**CTX-M \(\beta\)-Lactamase Production and Virulence of *Escherichia coli* K1**

Damien Dubois, Nemani V. Prasadarao, Rahul Mittal, Laurent Bret, Marie Roujou-Gris, and Richard Bonnet

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 \(\beta\)-lactamases (ESBLs) in *E. coli* has occurred (2). These enzymes confer resistance to nearly all \(\beta\)-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 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 administrated 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

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the gene encoding CTX-M-1. This strain was also resis-
tant to tetracycline, trimethoprim, and sulfamethaxazole
and susceptible to cefotaxime, imipennem, 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 DH5α. Three resistance profiles that enabled detec-
tion 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 (≈180 kb), con-
jugative plasmid (pOrl-1-Te). The 2 other plasmids were
pOrl-1-CTX-M-1, the CTX-M-1–encoding large (≈150
kb) conjugative plasmid carrying resistance to trimethop-
rim and sulfamethaxazole, 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 DH5α harboring pOrl-1-
CTX-M-1 were tested for invasiveness in human brain mi-
crovascular 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 strain RS218, was
used as a positive control (10).

Orl-1 and Orl-c strains exhibited 3.5× lower inva-
siveness than strain E44. However, their ability to invade
human brain microvascular endothelial cells was 400×
higher than that of strain DH5α–CTX-M-1. In the mouse
model, DH5α–CTX-M-1 did not cause bacteremia or men-
ingitis. 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 suggest-
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

<table>
<thead>
<tr>
<th>Integrative elements* (1)</th>
<th>Virulence genes</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI IIIimated–like</td>
<td>iroN</td>
<td>Yes</td>
</tr>
<tr>
<td>PAI IIaer–like</td>
<td>hek, hlyC, cnf1</td>
<td>No</td>
</tr>
<tr>
<td>PAI Iorl–like</td>
<td>aer (iucC)</td>
<td>Yes</td>
</tr>
<tr>
<td>HPI–like</td>
<td>fyuA, irp-2</td>
<td>Yes</td>
</tr>
<tr>
<td>GIMB–like</td>
<td>gimB</td>
<td>Yes</td>
</tr>
<tr>
<td>Pks island†</td>
<td>clbA, clbK-J, clbP, clbQ</td>
<td>Yes</td>
</tr>
<tr>
<td>Others</td>
<td>chuA</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>ompA</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>hek</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>iss</td>
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</tr>
<tr>
<td></td>
<td>malX</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>cdtB-I–V</td>
<td>No</td>
</tr>
</tbody>
</table>

*PAI, pathogenicity island; HPI, high-pathogenicity island.
†Positive cytopathogen effect with transient infection of HeLa cells.

Mice with E44- and Orl-1–induced neonatal menin-
gitis were treated with the third-generation cephalosporin
cefotaxime, as recommended for humans. Despite anti-
microbial 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 pre-
dominant organisms responsible for sepsis at any gesta-
tional 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 pro-
ducing ESBLs have been isolated from patients with neo-
natal 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-
phalosporins for treatment of patients with infected with NMEC. Carbapenems are usually recommended for treatment of infections with ESBL-producing \textit{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 \textit{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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**References**


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