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CTX-M-producing *Escherichia coli* in a maternity ward: a likely community importation and evidence of mother-to-neonate transmission

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Objectives: To investigate the high prevalence of extended-spectrum β-lactamase (ESBL)-producing strains of *Escherichia coli* (4%, 10/250 consecutive isolates) recovered during a 5 month period in the maternity ward of the University Hospital of Bordeaux, France.

Methods: β-Lactam resistance transfer was analysed by conjugation and transformation. ESBLs were characterized by isoelectric focusing, PCR amplification and sequencing. The relatedness of the strains was examined by PFGE and phylogenetic group determination. Plasmids were characterized by incompatibility group and restriction analysis.

Results: Ten ESBL-producing *E. coli* were isolated from urinary or genital samples of eight mothers and from gastric fluids of two newborns of carrier mothers. The patients were hospitalized in five different units of the maternity ward. Transconjugants, obtained for 7 of the 10 strains, and wild-type strains exhibited various antibiotypes. Different CTX-M enzymes were characterized: CTX-M-1 ($n=4$); CTX-M-14 ($n=3$); CTX-M-32 ($n=2$); and CTX-M-28 ($n=1$). The strains recovered from two mothers and their respective babies were identical. All the other strains were epidemiologically unrelated. Furthermore, various plasmids were identified. Environmental samples from the common echographic and sampling rooms did not reveal the presence of ESBL-producing enterobacteria.

Conclusions: The data argue against the occurrence of a nosocomial outbreak and support the hypothesis of an importation of community-acquired ESBL-producing strains into the hospital through colonized/infected patients. At present, not only patients transferred from other hospitals or long-term care facilities are at risk of carrying ESBL-producing enterobacteria on hospital admission, but also community patients.

Keywords: extended-spectrum β-lactamases, PFGE, phylogenetic groups, plasmid incompatibility groups

Introduction

In Gram-negative bacteria, β-lactamase production remains the most frequent mechanism of resistance. Among these enzymes, extended-spectrum β-lactamases (ESBLs) are particularly worrisome. The first to be described were derivatives of the TEM and SHV penicillinases, and were associated with nosocomial outbreaks predominantly due to *Klebsiella pneumoniae* and *Enterobacter aerogenes* infections.[1] In 1989, CTX-M ESBLs emerged.[1] They have become the most prevalent since 2000 and are essentially harboured by *Escherichia coli* isolates from community patients suffering from urinary tract infections.[1–3]

E. coli is responsible for nosocomial- and community-acquired infections. It is, together with *Streptococcus agalactiae*, the most common causative agent for severe neonatal diseases. Indeed, recently, ESBL-producing *E. coli* have been described as the cause of neonatal sepsis and meningitis.[4]

In this study we characterized strains responsible for an unexpectedly high rate of ESBL-producing *E. coli* in a maternity ward.

Materials and methods

Bacterial strains and culture conditions

Between November 2007 and April 2008, 10 ESBL-producing *E. coli* were collected from 10 patients of the maternity ward of the University Hospital of Bordeaux. All isolates were identified by API 20E (bioMérieux, Marcy l'Étoile, France) or the Phoenix system (Becton Dickinson,

Le Pont-de-Claix, France). All strains were routinely cultured at 37°C on Mueller–Hinton (MH) agar (Diagnostics Pasteur, Marnes-la-Coquette, France), or in brain heart infusion broth (GibcoBRL, Cergy Pontoise, France) or trypticase soy broth (Diagnostics Pasteur).

Antibiotic susceptibility testing and isoelectric focusing (IEF)

Antibiotic susceptibility was determined using 23 antimicrobial agents, by the disc diffusion method, according to the French Society of Microbiology guidelines (http://www.sfm.asso.fr). ESBL production was detected by the double-disc synergy test between clavulanic acid and ceftazidime, cefotaxime and cefepime on MH agar.

β-Lactamases were released by sonication, and their isoelectric points (pIs) determined by IEF as previously described.[5] β-Lactamases of known pIs [TEM-1 (pI 5.4), OXA-1 (pI 7.4) and CTX-M-15 (pI 8.6)] were used as markers.

Plasmid content analysis, and conjugation and transformation experiments

Resistance transfer to *E. coli* K12 (Rif^R-Nal^R) was attempted by a filter mating technique. Transconjugants were selected on MH agar plates containing ticarcillin (100 mg/L), cefotaxime (2 mg/L), or ceftazidime (2 mg/L) plus rifampicin (200 mg/L). For the strains isolated from a mother–baby couple, one transconjugant was analysed. Plasmid DNA extracted using an alkaline lysis method[5] and the Qiagen plasmid DNA midi kit (Qiagen, Courtaboeuf, France) was electroporated into *E. coli* JM109 with selection on ticarcillin (100 mg/L), and was restricted with EcoRI or HindIII enzymes. Incompatibility group was determined by PCR-based replicon typing.[6] When several plasmids were detected in a transconjugant, they were electroporated into *E. coli* JM109 to obtain transformants bearing single plasmids.

PCR amplification and sequencing

Total DNA was extracted as previously described,[5] and resistance genes were detected using primers specific for bla_{TEM}, bla_{SHV}, bla_{OXA-1} and bla_{CTX-M}.[3] For sequencing purposes, PCR products were used as templates using laboratory-designed primers and the automatic ABI 3130xl sequencer (Applied Biosystems, Courtaboeuf, France).[5] Sequences were analysed using the 'BioEdit' software.

Epidemiological typing

The relatedness of the clinical isolates was investigated by PFGE using the XbaI enzyme and the CHEF-DRIII apparatus (Bio-Rad, Marnes-la-Coquette, France). Phylogenetic groups and subgroups were determined by a multiplex PCR.[7]

Results and discussion

In November 2007, over a period of 1 week, two ESBL-producing *E. coli* were isolated from two patients (patients no. 1 and no. 3) on the maternity ward. Subsequently, antimicrobial susceptibility testing of all *E. coli* detected, even in non-pathological situations, was implemented. During the following 5 months and of 250 consecutively collected *E. coli* strains, 8 additional ESBL-producing *E. coli* were recovered, raising their prevalence on this ward to 4%. No other ESBL-producing Enterobacteriaceae (ESBLE) were detected. For comparison, in 2007, the ESBLE prevalence for the entire hospital was 3.69% and only 50% were *E. coli*. This finding led us to further characterize these strains, suspected to be responsible for a nosocomial outbreak.

The strains were recovered from urinary (five) or genital samples (three) of eight women, ranging in age from 27 to 39 years (mean age, 33.25 years) and from gastric fluid of two babies born to carrier mothers. Five women were hospitalized for 2–60 days and received antibiotic treatment for 51–70 days (Table 1). The others were admitted to the outpatient ward or to the emergency unit but did not stay in the maternity ward and did not receive any antibiotic. Four patients were infected by an ESBL-producing *E. coli* (patient no. 1, bacteraemia 9 days after the isolation of ESBL-producing *E. coli* in urine; patients no. 3, no. 4 and no. 9, urinary tract infections). Two of the four babies born during the study were colonized by the ESBL-producing *E. coli*. The patients were hospitalized in five different sections of the maternity ward, but they all came into contact with the same echography and urinary sampling rooms. Environmental samples taken from these premises did not reveal ESBL-producing strains.

All clinical isolates were resistant to β-lactams, excluding cefoxitin, moxalactam and imipenem, and produced ESBLs. They exhibited various resistance profiles to other antibiotics (Table 1), except for the strains that originated from two mothers and their babies.

β-Lactam resistance transfer to Rif^R-Nal^R *E. coli* K12 was successful in seven strains, with rates varying from 5.5×10^{-4} to 4.2×10^{-8}. For the three remaining strains, only two gave transformants exhibiting an ESBL phenotype (Table 1).

bla_{CTX-M} was detected in all strains, alone or in combination with bla_{TEM} or $bla_{OXA-1-like}$. IEF revealed two TEM-1-like enzymes (pI 5.4), one TEM-2-like enzyme (pI 5.6) and one OXA-1-like enzyme (pI 7.4). For the CTX-M β-lactamases, the pIs ranged between 8.1 and 8.9. Sequencing demonstrated the presence of CTX-M-1 (four strains, including one colonizing a mother and her baby), CTX-M-14 (three strains), CTX-M-32 (the second mother–baby pair) and CTX-M-28 (one strain). CTX-M-1 and CTX-M-14 are frequently detected in *E. coli* isolates in Spain and France.[3,8] CTX-M-32, despite its high prevalence in most European countries,[1] and CTX-M-28 are reported here for the first time in France. Strikingly, CTX-M-15, the most widely spread variant,[1,3] was not detected in our study.

Although, the presence of various resistance phenotypes and different CTX-M enzymes did not argue for a nosocomial outbreak, the relatedness of the strains was investigated. The four main phylogenetic groups, A, B1, B2 and D, were found. The four patients suffering from urinary tract infection or bacteraemia carried *E. coli* of the virulent groups D and B2, and commensal groups A and B1. The strains were distributed in different groups, except those collected from mother–neonate pairs and two CTX-M-14-producing *E. coli* (Table 1). Specific association between phylogenetic groups and CTX-M has been suggested, particularly group A with CTX-M-9 and group B2 with CTX-M-15.[1] However, a recent study showed that CTX-M-14 was related to a greater diversity of phylogroups.[9] PFGE (Figure 1, profiles I–VII) shows the strains to be unrelated except for the mother–neonate pairs, where transmission was confirmed. PFGE also proved that both CTXM-14-producing strains of the same phylogenetic group D were distinct (Figure 1). These results excluded a clonal strain outbreak. Dissemination of CTX-M-9, CTX-M-14, CTX-M-15 and CTX-M-32

Table 1. Patient, strain, plasmid and enzyme characteristics

Patient no.	Strain no. (date of isolation)	Unit	DH	Pathology and treatment	Phylogenetic group	PFGE type	β-Lactamase content and antibiotype	pI(s)	Plasmid incompatibility group
1 mother	**Ec4186 (23/11/07)**	**PP1**	**D15**	**PRM, amoxicillin (08/11/07) and ceftriaxone (23/11/07)**	**A**	**I**	**CTX-M-1, SXT, (TET)**	**8.4**	**I1-FIB**
	Ec4224 (Tc of Ec4186)						**CTX-M-1, SXT**	**8.4**	**I1-FIB**
	Tf of 4224						**CTXM-1, SXT**	**ND**	**I1**
2 baby	**Ec4187 (30/11/07)**	**NN**	**D1**	**birth 29/11/07**	**A**	**I**	**CTX-M-1, SXT, (TET)**	**8.4**	**I1-FIB**
3	Ec4189 (19/11/07)	Ow	D1	abdominal pain, bleeding, no ATB	B1	NT	CTX-M-14, TET, FQ	8.1	FIB
	Tf of Ec4189						CTX-M-14	ND	NT
4	Ec4190 (27/11/07)	EU	D0	conjugal violence, no ATB	D	II	CTX-M-1, TEM-1, TET, (SXT)	8.4; 5.4	I1-FIB
	Ec4223 (Tc of Ec4190)						CTX-M-1, (SXT)	8.4	I1
5	Ec4192 (07/01/08)	PP1	D2	PRM, spiramycin	B2	III	CTX-M-1, TET, CHL	8.4	I1-P
	Ec4226 (Tc of Ec4192)						CTX-M-1, TET	8.4	I1
6	Ec4193 (19/01/08)	PP1	D60	PRM, amoxicillin (10/11/07) and cefpodoxime (18/01/07)	D	IV	CTX-M-14, TEM-2, GEN, TOB, NET, TET, CHL, SXT, (FQ)	8.1; 5.6	I1
	Tf of Ec4193						CTX-M-14	ND	NT
7 mother	**Ec4195 (03/03/08)**	**Mat**	**D41**	**PRM, amoxicillin (20/01/08)**	**D**	**V**	**CTX-M-32, (TET)**	**8.9**	**N**
	Ec4225 (Tc of Ec4195)						**CTX-M-32**	**8.9**	**N**
8 baby	**Ec4194 (03/03/08)**	**Mat**	**D0**	**birth 03/03/08**	**D**	**V**	**CTX-M-32, (TET)**	**8.9**	**N**
9	Ec4196 (17/03/08)	EU	D0	abdominal pain, no ATB	B2	VI	CTX-M-28, OXA-1, TOB, NET, AMK, TET, SXT, FQ	8.9; 7.4	FIA
	Ec4256 (Tc of Ec4196)						CTX-M-28, OXA-1, TOB, NET, AMK, TET	8.9; 7.4	FIA
10	Ec4305 (11/04/08)	PP2	D20	haemorrhage, amoxicillin (22/03/08)	D	VII	CTX-M-14, TEM-1, FQ	8.1; 5.4	F
	Tf of Ec4305						TEM-1	ND	F

Tc, transconjugant of *E. coli* K12; Tf, transformant of *E. coli* JM109; DH, days of hospitalization; PP, pathological pregnancy; NN, neonatal unit; Ow, outpatient ward; EU, emergency unit; Mat, maternity; PRM, premature rupture of membranes; ATB, antibiotic treatment; ND, not determined; NT, non-typeable; pI, isoelectric point; SXT, co-trimoxazole; TET, tetracycline; FQ, fluoroquinolones; CHL, chloramphenicol; GEN, gentamicin; TOB, tobramycin; NET, netilmicin; AMK, amikacin; (), low-level resistance.
The mother–baby pairs are indicated in bold.

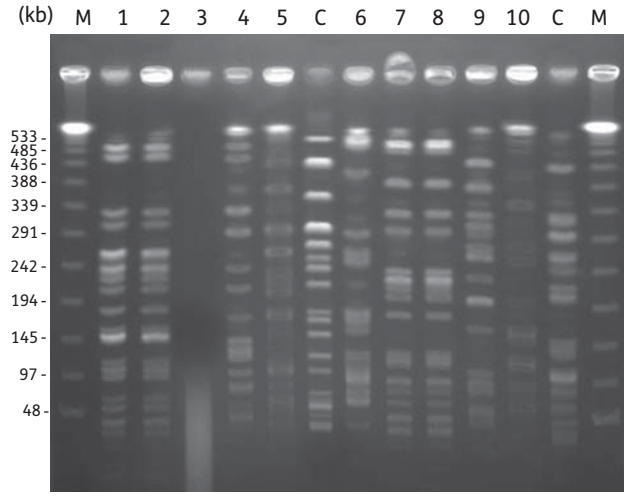

Figure 1. PFGE profiles of XbaI-digested whole-cell DNA of the 10 *E. coli* isolates. Lane 1, Ec4186 (mother); lane 2, Ec4187 (her baby); lane 3, Ec4189; lane 4, Ec4190; lane 5, Ec4192; lane 6, Ec4193; lane 7, Ec4194 (baby); lane 8, Ec4195 (his mother); lane 9, Ec4196; and lane 10, Ec4305. M, DNA ladder. C, DNA control of unrelated *E. coli* strains. Despite three successive experiments, one strain (Ec4189) was non-typeable, probably due to a high DNase activity.

has been linked with epidemic plasmids.[1] Thus, the plasmid incompatibility group was determined.[6] As previously described,[10] CTX-M-32 was found associated with the broad host range incompatibility group IncN plasmid. All CTX-M-1 genes were located on plasmids of incompatibility group I1. Interestingly, for the three CTX-M-14-producing strains, for which mating experiments were unsuccessful, CTX-M-carrying plasmids were non-typeable. In one case, CTX-M transconjugants could not be obtained, suggesting that $bla_{\text{CTX-M-14}}$ was either present on non-conjugative plasmids or was chromosomal; alternatively, donor strains might produce colicin. $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-14}}$ have been reported on plasmids of various incompatibility groups.[10] The location of $bla_{\text{CTX-M-28}}$ on a plasmid of the FIA group is reported here for the first time. Plasmids of this group usually carry $bla_{\text{CTX-M-15}}$, the encoded enzyme differing from CTX-M-28 by a single substitution (Asp288Asn) at the protein's C-terminal extremity, suggesting an *in vivo* evolution of CTX-M-15 towards CTX-M-28. Plasmids of the same incompatibility group and carrying the same $bla_{\text{CTX-M}}$ were further digested by EcoRI and HindIII. Plasmids harbouring $bla_{\text{CTX-M-1}}$ shared common bands, and those carrying $bla_{\text{CTX-M-14}}$ were indistinguishable (data not shown), suggesting their concomitant dissemination in the area. These plasmids frequently transport multiple resistance determinants, facilitating ESBL dissemination by co-selection processes. In our study, all CTX-M-encoding plasmids except one (patient no. 9) conveyed none or few additional resistances (Table 1); notably no plasmid-mediated quinolone resistance was detected.

In 2006, a French nationwide study highlighted a significant increase in ESBLE in the community (1.1% in France and 1.4% in the Bordeaux area).[3] CTX-M-15 was the most frequent β-lactamase found. Only 11 CTX-M-1 or CTX-M-14 were detected, including 2 in the area of Bordeaux, of which 1 presented the same resistance profile and phylogenetic group as one of the present strains (Ec4193). CTX-M-producing *E. coli* have been increasingly identified as a cause of community-onset infections,[2] and empirical therapy involving cephalosporins was associated with a higher mortality.[2,8] Clinicians should be aware of the risk of a possible treatment failure associated with these strains in invasive infections such as neonatal sepsis.

Finally, this study demonstrates the transmission of CTX-M-producing *E. coli* between mothers and their newborns, and strongly suggests the introduction of these strains from the community to the hospital. Awareness of possible ESBL carriage is now required for all patients on hospital admission.

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Transparency declarations

None to declare.

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