Colonization with Extraintestinal Pathogenic *Escherichia coli* among Nursing Home Residents and Its Relationship to Fluoroquinolone Resistance

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In a cross-sectional fecal prevalence survey involving 49 residents of a Veterans Affairs nursing home, 59% of subjects were colonized with extraintestinal pathogenic *Escherichia coli* (ExPEC), 22% were colonized with adhesin-positive *E. coli*, and 51% were colonized with fluoroquinolone-resistant *E. coli*. Among 80 unique isolates, adhesins correlated negatively and aerobactin correlated positively with fluoroquinolone resistance.

Nursing home (NH) residents are at increased risk for urinary tract infections (UTIs) and bloodstream infection (12). Gram-negative bacteria (GNB), in particular the subgroup of extraintestinal pathogenic *Escherichia coli* (ExPEC) strains, cause most such infections (12). ExPEC strains typically express adhesins that promote binding to the uroepithelium and hemolysins that inhibit normal phagocytic killing (1, 10). Conversely, ExPEC isolates are infrequent among collections of normal fecal flora (1, 6, 13).

Antimicrobial resistance among GNB is an increasing problem among hospitalized and ambulatory patients (2) and residents in NHSs (16). Resistance to fluoroquinolones (FQs), commonly prescribed oral agents, is also an increasing problem, including for *E. coli* (7–9). Since the fecal flora is the presumed reservoir from which extraintestinal infection isolates arise, we studied whether NH residents manifest high rates of fecal carriage of ExPEC and, if present, whether they are FQ resistant (FQR).

Subjects were prospectively recruited at a 240-bed Veterans Affairs NH. The demographic mix is 50% minority and 1% female, with an average age of 75 years. Informed consent was obtained from the resident’s legal guardian if the patient was cognitively impaired. The study was reviewed and approved by the facility Institutional Review Board.

Between March and July 2002, 77 patients were approached for participation: 60 provided informed consent and were enrolled. Five subjects died or were discharged prior to the first sample collection. Rectal swabs were obtained at the time of recruitment and inoculated onto MacConkey agar without antimicrobial additives (nonselective medium). Twenty-five lac-tose-positive colonies (as available) were arbitrarily selected and inoculated onto nonselective medium and MacConkey agar containing either 8-μg/ml ofloxacin or 1-μg/ml ceftazidine. Antimicrobial susceptibilities and confirmation of species as *E. coli* were performed by automated testing with the Vitek GNI card (BioMerieux, Hazelwood, Mo.). Expression of extended-spectrum β-lactamases (ESBLs) was determined by double-disc diffusion testing. The stools from six subjects did not yield *E. coli*. Thus, swabs from 49 subjects yielded *E. coli* and were further analyzed. FQR *E. coli* (FQREC) strains were detected in samples from 25 (51%) subjects; for 11 subjects, all colonies represented FQREC. ESBL expression was detected for only a single strain of FQREC.

Endonuclease analysis of XbaI macrorestriction fragments was performed by pulsed-field gel electrophoresis (PFGE) as described previously (11). Clonal relationships were as defined by Tenover et al. (14). PFGE was performed for all study isolates and yielded a single strain of *E. coli* for 27 (55%) of the subjects. Twenty-two samples (45%) yielded ≥2 distinct strains of *E. coli* for a total of 80 isolates.

For each patient, one isolate of each clonal type was subjected to phylogenetic and virulence factor analysis. Isolates were assigned to one of four phylogenetic groups (A, B1, B2, and D) by a triplex PCR-based method (3). Isolates were screened for five key virulence markers: *i.e., papA and papC* (P fimbriae), *sfa/foc* (S and F1C fimbriae), *afa/dra* (DR adhesin), *iutA* (aerobactin), and *kpsM* (group 2 capsule) and were defined as ExPEC if positive for ≥2 screening markers (4). Isolates determined to represent ExPEC were tested for 35 virulence markers with established PCR and dot blot-based assays (4, 5). Thirty-six (45%) strains from 29 (59%) subjects qualified as ExPEC (Table 1). Adhesin-encoding genes *pap*, *sfa/foc*, and *afa/dra* were identified among 12 (15%) strains from 11 (22%) subjects.

The frequency of FQR was analyzed in relation to each virulence factor (Table 1). Aerobactin (*iutA*)-positive strains were significantly more likely to be FQR (*P* < 0.001). Conversely, strains that were *sfa* and adhesin positive were more likely to be FQ susceptible (FQS; *P* = 0.045 and *P* = 0.063,
was due to increased fecal carriage of virulent strains of *E. coli* of urinary tract and bloodstream infection among NH residents between FQR and the presence of virulence factors. B2-2 and carried FQREC and whether such organisms represent ExPEC. Additionally, since there are data suggesting that the presence of aerobactin (hly) to nalidixic acid resistance (121.7) 3.0 61.7 0.67 (0.26, 1.68) 0.200

**TABLE 1. Prevalence of virulence factors among NH fecal strains**

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>No. (%) positive</th>
<th>% Virulence factor positive</th>
<th>OR (95% CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>afa/dra</em></td>
<td>6 (7.4)</td>
<td>12.5 0</td>
<td>(1.17, —)</td>
<td>0.076</td>
</tr>
<tr>
<td><em>hlyD</em></td>
<td>6 (7.4)</td>
<td>10.4 3.0</td>
<td>(0.38, 181.37)</td>
<td>0.393</td>
</tr>
<tr>
<td><em>papC</em></td>
<td>8 (10)</td>
<td>12.5 6.1</td>
<td>2.21 (0.36, 23.65)</td>
<td>0.462</td>
</tr>
<tr>
<td><em>sfa/foc</em></td>
<td>7 (8.6)</td>
<td>12.5 0</td>
<td>(1.17, —)</td>
<td>0.076</td>
</tr>
<tr>
<td>Any adhesin</td>
<td>12 (15)</td>
<td>22.9 6.1</td>
<td>0.41 (0.09, 4.40)</td>
<td>0.676</td>
</tr>
<tr>
<td><em>iutA</em></td>
<td>34 (42.5)</td>
<td>25.0 67.7</td>
<td>0.17 (0.06, 0.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>kpsIII</em></td>
<td>50 (62.5)</td>
<td>72.9 60.6</td>
<td>0.67 (0.26, 1.68)</td>
<td>0.200</td>
</tr>
<tr>
<td>ExPEC</td>
<td>34 (42.5)</td>
<td>37.5 54.5</td>
<td>0.50 (0.18, 1.35)</td>
<td>0.263</td>
</tr>
<tr>
<td><em>cnf</em></td>
<td>4 (11.1)</td>
<td>22.2 0</td>
<td>(1.19, —)</td>
<td>0.104</td>
</tr>
<tr>
<td><em>ire</em></td>
<td>4 (11.1)</td>
<td>16.7 3.0</td>
<td>3.40 (0.24, 188.77)</td>
<td>0.603</td>
</tr>
<tr>
<td><em>iroN</em></td>
<td>7 (17.9)</td>
<td>33.3 3.0</td>
<td>8.50 (0.81, 413.37)</td>
<td>0.088</td>
</tr>
<tr>
<td><em>malX</em></td>
<td>32 (88.8)</td>
<td>94.4 48.5</td>
<td>2.13 (0.10, 132.61)</td>
<td>0.999</td>
</tr>
<tr>
<td><em>sat</em></td>
<td>29 (74.4)</td>
<td>72.2 54.5</td>
<td>0.00 (0.00, 0.62)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

* A strain is designated as FQR if any of the examined colonies were observed as resistant.

* Odds ratios (OR) for strains with one cell as 0% reached in FQR— as uncalculable.

* The presence of these virulence factors was determined only for the 34 isolates of ExPEC. Virulence factors not represented in this collection of isolates included bma, gei, F17a, clpG, cdts, and iss, while FtsL, rfc, cva, and kpsIII were noted in a single isolate each.

respectively). Otherwise, there were no significant associations between FQR and the presence of virulence factors.

In this study, we sought to determine whether the increase risk of urinary tract and bloodstream infection among NH residents was due to increased fecal carriage of virulent strains of *E. coli*, i.e., ExPEC. Additionally, since there are data suggesting that antimicrobial resistance is problematic in this population, we sought to determine whether NH residents frequently carry FOREC and whether such organisms represent ExPEC.

ExPEC strains were observed to colonize the majority (54%) of study subjects, of which the majority represented clonal group B2-2 and carried *iutA* and *kpsIII*. In contrast, adhesins and hemolysis, virulence factors classically associated with bacteremia and pyelonephritis (10), were detected in a minority of isolates (15%) from 22.4% of subjects, a result similar to those from prior studies of normal fecal flora (1, 6, 13), which suggests that carriage of adhesin-positive strains was not spread within the NH population.

One study reported an inverse relationship between the presence of *cnfI* and *hly* (hemolysin) to nalidixic acid resistance (15). In this study, the presence of aerobactin (aer) correlated with FQR, while *sat* and adhesins correlated with an isolate being FQS. No other virulence factor or group of virulence factors was significantly associated with FQR, although conclusions are limited by the small sample size.

The increased prevalence of *iutA*- and *kpsIII*-positive (and adhesin-negative) strains has unknown clinical ramifications, primarily since isolates of *E. coli* causing UTIs and bloodstream infections typically express adhesins and/or hemolysis (10). An unanswered question is whether the presence of *iutA*- and *kpsIII*-positive strains may increase the risk for future infectious events.

In summary, while adhesin-positive strains are uncommon in NH residents, FQR ExPEC were frequently observed. Since the subjects were from a single Veterans Affairs NH, our study may not be applicable to all NH populations. The clinical importance of adhesin-negative isolates of FQR-ExPEC remains to be determined.

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REFERENCES


