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Occurrence of ST23 Complex Phylogroup A *Escherichia coli* Isolates Producing Extended-Spectrum AmpC β-Lactamase in a French Hospital[▽]

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Extended-spectrum AmpC β-lactamase (ESAC) *Escherichia coli* producers were investigated over a 5-year period. Eleven isolates presenting a strong *ampC* promoter and different strategic AmpC mutations, including two newly described modifications (A292V and an L-A-A insertion at 295), were characterized. All the isolates belonged to phylogenetic group A and to the ST23 complex.

In clinical *Escherichia coli* isolates, overexpression of the chromosomal AmpC β-lactamase was first largely attributed to different mutations located in strategic regions of the promoter, conferring various levels of resistance to cephalosporins (2). Structural modifications in the vicinity of the active site of the enzyme have also been recently described for extended-spectrum AmpC (ESAC) β-lactamases. They are characterized by increased catalytic efficiency against oxyiminocephalosporins, including cefepime and cefpirome (1, 5, 6, 7). The prevalence of ESAC-producing *E. coli* clinical isolates was investigated at Nantes University Hospital during the 5-year period of 2004 to 2008. Multilocus sequence types (MLST) and phylogenetic groups were determined for all the ESAC-producing isolates. A total of 41 strains (0.16% of the *E. coli* clinical isolates collected during the study period) were analyzed on the basis of their β-lactam resistance phenotypes compatible with AmpC overproduction (MIC ≥ 64 mg/liter for ceftazidime using the Vitek2 system [bioMérieux] and negative double-disk synergy test). MICs of cephalosporins were determined by the Etest method. A search for several plasmid-mediated AmpC β-lactamases (bla_{CMY}, bla_{ACC}, and bla_{DHA}) was performed under standard PCR conditions, using specific primers (8). PCR amplifications of the chromosomal *ampC* promoter and gene were performed with the primers AB1/ampC2 and Int-B1/Int-HN (2, 5). The nucleotide and deduced protein sequences were analyzed to search for nucleotide or amino acid replacements leading to extension of the hydrolysis spectrum.

Out of the 41 *E. coli* clinical isolates, 23 produced a plasmid-mediated CMY-2-type cephalosporinase associated with a wild-type chromosome-borne AmpC β-lactamase. In fact, no reliable and validated phenotypic method exists to differentiate chromosomal from plasmidic AmpC-overproducing isolates (8).

Five of the 18 remaining isolates, which had strategic mutations in the *ampC* promoter but not in the coding sequence, overproduced their chromosomal cephalosporinase. The isolates remained susceptible to cefepime (MIC ≤ 0.25 μg/ml). The 13 other strains were resistant to ceftazidime and cefotaxime, with reduced susceptibility to cefepime (MIC > 0.25 μg/ml). Among them, two were suspected either to produce an oxacillinase or to present altered permeability to drug in combination with overproduction of the AmpC β-lactamase. Eleven isolates (0.04% from the total collection) presented strategic amino acid mutations or insertions in the H-9 helix or H-10 helix or in the R2 loop of the chromosomal β-lactamase (Table 1). The presence of a strong *ampC* promoter was systematically demonstrated for the 11 strains (2). One mutation, at position −42, known to strongly increase *ampC* gene transcription, was detected in association with polymorphisms at positions −88, −82, −18, −1 and +58. Additional genetic events modifying the *ampC* attenuator (positions +17 to +37) were also detected for seven strains (Table 1). Thus, mutations in the promoter region contributing to overexpression of the AmpC β-lactamase could constitute a logical background for selection of modifications involved in the extension of the hydrolysis spectrum of the enzyme.

Among the 11 strains presenting strategic modifications in the vicinity of the active site of the enzyme, 9 harbored mutations previously reported as leading to an ESAC phenotype (H296P mutation for 6 isolates and S287N mutation for 3 isolates) (Table 1) (5). Furthermore, one strain presented a new mutation in the H-10 helix (the A292V mutation), and another strain revealed an undescribed structural modification in the R2 loop (three amino acid insertions [L-A-A] between residues 295 and 296, leading to the tandem duplication of the motif L-A-A) (Table 1). The residues 295 and 296 of the R2 loop could probably be a hot spot of the AmpC β-lactamase, since amino acid insertions responsible for an extended substrate spectrum have already been found at this site (6). In order to investigate the pattern of resistance conferred by the two newly described β-lactamases, cloning experiments using only the bla_{AmpC}-encoding genes without their own promoters

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TABLE 1. Characteristics of the 11 ESAC-producing *E. coli* strains isolated in our study

Isolate	Date	MIC (μg/ml)			Phylogenetic group	Allele no.							ST complex (ST)	*ampC* promoter mutations	Overproduced AmpC type (mutation)
		CAZ	CTX	FEP		*adk*	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*			
4009273	Oct. 2004	≥64	8	4	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +58	ESAC (S287N)
5000869	Mar. 2005	≥64	4	4	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +58	ESAC (H296P)
7001178	May 2007	≥64	8	2	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +20, +58	ESAC (S287N)
7008153	Jul. 2007	≥64	16	16	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +31, +58	ESAC (H296P)
7008211	Jul. 2007	≥64	8	16	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +31, +58	ESAC (H296P)
7008710	Aug. 2007	≥64	16	8	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +20, +58	ESAC (S287N)
7010738	Sept. 2007	≥64	8	8	A	6	4	12	1	20	18	7	STC23 (ST410)	−88, −82, −42, −18, −1, +22, +58	ESAC (H296P)
7014517	Dec. 2007	≥64	16	8	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +58	ESAC (295 Ins LAA)
8009162	Aug. 2008	≥64	8	0.75	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +23, +58	ESAC (A292V)
8009389	Sept. 2008	≥64	8	16	A	6	4	12	1	20	12	7	STC23 (ST88)	−88, −82, −42, −18, −1, +58	ESAC (H296P)
8009989	Dec. 2008	≥64	8	8	A	6	4	12	1	20	18	7	STC23 (ST410)	−88, −82, −42, −18, −1, +22, +58	ESAC (H296P)

CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime.

were performed as previously described (5). MICs of ceftazidime, cefotaxime, and cefepime determined for recombinant strains emphasized an ESAC phenotype (resistance to ceftazidime and cefotaxime and reduced susceptibility to cefepime) (Table 2).

Phylogenetic analysis, using a triplex PCR targeting *chuA*, *yja*, and TSPE4 (3), revealed that the 11 ESAC-producing isolates belonged to the commensal, likely less virulent group A (3, 7). The study of their genetic relatedness was performed by pulsed-field gel electrophoresis (PFGE) after digestion with NotI and by MLST analysis according to the instructions of the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli). One strain was nontypeable by PFGE due to high DNase activity. Seven different PFGE patterns (<90% similarity) were identified among the 10 other strains, indicating that ESAC-producing *E. coli* clinical isolates constituted a relatively diverse population. Nevertheless, all the strains belonged to the sequence type (ST) complex 23 and shared six of seven alleles (two ST410 isolates showing indistinguishable PFGE profiles and nine ST88 isolates) (Table 1). These results correlate with previous reports that *E. coli* strains overproducing chromosomal AmpC β-lactamase were significantly more likely to belong to the less-pathogenic phylogenetic group A and ST23 complex (3, 4).

The ESAC-producing isolates were predominantly collected in the 2007-to-2008 period (one in 2004, one in 2005, six in 2007, and three in 2008). All the isolates were recovered from urine samples. The 11 cases were hospital-acquired or health care-associated infections or colonizations, with in most cases prior antibiotic administration.

In conclusion, our study, although based on a limited number of isolates, underlines the emergence in our hospital of *E. coli* clinical isolates expressing ESAC β-lactamases, particularly within the last 2 years, with the discovery of two new variants. Development of resistance upon antimicrobial selection pressure is of great concern. As other β-lactamases, such as TEM or SHV, have given rise to extended-spectrum enzymes by amino acid replacements, the chromosomal AmpC β-lactamase of *E. coli* is also evolving. Further epidemiological surveys are needed to understand why that evolution seems to be limited to the less-pathogenic *E. coli* strains of particular genetic lineages, like the ST23 complex.

Nucleotide sequence accession numbers. The sequence encoding the AmpC β-lactamase with a new mutation in the H-10 helix (the A292V mutation) has been deposited in GenBank under accession number GQ463706, and that for an undescribed structural modification in the R2 loop (three amino acid insertions [L-A-A] between residues 295 and 296) has been deposited under accession number GQ463705.

TABLE 2. MIC values of several cephalosporins for *E. coli* 7014517, *E. coli* 8009162, *E. coli* ATCC 25922, and their corresponding recombinant clones

Cephalosporin	MIC value (μg/ml) for strain						
	E. coli 7014517 (AmpC with LAA insertion at position 295)	*E. coli* Top10 producing AmpC 7014517	*E. coli* 8009162 (AmpC with A292V mutation)	*E. coli* Top10 producing AmpC 8009162	*E. coli* ATCC 25922 (wild-type AmpC)	*E. coli* Top10 producing AmpC 25922	*E. coli* Top10
Ceftazidime	64	>256	64	>256	0.09	4	<0.06
Cefotaxime	16	32	8	12	<0.06	0.75	<0.06
Cefepime	8	4	0.75	0.75	<0.06	0.06	<0.06

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