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Characterization of CTX-M-14 and CTX-M-15 Producing *Escherichia coli* Strains Causing Neonatal Sepsis

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Neonatal sepsis is a disease affecting newborns ≤1 month of age with clinical symptoms and positive blood cultures. The number of *Escherichia coli* strains causing neonatal sepsis resistant to the antibiotics used in the treatment is increasing. In this study, two *E. coli* strains causing sepsis in neonates of mothers infected with an *E. coli* strain harboring extended spectrum beta-lactamases were characterized. The $bla_{\text{CTX-M-15}}$ and the $bla_{\text{CTX-M-14}}$ genes were found in an *Inc*FIA and nontypeable transferable plasmids, respectively. In addition, these highly virulent strains belonged to ST705 and ST156 clonal groups, respectively. The presence of strains, which are highly virulent and resistant to ampicillin, gentamicin, and cephalosporins, makes a change in empirical treatment necessary as well as an increase in the surveillance of these infections.

Introduction

Bacterial infection is an important cause of morbidity and mortality in neonates despite the great improvements in intensive neonatal care and the use of extended spectrum antimicrobial agents. Neonatal sepsis is a disease affecting newborns ≤1 month of age with clinical symptoms and positive blood cultures. Neonatal sepsis can be subdivided into early-onset neonatal sepsis (EONS) that is caused by microorganisms acquired from the mother before or during birth and late-onset neonatal sepsis (LONS) when the infection is presented 4 days or more after birth, due mainly to the microorganisms of the environment.

Group B *Streptococcus* is considered to be the most common microorganism causing EONS, but there have been reports of an increase in the incidence of EONS by *Escherichia coli,* especially in premature or very low birth weight neonates.[3,26,27]

Newborns presenting sepsis are treated with ampicillin, gentamicin, or cephalosporins. However, the number of *E. coli* strains causing neonatal sepsis resistant to these antibiotics is increasing.[3,12,19,29] The emergence of extended spectrum beta-lactamases (ESBL)-producing *E. coli* in neonates[1,12,13,23,24] makes epidemiological surveillance for vertical transmission of neonatal sepsis necessary, being of special interest in the cases of newborns from mothers with obstetric risk factors.

The aim of this study was to characterize *E. coli* strains harboring ESBL causing neonatal sepsis.

Materials and Methods

Bacteria

Two *E. coli* strains causing EONS (*Ec*-EONS) and LONS (*Ec*-LONS) collected in 2008 from neonates whose mothers presented an intra-amniotic infection also caused by an *E. coli* ESBL producer were studied.

Antimicrobial susceptibility

Minimal inhibitory concentrations (MICs) were determined using the MicroScan-Negative MIC Panel Type 37 (NM37; Siemens). Results were interpreted following the CSLI guidelines.[9] The *E. coli* ATCC25922 strain was used as control.

Detection of resistance genes

We detected the following selected antimicrobial resistance genes: $bla_{\text{TEM1-like}}$ and $bla_{\text{OXA1-like}}$ in all ampicillin-resistant strains; *tet*A, *tet*B, *tet*C, *tet*D, and *tet*G in tetracycline-resistant strains; *dfr*A, *dfr*Ib, and *dfr*VII in trimethroprim-resistant strains; *sul*1, *sul*2, and *sul*3 in sulfadiazine (Sd)-resistant strains; *aac*(3)-IV and *aac*(3)-II in gentamicin-resistant strains; and *cat*A, *cml*A, and *flo*R in choramphenicol-resistant strains. Mutations in the quinolone resistance-determining region (QRDR) of the genes encoding the essential enzymes, DNA gyrase and topoisomerase IV, were determined by polymerase chain reaction (PCR) and sequencing.[10] The $bla_{\text{CTX-M}}$ genes and their link with

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the IS*Ecp*1 insertion sequence were analyzed by PCR using specific primers and sequencing of the resulting fragments.[5,11] The sequences obtained were compared with those registered in GenBank.

Gene location

Plasmid DNA was extracted from the *E. coli* isolates using the Kado and Liu and S1 method,[15] transferred onto membranes, and hybridized with probes for $bla_{\text{CTX-M}}$ genes as described.[10] Conjugation experiments were performed as reported,[10] using the kanamycin-resistant strain *E. coli* K7 759Lac- as recipient. The incompatibility group of the plasmids was determined using the PCR-based inc/rep typing method described by Carattoli *et al.*[6]

Epidemiological characterization

The phylogenetic group of the isolates was analyzed by a triplex-PCR.[8] MLST was analyzed by amplification of seven housekeeping genes (*adk*, *fum*C, *icd*, *pur*A, *gyr*B, *rec*A, and *mdh*) (www.web.mpiib-berlin.mpg.de/mlst). Virulence profiles were analyzed by PCR using gene-specific primers for 17 virulence genes.[25] The PCR conditions used have been described elsewhere.[14]

Results

The two cases presented in this study were independent episodes that happened over 10 years in our hospital, from 1998 to 2008. No outbreaks by *E. coli* harboring ESBLs were reported in the period under study. The *Ec*-EONS strain was collected from a newborn within the first 24 hr of life, and the *Ec*-LONS strain was isolated from a child 4 days after birth.

The *Ec*-EONS strain presented the $bla_{\text{CTX-M-15}}$ gene and the *Ec*-LONS strain presented the $bla_{\text{CTX-M-14}}$ gene. The $bla_{\text{CTX-M}}$ gene was linked with the IS*Ecp*1 insertion sequence in both cases. In addition to cephalosporin resistance, the *Ec*-EONS strain showed resistance to ciprofloxacin due to a mutation in the QRDR region of the *gyr*A gene (Asp87 to Lys) and two mutations in the QRDR region of the *par*C gene (Ser80 to Ile and Glu84 to Val), and gentamicin due to the presence of the *aac*(3)-II. The *Ec*-LONS strain presented resistance to chloramphenicol caused by the presence of the *cml*1 gene, ciprofloxacin due to a mutation in the QRDR region of the *gyr*A gene (Ser83 to Leu), tetracycline by the presence of the *tet*A gene, and trimethroprim-sulfamethoxazole encoded by the *dfr*A12 and *sul*2 genes, respectively.

Ec-EONS presented two transferable plasmids of about 400- and 145-kb. The *Ec*-LONS presented three plasmids of about 95-, 120-, and 205-kb (Fig. 1a, b), but only the plasmid of about 120-kb was present in the transconjugant isolates. Hybridization experiments demonstrated the presence of the $bla_{\text{CTX-M-14}}$ gene in the nontypeable plasmid of 120-kb (Fig. 1c), whereas the $bla_{\text{CTX-M-15}}$ gene was found in the plasmids of 145-kb belonging to the *Inc*FIA incompatibility group (Fig. 1d).

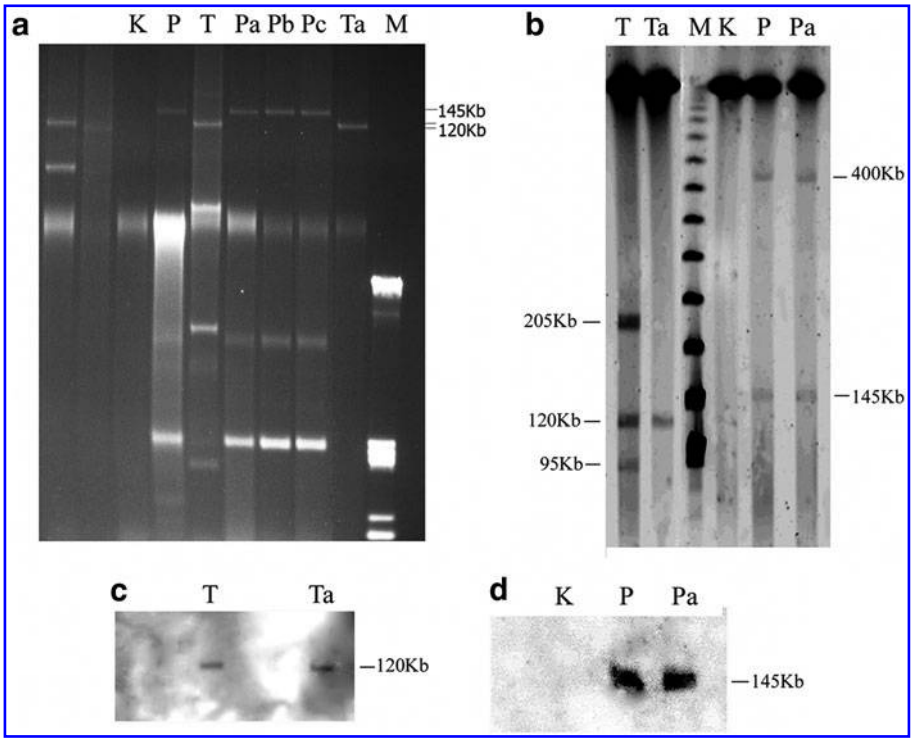

FIG. 1. Plasmid analysis of the *Escherichia coli* strains under study. **(a)** Plasmid profile obtained by the Kado-Liu protocol. **(b)** Plasmid profile obtained by S1 protocol. **(c)** Hybridization with the $bla_{\text{CTX-M-14}}$ gene probe. **(d)** Hybridization with the $bla_{\text{CTX-M-15}}$ gene probe. M in **a**, Lambda-DNA digested with PstI. M in **b**, Lambda ladder PFGE marker (New England Biolabs). K, kanamycin-resistant strain *E. coli* strain K12 J53 used as recipient. P, *Ec*-EONS wild-type strain; Pa to Pc, transconjugants obtained from *Ec*-EONS. T, *Ec*-LONS wild-type strain; Ta, transconjugant obtained from *Ec*-LONS. *Ec*-EONS, *E. coli* strains causing early-onset neonatal sepsis; *Ec*-LONS, *E. coli* strains causing late-onset neonatal sepsis.

TABLE 1. FEATURES OF THE *ESCHERICHIA COLI* HARBORING *BLA*$_{CTX-M}$ GENES

Strain	bla$_{CTX-M}$ *gene*	*MLST*	*Phylogenetic group*	*Virulence factors*
Ec-EONS	*bla*$_{CTX-M-15}$	ST705	B2	*hly*A, *sat*1, *pap*A, *pap*C, *pap*EF, *pap*GII, *pap*GIII, *prs*, *mal*X, *foc*G, *iha*, *fyu*A, *fim*A, *hra*, *iut*A, *iuc*C, *sfa*S.
Ec-LONS	*bla*$_{CTX-M-14}$	ST156	D	*hly*A, *sat*1, *pap*A, *pap*GII, *prs*, *mal*X, *foc*G, *fim*A, *iut*A, *iuc*C, *iro*N.

Ec-EONS, *E. coli* strains causing early-onset neonatal sepsis; *Ec*-LONS, *E. coli* strains causing late-onset neonatal sepsis.

A further characterization of the strains was carried out. The *Ec*-EONS strain belonged to the virulent phylogenetic group B2, ST705, and presented 14 virulence factors. On the other hand, the *Ec*-LONS was less virulent and belonged to the phylogenetic group D, ST156, and presented 10 virulence factors (Table 1).

Discussion

The increase of antimicrobial resistance among *E. coli* strains causing neonatal sepsis is becoming an important problem.[12] The percentage of strains resistant to ampicillin and gentamicin is on the rise, cephalosporins being the alternative treatment in these cases. However, the number of strains resistant to cephalosporins is also becoming an increasingly notable problem in developing countries.[1,21,24]

Several studies on the presence of ESBLs among neonates or in neonatal intensive care units (NICUs) in India have been reported, but only reporting percentages of ESBL-producing *E. coli*.[1,13,23] The percentages of neonatal sepsis caused by ESBL-producing *E. coli* were found to be between 52–65.3%.[1,7,13,23] Shakil *et al.,*[23] found *bla*$_{CTX-M-15}$ in five preterm neonates from NICU. All of these *E. coli* strains presented transferable plasmids. *E. coli* belonging to ST131 and harboring *bla*$_{CTX-M-15}$ was also associated with cases of neonatal meningitis.[22] In contrast, the strain studied in the present study belonged to ST705.

In developed countries, only a few cases of ESBL-producing *E. coli* have been reported in NICUs.[17,28] Boyer-Mariotte *et al.*[4] reported a case of fatal meningitis and sepsis in a newborn caused by an *E. coli* strain belonging to the phylogenetic group B2 and harboring *bla*$_{CTX-M-15}$. In contrast to our findings, in which the strains presented more than 10 virulence factors, this strain only presented aerobactin and yersiniabactin as virulence factors.

The CTX-M-14 enzyme has been reported worldwide, but has been substituted by CTX-M-15 in the last years.[2] An outbreak caused by a strain of *E. coli* presenting the CTX-M-14 enzyme has been reported in the literature.[20] The *bla*$_{CTX-M-14}$ gene was found in an *Inc*K plasmid of about 80-kb. This strain belonged to ST23 and phylogenetic group A. In addition, the *bla*$_{CTX-M-14}$ gene was linked to an IS*Ec*p1 insertion sequence. The strain characterized in the present study also presented the *bla*$_{CTX-M-14}$ gene, but was different to that described in the previously commented study in both clonal and phylogenetic groups. Other *E. coli* strains belonged to ST156, but carrying different ESBL genes were found in China and United Kingdom.[18,21]

The presence of antimicrobial-resistant strains in neonates makes it necessary to avoid empirical treatment in these cases. López-Cerero *et al.*[16] studied the case of a neonate whose mother presented premature membrane rupture and fever and was treated with ampicillin and gentamicin. The neonate was also treated with these two antimicrobial agents and his condition deteriorated due to the sepsis caused by a CTX-M-32-positive *E. coli* strain.

In summary, this is the first time that a CTX-M-14 producing *E. coli* strain belonging to ST156 and phylogenetic group D causing neonatal sepsis has been studied. The presence of strains that are highly virulent and resistant to ampicillin, gentamicin, and cephalosporins makes a change in empirical treatment necessary as well as an increase in the surveillance of these infections.

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