Genotyping May Provide Rapid Identification of *Escherichia coli* K1 Organisms That Cause Neonatal Meningitis

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*Escherichia coli* K1 is the most common cause of gram-negative neonatal bacterial meningitis and sepsis. In an attempt to identify genetic markers in *E. coli* K1 that are associated with the capacity of the organism to cause neonatal meningitis, we used rRNA gene restriction patterns. *E. coli* strains isolated from the CSF of neonates with meningitis (*n* = 43) on two continents were compared to strains isolated from the blood of neonates with bacteremia who did not have meningitis (*n* = 29) and to isolates from the vaginas of asymptomatic pregnant women whose neonates remained without infection (*n* = 39). *E. coli* strains from CSF are genetically less heterogeneous than isolates from blood and the vagina: 44.2% of the CSF isolates belonged to only two types, whereas no more than two blood vaginal strains were of the same type. After *Hind*III digestion, a 14.9-kb rDNA-containing fragment was found in 81.3% of the strains from CSF vs. 28.0% of the isolates from blood and only 12.8% of the vaginal isolates (*P* = .001). Thus, genotyping might provide markers to identify organisms in the maternal vaginal flora that are highly likely to cause neonatal meningitis. This observation may have very practical implications for the early identification of these organisms in pregnant women and thus for the selective establishment of preventive measures *per partum* or for the early treatment of colonized neonates.

Septicemia and meningitis play an increasing part in the morbidity and mortality of newborns. The incidence of neonatal sepsis and meningitis in newborn infants has been estimated to be somewhere between 1 and 5 per 1,000 live births [1]. *Escherichia coli* is the most common cause of gram-negative bacillary neonatal meningitis and sepsisemia [2]. Organisms bearing K1 capsular polysaccharide account for 80% of the *E. coli* involved in neonatal meningitis [2]. Despite antibiotic therapy, morbidity and mortality rates are high and few survivors function normally [3]. It has been estimated that up to 50% of the survivors sustain neurological sequelae and developmental disorders [4]. Thus, neonatal meningitis caused by *E. coli* remains a significant health problem. A few of the colonized infants are born to K1-negative mothers and presumably acquire *E. coli* K1 via horizontal transmission from nursery staff members or other infants. But the majority of the infants with meningitis acquire the pathogen from their mother, either at the time of delivery or during the neonatal period [5].

Previous studies have shown that neonatal sepsis and meningitis are associated with a limited number of clonal groups, in high contrast with the wide genetic heterogeneity of *E. coli* K1 strains isolated from the feces of children [6–9]. Thus, we hypothesized that genotyping with the currently available DNA analysis methods might provide markers to rapidly identify organisms in the maternal vaginal flora that might be highly likely to cause neonatal bacteremia and meningitis. To test this hypothesis, we used ribotyping to compare *E. coli* strains isolated in cases of neonatal meningitis, in cases of neonatal bacteremia, and from cultures of vaginal specimens from asymptomatic pregnant women whose neonates remained without infection.

**Materials and Methods**

*Bacteriology.* A total of 111 *E. coli* isolates were studied. Forty-three strains were recovered from the CSF of 43 neonates with meningitis in different countries on two continents. Of these 43 strains, 24 were isolated from 1990 through 1993 in different regions of France: Paris (14 strains, from three different hospitals), Lyon (1), Pontoise (2), Quimper (1), Nancy (4), Toulouse (1), and Aix-en-Provence (1). Five other strains were kindly provided by Prof. J. Hacker (Institut für Genetik un Mikrobiologie, Würzburg, Germany): strains IHE 3034 and IHE 3036 were isolated in Finland in 1977, strains A21 and RS176 in the United States in 1974, and strain A1521 in Germany in 1974 [10]. The last 14 CSF strains were kindly provided by Dr. R. Bortolussi (Children’s Hospital, Halifax, Nova Scotia, Canada), from North America [11]. For comparison, we studied 29 strains isolated from the blood of 29 neonates (age range, 1–21 days) who had bacteremia but not meningitis. All were born at the Robert Debré Hospital in Paris during the period 1989 through 1993. We also studied 39 *E. coli* K1 strains recovered in the 38th week of pregnancy in cultures of...
vaginal specimens from 39 asymptomatic pregnant women (age range, 17–34 years) attending the Robert Debré Hospital over the same period of time and whose newborns remained healthy. Finally, a reference strain of the species (ATCC 11775) was also included in the study.

Capsular typing. K1 antigen determinations were made with an antiserum to Neisseria meningitidis group B [12].

Ribotyping. Total E. coli DNA was prepared as previously described [13]. It was digested with HindIII or EcoRI and subjected to Southern blotting analysis with ribosomal 16 + 23S RNA from E. coli as a probe [13, 14]. The probe was labeled by random oligopriming with use of a mixture of hexanucleotides (Pharmacia, Uppsala, Sweden) and cloned M-MLV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) in the presence of 0.35-mM DIG-11-dUTP (digoxigenin-11-deoxyuridine-5’ triphosphate; Boehringer, Mannheim, Germany). The procedure for chemiluminescence detection was as previously reported [15]. Isolates showing two or more rDNA-containing fragment differences were considered different types [16].

Statistical analysis. DNA fragments that were differently distributed among the three groups of strains (CSF, blood, and vaginal isolates) were identified, and the statistical significance of this difference was tested by the χ² test.

Results

Capsular K1 antigen was found in 80% of E. coli isolates from blood and in 100% of those from CSF samples. Depending upon the strain, EcoRI and HindIII digestions generated 7 to 12 0.7–24-kb and 6 to 11 1.5–22.5-kb rDNA-containing fragments, respectively. For the 111 E. coli strains, EcoRI produced 29 fragmentation patterns and HindIII produced 42 patterns. EcoRI and HindIII fragments were combined to define a ribotype. Altogether, 75 different types were observed. Distribution of these types was unequal among the three groups of strains: 23 types were found for the 43 CSF strains, as compared with 25 types for the 29 blood isolates and 35 types for the 39 commensal isolates. Remarkably, 42% of the CSF strains belonged to two types only (11 and 8 strains, respectively). These two common ribotypes were found for strains isolated both in Europe (France, Germany, and Finland) and in North America. Two other types were found twice; in both cases the strains were isolated in the same institutions (Paris and North America, respectively). The remaining 47% of the CSF strains exhibited unique ribotypes. In contrast, for the two other groups of strains (blood and vaginal isolates), no types were shared by more than two strains. Among strains from blood, 72% exhibited unique ribotypes and four ribotypes were found twice. As for the vaginal strains, 79% belonged to unique ribotypes and four ribotypes were found twice.

We then conducted a strain-to-strain comparison based upon individual fragments, regardless of ribotype. A 3.6-kb EcoRI fragment was found in 69.7% of the CSF strains but in 43.3% of E. coli strains obtained from vaginal samples (P = .01) (figure 1). CSF strains harbor ing this fragment were obtained from Paris (12), Pontoise (2), Nancy (4), Lyon (1), Toulouse (1), Quimper (1), and Quimper (1), in France; Finland (2); Germany (1); and North America (6). This same fragment was found in 44.0% of E. coli strains obtained from blood. Two of the fragments described by Picard et al. [17] as being associated with highly virulent E. coli carboxylesterase B type B2 strains were found in our study. It is interesting that these 7.0-kb EcoRI and 5.5-kb HindIII fragments were found simultaneously in 62.7% of the isolates from CSF but in only 34.0% and 33.3% (P = .01) of the blood and vaginal isolates, respectively. However, the most striking results were obtained with regard to a 14.9-kb HindIII fragment. This fragment was present in 81.3% of the CSF strains but in only 12.8% of the vaginal strains (P = .001) (figure 2). CSF strains harboring this fragment were obtained from Paris (12), Pontoise (2), Nancy (4), Lyon (1), Toulouse (1), Quimper (1), and Aix-en-Provence (1) in France; Finland (2), Germany (1); and North America (10). The 14.9-kb HindIII fragment was part of the two common ribotypes observed for the CSF strains. Its frequency in strains obtained from blood (28.0%) was not statistically different from that in vaginal strains.

Discussion

Vertical transmission from mother to infant, most likely through the cervix or vagina during delivery, seems to be the most common means of acquisition of E. coli [2, 5]. E. coli K1 is present in the cervix of 5%–7% of pregnant women, and 70% of them convey E. coli to their neonates [18]. Among those infants, about 1% will have a neonatal infection. To date, there is no means of prevention because systematic eradication is unfeasible. Thus, identification of the women (among those carrying the organism) at risk of delivering infants with symptomatic E. coli K1 disease is of the utmost interest, because they could be the target of a prevention strategy.

O and H serotyping and multilocus enzyme electrophoresis (MEE) analysis might enable identification of E. coli virulent clones [19]. However, MEE is labor-intensive, and serotyping requires the availability of specific reagents. Because they also explore genetic diversity but at the DNA level, the newly developed genotyping techniques might provide the same type of information while being more appropriate for the routine microbiology laboratory.

In this work, as judged from rDNA restriction patterns, we found that E. coli strains from cases of neonatal meningitis are genetically less heterogeneous worldwide than strains isolated from blood and vaginal samples from a single hospital. Indeed, 44.2% of CSF strains belonged to only two types (both present on two continents), whereas no more than two blood or vaginal strains were of the same type. In addition, the frequency of some rDNA-containing restriction fragments was significantly increased in the group of CSF strains. These results are in
agreement with those obtained by MEE analysis and strongly suggest that neonatal meningitis is caused by a restricted number of highly virulent clones or groups of strains sharing common genetic determinants. This conclusion is strengthened by the fact that, on the contrary, strains isolated from the blood of neonates <1 month of age were as heterogeneous as the commensal strains. Indeed, these strains do not require specific pathogenic potential to cause bacteremia in naturally immunologically immature neonates [20].

Markers derived from studies such as ours might lead the way by genetic linkage to the identification of virulence factors in \textit{E. coli}. But they also have very practical implications. The

\textbf{Figure 1.} Representative rDNA restriction fragment length polymorphism patterns obtained for nine clinical strains of \textit{E. coli} and the type strain of the species after digestion by \textit{EcoRI}. Lane 1: strain ATCC 11775; lanes 2–9: patterns representative of eight different profiles obtained with \textit{E. coli} CSF strains; lane 10: pattern obtained with one commensal strain. The arrow indicates the 3.6-kb fragment, which can be seen in lanes 1, 2, 3, 4, 6, 8, and 9.

\textbf{Figure 2.} Representative rDNA restriction fragment length polymorphism patterns obtained for 10 clinical strains of \textit{E. coli} and the type strain of the species after digestion by \textit{HindIII}. Lane 1: strain ATCC 11775; lanes 2–9: patterns representative of eight different profiles obtained with \textit{E. coli} CSF strains; lanes 10 and 11: patterns obtained with two commensal strains. The arrow indicates the 14.9-kb fragment, which can be seen in lanes 1, 2, 3, 4, 6, 8, and 9.
presence of the 14.9-kb HindIII fragment was a significant feature of CSF E. coli K1 strains, as compared with commensal E. coli K1 strains. This raises the possibility that children of mothers who carry this type of strain may be at increased risk of meningitis. If this is true, then the prior identification of these mothers could lead to effective prevention strategies— including eradication per partum, in an attempt to prevent mother-to-infant transmission—and/or early treatment of their neonates. It is feasible to screen the vaginal flora and colonized neonates for strains of E. coli that have K1 antigen. Such a routine screening has been performed for more than 6 years in our hospital (at which there are 2,500 deliveries/year), and an average of 130 mothers/year are found to carry E. coli K1. Thus, 2–3 strains per week would have to be tested by ribotyping for the presence of the 14.9-kb HindIII fragment. Availability of nonradioactive probe labelling kits from commercial sources has now made ribotyping relatively easy to implement in the clinical laboratory, and thus we believe that such an approach is feasible.

Over the past few years, DNA analysis techniques developed at research laboratories and increasingly used on a routine basis have become powerful tools in human genetics and microbiology. In the latter field, they have shown their usefulness for epidemiological investigations and, mostly since the advent of PCR, for diagnostic purposes [14, 21, 22]. In human genetics, markers linked to the gene involved in a given disease are used for diagnosis long before the gene itself is identified. Because they are indirect markers and thus subject to an absence/loss of linkage in some individuals, they do not allow a positive diagnosis but an estimation of the risk. The situation we describe here is very similar, and, to our knowledge, this potentiality for genetic markers in infectious diseases has not been addressed yet. Our results illustrate the fact that developing such genetic markers is indeed feasible, even when the nature of virulence is unknown. The 14.9-kb HindIII fragment was also present in 12.8% of the commensal strains; however, one should keep in mind that regardless of the approach one uses, the identification of markers associated with virulence will undoubtedly be obscured by variability of the host’s susceptibility or response to the pathogen.

In E. coli, ribotyping provides results similar to those of MEE [23]. However, it might not be the best or the most suitable method in the future. The 14.9-kb HindIII fragment was found in 81.3% of the CSF strains. It might be that in some strains, mutational events have altered the suggested genetic linkage between this specific polymorphism and a putative virulence factor, but it might also be that ribotyping cannot generate more specific markers. It is possible that pulse-field gel electrophoresis or random PCR will be more sensitive and more powerful methods to generate such markers. For routine use in laboratories, PCR-based techniques would have the advantage of rapidity and of the fact that only minute quantities of DNA are necessary and not in a purified form. Prospective studies are needed (1) to appreciate the ultimate value of this type of genetic marker for evaluating risk, (2) to determine if identification of a strain with markers of risk could lead to effective preventive measures that would indeed reduce morbidity and mortality rates, and (3) to evaluate the cost-benefit of such analysis.

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References


