Host factors *versus* virulence-associated bacterial characteristics in neonatal and infantile bacteraemia and meningitis caused by *Escherichia coli*

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Summary. Possession of P fimbriae, virulence-associated O and K antigens, haemolysin and aerobactin production, and susceptibility to 10 antimicrobial agents were studied in 63 *Escherichia coli* strains isolated from blood or CSF of infants who were grouped according to their clinical characteristics. These isolates were compared with 35 faecal *E. coli* strains from healthy infants. Individual virulence factors showed a relatively weak association with invasive infection except for P fimbriae in urosepsis and aerobactin production in meningitis. Combinations of factors were generally more predictive for defining virulent clones, particularly in infants defined as being at normal risk of developing septicaemia. Thus, 62% of isolates from such infants had characteristics typical of previously described uropathogenic or meningitis-associated clones of *E. coli*, compared with 32% of the isolates from high-risk infants (i.e., those defined as being at high risk of developing septicaemia) and only 9% of the faecal isolates (p<0.001 and <0.05, respectively). Overall, 45% of the episodes of invasive infection were caused by such clones, whereas risk factors (conditions considered to be associated with increased risk of invasive infection) were present in 59% of the infected infants (39% in meningitis and urosepsis, 78% in cryptogenic septicaemia and untreated bacteraemia). The results indicated that bacterial factors played a significant causative role in neonatal meningitis and urosepsis, particularly in normal-risk infants, whereas predisposing host factors contributed greatly to cryptogenic septicaemia and untreated bacteraemia.

Introduction

Invasive bacterial infection is an important problem in infants, particularly in neonates, and *Escherichia coli* continues to be one of the dominating pathogens in septicaemia and meningitis during the first year of life. Invasive bacterial infection is a consequence of a disturbed balance between the host defences and the colonising micro-organism. Infants most at risk from invasive bacterial infection, i.e., high-risk infants such as prematurely born babies, are not fully immunocompetent and are often exposed to invasive procedures in the hospital. Thus, they may become infected with bacteria of lower virulence than those which are able to attack healthy children.

During the last decade our knowledge of the bacterial characteristics associated with virulence in *E. coli* has greatly increased. P fimbriae, certain O and K antigens, haemolysin and aerobactin production are typical of *E. coli* strains causing pyelonephritis and bacteraemia, and such strains appear to belong to a limited number of widespread *E. coli* clones. Clones causing meningitis seem to differ somewhat from those causing pyelonephritis.

In previous studies of *E. coli* virulence in neonatal septicaemia and meningitis, the isolates have been examined without relating bacterial characteristics to the clinical status of the infected infants. In the present study, the virulence characteristics of invasive isolates of *E. coli* from infants were compared with those of isolates from the faecal flora of healthy infants. The presence of the virulence characteristics in the infecting strains was also assessed in relation to clinical data of the babies.

Materials and methods

Patients and isolates

Of the 33 bacteriological laboratories in Sweden, 26 contributed recent *E. coli* isolates from the blood or CSF of infants. Clinical data were obtained from
hospital records. The 63 infants studied were assigned to one of four diagnostic categories: meningitis (organism in CSF), urosepsis (same organism in urine and blood), crypto- genic septicaemia (without a known focus of the invasive infection), and untreated bacteraemia (a child who had been regarded, by the paediatrician in charge of the child, as not having septicaemia and therefore left untreated) (table I).

According to clinical criteria, the infants were grouped by one of us (KT) as infants either with high risk for developing septicaemia or with normal risk. The conditions considered to be associated with increased risk of invasive infection were prematurity (18), hyaline membrane disease (10), severe asphyxia (4), premature rupture of the amniotic membranes with intra-uterine infection (4), small for gestational age (3), myelomeningocele (3), vesico-ureteric reflux grade III or more (2), Down's syndrome (2), leukaemia, exchange transfusion, incarcerated hernia, posterior urethral valve, Hirschsprung's disease, necrotising enterocolitis and a meningeal fistula (one case each). Many infants had several of these risk factors.

Faecal isolates of *E. coli* were obtained from healthy infants aged 0–18 months and without a history of septicaemia or urinary tract infection.

**Bacteriological methods**

O serogroups were determined by bacterial agglutination with antisera to 69 O antigens. As previously postulated, the O groups 1, 2, 4, 6, 7, 16, 18 and 75 were considered as "virulent" O groups. K antigens were typed by a modification of the serum agar technique in which 22 different antisera to capsular acidic polysaccharide K antigens were used. The K5 antigen was identified with a K5-specific phage. Isolates with K antigens 1, 2, 3, 5, 12, 13 were regarded as "virulent".

Expression of P fimbriae was tested, after cultivation of isolates on colonisation factor antigen (CFA) agar, with the P-fimbriae-specific latex particle agglutination test (PPA-test). Haemolysin production was determined after incubation overnight on blood agar plates containing Blood Agar Base No. 2 (Oxoid) and thrice-washed sheep red blood cells at a final concentration of 5% v/v. The production of aerobactin was determined with the test *E. coli* strain LG 1522.

The strains were defined as uropathogenic if they expressed P fimbriae and haemolysin and shared O serogroup and K antigen with eight previously described uropathogenic clones. Strains expressing the K1 antigen and of O groups 7, 16, 18, 83 or rough were defined as meningitis strains. The K1 antigen was identified in 69% of the meningitis isolates.

P fimbriae were expressed more often by urosepsis isolates than by the other invasive or faecal isolates, but were found in none of the isolates from the untreated infants (table II). Haemolysin and aerobactin production were least prevalent amongst isolates from the untreated group (table II). Aerobactin production was associated with isolates from meningitis (table II). P-fimbriate strains produced aerobactin more often than P-negative strains (78% versus 48%; **χ²** test, p < 0.05) whereas aerobactin production was evenly distributed amongst haemolysin-positive and -negative strains (58% and 58%, respectively).

**Statistical analyses**

**χ²** test, Fisher's exact test and the Mann-Whitney test were used when appropriate.

**Results**

**Clinical characteristics**

The isolates of *E. coli* from blood and CSF of infants were associated with meningitis (21%), urosepsis (29%), crypto- genic septicaemia (40%) or untreated bacteraemia (11%) (table I). Children with crypto- genic septicaemia had lower gestational age, birth weight and Apgar score and developed their infection earlier than the other groups of patients with invasive infection (table I). Infants with untreated bacteraemia tended to have similar characteristics whereas infants with meningitis and urosepsis were more mature, healthier at birth, and older when they developed the infection. A majority of infants with crypto- genic septicaemia (80%) or untreated bacteraemia (71%) were classified as high-risk infants, compared to 46% and 33% of infants with meningitis and urosepsis, respectively.

Of the patients in the crypto- genic and the untreated groups, 25% and 29% respectively, had received antibiotics before the infection studied. Those who had received antibiotic treatment had a lower birth weight (average 948 g versus 3099 g; Mann-Whitney test, p < 0.001) and a higher proportion of risk factors (100% versus 52%; Mann-Whitney test, p < 0.01) than the other infants.

**Virulence-associated characteristics**

O serogroups and K antigens reported to be associated with virulence showed similar prevalences amongst isolates from clinical infection and faeces but were less prevalent in the untreated bacteraemia group (Fisher's exact test, p < 0.05; table II). The K1 antigen was identified in 69% of the meningitis isolates.

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Table I. Host factors in the different diagnostic groups

<table>
<thead>
<tr>
<th>Host factors</th>
<th>Meningitis</th>
<th>Urosepsis</th>
<th>Cryptogenic septicemia</th>
<th>Untreated bacteraemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls/boys</td>
<td>7/6</td>
<td>5/13</td>
<td>6/19</td>
<td>4/3</td>
</tr>
<tr>
<td>Age (days) at infection</td>
<td>12 (1–240)</td>
<td>18 (5–436)</td>
<td>4* (0–134)</td>
<td>5 (1–40)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40 (27–42)</td>
<td>40 (37–42)</td>
<td>33* (25–41)</td>
<td>37 (25–42)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3040</td>
<td>3480</td>
<td>1900*</td>
<td>2720</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>9 (5–10)</td>
<td>9 (8–10)</td>
<td>7* (0–10)</td>
<td>8 (4–10)</td>
</tr>
<tr>
<td>Percentage of patients receiving antibiotics before infection</td>
<td>46%</td>
<td>33%</td>
<td>80%*</td>
<td>71%</td>
</tr>
<tr>
<td>Percentage of high-risk infants</td>
<td>0%</td>
<td>0%</td>
<td>25%*</td>
<td>29%</td>
</tr>
</tbody>
</table>

*Mann-Whitney test, p ≤ 0.01 versus the other isolates.
†For definition of a high-risk infant see Materials and methods.

"Virulent" clones

Twenty-five isolates (40%) were classified as "virulent" strains. Uropathogenic strains were more common in urosepsis than in the other diagnostic groups and meningitis-associated strains were found in meningitis and a few cases of cryptogenic septicemia (table II). The serotypes of the uropathogenic strains found in this study were: O2:K1:P+:Hly−, O4:K3:P+:Hly+, O6:K5:P+:Hly+, O7:K1:P+:Hly−, O16:K1:P+:Hly+ and O18:K5:P+:Hly+. Both categories of "virulent" strains produced aerobactin more often than the other strains (85% and 84% versus 42%; χ² test, p < 0.001).

Co-expression of virulence characteristics

Isolates from urosepsis or cryptogenic septicemia exhibited three or more virulence characteristics more often amongst those from normal-risk infants than amongst those from high-risk infants and faecal isolates (71% versus 39% and 43%, respectively; Fisher’s exact test, p < 0.05; table III). The isolates from the untreated infants were too few to split into two groups but they never exhibited more than two virulence characteristics (Fisher’s exact test, p < 0.05 compared to faecal isolates).

Normal-risk versus high-risk infants

The occurrence of "virulent" O serogroups and K antigens and aerobactin production was similar amongst isolates from infections in infants with or without risk factors and those from the faecal flora (table IV). Expression of P fimbriae was twice as common amongst isolates from infants without apparent risk factors than amongst isolates from high-risk infants and faeces (table IV). Isolates from normal-risk infants with urosepsis, cryptogenic septicemia or meningitis expressed P fimbriae in 83%, 40% and 29% of instances, respectively, whereas isolates from high-risk infants in these diagnostic groups were P fimbriate in 17%, 30% and 33% of instances, respectively (Fisher’s exact test, p < 0.05 and p > 0.05, respectively). Haemolysin production was less common amongst isolates from normal-risk infants than amongst those from high-risk infants or faeces (table IV).

“Virulent” strains caused 62% of the infections in normal-risk infants, 32% of the infections in high-risk infants (Fisher’s exact test, p < 0.05), but represented only 9% of the faecal isolates (Fisher’s exact test, p < 0.001; table IV).

Of the infecting strains, 24% were resistant to ampicillin, 14% to co-trimoxazole, 5% to cefuroxime, 5% to chloramphenicol and none to aztreonam, cefotaxime, cepfirome, ceftazidime, gentamicin or imipenem. All but one of the 10 isolates with antimicrobial multiresistance were collected at a referral centre. Antibiotic resistance correlated neither with virulence characteristics nor host factors.

Discussion

The present results indicated that bacterial factors played a significant causative role in neonatal meningitis and urosepsis, particularly in normal infants, whereas predisposing host factors contributed greatly to cryptogenic septicemia and untreated bacteraemia.
Table II. Distribution of virulence characteristics among 63 isolates of E. coli from blood or CSF in relation to clinical diagnosis, and 35 faecal isolates

<table>
<thead>
<tr>
<th>Virulence characteristic</th>
<th>Percentage of isolates from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>meningitis (13)</td>
</tr>
<tr>
<td>O serogroups</td>
<td></td>
</tr>
<tr>
<td>1, 2, 4, 6, 7, 16, 18, 75</td>
<td>54</td>
</tr>
<tr>
<td>K antigens</td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 5, 12, 13</td>
<td>77</td>
</tr>
<tr>
<td>P fimbriae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Haemolysin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Aerobactin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77*</td>
</tr>
<tr>
<td>&quot;Virulent&quot; isolate†</td>
<td></td>
</tr>
<tr>
<td>uropathogenic meningitis</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>62†</td>
</tr>
</tbody>
</table>

* Fisher's exact test, p < 0.05 compared to faecal isolates.
† Fisher's exact test, p < 0.01 compared to faecal isolates.
‡ For definition see Materials and methods.

Table III. Relationship between the number of bacterial virulence characteristics, diagnostic group and risk status of the infants

<table>
<thead>
<tr>
<th>Number of virulence characteristics per isolate</th>
<th>Number of isolates from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>meningitis (HRI 6, NRI 7)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>One</td>
<td>0</td>
</tr>
<tr>
<td>Two</td>
<td>2</td>
</tr>
<tr>
<td>Three</td>
<td>1</td>
</tr>
<tr>
<td>Four</td>
<td>2</td>
</tr>
<tr>
<td>Five</td>
<td>1</td>
</tr>
<tr>
<td>Median number of characteristics</td>
<td>3.5</td>
</tr>
</tbody>
</table>

HRI, high-risk infant; NRI, normal-risk infant.

However, the association between the classical virulence-associated O and K antigens and invasive infection was weak in the present study, partly due to an unusually high prevalence of these antigens amongst the faecal isolates. Such O serogroups occurred at prevalences of 36–56% in the different diagnostic groups which is similar to published data from adults and children with septicaemia (36–83%) and for urinary isolates from children with vesico-ureteric reflux cystitis and asymptomatic bacteriuria (8–59%), but lower than for isolates from pylonephritis in children (58–80%).

The virulence-associated K antigens have been found previously in 41–83% of blood isolates and 44% of faecal isolates. Although these studies did not determine the presence of the K5 antigen, the percentages found here were of the same order. The prevalence of the K1 antigen in meningitis isolates (69%) was somewhat lower than previously reported.

P fimbriation was more common amongst blood isolates from infants with urosepsis without risk factors (83%) than amongst the other blood or CSF isolates (0–49%) and the faecal isolates (29%). This is logical as P fimbriation is a virulence factor presumed to be of importance mainly in the urinary tract. Previous studies have shown P fimbriation (or mannose-resistant haemagglutination) in 62–74% of urosepsis isolates, in 2–15% of isolates from cryptogenic or unspecified bacteraemia, and in 7% of neonatal meningitis isolates.

Haemolysin production was surprisingly common amongst our faecal strains, 60% versus 5–10% in strains from other sources, whereas our data for
invasive isolates were closer to those published previously; 36% versus 25% in neonatal septicaemia,\textsuperscript{11} and 50% in urosepsis versus 60–72% in pyelonephritogenic urine isolates from children.\textsuperscript{11,12} Aerobactin production occurred at frequencies similar to those previously reported for bacteraemic strains (56–77% versus 55–69%).\textsuperscript{7,12}

Combinations of factors were generally more predictive for defining “virulent” clones than the single factors, particularly in normal-risk infants. Thus, 62% of their isolates had characteristics typical of previously described uropathogenic or meningitis clones of \textit{E. coli} compared to 32% of isolates from high-risk infants and only 9% of the faecal isolates. Previous studies have reported that uropathogenic clones comprise 43% of urine isolates from children with acute pyelonephritis\textsuperscript{18} and 29% of isolates from neonatal septicaemia.\textsuperscript{11}

To our surprise, as many as seven cases of bacteraemia had, by the clinician in charge of the child during hospitalisation, been regarded as not relevant for the infant’s clinical condition and left untreated. Five of these seven cases occurred in high-risk infants. The isolates were unusual, also, in lacking nearly all of the virulence-associated factors and none belonged to a “virulent” clone. These isolates seem to represent a selection of low-virulent faecal strains that, even amongst high-risk infants, had been able to cause only transient bacteraemia.

The invasive isolates rarely showed antibiotic multiresistance. The rate of ampicillin resistance (24%) was similar to that previously found in faecal \textit{E. coli} from neonates in 22 Swedish neonatal wards (26%).\textsuperscript{35,36}

The present study emphasises the growing importance of considering host factors when evaluating possible virulence factors in bacteria. This has not been done in previous studies of neonatal \textit{E. coli} septicaemia. Conflicting data regarding the prevalence, and thus the significance, of virulence factors may be explained by differences between the patient populations studied. Our data also support the view that \textit{E. coli} isolates causing bacteraemia in compromised patients are of lower virulence than other bacteraemic isolates.\textsuperscript{8,12} similar to the strains which cause pyelonephritis in children with anatomical risk factors such as reflux.\textsuperscript{19}

Recognising the relative importance of bacterial virulence and impaired host defence has significant implications for improved prevention of invasive gram-negative infections in the neonate. Thus, attempts to reduce colonisation with “virulent” \textit{E. coli} strains in neonatal units would, during non-epidemic periods, potentially influence only half (in this study 45%) of the episodes of \textit{E. coli} septicaemia. Current preventive measures directed at the individual infant at risk and future methods to enhance host defences will probably be of growing importance for the prevention of these neonatal diseases.

This study was supported by grant B88–16X–08302–01A from the Swedish Medical Research Council, and grants from the Swedish Society for Medical Sciences, the First May Flower Campaign for Children’s Health, the Karolinska Institute, the General Maternity Hospital Foundation and the Samariten Foundation.

We thank the staffs of the participating Departments of Pediatrics and Bacteriology for their kind interest and contribution to the study and Professor B. Kaijser for serotyping the bacterial isolates.
References