Molecular Epidemiology Unravels the Complexity of Neonatal *Escherichia coli* Acquisition in Twins

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Septicemia and meningitis are increasingly involved in the morbidity and mortality of newborns. Most cases of gram-negative bacillary bacteremia and meningitis in neonates are caused by *Escherichia coli* (17). Sarrf et al. have suggested that 70% of infants with meningitis due to *E. coli* K1 acquire the pathogen from their mothers either at the time of delivery or in the neonatal period (18). Molecular markers now provide improved tools for epidemiological and virulence studies. Here, we used the combined analysis of restriction fragment length polymorphism (RFLP) of regions of genes coding for rRNA (rDNA) (ribotyping) and esterase electrophoretic typing to document neonatal acquisition of *Escherichia coli* in twins. Our study shows vertical mother-to-infant transmission of one strain of *E. coli* to one twin and the development of neonatal septicemia with a distinct nonvirulent carboxylesterase type B, *E. coli* strain for the other twin.

**Case study.** A 30-year-old multiparous mother was admitted for premature rupture of the ovular membranes at 36 weeks of gestation of a twin pregnancy. Two hours later, the spontaneous onset of labor was followed by a rapid delivery. Cervical culture obtained immediately postpartum grew a pure culture of *E. coli*. The clinical course of the firstborn twin (twin 1), a 2,240-g female, was uneventful. Although cultures of a gastric aspirate were positive for *E. coli*, with more than 30 colonies, blood cultures were negative and twin 1 remained well without antimicrobial therapy. Soon after birth, the second twin (twin 2), a 2,050-g male, developed a mild respiratory distress syndrome requiring mechanical ventilation. Two initial blood cultures were negative for *E. coli*. Partial blood exchange transfusion with an umbilical vein catheter was soon performed in order to decrease the hematocrit from 69% to about 55%. The baby, who was polycythemic, became alert. The gastric aspirate also grew *E. coli*. However, on the fifth day of twin 2’s life, a severe septic shock occurred, with tachycardia, systemic hypotension, anuria, and metabolic acidosis. *E. coli* was found in four successive blood cultures, and 10⁷ CFU/ml were found on the umbilical vein catheter immediately after removal. Appropriate antimicrobial therapy (cefotaxime and amikacin) was immediately given, leading to negative blood cultures 24 h after the beginning of therapy and controlling the infection. Unfortunately, severe intracranial hemorrhage leading to brain death occurred, and the baby expired 5 days later.

A total of 11 *E. coli* isolates were selected for the study. Five isolates relating to the reported case were studied. One was recovered from the mother (cervical culture [TM1]), one was recovered from twin 1 (gastric aspirate [T1]) and three were recovered from twin 2 (gastric aspirate [T2], blood culture [T3], and umbilical vein catheter [T4]). For comparison, we also studied the type strain of the species, ATCC 11775, and five epidemiologically unrelated *E. coli* strains isolated from neonates hospitalized in the same unit during the 6 months which preceded the reported case. Identification of *E. coli* was based on colony morphology and standard biochemical tests included in the API 20E system (API, La Balmes-les Grottes, France). K1 antigen determinations were performed with antiserum to *Neisseria meningitidis* group B (5). Susceptibility testing was performed on Mueller-Hinton agar plates by the disk diffusion method (Diagnostics Pasteur, Marnes-La-Coquette, France).

Total *E. coli* DNA was prepared by the method previously described (16). DNA was digested with *Hind* III or EcoRI and subjected to Southern blotting analysis with 16+23S rRNA from *E. coli* as a probe (4), labeled by random oligonucleotide priming with a mixture of hexanucleotides (Pharmacia, Uppsala, Sweden) and cloned mouse mammary leukemia virus reverse transcriptase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in the presence of 0.35 mM digoxigenin-11-dUTP (Boehringer, Mannheim, Germany). Chemiluminescence detection procedures were as already reported (3). Esterase electrophoretic typing, including conditions for bacterial growth, preparation of extracts, horizontal polyacrylamide slab gel electrophoresis, estimation of electrophoretic mobility (Mₑ value), and esterase staining, was as described previously (8, 9).

Two *E. coli* strains from the mother and strain T1 from twin 1 exhibited identical biochemical patterns and susceptibility profiles, but their patterns were different from those observed for the strains isolated from twin 2 (Table 1). All isolates were negative for the K1 antigen. The four strains isolated from the four blood cultures from twin 2 were phenotypically indistinguishable. Thus, only one of them, T3, was genotypically characterized. EcoRI and *Hind* III gave eight and six different rDNA RFLP patterns, respectively, for the 11 studied strains, thus defining eight ribotypes (Fig. 1; Table 1). Esterase electrophoretic typing

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allowed the distinction of eight zymotypes among the strains (Table 1). The additional esterase type B1, corresponding to $M_F$ values of $=66$ to 74, was observed for all strains from twin 2, whereas the electrophoretic type B2 ($M_F$ values of $=57$ to 63) was observed for the strains from the mother and twin 1 (Fig. 2; Table 1). The two sets of molecular data were found to be correlated and allowed the determination of eight types among the studied strains, a type being defined as the zymotype-ribotype association. Strains from the mother and twin 1 shared the same type (zymotype 1 and ribotype A), which was different from that of the several strains obtained from twin 2 (zymotype 2 and ribotype B). The strains studied for comparison clearly exhibited types distinct from those of the clinically related strains (Table 1).

*E. coli* is the most common cause of gram-negative neonatal bacterial sepsis and meningitis (13). Vertical transmission from mother to infant seems to be the most common way of acquiring *E. coli* (18). If neonatal acquisition of *E. coli* is the result of contamination during delivery, the most likely contaminating site for that organism would be the cervix or vagina (1). Indeed, it is generally accepted that *E. coli* is present in the cervix of 3 to 20% of normal women (7). On the other hand, nosocomial acquisition of *E. coli* in neonates has also been reported (10, 11). Valuable epidemiologic markers are necessary for the understanding of the mode of acquisition in neonatal *E. coli* infection. Esterase electrophoretic typing (9) and ribotyping (2, 15) have already

### TABLE 1. Origins and phenotypic and genotypic characteristics of the 10 clinical *E. coli* strains studied and of the type strain of the species

<table>
<thead>
<tr>
<th>Patient</th>
<th>Strain</th>
<th>Origin</th>
<th>API profile</th>
<th>Antibiotic susceptibility</th>
<th>Esterase electrophoretic type</th>
<th>Carboxylesterase type</th>
<th>rDNA RFLP pattern</th>
<th>Ribotype</th>
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<tr>
<td>Twins' mother</td>
<td>TM1</td>
<td>Cervix</td>
<td>5144552</td>
<td>S</td>
<td>1</td>
<td>B2</td>
<td>EcoRI HindIII</td>
<td>A</td>
</tr>
<tr>
<td>Twin 1</td>
<td>T1</td>
<td>Gastric</td>
<td>5144552</td>
<td>S</td>
<td>1</td>
<td>B2</td>
<td>a</td>
<td>A</td>
</tr>
<tr>
<td>Twin 2</td>
<td>T2</td>
<td>Gastric</td>
<td>5044552</td>
<td>R</td>
<td>2</td>
<td>B1</td>
<td>b</td>
<td>B</td>
</tr>
<tr>
<td>Twin 2</td>
<td>T3</td>
<td>Blood</td>
<td>5044552</td>
<td>R</td>
<td>2</td>
<td>B1</td>
<td>b</td>
<td>B</td>
</tr>
<tr>
<td>Twin 2</td>
<td>T4</td>
<td>Catheter</td>
<td>5044552</td>
<td>R</td>
<td>2</td>
<td>B1</td>
<td>b</td>
<td>B</td>
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<td>ATCC 11775</td>
<td>159</td>
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* Susceptibility to ampicillin and co-trimoxazole. S, sensitive; R, resistant.

FIG. 1. *E. coli* rDNA RFLP patterns obtained after HindIII digestion. Lanes contain strains as follows: 1, ATCC 11775 (pattern c); 2, 159 (pattern c); 3, 161 (pattern d); 4, 164 (pattern e); 5, T2 (pattern b); 6, T3 (pattern b); 7, T4 (pattern b); 8, TM1 (pattern a); 9, T1 (pattern a); 10, 163 (pattern f); 11, 154 (pattern f). See Table 1 for origins of the strains.

FIG. 2. Esterase electrophoretic patterns of three epidemiologically related *E. coli* strains. Lanes: 1, strain T1; 2, strain TM1; 3, strain T4. B1, carboxylesterase B type B1 (fast moving); B2, carboxylesterase B type B2 (slowly moving). Plus sign and arrow indicate the cathode and the direction of migration, respectively. See Table 1 for origins of the strains.
been shown individually to be powerful and readily applicable epidemiological tools for *E. coli* typing. It was reported earlier that using at least two molecular typing systems improves epidemiologic analysis (6). We used the combination of esterase electrophoresis and RFLP analysis of the rDNA regions for the epidemiological evaluation of *E. coli* strains obtained from a mother and her twins. For this, we used a nonradioactive probing system which has several advantages over radioactive probes, including safety, easier disposal, and stability (3). Our results show a good correlation between the genotypic and phenotypic data (Table 1).

Concerning the mode of neonatal acquisition of *E. coli*, our molecular approach yields evidence for a vertical mother-to-infant transmission to twin 1 only. It shows that twin 2 had been colonized and then infected by a distinct strain of *E. coli* (T2). The fact that T2 was not present in the cervical culture of the mother can be interpreted in two ways. First, nosocomial acquisition of *E. coli* T2 could have occurred independently, but the finding of T2 in the gastric aspirate favors the hypothesis that it was maternally acquired. Thus, another possibility is that the two strains were indeed both present in the vagina of the mother but that T2 was not found in the cervical culture either because it was at a level below the sensitivity of the technique or because it was present at a location other than that of the sampling.

An other question raised by this case study relates to the virulence of strain T2 that was responsible for sepsis. Previous studies (12, 14) have established that *E. coli* strains showing the electrophoretic carboxylesterase type B2 are highly pathogenic, whereas strains of type B1 are responsible of bacteremia only in patients with an underlying disease or who are immunosuppressed. In our study, strains from the mother and twin 1 were of type B2, but twin 1 was only colonized and did not develop infection. In contrast, strain T2 was of type B1, and twin 2 died at 10 days of age. Thus, septicemia was more likely related to the use of an aggressive instrument, i.e., the umbilical vein catheter, on a weakened infant than to a particular virulence of the organism. This fact illustrates the importance of the patient’s characteristics and invasive devices in the pathogenesis of nonviral *E. coli* septicemia in neonates.

Our work shows the complexity of *E. coli* neonatal infection. The molecular approach that we have developed for strain typing provides precise markers that will be helpful in the understanding of neonatal *E. coli* acquisition and physiopathology of infections.

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REFERENCES