

Molecular Epidemiology of *Escherichia coli* Producing Extended-Spectrum β -Lactamases Isolated in Rome, Italy[∇]

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Received 2 August 2007/Returned for modification 16 August 2007/Accepted 17 October 2007

Escherichia coli strains producing extended-spectrum β -lactamases (ESBLs) are a major problem in many different hospitals worldwide, causing outbreaks as well as sporadic infections. The prevalence of *Escherichia coli* ESBL producers was analyzed in a surveillance study performed on the population attending the Policlinico Umberto I, the largest university hospital in Rome, Italy. We also investigated genotypes, pathogenicity islands, and plasmids in the ESBL-positive *E. coli* isolates as further markers that are useful in describing the epidemiology of the infections. In this survey, 163 nonreplicate isolates of *Escherichia coli* were isolated from patients from 86 different wards, and 28 were confirmed as ESBL producers. A high prevalence (26/28) of CTX-M-15 producers was observed within the bacterial population circulating in this hospital, and the dissemination of this genetic trait was associated with the spread of related strains; however, these do not have the characteristics of a single epidemic clone spreading. The dissemination was also linked to horizontal transfer among the prevalent *E. coli* genotypes of multireplicon plasmids showing FIA, FIB, and FII replicons in various combinations, which are well adapted to the *E. coli* species. The analysis of related bacteria suggests a probable interpatient transmission occurring in several wards, causing small outbreaks.

A rapid dissemination of isolates producing CTX-M-type extended-spectrum β -lactamases (ESBLs) has recently been reported in some European countries, including Italy, and is a matter of major concern. The *bla*_{CTX-M} genes have been captured on transferable plasmids from the chromosomes of *Kluyvera* spp., and their products are becoming the most prevalent ESBLs encountered in *Enterobacteriaceae* (19). The prevalent *bla*_{CTX-M}-type genes in Europe have been identified as *bla*_{CTX-M-1}, *bla*_{CTX-M-3}, *bla*_{CTX-M-9}, *bla*_{CTX-M-14}, and *bla*_{CTX-M-15} (2, 19). Infections caused by enterobacteria producing ESBLs are associated with increased morbidity, mortality, and health care-associated costs (9, 16).

In Italy, the presence of CTX-M-type ESBLs in clinical isolates of *Enterobacteriaceae* examined in a Italian nationwide survey (23, 27), as well as in isolates from companion animals (7), was previously reported, and the results showed that CTX-M-type enzymes were common overall (around 20%) among ESBL producers (20). However, the local epidemiology of *Escherichia coli* producing ESBLs may differ from the national picture, with dominance of different strains. We therefore studied the prevalence and the clonality of the *Escherichia coli* ESBL producers in the population attending the Policlinico Umberto I, the largest university hospital in Rome, Italy, which had not been included in the nationwide survey. We also investigated virulence factors encoded on pathogenicity islands (PAIs) and plasmids carried by the *E. coli* ESBL producers as

further markers that are useful in describing the epidemiology of nosocomially acquired infections.

MATERIALS AND METHODS

Clinical isolates and antibiotic susceptibilities. A total of 4,769 various clinical samples from 86 different wards were sent for bacterial isolation to the microbiology laboratory of the Policlinico Umberto I hospital in Rome from 1 April to 15 September 2006. A total of 163 nonreplicate clinical isolates of *Escherichia coli* were isolated by routine procedures. In vitro antimicrobial activities were determined by the Vitek2 system with AST-N041 cards (BioMérieux, Mercy l'Etoile, France). Strains with suspected ESBL production were tested by the double-disk (BioMérieux, Mercy l'Etoile, France) synergy test between amoxicillin-clavulanic acid (20/10 μ g) and cefotaxime (30 μ g), ceftazidime (30 μ g), aztreonam (30 μ g), and cefepime (30 μ g) (12). A cefepodoxime disk (10 μ g) was used as an additional test for analyzing suspected ESBL production.

Extraction of β -lactamases and analysis by IEF. Crude extracts of β -lactamase were obtained by ultrasonication. Analytical isoelectric focusing (IEF) of these extracts was performed on polyacrylamide gels at pH 3.5 to 11 (Amersham Pharmacia Biotech, Uppsala, Sweden). Enzymatic activity was assayed by the iodometric method with 250 mg/liter of ceftriaxone and 250 mg/liter of penicillin G (1). Crude extracts of the TEM-1 (pI 5.4), SHV-2 (pI 7.6), CTX-M-14 (pI 8.0), SHV-12 (pI 8.2), and CTX-M-15 (pI 8.6) plasmid β -lactamases were used as controls.

Molecular typing, phylogenetic groups, and PAI detection. Chromosomal DNA was prepared for pulsed-field gel electrophoresis (PFGE) as previously described (28). After digestion with XbaI (Roche Diagnostics, Monza, Italy), the DNA fragments were separated by electrophoresis in 1% agarose gels (pulsed-field agarose certified; Bio-Rad) and 0.5 \times Tris-borate-EDTA buffer by using a contour-clamped homogeneous electric field (CHEF-Mapper system; Bio-Rad). Electrophoresis conditions were 14°C at 6 V/cm (200 V) for 22 h with switch times ranging from 2 to 64 s of linear ramping. To perform phylogenetic analysis of restriction profiles, TIFF files were analyzed with BioNumerics software (v.3.5; Applied Maths, Belgium). Strains showing >80% similarity were classified as genetically related and assigned to the same lineage, identified as PFGE types A to G (33). Different numbers were assigned to more closely related strains to differentiate members of the two prevalent A and B PFGE types as subtypes (e.g., A1 to A3).

Total DNA of higher purity was prepared with the Wizard genomic DNA purification kit (Promega, Milan, Italy) according to the manufacturer's proce-

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[∇] Published ahead of print on 24 October 2007.

ture. The phylogenetic groups of these isolates were determined by a previously described PCR-based method (8). Two multiplex PCR assays were applied to detect eight PAI markers as previously described (29), and uropathogenic *E. coli* (UPEC) strains 536 and J96 were used as positive controls.

ESBL gene identification. ESBL genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} type) were characterized by PCR and sequencing using specific primers previously published (11, 21, 24, 34). New primers were designed to amplify and sequence the complete *bla*_{CTX-M-15} gene: CTX-M-1g Fw (5'-CCCATGGTTAAAAAATC ACTGC-3') and CTX-M-1g Rv (5'-CAGCGCTTTGCCGTCTAAG-3').

Plasmid analysis. Plasmids were analyzed by the extraction method described by Kado and Liu (13), separated by 0.8% agarose gel electrophoresis and blotted onto positively charged nylon membrane (Roche Diagnostics, Monza, Italy) by standard procedures (30), and cross-linked to the membrane with UV light. Filters were consecutively hybridized with the *repF* amplicon (6) used as a probe for the FII replicon and with the amplicon obtained with the CTX-M-1g FW and CTX-M-1g RV primers mentioned above, recognizing the *bla*_{CTX-M-15} gene. DNA probes were digoxigenin labeled, using the digoxigenin PCR DIG probe synthesis kit (Roche Diagnostics GmbH, Mannheim, Germany). Hybridization and detection were performed according to the manufacturer's instructions.

For transformation and restriction analysis of the plasmids, plasmid DNA was purified with the Qiagen plasmid midikit (Qiagen Inc., Milan, Italy). Purified plasmids were used to transform competent *E. coli* DH5 α cells (MAX Efficiency DH5 α chemically competent cells; Invitrogen, Milan, Italy). Selection of the transformants was performed on LB agar plates containing ampicillin (100 μ g/ml). Plasmids were typed by the PCR-based replicon typing (PBRT) method as previously described (6).

RESULTS

Identification and epidemiology of ESBL producers. A prospective study was carried out from April to September 2006 at the Policlinico Umberto I Hospital in Rome. In that survey, 163 nonreplicate clinical isolates of *Escherichia coli* were isolated from 162 patients; two *E. coli* strains showing different resistance profiles were isolated from the same patient, and both were included within the collection. Twenty-five patients were from emergency units, seven patients were recovering in the intensive care unit of the hospital and the remaining were recovering in medical, surgical, or geriatric units. The majority of *E. coli* strains was isolated from urine specimens (53%); others were from blood, central vein catheters, tracheal/bronchial aspirates, wound swabs, and other various clinical samples. All the 163 *E. coli* isolates were analyzed for antimicrobial resistance by Vitek2 automatic screening and 32 were found to be suspected ESBL producers. Of these, 28 were confirmed using the double-disk diffusion assay, indicating a prevalence of 17.2% (28/163) in this study. Eight (28%) *E. coli* ESBL producers were from medical wards, seven (25%) were from surgical wards, seven (25%) were from the geriatric ward, three (11%) were from the intensive care unit, and three (11%) were from emergency units (Table 1). The clinical sites of isolation were as follows: 13 (46%) were from urine specimens, 2 (7%) were from blood specimens, 2 (7%) were from the respiratory tract, 1 (4%) was from a central vein catheter, and the remaining 10 (36%) were from wound swabs and various other clinical samples (Table 1). For 10 patients the ESBL-producing strain was isolated in the first 24 h after admission. Six of these patients had been admitted for first aid at the surgical, emergency, or intensive care unit, suggesting that these infections were incubating prior to admittance. However, past clinical information for these patients is lacking, and thus we cannot exclude hospitalization in the previous months. The remaining patients presented the infection from 5

days up to 6 months after admission, suggesting that these were hospital-acquired infections (Table 1).

All 28 identified ESBL producers were resistant to cefpodoxime. All strains but two were resistant to ceftazidime, cefotaxime, and cefepime; strain 47242 was susceptible to ceftazidime and showed intermediate resistance to aztreonam, and strain 46733 was susceptible to cefotaxime and cefepime. All the isolates except five showed resistance to amoxicillin-clavulanic acid, suggesting the presence of an oxacillinase gene.

Genotyping of the ESBL producers. Twenty-seven of the 28 ESBL producers were assigned to the group B2, which has been more frequently associated with extraintestinal *E. coli* isolates (22), and one strain was group D (Table 1).

Strains were genotyped by PFGE of genomic DNA restricted by XbaI (Fig. 1). Seven different clonal lineages were identified and classified as PFGE types A to G. PFGE types A and B were prevalent within this collection (Table 1; Fig. 1). Three and two subtypes were further discerned within the A and B PFGE types; these were named A1, A2, and A3 and B1 and B2, respectively, with each subtype including the strains showing higher levels of similarity (85 to 100%).

Distribution of PAIs in the *E. coli* ESBL producers. Strains were tested by multiplex PCR (29) for the presence of specific markers recognizing the major PAIs associated with UPEC.

Only three types of PAIs were detected, with the most prevalent being PAI IV₅₃₆, which was detected in all of the isolates. The majority of the strains also carried PAI I_{CFT073} and PAI II_{CFT073}; only two strains lacked I_{CFT073}, and four strains lacked II_{CFT073}. The strains showing one or two PAIs belong to rare PFGE types, while those showing the three PAIs were mainly of the prevalent A and B genotypes (Table 1).

β -Lactamase gene identification. The 28 ESBL producers were first tested by IEF to determine the ESBLs contained in each strain. Most of the strains showed enzymes migrating at pI 8.6 and pI 5.4; a few strains showed different β -lactamases migrating at pI 8.0, 8.2, or 7.6 (Table 2). All of the strains were then screened for the *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} type ESBL genes and for the *bla*_{OXA-1} gene, and amplicons were sequenced. The most widespread class A ESBL in this collection was CTX-M-15 (pI 8.6), which was detected in 26/28 *E. coli* strains. The two strains lacking CTX-M-15 produced CTX-M-14 (isolate 47242, showing a β -lactamase at pI 8.0) and SHV-12 (isolate 46733, showing a β -lactamase at pI 8.2) (Table 2). Two strains carried both SHV-11 (pI 7.6) and CTX-M-15. Most of the strains (79%) were also positive for the *bla*_{TEM} gene (pI 5.4), and 75% were positive for the *bla*_{OXA-1} gene; the latter was found in all isolates showing amoxicillin-clavulanic resistance. The *bla*_{CTX-M-15} gene was found in different PFGE genetic lineages. Strains carrying the *bla*_{SHV-12} or *bla*_{CTX-14} gene were of the rare D and G PFGE types, respectively.

Plasmid analysis and PBRT. The *bla*_{CTX-M-15} gene was successfully transferred by transformation from several PFGE type A and B prototypic strains, demonstrating a plasmid localization of this ESBL gene (data not shown). Plasmids obtained from all the strains and from the transformants were classified by PBRT (Table 2). All the strains were positive for plasmids belonging to the IncF incompatibility group, since all carried the FII replicon. The *bla*_{CTX-M-15} gene was invariably associated with the FII plasmids by Southern blot hybridization experiments performed on crude plasmid extracts obtained

TABLE 1. ESBL-producing *E. coli* isolates analyzed in this study

Isolate	Date (day/mo/yr) of:		Ward ^a	Site of isolation	PFGE type	Phylogenetic group	PAI		
	Patient admission	Isolation					IV	II	I
45545	30/01/06	06/04/06	Cardiology (MED 1)	Wound swab	A1	B2	536	CFT073	CFT073
45606	30/03/06	07/04/06	Oncology (SUR 1)	Bile	B1	B2	536	CFT073	CFT073
45852	12/04/06	13/04/06	General surgery (SUR 2)	Blood	B1	B2	536	CFT073	CFT073
45913	06/04/06	15/04/06	Internal medicine (MED 2)	Urine	B1	B2	536	CFT073	Negative
46214	14/04/06	26/04/06	General surgery (SUR 2)	Venous catheter	A1	B2	536	CFT073	CFT073
46427	12/04/06	03/05/06	GER	Wound swab	C	B2	536	CFT073	CFT073
46595	01/03/06	06/05/06	Angiology (MED 3)	Wound swab	A1	B2	536	CFT073	CFT073
46644	07/05/06	08/05/06	Neurology (MED 4)	Urine	ND ^b	B2	536	CFT073	CFT073
46688	10/05/06	10/05/06	Surgery day hospital (SUR 3)	Abdominal abscess	B1	B2	536	CFT073	CFT073
46733	11/05/06	11/05/06	ICU	Tracheal aspirate	D	B2	536	Negative	Negative
46844	14/05/06	15/05/06	General surgery (SUR 4)	Wound swab	A1	B2	536	CFT073	CFT073
47242	10/03/06	24/05/06	EME 1	Blood	G	B2	536	Negative	Negative
47731	15/05/06	09/06/06	General surgery (SUR 2)	Bile	B1	B2	536	CFT073	CFT073
47736	08/06/06	09/06/06	Angiology (MED 3)	Wound swab	A2	B2	536	CFT073	CFT073
47849	11/05/06	13/06/06	ICU	Urine	B2	B2	536	CFT073	CFT073
47856	30/05/06	13/06/06	GER	Urine/blood	F	D	536	Negative	CFT073
48112	20/06/06	20/06/06	EME 1	Wound swab	A3	B2	536	CFT073	CFT073
48115	19/06/06	20/06/06	Internal medicine (MED 5)	Urine	A2	B2	536	CFT073	CFT073
48165	20/06/06	21/06/06	Internal medicine (MED 6)	Urine	A3	B2	536	CFT073	CFT073
49107	18/07/06	18/07/06	ICU	Tracheal aspirate	A3	B2	536	CFT073	CFT073
49403	14/01/06	27/07/06	GER	Urine	E	B2	536	Negative	CFT073
50055	09/05/06	18/08/06	GER	Urine	A2	B2	536	CFT073	CFT073
50068	22/05/06	18/08/06	General surgery (SUR 2)	Urine	B2	B2	536	CFT073	CFT073
50252	14/07/06	24/08/06	GER	Urine	A1	B2	536	CFT073	CFT073
50419	09/08/06	30/08/06	GER	Urine	A2	B2	536	CFT073	CFT073
50552	29/08/06	04/09/06	EME 2	Wound swab	A1	B2	536	CFT073	CFT073
50811	01/09/06	09/09/06	Internal medicine (MED 2)	Urine	A1	B2	536	CFT073	CFT073
50814	08/08/06	09/09/06	GER	Urine	A1	B2	536	CFT073	CFT073

^a MED, medical; SUR, surgical; GER, geriatric unit; ICU, intensive care unit; EME, emergency unit.

^b ND, not determined because of DNA self-degradation.

from the 26 positive strains (data not shown). The DNA sequence of the FII replicon showed the IncF plasmids in two types, identified as FII[1] and FII[2], as previously described (10). The FII[2] plasmid group was characterized by association with the FIA and FIB replicons in multireplicon plasmids (32). The FII[1] plasmid group was more frequently (80%) associated with the FIA replicon only (Table 2).

Seven strains were also positive for the simultaneous presence of additional plasmids, more frequently of the IncA/C or IncI1 group (Table 2).

DISCUSSION

E. coli strains with the CTX-M-15 ESBL are a major problem in many different hospitals worldwide, causing outbreaks as well as many sporadic infections. The impressive prevalence of this genetic trait in the hospital analyzed in this study was associated with the spread of related strains; these, however, did not have the characteristic of the spread of a single epidemic clone, because the bacterial population was not perfectly homogeneous and very

few identical patterns were observed by PFGE analysis. The majority of the strains in our study belonged to two closely related clusters that probably originate from a common clone that was widely distributed in the hospital. Multireplicon plasmids showing FIA, FIB, and FII replicons in various combinations were identified by replicon typing in these isolates as the horizontal vehicle for *bla*_{CTX-M-15} gene dissemination. IncF plasmids have been previously identified as the prevalent carriers of the *bla*_{CTX-M-15} gene in other countries also, suggesting an impressive worldwide distribution of these plasmids, prevalently associated with the *E. coli* species (3, 10, 14, 18, 25). The first evidence of the association of the FII plasmid with the *bla*_{CTX-M-15} gene was obtained by the determination of the full sequence of the pC15-1a plasmid from epidemic *E. coli* isolated in Canada in 2000 (EMBL no. NC_005327) (3). The pC15-1a plasmid did not contain additional FIA or FIB replicons. IncF plasmids carrying the FII replicon in association with the FIA or FIB replicon were later identified as associated with the *bla*_{CTX-M-15} gene in several studies performed on *E. coli* strains from Tunisia, France, Spain, and the United Kingdom, demonstrating that these multireplicon plasmids were

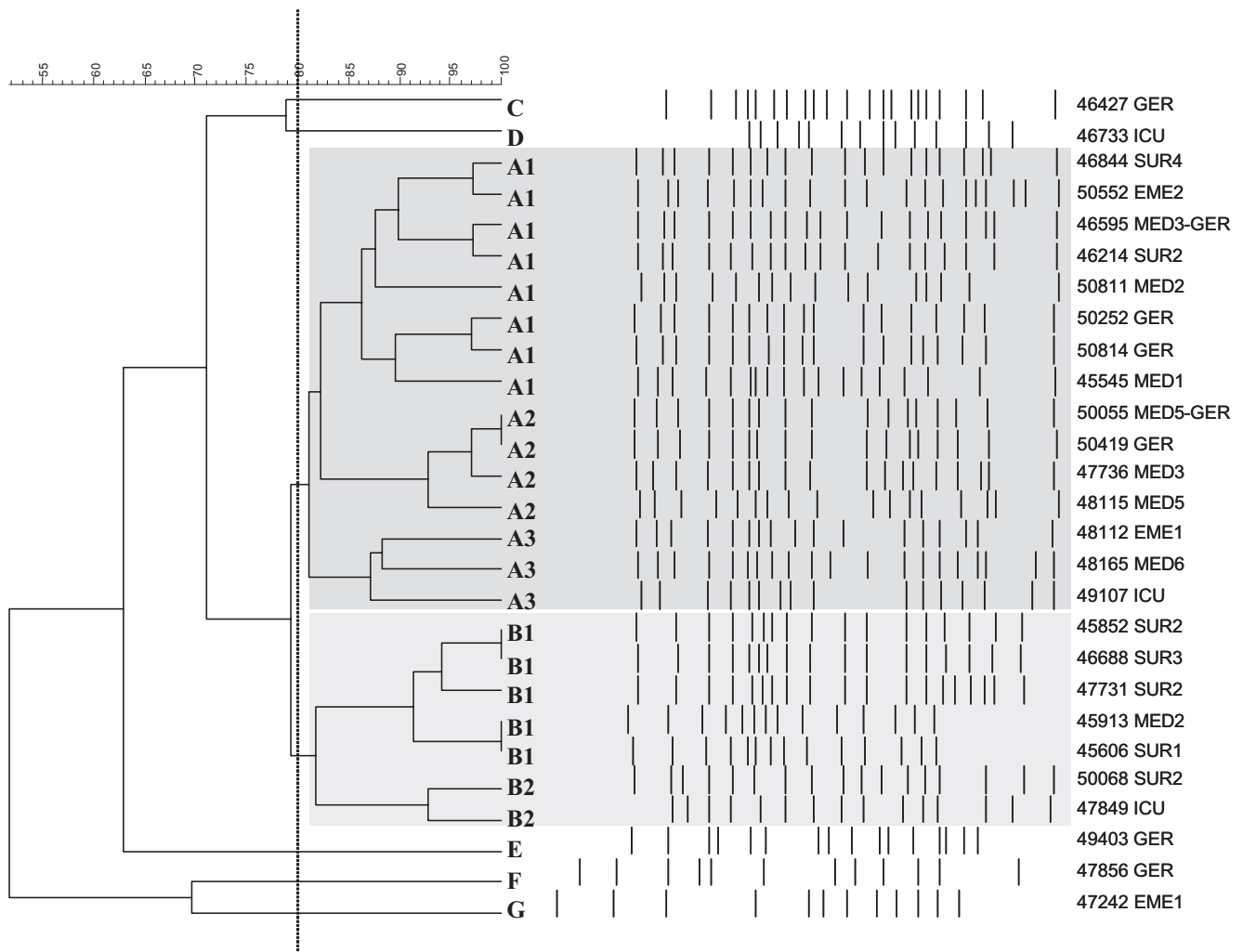


FIG. 1. Dendrogram (unweighted-pair group method using average linkages and Dice coefficient) showing the relatedness of PFGE patterns for the 28 *E. coli* ESBL producers. The dotted line indicates 80% similarity. Related A and B clusters showing >80% similarity are shaded dark gray and light gray, respectively. The isolate numbers and the wards where the patients recovered are indicated and refer to Table 1.

also important vehicles for the dissemination of this gene variant (10, 14, 18, 25). A recent study performed on a nonbiased *Enterobacteriaceae* population demonstrated that plasmids belonging to the IncFII group are unevenly distributed between the different species, being more prevalent in *E. coli* (58%) than in other genera (31). These data could suggest that the *bla*_{CTX-M-15} gene has been acquired on a plasmid type that frequently occurs in the *E. coli* species and is now spreading in association with it.

It is interesting to note the role played by IncFII plasmids in intestinal bacterial pathogens. There are very few biochemical properties that distinguish intestinal pathogens from normal inhabitants of the human gastrointestinal tract. Diseases are often due to the presence of specific virulence genes harbored on the so-called virulence plasmids or on chromosomally located PAIs (15). *Salmonella enterica*, *Shigella* spp., *Yersinia* spp., and enteropathogenic, enteroinvasive, and enterohemorrhagic O157:H7 *E. coli* all show virulence plasmids, and these virulence plasmids are in the range of 60 to 200 kb in size and carry replicons belonging to the IncFII group (4). The current information on the *bla*_{CTX-M-15} gene-carrying plasmids seems

to exclude the possibility that these IncFII plasmids are related to the virulence plasmids associated with intestinal pathogens, since they are more related to the R100 IncFII reference plasmid (EMBL no. AP000342) (3) or to the pRSB107 IncF multireplicon plasmid isolated from a sewage treatment plant in Germany (EMBL no. AJ851089) (18, 32). These plasmids could be characteristic of UPEC *E. coli*, carrying important but still unknown functions promoting their large prevalence within this kind of *E. coli* strain.

From this and previous studies, the role of PAIs is not completely clear. In a previous study (29) PAI IV₅₃₆ was one of the most frequently encountered in both commensal and UPEC *E. coli* strains, indicating that it is the first to be acquired and fixed on the chromosome, making it the most stable PAI. PAI IV₅₃₆ was frequently found with the PAI I and PAI II of the CFT073 locus, and these two considered the second and third to be acquired, respectively. This study for the first time describes uropathogenic PAIs associated with resistant *E. coli* carrying the worldwide distributed *bla*_{CTX-M-15} gene. Since the strains analyzed in this study belong to phylogenetic group

TABLE 2. Characterization of ESBL genes and PBRT of plasmids

Isolate	PFGE type	ESBL pI by IEF	β-Lactamase genes				PBRT
			CTX-M	TEM	SHV	OXA	
45545	A1	8.6	CTX-M-15	Negative	Negative	OXA-1	FIA, FIB, FII[2]
46214	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	Negative	FIA, FII[1]
46595	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
46644	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2], A/C, I1
46844	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	Negative	FIA, FIB, FII[1], I1
50252 ^a	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
50552	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2]
50811	A1	8.6, 7.6, 5.4	CTX-M-15	TEM-1	SHV-11	OXA-1	FIA, FII[1]
50814 ^a	A1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
47736	A2	8.6, 5.4	CTX-M-15	TEM-1	Negative	Negative	FIA, FII[1], A/C, T
48115	A2	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
50055 ^a	A2	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
50419 ^a	A2	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
48112	A3	8.6	CTX-M-15	Negative	Negative	OXA-1	FIA, FIB, FII[2]
48165	A3	8.6	CTX-M-15	Negative	Negative	OXA-1	FII[1]
49107	A3	8.6	CTX-M-15	Negative	Negative	OXA-1	FII[2]
45606	B1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2]
45852	B1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2]
45913	B1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2]
46688	B1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2]
47731	B1	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2], A/C, I1
47849	B2	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FIB, FII[2], A/C, I1
50068	B2	8.6, 5.4	CTX-M-15	TEM-1	Negative	Negative	FIA, FII[1]
46427 ^a	C	8.6, 5.4	CTX-M-15	TEM-1	Negative	OXA-1	FIA, FII[1]
46733	D	8.2	Negative	Negative	SHV-12	Negative	FII[1], A/C
49403 ^a	E	8.6, 7.6, 5.4	CTX-M-15	TEM-1	SHV-11	OXA-1	FIA, FIB, FII[2]
47856 ^a	F	8.6	CTX-M-15	Negative	Negative	Negative	FIA, FIB, FII[2]
47242	G	8.0, 5.4	CTX-M-14	TEM-1	Negative	Negative	FIA, FIB, FII[2], A/C, I1

^a Strain isolated in the geriatric unit (see text).

B2 and show the combination of these three PAIs, they probably cause urinary tract infections, and in fact the majority of our ESBL producers were isolated from urine specimens. However, several isolates, which were indistinguishable by PFGE type, phylogenetic group, plasmid type, and PAI profile, were from invasive infections and isolated from blood or other sterile sites, thus indicating that these strains can cause more serious infections probably affecting immunocompromised hosts.

Among the epidemiological results obtained in this study, we noted that the ESBL producers isolated from May to July in the geriatric unit belonged to the rare C (isolate 46427), E (isolate 49403), and F (isolate 47856, phylogenetic group D) PFGE types, while those isolated in the same ward in August belonged to the A1 (isolates 50252 and 50814) and A2 (isolates 50055 and 50419) PFGE types, which largely spread in the other wards of the hospital. Irrespective of the PFGE type, all these isolates showed the *bla*_{CTX-M-15} gene, and this gene was linked to IncFII multireplicon plasmids. The E and F type strains were associated with two PAIs (IV₅₃₆ and I_{CFT073}), while the four cases observed in August also carried the third PAI II_{CFT073}. The epidemiological investigation of these cases showed that the introduction of the prevalent A2 PFGE type *E. coli* within the geriatric unit correlated with the admission of patient 50055, who was previously recovering in June in a medical ward where this A2 clone was isolated. The patient was transferred to the geriatric unit in August and there developed a urinary tract infection caused by the *E. coli* ESBL producer. The introduction of the A1 PFGE type in the geri-

atric unit could also be due to the transfer of patient 46595 to this unit: a wound swab taken in May from the patient was found to be positive for a CTX-M-15 producer of this genotype.

The two unique ESBL producers not positive for the *bla*_{CTX-M-15} gene in our collection were of the D (isolate 46733) and G (isolate 47242) PFGE types, showing only PAI IV₅₃₆ and coming from patients recovering in emergency units; thus, they were not related to the prevalent strains circulating within the hospital.

Analyzing the time of admission to the hospital and the date of the first isolation of the ESBL-producing strain (Table 1), we observed that a large proportion of the infections occurred after a long period of hospitalization, indicating that these infections were acquired within the hospital. The sparse dissemination of related bacteria suggests a probable contamination of foreign materials (water, catheter, or disinfectants) and an interpatient transmission occurring in the wards, in several cases causing small outbreaks involving two or three patients. The dissemination of relevant ESBL genes is linked to the spread of specific plasmids, well adapted to the *E. coli* species, by horizontal genetic exchange among bacteria of different genotypes.

The eruptive dissemination of the ESBL producers in this hospital is comparable to that observed in other hospitals in Italy and worldwide (5, 17, 19, 26) and emphasizes the necessity of continuous epidemiological monitoring followed by immediate intervention to prevent severe nosocomial infections.

ACKNOWLEDGMENTS

We are grateful to Anna Maria Dionisi for her work on the PFGE pattern analysis, to Tonino Sofia for manuscript revision, and to G. Prats for kindly providing us with positive control UPEC strains 536 and J96.

This work was supported by the DRESP2 (LSHM-CT-2005-018705) contract with the European Commission and by the FIRB project of the Italian Ministry of Research and University.

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