Phylogenetic distribution of virulence-associated genes among Escherichia coli isolates associated with neonatal bacterial meningitis in the Netherlands

Johnson, J.R.; Oswald, E.; O’Bryan, T.T.; Kuskowski, M.A.; Spanjaard, L.

Published in:
The Journal of Infectious Diseases

DOI:
10.1086/339343

Citation for published version (APA):
Phylogenetic Distribution of Virulence-Associated Genes among Escherichia coli Isolates Associated with Neonatal Bacterial Meningitis in The Netherlands

James R. Johnson,1,3 Eric Oswald,5 Timothy T. O’Bryan,1,3 Michael A. Kuskowski,2,4 and Lodewijk Spanjaard6

Seventy cerebrospinal fluid Escherichia coli isolates from infants with neonatal bacterial meningitis (NBM), as submitted to the Netherlands Reference Laboratory for Bacterial Meningitis from 1989 through 1997, were assessed for phylogenetic background and extended virulence genotypes, in comparison with the E. coli reference collection, by using molecular methods. Phylogenetic group B2 significantly predominated overall (81%). The 4 major phylogenetic clusters exhibited distinctive virulence genotypes, suggesting diverse evolutionary histories for the individual genes. Many genes not previously studied in NBM, notably diarrhea-associated cdtB (cytotoxic lethal distending toxin [46%]) and urinary tract infection–associated ompT (outer membrane protease T [96%]), were as or more prevalent than traditional NBM-associated traits, such as ibeA (invasion of brain endothelium [33%]), sfaS (S-fimbriae [59%]), and K1 capsule (81%). These findings provide novel insights into the phylogenetic origins of NBM-associated E. coli and suggest numerous new potential targets for preventive interventions against this dire disease.

Escherichia coli is one of the main causes of neonatal bacterial meningitis (NBM), a rare but potentially devastating illness that is associated with 17%–38% mortality and 58% long-term neurological sequelae [1–5]. Improved understanding of the causative E. coli strains is needed to guide the development of effective preventive measures.

Previous studies indicated that E. coli NBM isolates can be distinguished from the general E. coli population by their high prevalence of certain serotypes, such as O18:K1:H7, O83:K1, and O7:K1, and of certain virulence-associated factors (VF;), such as S-fimbriae (sfa), K1 capsule, and invasion of brain endothelium (ibeA) [6–9]. These meningovirulent strains appear to derive predominantly from E. coli phylogenetic group B2 [10], which also is the source of most of the extraintestinal pathogenic E. coli (ExPEC) strains that cause urinary tract infections (UTIs) and bacteremia [11–16].

However, previous studies of NBM-derived E. coli isolates have several epidemiologic and methodologic limitations. They either have included only small numbers of definite NBM isolates or have pooled NBM isolates from diverse locales [6–10]. In addition, they have examined comparatively few extra-intestinal VFs, compared with the broad range that is now recognized [15, 17]. The latter include properties such as cytotoxic lethal distending toxin (CDT) and the gene encoding it (cdt), which, although first described among diarrheogenic E. coli and in other species [18–24], are increasingly recognized as prevalent also among ExPEC, including strains causing UTI and/or septicemia in humans, dogs, cats, lambs, and calves [15, 17, 25, 26]. Finally, no previous study of NBM isolates has examined the population’s clonal structure both broadly, in comparison with the E. coli reference (ECOR) collection, which provides a phylogenetic “benchmark” for clinical isolates [10, 27], and narrowly, according to individual clonal groups or serotypes within the broader phylogenetic divisions [7, 9].

Thus, we undertook the present study to define the phylogenetic background and VF profiles of a comparatively large population (n = 70) of recent consecutive NBM isolates from The Netherlands. Specifically, we sought to define the population’s clonal structure and extended virulence genotypes to assess the phylogenetic distribution of VFs within the population.
and to identify associations between individual VFs, all in comparison with the ECOR strains.

Materials and Methods

Strains. Seventy anonymous cerebrospinal fluid (CSF) isolates from newborns (<28 days old) with NBM were analyzed.

Table 1. Phylogenetic origin of 70 Escherichia coli bacterial meningitis (NBM) isolates from neonates in The Netherlands and of E. coli reference (ECOR) collection strains.

<table>
<thead>
<tr>
<th>E. coli phylogenetic group</th>
<th>Prevalence of phylogenetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBM (n = 70)</td>
</tr>
<tr>
<td>A</td>
<td>1 (1)</td>
</tr>
<tr>
<td>B1</td>
<td>7 (10)</td>
</tr>
<tr>
<td>B2</td>
<td>57 (81)</td>
</tr>
<tr>
<td>D</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Ungrouped</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of isolates, except where noted.

<sup>a</sup> Defined by Herzer et al. [33] on the basis of the electrophoretic mobility for 38 metabolic enzymes.

<sup>b</sup> Isolates from the present study.

<sup>c</sup> Fisher’s exact test.

**Table 1.** Phylogenetic origin of 70 Escherichia coli bacterial meningitis (NBM) isolates from neonates in The Netherlands and of E. coli reference (ECOR) collection strains.

The 70 isolates were tested for 25 different VFs of ExPEC, using membrane dot-blot hybridization under stringent conditions, as described elsewhere [17, 35, 36]. Probes were generated and digoxigenin-labeled, using primers de-
and then by examining the HeLa cells for distension, growth arrest in G2/M phase, nuclear swelling, and chromatin fragmentation, in comparison with appropriate positive and negative controls, as described elsewhere [24, 26].

Statistical methods. Comparisons involving the prevalence of a given trait in different populations were tested using Fisher’s exact test. Comparisons involving the prevalence of different traits within the same population were tested using McNemar’s test [43]. Associations between traits were assessed using Cramer’s Φ coefficient [15]. Because of multiple comparisons, the threshold for statistical significance was P < .01, with P < .05 considered to reflect borderline statistical significance.

Results

Phylogenetic structure and serotypes. Composite RAPD analysis indicated that 57 (81%) of the 70 NBM isolates corresponded with E. coli phylogenetic group B2, 7 (10%) corresponded with group B1, 5 (7%) corresponded with group D, and 1 (1%) corresponded with group A (P < .01, prevalence of group B2 vs. all other groups, singly or combined, McNemar’s test; table 1). Among the NBM isolates, the prevalence of groups B2 and A was significantly greater and significantly lower, respectively, than that among the members of the ECOR collection, which predisposed a surrogate for the E. coli population as a whole (table 1).

Cluster analysis by UPGMA revealed 4 major clusters among the NBM isolates (figure 1). The first 2 clusters (I and II), which were the most populous, were linked directly to one another and contained all the phylogenetic group B2 NBM isolates. Cluster I comprised predominantly O83:K1 NBM isolates (6 of 10 O-typeable isolates in this cluster). It also included a small subcluster comprising ECOR 52 (O25:[non-K1]:H1, the only ECOR strain in cluster I) and 2 O6:[non-K1] NBM isolates (figure 1). Cluster II, which included ECOR 62 (O2:[K1]:H4), comprised predominantly O18:K1 NBM isolates (14 of 24 O-typeable isolates). It also included 2 NBM isolates each of serotypes O1:[K1] and O12:[K1 or K−], which were intermingled with the O18:K1 isolates rather than forming a separate subcluster (data not shown).

Cluster III consisted of ECOR 39 (O7:[K1]:H−, group D) and 4 O7:[K1] NBM isolates, all putatively from group D (figure 1). Cluster IV consisted of various non–group B2 ECOR strains, 7 serologically diverse putative group B1 NBM isolates, and 1 NBM isolate each from groups A and D (figure 1).

Prevalence of VFs. In the population as a whole, the various VFs ranged in prevalence from >90% (kpsMT II, ompT, and fyuA) to 1% (papG allele III, focG, and rfj; table 2), with only papG allele I, nfaE, and kpsMT III not being detected in any strain. Additional VFs that occurred in >50% of the population included sfa/foc DE, sfaS, iroN, intA, the K1 kpsMT variant, cvaC, traT, and the PAI marker malX (table 2). Of note, pap elements and ibeA were present in only 29% and 33% of all isolates, respectively, whereas the diarrhea-associated toxin gene cdT occurred in 46% of isolates. Because the observed prevalence of cdT was so unexpectedly high, the dot-blot results were confirmed by PCR detection, which yielded highly comparable results (data not shown). In addition, all 12 cdT-positive strains that were assessed phenotypically produced a CDT similar to that previously isolated from an enteropathogenic E. coli strain [44] (data not shown).

Phylogenetic distribution of VFs. Most of the VFs studied exhibited a significant nonrandom distribution among the 4 major phylogenetic clusters; the only exceptions involved VFs that were present in <10% or >90% of the isolates, plus iha, intA, and traT (table 2). Conversely, each of the 4 clusters exhibited a significantly higher and/or lower prevalence of ≥1 virulence genes than did the remainder of the study population (table 2). Traits that were significantly concentrated in cluster I included sfa/foc, cdT, and ibeA, with similar borderline significant trends for sfaS, focG, iroN, and malX (table 2). Traits concentrated in cluster II included the F11 papA allele and K1 kpsMT variant (both with borderline significance; table 2). Cluster III was significantly associated with papA, the F10 papA allele, papG, and papG allele II, whereas cluster IV was significantly associated with sfa/dra, bmaE, and the K2 kpsMT variant (table 2). As for negative associations, cluster I exhibited a decreased prevalence of papG allele II (borderline significance); cluster III was significantly negatively associated with iroN, cvaC, and malX; and cluster IV was significantly negatively associated with sfa/foc, sfaS, cdT, kpsMT II, the K1 variant, ibeA, and malX (table 2).

When the population was stratified dichotomously as group B2 (clusters I and II combined) versus non–group B2 (clusters III and IV combined), VFs significantly associated with B2 status included the F11 papA allele, sfa/foc, sfaS, cdT, iroN, fyuA, kpsMT II (by PCR), K1, cvaC, ibeA, and malX (table 2). In contrast, VFs associated with non–group B2 status included the F10 papA allele, sfa/dra, iha, bmaE, and the K2 kpsMT variant (table 2).
Comparisons between individual clusters with respect to the prevalence of individual VFs revealed substantial differences even within the same phylogenetic group. The 2 group B2 clusters (I and II) differed from one another by $\geq 20\%$ with respect to the prevalence of several pap elements plus sfa/foc, cdt, iroN, and ibeA (table 2). Likewise, the 2 non–group B2 clusters (III and IV) differed from one another by $\geq 20\%$ with respect to the prevalence of several pap elements plus afa/dra, bmaE, iroN, the K1 and K2 kpsMT variants, cvaC, and traT (table 2).

### Table 2. Phylogenetic distribution of virulence factors (VFs) among 70 meningitis-associated Escherichia coli isolates from neonates.

<table>
<thead>
<tr>
<th>VF</th>
<th>Total (n = 70)</th>
<th>I (n = 16)</th>
<th>II (n = 41)</th>
<th>III (n = 4)</th>
<th>IV (n = 9)</th>
<th>I + II (n = 57)</th>
<th>III + IV (n = 13)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>papA</td>
<td>20 (29)</td>
<td>1 (6)b</td>
<td>12 (29)</td>
<td>4 (100)c</td>
<td>3 (33)</td>
<td>13 (23)</td>
<td>7 (54)</td>
<td>.001</td>
</tr>
<tr>
<td>F10</td>
<td>4 (6)</td>
<td>0</td>
<td>0</td>
<td>4 (100)d</td>
<td>0</td>
<td>0</td>
<td>4 (31)</td>
<td>.04</td>
</tr>
<tr>
<td>F11</td>
<td>9 (13)</td>
<td>0</td>
<td>9 (22)b</td>
<td>0</td>
<td>0</td>
<td>9 (16)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>papG</td>
<td>16 (23)</td>
<td>1 (6)</td>
<td>10 (24)</td>
<td>4 (100)f</td>
<td>1 (11)</td>
<td>11 (19)</td>
<td>5 (38)</td>
<td></td>
</tr>
<tr>
<td>Allele II</td>
<td>14 (20)</td>
<td>0*</td>
<td>10 (24)</td>
<td>4 (100)f</td>
<td>0</td>
<td>10 (18)</td>
<td>4 (31)</td>
<td></td>
</tr>
<tr>
<td>Allele III</td>
<td>1 (1)</td>
<td>1 (6)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sfa/focDE</td>
<td>43 (61)</td>
<td>15 (94)c</td>
<td>27 (66)</td>
<td>1 (25)</td>
<td>0d</td>
<td>42 (74)</td>
<td>1 (8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>sfaS</td>
<td>41 (59)</td>
<td>13 (81)b</td>
<td>27 (66)</td>
<td>1 (25)</td>
<td>0d</td>
<td>40 (70)</td>
<td>1 (8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>fucG</td>
<td>1 (1)</td>
<td>1 (6)b</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>afa/draBC</td>
<td>18 (26)</td>
<td>3 (19)</td>
<td>7 (17)</td>
<td>0</td>
<td>8 (89)d</td>
<td>10 (18)</td>
<td>8 (62)</td>
<td>.003</td>
</tr>
<tr>
<td>iha</td>
<td>13 (19)</td>
<td>2 (13)</td>
<td>5 (12)</td>
<td>2 (50)</td>
<td>4 (44)</td>
<td>7 (12)</td>
<td>6 (46)</td>
<td>.011</td>
</tr>
<tr>
<td>bmaE</td>
<td>4 (6)</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td>3 (33)c</td>
<td>1 (2)</td>
<td>3 (23)</td>
<td>.019</td>
</tr>
<tr>
<td>gapD</td>
<td>2 (3)</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td>1 (11)</td>
<td>1 (2)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>hlyA</td>
<td>6 (9)</td>
<td>2 (13)</td>
<td>3 (7)</td>
<td>0</td>
<td>1 (11)</td>
<td>5 (9)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>cnfI</td>
<td>6 (9)</td>
<td>2 (13)</td>
<td>3 (7)</td>
<td>0</td>
<td>1 (11)</td>
<td>5 (9)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>cdtB</td>
<td>32 (46)</td>
<td>13 (81)i</td>
<td>19 (46)</td>
<td>0</td>
<td>0*</td>
<td>32 (56)</td>
<td>1 (8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>iroN</td>
<td>45 (64)</td>
<td>14 (88)b</td>
<td>28 (68)</td>
<td>0b</td>
<td>3 (33)</td>
<td>42 (74)</td>
<td>3 (23)</td>
<td>.001</td>
</tr>
<tr>
<td>fyuA</td>
<td>66 (94)</td>
<td>16 (100)</td>
<td>40 (98)</td>
<td>3 (75)</td>
<td>7 (78)</td>
<td>56 (98)</td>
<td>10 (77)</td>
<td>.019</td>
</tr>
<tr>
<td>ivaT</td>
<td>43 (61)</td>
<td>10 (63)</td>
<td>27 (66)</td>
<td>2 (50)</td>
<td>4 (44)</td>
<td>37 (65)</td>
<td>6 (46)</td>
<td></td>
</tr>
<tr>
<td>kpsMT II (b)</td>
<td>68 (97)</td>
<td>16 (100)</td>
<td>40 (98)</td>
<td>4 (100)</td>
<td>8 (89)</td>
<td>56 (98)</td>
<td>12 (92)</td>
<td></td>
</tr>
<tr>
<td>K1</td>
<td>60 (86)</td>
<td>16 (100)</td>
<td>38 (93)</td>
<td>4 (100)</td>
<td>2 (22)d</td>
<td>54 (95)</td>
<td>6 (46)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>“K2”</td>
<td>57 (81)</td>
<td>14 (88)</td>
<td>37 (90)b</td>
<td>4 (100)</td>
<td>2 (22)d</td>
<td>51 (89)</td>
<td>6 (46)</td>
<td>.001</td>
</tr>
<tr>
<td>rfc</td>
<td>8 (11)</td>
<td>0</td>
<td>2 (5)</td>
<td>0</td>
<td>6 (67)d</td>
<td>2 (4)</td>
<td>6 (46)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>cvaC</td>
<td>47 (67)</td>
<td>12 (75)</td>
<td>31 (76)</td>
<td>0*</td>
<td>4 (44)</td>
<td>43 (75)</td>
<td>4 (31)</td>
<td>.006</td>
</tr>
<tr>
<td>trA</td>
<td>58 (83)</td>
<td>12 (75)</td>
<td>36 (88)</td>
<td>2 (50)</td>
<td>8 (89)</td>
<td>48 (84)</td>
<td>10 (77)</td>
<td></td>
</tr>
<tr>
<td>ibeA</td>
<td>23 (33)</td>
<td>10 (63)c</td>
<td>13 (32)</td>
<td>0</td>
<td>0b</td>
<td>23 (40)</td>
<td>0</td>
<td>.003</td>
</tr>
<tr>
<td>ompT</td>
<td>67 (96)</td>
<td>16 (100)</td>
<td>40 (98)</td>
<td>3 (75)</td>
<td>8 (89)</td>
<td>56 (98)</td>
<td>11 (85)</td>
<td></td>
</tr>
<tr>
<td>malX</td>
<td>55 (79)</td>
<td>16 (100)b</td>
<td>38 (93)d</td>
<td>0d</td>
<td>1 (11)d</td>
<td>54 (95)</td>
<td>1 (8)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of isolates, except where noted. afa/dra, Dr-binding adhesins; bmaE, M fimbr; cdfB, cytotoxic necrotizing factor 1; cfaC, colicin V; fucG, F1C fimbr; fyuA, Versiniabactin receptor; gapD, G fimbr; hlyA, hemolysin; ibeA, invasion of brain endothelium A; iha, putative adhesin-siderophore; iroN, putative catecholate siderophore; ivaT, aerobactin receptor; K1, K1 kpsMT II variant; “K2,” putative K2 kpsMT II variant; kpsMT II, group II capsule synthesis; malX, marker for pathogenicity-associated island from strain CFT073; ompT, outer membrane protease T; papA, P fimbral structural subunit; papG, P fimbral adhesin molecule, with variants II and III; rfc, O4 lipopolysaccharide synthesis; sfa/focDE, S and F1C fimbr; sfaS, S fimbral adhesin; traT, serum resistance-associated outer membrane protein.

*P* Values (for comparison of combined groups [I + II] vs. [III + IV]) shown only when *P* < .05.

bP < .05, indicated group vs. all other strains.

cP < .01, indicated group vs. all other strains.

bP < .001, indicated group vs. all other strains.

Detected by blot hybridization (b).

Detected by polymerase chain reaction (P).

higher prevalence of multiple VFs, including the F11 papA allele, sfa/foc, sfaS, afa/dra, cdtB, iroN, fyuA, ivaT, group II and K1 capsule genes, cvaC, traT, ibeA, ompT, and malX. In contrast, they exhibited a lower prevalence of papG allele III and fucG (table 3).

However, these findings could simply reflect the disproportionately greater contribution of (virulence-associated) phylogenetic group B2 to the present NBM population (compared with the ECOR collection) in conjunction with the known concentration of many extraintestinal VFs in phylogenetic group B2. Consequently, a stratified analysis was done in which the 57 putative group B2 NBM isolates (clusters I and II) were compared with the 15 group B2 ECOR strains (table 2). Even with this group B2–specific analysis, the NBM isolates still differed substantially from the ECOR strains with respect to mul-
multiple VF(s) other than the previously noted elements and associations with one another, many of which contrasted with associations previously described among isolates from patients with urosepsis or cystitis or within the ECOR collection as a whole (figure 2). Of note were the negative associations of sfa and foc with pap elements, hly, and cnf; the negative association of cdhB with pap elements but its positive associations with sfa elements and ihe; and the negative associations of iroN with pap elements (figure 2).

**Discussion**

This clonal and pathotypic analysis of 70 NBM *E. coli* isolates from The Netherlands represents the largest and the only population-based study of its type to date. Our findings confirm the previously described predominance of phylogenetic group B2 and the rarity of phylogenetic group A among NBM isolates.
Figure 2. Correlations between different virulence-associated factors (VFs) among 70 meningitis-associated *Escherichia coli* isolates from neonates. Only those VFs that yielded $\geq 1$ association at the $P < .01$ level are shown. Significance codes: $-$, $P > .01$; $+$, $P < .01$; $++$, $P < .001$; parentheses indicate a negative association. For *kpsMT* (group II capsule synthesis), “blot” denotes detection by dot-blot, “PCR” denotes detection by polymerase chain reaction, “K1” indicates the K1 *kpsMT* variant, and “K2” indicates detection of putative *kpsMT* II by blot but not by PCR, consistent with the K2 *kpsMT* variant [17]. Identity of other VFs is as follows: *afa/draBC*, Dr-binding adhesins; *bmaE*, M fimbriae; *cdtB*, cytolethal distending toxin; *cnf1*, cytotoxic necrotizing factor 1; *cvaC*, colicin V; *focG*, F1C fimbriae; *fyuA*, yersiniabactin receptor; *gafD*, G fimbriae; *hlyA*, hemolysin; *ibeA*, invasion of brain endothelium A; *iha*, putative adhesin-siderophore; *ireN*, putative catecholate siderophore; *iutA*, aerobactin receptor; *malX*, marker for pathogenicity-associated island from strain CFT073; *papA*, P fimbrial structural subunit; *papG*, P fimbrial adhesin molecule, with variants II and III; *sfaS*, S fimbrial adhesin; *rfc*, O4 lipopolysaccharide synthesis; *sfa/focDE*, S and F1C fimbrial; and *traT*, serum resistance-associated outer membrane protein.
The findings provide novel insights into the prevalence of and associated VFs of specific clonal groups within the NBM population, and they greatly expand the range of VFs studied in this clinical context, including several “new” VFs (e.g., cdt, fyuA, traT, and ompT) that were as or more prevalent than certain traditional NBM-associated VFs, such as sfaS, the K1 capsule, and ibeA [6–10].

Previous studies of NBM-associated E. coli either have included only a small number of confirmed NBM isolates within a larger population of predominantly neonatal septicemia isolates [6, 7, 9] or have pooled NBM isolates from diverse locales [8, 10]. They also have analyzed convenience samples assembled on the basis of unspecified selection criteria, in some instances including previously published reference strains [6–10]. In contrast, the present population consisted of 70 randomly selected strains representing 80% of the CSF isolates from Dutch neonates as submitted to The Netherlands Reference Laboratory for Bacterial Meningitis between 1989 and 1997. All isolates were previously uncharacterized except for O:K serotype. Thus, this study provides the first opportunity to examine the prevalence of clonal groups and VFs within a defined E. coli NBM population in the absence of selection bias. However, because of the geographically circumscribed study population, the ability to more broadly generalize these results needs to be assessed in future population-based studies.

By including 24 different VFs plus the 3 papG alleles and the 12 papA alleles, the present study greatly exceeded the scope of previous studies of NBM-derived E. coli, which addressed no more than 8 VFs each [6–10]. In addition, to our knowledge, the present study is the first to resolve NBM isolates both into the major phylogenetic groups of E. coli and into constituent clonal groups and subclones thereof. Correlation of these bacterial traits with host characteristics and clinical outcomes would make a valuable addition to future such studies.

The observed striking predominance of phylogenetic group B2 among the present NBM isolates is consistent with previous findings from every analyzed collection of extraintestinal infection isolates of E. coli, regardless of clinical syndrome [11, 12, 14, 16, 25, 45, 46], and contrasts markedly with the minority contribution of group B2 to the general E. coli population [33]. This skewed distribution of phylogenetic groups among extraintestinal clinical isolates has been thought to reflect a special virulence capability of B2 strains for causing extraintestinal infections [12, 15, 47]. The 81% prevalence of group B2 is the highest noted to date in any collection of extraintestinal E. coli and contrasts with the 68% prevalence documented by Bingen et al. [10] among 69 NBM isolates (P = .08). In contrast to group B2, group A, which is associated with commensal status in humans [27], was represented by only 1 isolate in the present population, similar to the 6% prevalence found by Bingen et al. [10] among their NBM isolates.

The seemingly greater virulence of group B2 strains among human clinical isolates has been presumed to be due to the numerous extraintestinal VFs that are concentrated within this phylogenetic group [13, 48, 49]. Indeed, direct experimental assessment of extraintestinal virulence [12] showed that, although the group B2 strains were the most virulent overall, VF repertoire actually was a stronger independent predictor of virulence than was phylogenetic background [49]. In the present study, the group B2 NBM isolates had a significantly higher prevalence of many VFs than even the group B2 ECOR strains, most (73%) of which are fecal rather than clinical isolates [27]. This suggests that the present population represents primarily an extra-high virulence subset from within phylogenetic group B2.

Whether the observed pathotypic differences between the group B2 NBM isolates and the group B2 ECOR strains actually reflect NBM-specific VFs (as has been inferred elsewhere [10]) or, instead, generic extraintestinal VFs, remains to be determined by direct comparisons between NBM isolates and clinical isolates from other clinical syndromes. It should be noted that the ECOR strains were not selected randomly [27]; hence, they are not an ideal substrate for epidemiologic analyses.

The 2 most prevalent clonal groups in the present population, represented by clusters I and II, correspond largely with the 2 predominant O:K serotypes that have been identified in previous studies of NBM and neonatal sepsis (i.e., O83:K1 and O18:K1). Cluster II (predominantly O18:K1) clearly corresponds with ECOR 62, a known O2:K1:H4 strain that exhibits a VF profile characteristic of the O1:K1 and O:K1 isolates from cluster II [15]. Previous analyses have shown that NBM-derived strains of serotypes O1:K1, O2:K1, and O18:K1 are quite closely related [7, 50]. However, consistent with our findings, European isolates of E. coli O18:K1 typically represent the outer membrane protein pattern (OMP) 9 subclone of E. coli O18:K1:H7 and lack pap, in contrast to the typically pap-positive members of the OMP 6 subclone of O18:K1:H7, which predominates in the United States in both NBM and cystitis [6, 30, 51–53]. Thus, it is noteworthy that, with few exceptions [52, 54], recent molecular analyses of O18:K1:H7 NBM isolates, including those from European investigators, have focused on representatives of the OMP 6 subclone of E. coli [55–58].

Cluster I, which consisted predominantly of O83:K1 strains, was characterized by a high prevalence not only of classic meningitis-associated traits, such as sfaS, K1, and ibeA, but also of cdt (81%). CDTs constitute a family of genetically related bacterial proteinic toxins that can block the proliferation of numerous cell lines by triggering a host-cell signaling pathway that prevents the transition between the G2 and M phases of the cell cycle [26, 59, 60]. Although CDT has yet to be conclusively shown to contribute to virulence in vivo, a possible antiproliferative effect on epithelial or endothelial membranes [59] and/or inhibition of T and B cell function [61] are possible mechanisms for such an effect.

CDT was originally described among enteropathogenic E. coli and other diarrhea-associated E. coli variants [18, 19, 23], but it recently has been found to occur also among E. coli iso-
lates from extraintestinal infections in humans and dogs [15–
17, 25, 35, 46]. Among extraintestinal isolates, 
*cdt* has been
encountered in only 2 clonal groups (i.e., *E. coli* O2:K5/K7:H1
and *E. coli* O6:K53:H1) [15–17, 25, 31, 62]. The present study
provides novel evidence of *cdt* also within the O83:K1 clonal
group and, to a lesser extent, the O18:K1 clonal group. Within
each of these group B2−derived clusters (i.e., clusters I and
II), the prevalence of *cdtB* (81% and 68%, respectively) was ac-
tually higher than that of *ibeA* (63% and 32%, respectively).

Whether CDT contributes directly to the pathogenesis of
NBM warrants empirical assessment. Of note, experimental
evidence supports another “non−meningitis−associated” cyto-
toxin, CNF, as a contributor to invasion of brain microvascular
endothelial cells by NBM isolate RS218, a member of the OMP 6
subclone of *E. coli* O18:K1:H7 [56]. Of interest, members
of the American OMP 6 subclone of *E. coli* O18:K1:H7, such
as archetypal NBM isolate RS218, characteristically lack *cdt*
[25, 30, 63].

In comparison with the members of clusters I and II, the
members of (non−group B2) clusters III and IV exhibited dif-
ferent rather than simply fewer VFs. This result, plus the bal-
anced phylogenetic distribution of certain VFs, such as *iutA*,
*traT*, and *ompT*, conflicts with previously proposed models for
the acquisition of extraintestinal VFs by *E. coli*. According to
the previously proposed models, phylogenetic group B2 was
the original source of extraintestinal VFs within the species,
with VFs later migrating horizontally from group G2 to other
lineages [13, 64]. Such models are based on a limited set of
VFs that indeed are concentrated within phylogenetic groups
B2 and/or D. Analysis of a broader array of VFs has suggested
considerably more complex evolutionary histories for the var-
ious extraintestinal VFs of *E. coli* than those posited for *pap*,
hly, *sfa*, and *kps* [15–17].

We found that many VFs were as or more prevalent in the
NBM population than were the major traditional NBM−associated
VFs. For example, more prevalent than the K1 *kpsMT* variant
(81%) were *fxuA* (94%), *traT* (83%), and *ompT* (96%); more
prevalent than *sfaS* (59%) were *iroN* (64%), *iutA* (61%), *cvaC*
(67%), and *malX* (79%); and approximating the prevalence of
*ibeA* (33%) were *cdtB* (46%, as discussed above), *papA* (29%),
and *afa/dra* (26%). The high prevalence of *ompT*, which en-
codes an outer membrane protease (OmpT) and has been epide-
miodologically associated with UTI [65, 66], is of interest in view
of the recent evidence that OmpT may contribute to invasion of
human endothelial cells, a necessary step in the pathogenesis of
NBM [67]. Of note, not included in our analysis was *fmhH*,
which is almost universally prevalent within *E. coli* [15].

These prevalence values do not mean that the traits studied
necessarily contribute to NBM. Nevertheless, they do suggest
the possibility of numerous additional targets for protective in-
terventions if a virulence role for any of these traits in NBM
can be confirmed in vivo. The results of subtractive hybridiza-
tion analysis of NBM isolate C5 [55, 56] and of genome map-
ping of strain RS218 [57, 68], together with the high preva-
lence of PAI marker *malX* noted here, suggest that numerous
additional VFs await discovery within the PAIs of NBM−
derived *E. coli*.

Acknowledgments

We thank Michèle Boury for invaluable technical assistance,
Dave Prentiss for help with preparing figure 1, and Ann Emery
for help preparing the manuscript.

References

1. Mulder CJ, Zanen HC. A study of 280 cases of neonatal meningitis in The
3. Franco SM, Cornelius VE, Andrews BF. Long-term outcome of neonatal
4. Louvois J. Acute bacterial meningitis in the newborn. J Antimicrob Che-
5. Pong A, Bradley JS. Bacterial meningitis and the newborn infant. Infect
6. Korhonen TK, Valtonen MV, Parkkinen J, et al. Serotypes, hemolysis pro-
duction, and receptor recognition of *Escherichia coli* strains associated
7. Selander RK, Korhonen TK, Väisänen-Rhen V, Williams PH, Pattison PE,
Caquent DA. Genetic relationships and clonal structure of strains of
*Escherichia coli* causing neonatal septicemia and meningitis. Infect
8. Bingen E, Bonacorsi S, Brahim N, Denamur E, Elion J. Virulence pat-
terns of *Escherichia coli* K1 strains associated with neonatal meningitis.
associated bacterial characteristics in neonatal and infantile bacteremia
and meningitis caused by *Escherichia coli*. J Med Microbiol 1992;36:
203–8.
chia coli* strains causing neonatal meningitis suggests horizontal gene
transfer from a predominant pool of highly virulent B2 group strains.
11. Picard B, Journet-Mancy C, Picard-Pasquier N, Goullet P. Genetic struc-
tures of the B2 and B1 *Escherichia coli* strains responsible for extra-
and virulence in *Escherichia coli* extraintestinal infection. Infect Immun
13. Boyd EF, Hartl DL. Chromosomal regions specific to pathogenic isolates
of *Escherichia coli* have a phylogenetically clustered distribution.
Association of carboxylesterase B electrophoretic pattern with presence
and expression of urovirulence factor determinants and antimicrobial
resistance among strains of *Escherichia coli* causing urosepsis. Infect
15. Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribu-
tion of extraintestinal virulence-associated traits in *Escherichia coli*.
16. Johnson JR, O’Bryan TT, Kuskowski MA, Maslow JN. Ongoing horizon-
tal and vertical transmission of virulence genes and *papA* alleles among
*Escherichia coli* blood isolates from patients with diverse-source bacte-


54. Poult R, Puustinen T, Virkola R, Hacker J, Klemm P, Korhonen TK. Amino acid residue Ala-62 in the FinH fimbrial adhesin is critical for the adhesiveness of meningitis-associated *Escherichia coli* to collo- 


