2], S. Ferreira[1,3] and S. Mendo[1]

1) *CESAM and Department of Biology, Department of Biology, University of Aveiro, Campus de Santiago, Aveiro,* 2) *Medinfar–Pharmaceutical Products SA, Amadora, Venda Nova and* 3) *Hospital Infante D. Pedro, EPE, Aveiro, Portugal*

Abstract

Three *Pseudomonas putida* strains containing bla_{VIM-2} were isolated from an inanimate surface of a female ward sanitary facility in the Hospital Infante D. Pedro, Aveiro. A novel class 1 integron was found in strain Pp2 (*aacA4*/bla_{VIM-2}/*aac6′-IIc* disrupted by an insertion sequence IS1382), and strain Pp1 was found to carry a class 1 integron (*aacA7*/bla_{VIM-2}/*aacC1*/*aacA4*), which is described for the first time in this species. Strain PF1 carries a class 1 integron associated with a *Tn5090*-like transposon, constituting the first finding of this type of arrangement in a strain from Portugal. This association highlights further dissemination of bla_{VIM-2} in environmental hospital isolates.

Keywords: Antibiotic resistance, class 1 integrons, Gram-negative bacteria, hospital environment, *Tn5090*-like transposon

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Corresponding author: S. Mendo, CESAM and Department of Biology, University of Aveiro, 3810 Aveiro, Portugal
E-mail: smendo@ua.pt

Pseudomonas putida is a Gram-negative opportunistic pathogen that is rarely involved in human infections [1] and is therefore considered to be a low-grade pathogen [2–4].

Carbapenems are broad-spectrum antibiotics that are frequently used in the treatment of *Pseudomonas* spp. infections [3]. Consequently, the emergence of metallo-β-lactamases (MBLs) of the IMP or VIM type among non-fermenting Gram-negative bacteria is becoming frequent and represents an epidemiological risk, as these enzymes confer resistance not only to carbapenems, but to virtually all β-lactams [4,5]. Moreover, bla_{IMP} and bla_{VIM} are usually carried on integrons, in association with aminoglycoside resistance cassettes. These mobile elements are easily spread horizontally between different species, owing to their association with transposons or plasmids [4–7].

The bla_{VIM-2} gene was first described in a *Pseudomonas aeruginosa* [8] isolate in France. Currently, the VIM-type enzymes constitute the second most dominant group of β-lactamases, and have been reported in different species from 23 countries worldwide [4,9], with the alleged 'index' strain being a Portuguese *P. aeruginosa* isolate recovered in 1995 [10,11]. The