Case Report

Neonatal sepsis caused by a CTX-M-32-producing *Escherichia coli* isolate

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We describe what we believe to be the first case of neonatal sepsis caused by CTX-M-producing *Escherichia coli*, in a low-weight preterm infant, born to a colonized mother who had received antibiotic treatment antepartum. Increased dissemination of extended-spectrum β -lactamase-producing *E. coli* in the community should be borne in mind for empirical therapy of sepsis in high-risk newborns.

Case report

A male preterm infant of 26 weeks and 4 days gestation was born by spontaneous vaginal delivery weighing 1140 g. The mother was admitted to hospital 10 days before labour because of fever and rupture of membranes. She was suspected of having chorioamnionitis and given ampicillin and gentamicin after collection of blood cultures. The infant was born in good condition with good Apgar scores. Because of prematurity and intrapartum antibiotic exposure, intravenous ampicillin and gentamicin treatment were commenced in the intensive neonatal care unit. His condition deteriorated on day 7 whilst still receiving the previous antimicrobial regimen, and a blood culture and cerebrospinal fluid (CSF) were obtained. At this time, the infant was switched to vancomycin and meropenem. The CSF analysis was normal and its culture was sterile. The blood culture was positive for extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* (isolate H344, 7th day after delivery). The patient was placed under contact precautions and intravenous meropenem was continued for a 7 day course. The child's course was further complicated by bronchopulmonary dysplasia, severe anaemia, intraventricular haemorrhaging and retinopathy of prematurity. He was discharged home on the 88th day after birth weighing 3190 g and with a favourable outcome for ocular and respiratory problems.

Microbiology

Maternal vaginal and rectal swab cultures were obtained immediately after the culture report of the infant, yielded

ESBL-producing *E. coli* (isolates P354 and P355, 9th day after delivery). Cultures from vaginal and rectal swabs were both grown on selective MacConkey agar with 2 mg cefotaxime l⁻¹. MICs and species identification of all three *E. coli* isolates were determined using standard methods. ESBL production was confirmed by the double-disc synergy test (CLSI, 2005).

The three *E. coli* isolates were resistant to amoxicillin (MIC >16 mg l⁻¹), cefotaxime (MIC $\geq$ 8 mg l⁻¹), ceftazidime (MIC >16 mg l⁻¹) and gentamicin (MIC $\geq$ 8 mg l⁻¹) and showed synergy between clavulanic acid and cefotaxime/ceftazidime compatible with ESBL production. β -Lactamase characterization was carried out by isoelectric focusing of the sonicated extract (Matthew *et al.*, 1975) and by PCR of the *bla* genes with specific primers for the CTX-M-1 ESBL group (Oteo *et al.*, 2006). Amplicons were further sequenced for the final characterization of the *bla* gene. Strains were assigned to a phylogenetic group by a multiplex PCR method based on three DNA marker fragments (*chuA*, *yjaA* and TspE4.C2), as described elsewhere (Clermont *et al.*, 2000).

All three *E. coli* isolates demonstrated a closely related *Xba*I PFGE pattern (http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf) (Fig. 1) and were assigned to the A₁ phylogenetic group (*chuA*⁻, *yjaA*⁺, TspE4.C2⁻). These strains expressed a β -lactamase with a pI of 9.0, suggesting the presence of a CTX-M-1-type enzyme. Sequence analysis of the PCR products obtained identified β -lactamase CTX-M-32, a recently identified variant of the CTX-M-1 group (Cartelle *et al.*, 2004). The *bla*_{CTX-M-32} gene was successfully transferred to *E. coli* J53 Az^R (Romero *et al.*, 2004) from H344, P354 and P355.

Abbreviation: ESBL, extended-spectrum β -lactamase.

Fig. 1. PFGE patterns of the three *E. coli* isolates obtained with *Xba*I. Lanes: 1 and 2, unrelated *E. coli* isolates; 3, *E. coli* isolate H344; 4, *E. coli* isolate P354; 5, *E. coli* isolate P355.

*Eco*RI restriction patterns of the plasmids of the three isolates were extremely similar (Fig. 2).

Discussion

The present report, to our knowledge, provides the first evidence of ESBL-producing *E. coli* spreading from mother to child and causing neonatal sepsis. Neonatal sepsis by ESBL-producing *E. coli* isolates used to occur as nosocomial infections in intensive care units (Jain *et al.*, 2003). Recently, in our area, ESBL-producing *E. coli* isolates have tended to emerge as community infections (Romero *et al.*, 2005) and a significant number of community faecal carriers have been detected (Miró *et al.*, 2005). Our case is unique in indicating the possibility of vertical transmission of a drug-resistant strain that was probably community-acquired. This is suggested by the clonal relatedness between isolates and the ESBL enzyme type. At present, isolates expressing CTX-M-32 have barely been detected in our country and every case has been in a non-hospitalized patient (Oteo *et al.*, 2006). Similarly, this enzyme was not identified in our hospital until this case.

Vertical confirmed neonatal sepsis is generally defined in terms of a positive blood culture along with clinical signs and laboratory abnormalities consistent with infection

Fig. 2. Plasmid *Eco*RI profiles of the three *E. coli* isolates. Lanes: 1, 2 and 3, *E. coli* isolates H344, P354 and P355, respectively; 4, lambda DNA *Hind*III digest.

diagnosed within the first 3 days of life (López-Sastre *et al.*, 2005). Although, in the present report, the infection was diagnosed after 3 days, the criteria for vertical neonatal sepsis were met: risk factors for vertical transmission, a positive blood culture for traditional pathogens of vertical transmission, absence of similar cases at the unit and the same strain of *E. coli* isolated in both mother and her newborn. Mothers are educated to follow exhaustive contact precautions. Although we cannot completely discard the possibility that the strain was transmitted by direct contact between mother and child, we consider that vertical transmission was much more likely.

It has been suggested that, in the absence of evidence for group B streptococcus disease, clinicians should consider the possibility of ampicillin-resistant *E. coli* infection in critically ill newborn of women with a history of intrapartum fever and ampicillin treatment (Stoll *et al.*, 2005). The problem is made worse because of the increasing dissemination of ESBL-producing *E. coli* in our area and a high rate of co-resistance to aminoglycosides observed for these strains (Romero *et al.*, 2005).

The increasing prevalence of community-acquired ESBL-producing *E. coli* suggests that further epidemiological surveillance is required in order to monitor the aetiology and management of vertical transmission of neonatal sepsis when obstetric risk factors are present.

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