JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2008, p. 1123–1125 Vol. 46, No. 3
0095-1137/08/$08.00+0 doi:10.1128/JCM.01682-07

Comparative Study of Virulence Traits of *Escherichia coli* Clinical Isolates Causing Early and Late Neonatal Sepsis[▽]

S. M. Soto, J. Bosch, M. T. Jimenez de Anta, and J. Vila*

Microbiology Department, Hospital Clinic, IDIBAPS, School of Medicine, University of Barcelona, Barcelona, Spain

Received 23 August 2007/Returned for modification 1 October 2007/Accepted 17 December 2007

Neonatal meningitis and septicemia caused by *Escherichia coli* are still major health problems in industrialized countries. Forty-seven *E. coli* strains causing neonatal sepsis were analyzed. Twenty-two and 25 strains caused early (detected from 0 to 3 days after birth) and late (detected from 4 to 28 days after birth) infections, respectively. Only the *ibeA* gene was significantly more prevalent in the strains causing early infections.

Neonatal risk factors for invasive bacterial disease and their diagnosis and therapy remain an important problem for obstetricians and pediatricians. Early-onset neonatal sepsis is caused by microorganisms acquired from the mother before or during birth (vertically transmitted and perinatally acquired); thus, microorganisms from the maternal genital tract may play an important role in early infection (6). Vaginal colonization by pathogenic microorganisms, observed in 3 to 20% of pregnant women, seems to be an important step in neonatal infection. Infections presenting 4 or more days after birth are considered late-onset infections and are generally caused by microorganisms acquired from the environment (nosocomial and horizontally transmitted) rather than from the mother. In late-onset neonatal infections, a low proportion of neonates are infected at the time of birth; others are born prematurely and/or have a very low weight (9). This may be due to the presence of only a low infectious dose of *Escherichia coli* strains that can complicate pregnancy or cause neonatal infection. For neonatal infection to take place, *E. coli* strains have to adapt to various ecological media; i.e., (i) the physicochemical conditions of the vaginal cavity are very different from those of the intestinal tract, (ii) the bacteria have to cross the endocervix and survive in the amniotic fluid, and (iii) the bacteria may be subjected to strong selection pressure by these conditions before generating neonatal septicemia (11).

Neonatal sepsis is associated with a limited number of *E. coli* phylogenetic groups, and the pathogenicity of these strains is correlated with the presence of several virulence factors (2).

The aim of this study was to perform a comparative analysis of the prevalence of virulence factors in *E. coli* clinical isolates causing early versus late neonatal sepsis.

A total of 47 *E. coli* strains causing septicemia were collected from neonates between 0 and 28 days old in the Hospital Clinic of Barcelona from 1987 to 2006. Sepsis was considered early when detected between 0 and 3 days after birth and was considered late when detected between 4 and 28 days after birth. Thus, 22 and 25 strains caused early and late infections, respectively. Clinical data are compiled in Table 1.

The *E. coli* phylogenetic group was determined by multiplex PCR as previously described (3). The presence of hemolysin (*hlyA* gene), cytotoxic necrotizing factor 1 (*cnf1* gene), toxin autotransporter (*sat1* gene), P fimbriae (*papA*, *-C*, *-G*, and *-EF* genes, *prs*), type 1 fimbriae (*fimA* gene), F1C fimbriae (*focG* gene), S fimbriae (*sfaS* gene), yersiniabactin (*fyuA* gene), aerobactins (*aer* and *iucC* genes), siderophore receptor (*iroN* gene), a putative adhesion siderophore (*iha* gene), the pathogenicity island marker (*malX* gene), invasion factor (*ibeA* gene), and heat-resistant agglutinin (*hra* gene) was determined by PCR. The PCR conditions used have been described elsewhere (5). Type 1 fimbria expression was tested by agglutination with a *Saccharomyces cerevisiae* strain (7). Pulsed-field gel electrophoresis was performed to examine the genetic relationship of the isolates. The profiles generated showed that all of them belonged to different clones (data not shown), according to the criteria of Tenover et al. (10). The differences in the presence of virulence genes were analyzed by the chi-square test.

The phylogenetic group of the 47 *E. coli* strains causing neonatal sepsis was analyzed. Five isolates were associated with meningitis episodes (three were associated with early sepsis, and two were associated with late sepsis) (Table 1), and two strains causing late sepsis were also isolated from a catheter and from peritoneal fluid. These two isolates were excluded from the virulence analysis. Finally, 10 *E. coli* strains from late sepsis also caused urinary tract infections whereas the remaining 21 isolates did not have a focus of infection and were therefore considered as probably translocated from the gut. No identical isolates were obtained from different samples from the same patient.

Twenty-four (51%), 15 (32%), 5 (11%), and 3 (6%) strains belonged to phylogenetic groups B2, D, A, and B1, respectively, with no statistically significant differences between the early and late sepsis groups. The number of virulence factors presented among the strains ranged from 0 to 13, with the factors most frequently shown by the *E. coli* strains being *fimA* (91%), *ibeA* (68%), *iucC* (60%), *papEF* (57%), and *papC* (47%). The virulence factors found least frequently were *cnf1* and *sat1* (both 2%) (Table 2).

When the strains were subgrouped into those causing early neonatal sepsis and those causing late neonatal sepsis (except for the isolates collected from peritoneal liquid and a catheter)

* Corresponding author. Mailing address: Department of Microbiology, Hospital Clinic of Barcelona, Villarroel 170, Barcelona 08036, Spain. Phone: 34-93-227-54-00. Fax: 34-93-227-93-22. E-mail: jvila@ub.edu.

▽ Published ahead of print on 26 December 2007.

TABLE 1. Features of the neonates with early or late sepsis

Time of infection (no. of neonates)	Age (days)	No. (%) CSF[a] positive	No. (%) urine culture positive	No. (%) who died	Total no. (%) not born prematurely	No. (%) born prematurely[b] who weighed:	
						<1,500 g	1,500–2,500 g
Early (22)	0–3	3 (14)	0	5 (23)	6 (27)	8 (36)	8 (36)
Late (25)	4–22	2 (8)	10 (40)	6 (24)	14 (56)	7 (28)	4 (16)

[a] CSF, cerebrospinal fluid.
[b] Less than 37 weeks of gestation.

and their virulence background was studied, no statistically significant differences were observed between the two groups, with the exception of the *ibeA* gene, which was more prevalent in the strains involved in early infection than in those involved in late infection (86% versus 52%, respectively; $P = 0.01$), and the *papGIII* allele, which was more prevalent in the strains involved in late infection than in those involved in early infection (28% versus 5%, respectively; $P = 0.03$). Hemolysin was also more frequent in the strains causing early infection (41% versus 20%, $P = 0.11$). Most of the isolates causing late infection also caused urinary tract infections. No significant differences in the presence of the other virulence factors were found (Table 2).

This study analyzed the virulence traits of *E. coli* strains causing neonatal sepsis collected in our hospital from neonates ranging from 0 to 28 days of age and compared the virulence genes present in the strains causing early- versus late-onset infection. Several studies have been performed concerning the virulence of *E. coli* strains causing neonatal sepsis. Bingen et al. (1) analyzed the virulence characteristics of 47 *E. coli* K1 strains collected from the blood of neonates with meningitis. The *ibeA* gene was present in 17% of the strains, which varies greatly from the percentage obtained in the present study (68%). The percentages of *sfa* and *pap* genes were similar to those found in the present study (30% versus 20% and 51% versus 44%, respectively). Watt et al. (11) also studied the virulence characteristics of 17 strains causing septicemia without meningitis in neonates and found that the 17 strains belonged to the B2 phylogenetic group. Nonetheless, the rate of virulence factors was very different from that obtained in the present study. They found a higher percentage of the *hly* and *sfa*/*foc* genes (62% versus 30% and 59% versus 21%, respectively) and a similar percentage of the *iucC* gene. On the other hand, they found a lower presence of P fimbriae and the *ibeA* gene (0% and 18%, respectively). However, Korhonen et al. (8) found that S fimbriae were associated with strains causing meningitis. To date, no studies have been performed on the differences between early and late neonatal sepsis. The higher frequency of the *ibeA* gene in the strains causing early infection is worthy of mention. The *ibeA* gene encodes a protein with 456 amino acids and a calculated molecular mass of 50 kDa. This protein mediates *E. coli* invasion of human endothelial cells and has been located in a genetic island named GimA (20.3 kb). This island contributes to *E. coli* invasion of the blood-brain barrier through a carbon-regulated process (4). Although the role of this protein in the invasion of epithelial cells has not been demonstrated, this protein may also be involved in the invasion of the cells of the chorioamniotic membrane, allowing the bacteria to cross this membrane and to reach the amniotic fluid with the consequent potential infection of the fetus.

TABLE 2. Virulence characteristics of *E. coli* strains causing neonatal sepsis

Strain feature	No. (%) of strains that caused:		*P* value
	Early infection ($n = 22$)	Late infection ($n = 25$)	
Phylogenetic group A	2 (9)	3 (12)	0.56
Phylogenetic group B1	1 (5)	2 (8)	0.54
Phylogenetic group B2	13 (59)	11 (44)	0.30
Phylogenetic group D	6 (27)	9 (36)	0.52
hlyA	9 (41)	5 (20)	0.11
cnf1	0 (0)	1 (4)	0.53
sat1	1 (5)	0 (0)	0.46
papA	9 (41)	12 (48)	0.62
papC	10 (45)	12 (48)	0.86
papEF	14 (63)	13 (52)	0.42
papGII	9 (41)	8 (32)	0.52
papGIII	1 (5)	7 (28)	0.03
prs	2 (9)	4 (16)	0.39
fimA	20 (91)	23 (92)	0.89
Type 1 fimbria expression	8 (36)	11 (44)	0.59
foc	6 (27)	4 (16)	0.27
sfa	2 (9)	7 (28)	0.1
fyuA	6 (27)	8 (32)	0.72
aer	8 (36)	14 (56)	0.17
iucC	13 (59)	15 (60)	0.94
iroN	10 (45)	7 (28)	0.21
iha	3 (14)	3 (12)	0.60
malX	1 (5)	5 (20)	0.12
ibeA	19 (86)	13 (52)	0.01
hra	12 (55)	9 (36)	0.20

In conclusion, there were no statistically significant differences in the content of virulence genes among the *E. coli* isolates in the two groups studied, with the exception of *ibeA*, which was more prevalent in the strains causing early infection.

This material is based upon work supported by grants FIS 05/0068 and FIS 05/0097 from the Ministry of Health, Spain, and 2005 SGR00444 from the Department d'Universitats, Recerca I Societat de la Informació de la Generalitat de Catalunya, Spain (to J.V.). Sara M. Soto is an investigator of the Sistema Nacional de Salud and belongs to the Fondo de Investigación Sanitaria of the Spanish Health and Consumption Ministry (CP05/00140).

REFERENCES

1. **Bingen, E., S. Bonacorsi, N. Brahimi, E. Denamur, and J. Elion.** 1997. Virulence patterns of *Escherichia coli* K1 strains associated with neonatal meningitis. J. Clin. Microbiol. **35:**2981–2982.
2. **Bingen, E., B. Picard, N. Brahimi, S. Mathy, P. Desjardin, J. Elion, and E. Denamur.** 1998. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J. Infect. Dis. **177:**642–650.
3. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.

4. **Huang, S., Y. Chen, G. Kong, S. H. M. Chen, J. Besemer, M. Borodovsky, and A. Jong.** 2001. A novel genetic island of meningitic *Escherichia coli* K1 containing the *ibe*A invasion gene (GimA): functional annotation and carbon-source-regulated invasion of human brain microvascular endothelial cells. Funct. Integr. Genomics **1:**312–322.
5. **Johnson, J. R., and A. Stell.** 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J. Infect. Dis. **181:**261–272.
6. **Kerur, B. M., B. Vishnu Bhat, B. N. Harish, S. Habeebullah, and C. Uday Kumar.** 2006. Maternal genital bacteria and surface colonization in early neonatal sepsis. Indian J. Pediatr. **73:**29–32.
7. **Korhonen, T. K.** 1979. Yeast cell agglutination by purified enterobacterial pili. FEMS Microbiol. Lett. **6:**421–427.
8. **Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Väisänen-Rhen, J. Finne, F. Ørskov, I. Ørskov, S. B. Svenson, and P. H. Mäkelä.** 1985. Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. **48:**486–491.
9. **Krohn, M. A., S. S. Thwin, L. K. Rabe, Z. Brown, and S. L. Hillier.** 1997. Vaginal colonization by *Escherichia coli* as a risk factor for very low birth weight delivery and other perinatal complications. J. Infect. Dis. **175:**3606–3610.
10. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
11. **Watt, S., P. Lanotte, L. Mereguetti, M. Moulin-Schouleur, B. Picard, and R. Quentin.** 2003. *Escherichia coli* strains from pregnant women and neonates: intraspecies genetic distribution and prevalence of virulence factors. J. Clin. Microbiol. **41:**1929–1935.