High prevalence of *bla*_{CTX-M-1}-group extended-spectrum β -lactamase genes in *Enterobacteriaceae* isolates from Emilia-Romagna, Italy

The production of extended-spectrum β -lactamases (ESBLs) by members of the *Enterobacteriaceae* is an increasing challenge in the treatment of infections. The ESBL type that has been detected most frequently over the last few years is CTX-M, which also has the particular ability to spread in the community (Pitout & Laupland, 2008). In Emilia-Romagna, a northern Italian region with a population of about 4 million, the rate of resistance to β -lactamase due to ESBLs increased rapidly in the period 2003–2006 (Gagliotti *et al.*, 2008a). No data on the type of circulating ESBL are available at the regional level. This study aims to describe the epidemiology of ESBLs by testing a sample of clinical ESBL-producing strains that were isolated in Emilia-Romagna.

Thirty isolates were randomly selected from the strain collection of a previous study, conducted in Emilia-Romagna in 2006, to evaluate the accuracy of automated systems in detecting ESBLs in *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. These isolates were collected in four of the five laboratories participating in the study (Gagliotti *et al.*, 2008b). The ESBL phenotype was confirmed by the combination disc method (CLSI, 2007). All strains underwent PCR amplification of *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} and *ampC*-type genes. Sequencing was performed to identify the subclasses of *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} genes (Al Naiemi *et al.*, 2006; D'Andrea *et al.*, 2006; Perilli *et al.*, 2002).

The 30 isolates studied were all clinical samples (26 *E. coli*, 2 *K. pneumoniae* and 2 *P. mirabilis*). Sequencing of ESBL-encoding genes showed that most strains (26/30; 87 %) carried *bla*_{CTX-M-1}-group genes, with *bla*_{CTX-M-15} and *bla*_{CTX-M-1} being the most prevalent variants (63 % and 20 %, respectively) whereas the *bla*_{CTX-M-32} gene was carried by only one strain (3 %) of *E. coli*. The GenBank/EMBL/DDBJ

accession numbers for the *bla*_{CTX-M-15}, *bla*_{CTX-M-1} and *bla*_{CTX-M-32} gene sequences of *E. coli* strains are AY0444436, X92506 and AJ557142, respectively.

Two *E. coli* isolates harboured *bla*_{SHV-5} genes while one *K. pneumoniae* isolate had multiple ESBL genes (*bla*_{CTX-M-15} + *bla*_{SHV-12}). Both *P. mirabilis* isolates harboured *bla*_{TEM-1} (non-ESBL β -lactamase gene), but the ESBL encoding genes were not identified. *bla*_{TEM-1} was also retrieved in three *E. coli* isolates, while only one isolate showed an *ampC*

genotype (CMY-2-like). Most patients had a urine infection (87 %), were ≥ 80 years (53 %) and female (60 %). A high proportion (43 %) of clinical samples were collected from outpatients (Table 1). The total prevalence of resistance to ciprofloxacin and gentamicin was 80 % and 30 %, respectively; 23 % of isolates (five *E. coli*, one *K. pneumoniae* and one *P. mirabilis*) were co-resistant to both agents.

The data show that CTX-M is, by far, the most common ESBL type in members of

Table 1. Genotype, sample and patient characteristics for 30 ESBL-producing strains

	<i>E. coli</i> (n=26)	<i>K. pneumoniae</i> (n=2)	<i>P. mirabilis</i> (n=2)	All organisms (n=30)
ESBL type				
CTX-M-15	17*	1	0	18 (60 %)
CTX-M-1	6*	0	0	6 (20 %)
CTX-M-32	1	0	0	1 (3 %)
CTX-M-15 + SHV-12	0	1	0	1 (3 %)
SHV-5	2†	0	0	2 (7 %)
Not identified	0	0	2‡	2 (7 %)
Sample				
Urine	22	2	2	26 (87 %)
Other/unknown	4	0	0	4 (13 %)
Patient				
Outpatient	12	0	1	13 (43 %)
Inpatient (hospital)	11	1	0	12 (40 %)
Inpatient (long-term facilities)	2	1	1	4 (13 %)
Unknown	1	0	0	1 (3 %)
Age category (years)				
0–19	1	0	0	1 (3 %)
20–59	4	0	0	4 (13 %)
60–79	8	0	0	8 (27 %)
≥ 80	12	2	2	16 (53 %)
Unknown	1	0	0	1 (3 %)
Gender				
Female	18	0	0	18 (60 %)
Male	8	2	2	12 (40 %)

*One *E. coli* strain also harboured a non-ESBL *bla*_{TEM} gene (*bla*_{TEM-1}).

†One *E. coli* strain also harboured a non-ESBL *bla*_{TEM} gene (*bla*_{TEM-1}); the other *E. coli* strain also harboured an *ampC* gene (*bla*_{CMY-2}-like).

‡The two *P. mirabilis* strains also harboured a non-ESBL *bla*_{TEM} gene (*bla*_{TEM-1}).

the *Enterobacteriaceae* in Emilia-Romagna. This finding confirms that CTX-M-producing strains (mainly *E. coli*) are widespread throughout Italy [i.e. Varese, Bergamo, Novara, Verona, Florence, Ancona, Naples, Sassari, Palermo, Catania (Mugnaioli *et al.*, 2006) and Pavia (Pagani *et al.*, 2003)]. CTX-M-positive isolates have also been reported previously in Italy at the community level (Brigante *et al.*, 2005) and in pets (Carattoli *et al.*, 2005). The *bla*_{CTX-M-32} gene, retrieved in one strain cultured from the urine of an outpatient, is a novel variant derived from the *bla*_{CTX-M-1} gene through a single Asp240→Gly substitution. Currently, isolates harbouring the *bla*_{CTX-M-32} gene variant have emerged in some European countries such as Spain, Portugal and Italy, being associated with community-acquired infections (Cantón *et al.*, 2008; López-Cerero *et al.*, 2008).

Another finding deserving attention was the absence of *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} ESBL genes in the two ESBL-producing *P. mirabilis* isolates, suggesting the presence of unknown ESBL genes. In fact, PCR amplification of *bla*_{VEB}, *bla*_{PER} and *bla*_{GES} was also negative. Moreover, the identification of an *E. coli* strain carrying a *bla*_{CMY-16}-like gene highlights the presence of acquired AmpC-type β -lactamases in members of the *Enterobacteriaceae*, which represents another emerging problem in Italy (Migliavacca *et al.*, 2007). Multifocal detection of *P. mirabilis* isolates carrying the *bla*_{CMY-16} AmpC-encoding gene has been reported in different cities in northern Italy (i.e. Bergamo, Milan, Novara and Varese) (D'Andrea *et al.*, 2006; Luzzaro *et al.*, 2009).

Finally, the majority of ESBL-producing strains included in this study were also resistant to fluoroquinolones and/or aminoglycosides, with a consequent limited number of effective options for antibiotic treatment.

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