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Virulence Patterns of *Escherichia coli* K1 Strains Associated with Neonatal Meningitis

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The prevalence of the *ibe10* gene, of the *pap*, *afa*, and *sfa* adhesin-encoding operons, and of a 14.9-kb *rrn*-containing *Hin*dIII fragment was studied for 67 *Escherichia coli* neonatal meningitis strains, 58 *E. coli* K1 commensal strains, and 47 *E. coli* blood isolates from neonates without meningitis. *ibe10*, *sfa*, and the 14.9-kb *Hin*dIII fragment were observed significantly more often in the meningitis strains than in blood or commensal strains.

Escherichia coli is the most common cause of gram-negative neonatal meningitis (NBM) (7). Most of the *E. coli* pathogens causing NBM carry known virulence factors, such as the capsule antigen K1 (8) and the S fimbria adhesins (8, 11, 12). S fimbriae promote efficient adherence of the bacterium to epithelial cells lining the choroid plexus and brain ventricles, and also to vascular endothelium in the brain (13). Recently, an 8.2-kDa protein (Ibe10) was shown to be associated with *E. coli* K1 invasion of brain microvascular endothelial cells (6).

We have studied the prevalence of the PCR-detected *ibe10* gene and *sfa* adhesin-encoding operon, as well as that of a 14.9-kb *rrn*-containing *Hin*dIII fragment, previously shown to be associated with NBM (1), in 67 *E. coli* strains isolated from the cerebrospinal fluid (CSF) of newborns with meningitis. The data were compared with those obtained for 58 commensal *E. coli* K1 strains from healthy neonates and 47 *E. coli* strains isolated from the blood of 47 neonates who had bacteremia but not meningitis. For the purpose of comparison, the prevalence of the *pap* (pyelonephritis-associated pili) operon and the *afa* (afimbrial adhesin) gene was also studied.

Bacterial strains. A total of 172 *E. coli* strains were tested. Sixty-seven strains were recovered from the CSF of 67 neonates (age range, 1 to 28 days) with meningitis on three continents. Among them, 42 strains were isolated from different regions of France. Five CSF strains were provided by J. Hacker (Würzburg, Germany) (11), and 16 CSF strains were isolated in North America by R. Bortolussi (Halifax, Nova Scotia, Canada) (4). Four CSF strains were isolates from Morocco (M. Benbachir, Casablanca). Forty-three of these strains had been previously studied by ribotyping (1). For comparison, we studied 47 *E. coli* strains consecutively isolated from the blood of 47 neonates with bacteremia but not meningitis. We also studied 58 selected *E. coli* K1 isolates from the feces of 58 healthy neonates (age range, 1 to 7 days). All were born at the Robert Debré hospital in Paris.

Capsular typing. Capsular typing was performed with an antiserum to *Neisseria meningitidis* group B polysaccharide (5).

Detection of the 14.9-kb *rrn*-containing *Hin*dIII fragment. The 14.9-kb *rnn*-containing *Hin*dIII fragment was detected by a classical ribotyping analysis by Southern blotting with 16S-23S rRNA from *E. coli* as a probe (2, 3).

Detection of the *pap*, *afa*, and *sfa* operons and of the *ibe10* gene. PCR with the primers and the amplification procedure described by Le Bouguenec et al. (10) was used to detect the *pap*, *afa*, and *sfa* operons. Detection of the *sfa* operon was performed with oligonucleotides derived from the sequences of the 0.8-kb *Pst*I internal fragment of the *sfaD* and *sfaE* genes. Detection of the *ibe10* gene was performed with the sense primer 5′ TTACCGCCGTTGATGTTATCA 3′ and the antisense primer 5′ TTACCGCCGTTGATGTTATCA 3′ at a temperature of 60°C for annealing in a standard amplification protocol that generated a 171-bp fragment. The positive control was DNA from strain C5 in which the *ibe10* gene had been detected earlier by Southern blotting (6).

Statistical analysis. Existence of a difference in the distribution of the studied determinants among the different groups of strains was tested by the χ^2 test. A P value below 0.05 was considered to indicate statistical significance.

The presence or absence of the studied determinants for the 172 CSF, blood, and commensal strains is given in Table 1. Capsular K1 antigen was found in 70% of the isolates from blood and in 83% of those from CSF. No difference in the prevalence of the *pap* or the *afa* operon was observed among the three groups of strains. The *sfa* operon and *ibe10* were observed more often in the meningitis strains than in blood or in commensal strains. Still, the most striking difference was observed for the 14.9-kb *Hin*dIII fragment. No difference among strains could be observed regarding country or year of isolation.

To be successful as a meningeal pathogen, an *E. coli* strain must possess traits that allow the organism to (i) colonize the host mucosal surfaces, (ii) translocate from mucosal epithelial surfaces into the bloodstream, (iii) avoid normal host defense mechanisms, thus surviving in the bloodstream, (iv) cross the blood-brain barrier, and (v) survive in CSF (14). In some cases, however, strains that do not possess all these pathogenic properties may still be successful as pathogens because of a specific susceptibility of the host to infection.

K1-encapsulated *E. coli* accounts for approximately 80% of *E. coli* meningeal disease (15). The virulence of *E. coli* K1 is related to the ability of the K1 capsule to inhibit phagocytosis and to resist antibody-independent serum bactericidal activity.

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TABLE 1. Prevalence of K1 antigen, the *pap*, *sfa*, and *afa* operons, *ibe10*, and the 14.9-kb *Hin*dIII *rrn*-containing fragment in *E. coli* strains associated with meningitis, bacteremia, and feces

Origin of isolates (*n*)	% of isolates with:						
	K1 antigen	*pap*	*sfa*	*afa*	*ibe10*	14.9-kb *Hin*dIII fragment	At least 1 virulence determinant[a]
Feces (58)	100[b]	46	12[c]	3.5	3.5[d]	19[d]	100[b]
Blood (47)	70	51	30	6	17	32	70
CSF (67)	83	50	44[e]	3	32[f]	73[g]	94

[a] Virulence determinants were the K1 antigen, the 14.9-kb *Hin*dIII fragment, the *sfa* operon, and the *ibe10* gene.
[b] Only K1-positive strains were selected for the study.
[c] $P < 0.01$ (compared with CSF strains).
[d] $P < 0.001$ (compared with CSF strains).
[e] Not significant (compared with blood strains).
[f] $P < 0.01$ (compared with blood strains).
[g] $P < 0.001$ (compared with blood strains).

The prevalence of the capsular K1 antigen found in our CSF isolates is in good agreement with the data in the literature.

To our knowledge, the distribution of other pathogenic determinants in large series of clinical isolates from the CSF of neonates of different origins has not been studied before. We found no correlation between the presence of the K1 antigen and that of the other determinants studied. For example, the frequency of occurrence of the *sfa* operon was significantly higher in the blood and CSF isolates than in the feces isolates, even though the latter were all K1 positive. S fimbria binding to epithelial cells of the choroid plexus and brain ventricles has been suggested to promote trapping of plasminogen and tissue-type plasminogen activator in the extracellular space (9). This may enhance the local formation of plasmin, which in turn would promote bacterial penetration into the tissues (9). In addition, S fimbria-mediated binding decreases after the neonatal period, paralleling the decrease in susceptibility to *E. coli* meningitis (14). However, even though the prevalence of *sfa* is higher in CSF than in blood isolates, the difference does not reach statistical significance. Our results can be compared to those previously reported for two short series of older children with meningitis in Finland. Korhonen et al. (8) report a frequency of 36% *sfa*-positive strains in 14 children (age, <6 months), and Siitonen et al. (16) report a frequency of 23% in 7 children (age range, 7 days to 23 months).

In contrast, the increased prevalence of *ibe10* in CSF strains compared to fecal and blood isolates is statistically significant. We confirm on a larger series our previous observation that the 14.9-kb *Hin*dIII fragment is the most discriminative marker reported to date with regard to *E. coli* commensal, blood, and meningitis isolates (1).

These results show that, in addition to K1, other known virulence determinants, such as *ibe10* (and probably *sfa*), together with as yet unidentified determinants that may be linked to the 14.9-kb *Hin*dIII fragment, are associated with neonatal meningitis strains. Experimental studies are required to determine if specific combinations of these determinants are associated with pathogenicity.

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