

CTX-M β-Lactamase Production and Virulence of *Escherichia coli* K1

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We report a patient with neonatal meningitis caused by a CTX-M-1-producing *Escherichia coli* K1 strain. The influence of CTX-M production on virulence was investigated in cell culture and a newborn mouse model of meningitis. CTX-M production had no influence on virulence but was a major factor in clinical outcome.

Escherichia coli is the second most common cause of neonatal meningitis. Neonatal meningitis *E. coli* (NMEC) belong mainly to phylogenetic group B2 and harbor numerous virulence factors (1).

Since the beginning of the 21st century, an explosive spread of CTX-M-type extended-spectrum β-lactamases (ESBLs) in *E. coli* has occurred (2). These enzymes confer resistance to nearly all β-lactam antimicrobial drugs, including third-generation cephalosporins, the first-line treatment for patients with serious *E. coli* infections. However, CTX-M-type ESBLs have been observed mainly in *E. coli* strains with few virulence factors or in strains causing minor infections (3–5). In addition, bacterial resistance to antimicrobial drugs is frequently reported as difficult to reconcile with bacterial virulence (6). Highly pathogenic *E. coli* such as NMEC are therefore considered susceptible to antimicrobial drugs (7). We report a clinical case of neonatal meningitis caused by CTX-M-producing NMEC and the influence of CTX-M production on virulence.

The Study

In April 2007, a 39-year-old pregnant woman with amniotic sac rupture was admitted to a hospital in Orleans, France, at 28 weeks and 4 days of gestation. Treatment

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was started with betamethasone (12 mg 1×/d) for fetal lung maturation and amoxicillin (1g 3×/d) for 4 days. Because of a high serum level of C-reactive protein, antimicrobial drug therapy was switched to amoxicillin with clavulanic acid (1g 3×/d) for 1 day. A cesarean delivery was performed at 29 weeks and 2 days of gestation. A lumbar puncture sample of the low-weight (1,560 g) newborn female was tinged with blood. Cerebrospinal fluid (CSF) protein and glucose values were 4.00 g/L and 3.5 mmol/L, respectively. Results of CSF Gram staining were negative.

The infant was admitted to the neonatal critical care unit and received amoxicillin (150 mg), cefotaxime (120 mg), and netilmicin (8 mg) 2×/d for 2 days. Culture of placenta, maternal and infant blood, and infant gastric fluid yielded *E. coli*. The isolate was resistant to antimicrobial drugs, including third-generation cephalosporins. Imipenem/cilastatin (25 mg 4×/d) and amikacin (15 mg 2×/d) were given for 2 days. Treatment with imipenem/cilastatin was given for 15 days and then stopped because of the infant's clinical improvement and return of C-reactive protein to the reference level. Similar drug treatment was administered to the mother.

One week after drug treatment was discontinued, the infant showed signs of septicemia. A second lumbar puncture sample had protein and glucose levels of 4.56 g/L and 0.1 mmol/L, respectively, and a leukocyte count of 4,700 cells/μL (54% polymorphonuclear cells). *E. coli* were isolated from blood and CSF cultures and showed a resistance pattern identical to that of the previous isolate. Meningitis was a complication of the initial sepsis or a relapse of initial unapparent meningitis (8).

Treatment was started with imipenem/cilastatin (30 mg 4×/d) for 25 days and amikacin (15 mg 2×/d) for 5 days. Because the infant had a seizure, phenobarbital (22.5 mg) and ciprofloxacin (15 mg 2×/d) were prescribed for 5 additional days. Her condition gradually improved and blood and CSF values returned to reference levels. The infant was discharged from the hospital 1 month later and treatment with the anticonvulsant was discontinued. She showed normal psychomotor development at a regular follow-up pediatric visit.

E. coli strains isolated from the mother and infant were indistinguishable by enterobacterial repetitive intergenic consensus sequence 2 PCR, random amplified polymorphic DNA analysis, and typing with a MALDI BioTyper (Bruker Daltonique, Wissembourg, France) (9). Thus, the isolates corresponded to the same strain, designated Orl-1. PCR-based phylogenetic analysis and serotyping showed that the strain belonged to group B2 and serotype O7:K1:H7, a major O antigen encountered worldwide in NMEC (1).

Resistance of the Orl-1 strain (MICs 128 μg/mL for cefotaxime and 8 μg/mL for ceftazidime) was caused by

the gene encoding CTX-M-1. This strain was also resistant to tetracycline, trimethoprim, and sulfamethoxazole and susceptible to cefoxitin, imipenem, aminoglycosides, quinolones, chloramphenicol, and fosfomycin. It harbored the major *E. coli* genes associated with neonatal meningitis (Table 1) (1,10).

Plasmids from Orl-1 were used to transform *E. coli* K-12 DH5 α . Three resistance profiles that enabled detection of 3 plasmids were obtained. On the basis of screening of plasmid transformants and Orl-1, most virulence factors genes were presumably chromosomally encoded. Three virulence factors (*aer*, *iss*, and a second copy of *iroN*) were mediated by a tetracycline-resistant, large (\approx 180 kb), conjugative plasmid (pOrl-1-Te). The 2 other plasmids were pOrl-1-CTX-M-1, the CTX-M-1-encoding large (\approx 150 kb) conjugative plasmid carrying resistance to trimethoprim and sulfamethoxazole, and pOrl-1-TEM-1, a TEM-1-encoding small (<40 kb) plasmid.

A derivative strain that did not harbor the 3 plasmids (Orl-c) was obtained by plasmid elimination with ethidium bromide. Orl-1, Orl-c, and *E. coli* DH5 α harboring pOrl-1-CTX-M-1 were tested for invasiveness in human brain microvascular endothelial cells (10) and in a newborn mouse (C57BL/6 wild-type) model of meningitis (R. Mittal et al., unpub. data) to investigate the influence of CTX-M-1 production on virulence. *E. coli* strain E44, a rifampicin-resistant mutant of archetypical NMEC K1 strain RS218, was used as a positive control (10).

Orl-1 and Orl-c strains exhibited 3.5 \times lower invasiveness than strain E44. However, their ability to invade human brain microvascular endothelial cells was 400 \times higher than that of strain DH5 α -CTX-M1. In the mouse model, DH5 α -CTX-M1 did not cause bacteremia or meningitis. In contrast, Orl-1, Orl-c, and E44 induced meningitis (prevalences of 100%, 84%, and 85%, respectively) (Table 2).

The difference between Orl-1 and Orl-c in the mouse model may be explained by loss of plasmid pOrl-1-Te from Orl-c. Plasmid pOrl-1-Te is likely similar to pS88-related plasmids of NMEC because they share 3 virulence factor genes (*iss*, *aer*, and *iroN*) and are large and conjugative. These plasmids contribute to virulence of NMEC (11). Orl-1 and Orl-c showed similar behaviors, which suggested that the CTX-M-1-encoding plasmid pOrl-1-CTX-M-1 does not alter virulence of the strain.

Table 1. Virulence factors of Orl-1 *Escherichia coli* K1 strain, France

Integrative elements* (1)	Virulence genes	Present
PAI III ₅₃₆ -like	<i>iroN</i>	Yes
	<i>sfa/foc</i>	Yes
GimA-like	<i>ibeA (gimA4), ptnC (gimA1)</i>	Yes
PAI II _{J96} -like	<i>hra</i>	Yes
	<i>hlyC, cnf1</i>	No
PAI I _{CF1073} -like	<i>hlyC</i>	No
	<i>aer (iucC)</i>	Yes
HPI-like	<i>fyuA, irp-2</i>	Yes
GimB-like	<i>gimB</i>	Yes
<i>pks</i> island†	<i>clbA, clbK-J, clbP, clbQ</i>	Yes
Others	<i>chuA</i>	Yes
	<i>ompA</i>	Yes
	<i>hek</i>	Yes
	<i>iss</i>	Yes
	<i>malX</i>	Yes
	<i>cdtB-I to -V</i>	No

*PAI, pathogenicity island; HPI, high-pathogenicity island.

†Positive cytopathogen effect with transient infection of HeLa cells.

Mice with E44- and Orl-1-induced neonatal meningitis were treated with the third-generation cephalosporin cefotaxime, as recommended for humans. Despite antimicrobial drug treatment, Orl-1, but not strain E44, caused meningitis, suggesting that drug resistance is a major factor in clinical outcomes.

Conclusions

Studies have reported emergence of *E. coli* as the predominant organisms responsible for sepsis at any gestational age and for increased rates of drug-resistant *E. coli* caused by intrapartum drug prophylaxis (12). Spread of ESBLs in *E. coli* and intrapartum exposure to antimicrobial drugs may favor emergence of NMEC strains resistant to third-generation cephalosporins.

Two other well-characterized *E. coli* K1 strains producing ESBLs have been isolated from patients with neonatal meningitis in Algeria and France. The ESBL was identified as CTX-M-15 in both patients, and 1 infection was lethal (13,14). Other putative ESBL-producing *E. coli* K1 have been recently isolated, especially in developing countries (15).

Emergence of ESBL-producing *E. coli* strains, which are frequently resistant to fluoroquinolone (2), highlights the need for possible alternatives to third-generation ce-

Table 2. Incidence of meningitis in a newborn mouse model by *Escherichia coli* strain, France*

Bacterial strain	No. animals	Mean \pm SD bacteremia, log CFU/mL blood	No. positive CSF cultures (% meningitis)
E44	20	6.95 \pm 0.6	17 (85)†
Orl-1	16	6.75 \pm 0.8	16 (100)†
Orl-c	17	6.60 \pm 0.5	14 (82)†
DH5 α -CTX-M1	10	0.10 \pm 0.1	0

*CSF, cerebrospinal fluid.

†p<0.005, significantly higher than the incidence of meningitis by DH5 α -CTX-M1 by χ^2 test.

phalosporins for treatment of patients with infected with NMEC. Carbapenems are usually recommended for treatment of infections with ESBL-producing *E. coli* (2). However, this case report shows the role of treatment duration and the need for additional pharmacokinetic and safety studies in neonates and for adjunctive therapies (8).

This characterization of a CTX-M-1–producing NMEC strain highlights the emergence of CTX-M–type ESBL in highly virulent *E. coli*. Because of worldwide spread of CTX-Ms, caution should be exercised in the management of patients with NMEC, and first-line treatment for neonatal meningitis may need to be reconsidered.

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