Extraintestinal Pathogenic and Antimicrobial-Resistant *Escherichia coli* Contamination of 56 Public Restrooms in the Greater Minneapolis-St. Paul Metropolitan Area

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How extraintestinal pathogenic *Escherichia coli* (ExPEC) and antimicrobial-resistant *E. coli* disseminate through the population is undefined. We studied public restrooms for contamination with *E. coli* and ExPEC in relation to source and extensively characterized the *E. coli* isolates. For this, we cultured 1,120 environmental samples from 56 public restrooms in 33 establishments (obtained from 10 cities in the greater Minneapolis-St. Paul, MN, metropolitan area in 2003) for *E. coli* and compared ecological data with culture results. Isolates underwent virulence genotyping, phylotyping, clonal typing, pulsed-field gel electrophoresis (PFGE), and disk diffusion antimicrobial susceptibility testing. Overall, 168 samples (15% from 89% of restrooms) fluoresced, indicating presumptive *E. coli*: 25 samples (2.2% from 32% of restrooms) yielded *E. coli* isolates, and 10 samples (0.9% from 16% of restrooms) contained ExPEC. Restroom category and cleanliness level significantly predicted only fluorescence, gender predicted fluorescence and *E. coli*, and feces-like material and toilet-associated sites predicted all three endpoints. Of the 25 *E. coli* isolates, 7 (28%) were from phylogenetic group B2 (virulence-associated), and 8 (32%) were ExPEC. ExPEC isolates more commonly represented group B2 (50% versus 18%) and had significantly higher virulence gene scores than non-ExPEC isolates. Six isolates (24%) exhibited ≥3-class antibiotic resistance, 10 (40%) represented classic human-associated sequence types, and one closely resembled reference human clinical isolates by pulsed-field gel electrophoresis. Thus, *E. coli*, ExPEC, and antimicrobial-resistant *E. coli* sporadically contaminate public restrooms, in ways corresponding with restroom characteristics and with-in restroom sites. Such restroom-source *E. coli* strains likely reflect human fecal contamination, may pose a health threat, and may contribute to population-wide dissemination of such strains.

*Escherichia coli* is a major cause of urinary tract and other extraintestinal infections, resulting in considerable morbidity, mortality, and costs (1). Most such infections are caused by distinctive *E. coli* strains called extraintestinal pathogenic *E. coli* (ExPEC) because of their enhanced ability to invade and cause disease at extraintestinal sites (2). Such strains’ main reservoir is the human intestinal tract, where they usually reside harmlessly as long-term colonizers (3). ExPEC strains can be distinguished from other *E. coli* strains by their extensive virulence gene repertoire and primarily group B2 phylogenetic background (2).

Dissemination of virulent and antimicrobial-resistant *E. coli* clones through the human population has been recognized recently as an important contributor to the overall burden of ExPEC-associated disease (4–7). However, the mechanisms of such clonal dissemination remain undefined. Flush toilets create microdroplets containing viable bacteria (8, 9), and both public and private restrooms have been shown to be variably contaminated with human-source bacteria (10–13). However, specific data are scant for *E. coli* and are absent for ExPEC and antimicrobial-resistant *E. coli*. Thus, although public restrooms conceivably could represent an environmental reservoir and dissemination point for human gut-source *E. coli* (14), including ExPEC and antimicrobial-resistant strains, leading to transmission among healthy individuals, further study is needed.

Accordingly, we surveyed public restrooms for environmental *E. coli* and ExPEC. Specifically, we sought to define (i) the overall prevalence of *E. coli* and ExPEC in public restrooms, (ii) restroom characteristics associated with positive cultures, (iii) the specific sites within the restroom at highest risk for *E. coli*, and (iv) the likely origins and potential human health implications of such organisms.

**MATERIALS AND METHODS**

**Epidemiological data.** During 2003, one-time culture surveillance was done in 56 public restrooms located in 33 different establishments in 10 different municipalities situated within 3 counties (Hennepin, Ramsey, and Dakota) in the greater Minneapolis-St. Paul, MN, metropolitan area. These counties are the three most populous in Minnesota, accounting for more than 30% of the state’s total population and collectively covering a surface area of 1,362 square miles.

The restrooms were selected as 10 each from 5 different types of establishments (fast-food outlets, gas stations, public parks, supermarkets, and...
the Minneapolis Veterans Affairs Medical Center (MVAMC)) and 6 from
malls/stores. For each restroom, in addition to geographic and institu-
tional location (category of establishment), the data recorded included
the restroom’s gender status (male, female, or unisex) and overall cleanliness
level, which was scored subjectively as 1 to 4 (where 1 is “excellent” and 4
is “poor”), based on visual inspection according to specific criteria (see
Table S1 in the supplemental material). Additionally, a traffic score rang-
ing from 0 to 6 was assigned depending on how many people used the
restroom during the sampling visit. For each site sampled within the re-
strom, the presence or absence of presumptive fecal material was re-
corded.

Sampling approach. Within each restroom, 20 swab samples were
collected from diverse prespecified sites that were considered likely to have
fecal contamination or to be touched with bare hands (15). Study personnel
were allowed some flexibility in selection of sampling
sites, depending on local conditions. Sites were classified as toilet associ-
ed (e.g., floor surfaces within 2 ft of the toilet, toilet seat, toilet surfaces,
toilet water, and in-stall sanitary napkin receptacle) versus non-toilet as-
associated (e.g., stall latch, sink drain, cold water tap, and handle of the
diaper changing table) (see Table S2 in the supplemental material). A
checklist was used in each restroom to guide sample collection from ap-
propriate sites.

Swabbing was done systematically by using sterile 6-in., wooden-shaft,
cotton-tipped applicators (Fisher Scientific Company, LLC). Swabs were
moistened with sterile saline for dry surfaces and were used dry for toilet
bowls (water and internal surfaces) and sink drains. The swabbing tech-
nique was adjusted for the nature of the surface (e.g., knobs, drains, faucets,
floor, toilet bowl, or seat) but was consistent for each type of surface.
For flat surfaces, an approximately 100-cm² area was swabbed. Firm pres-
sure was applied during swabbing. For each site, any substance resembling
feces was specifically targeted.

Cultures of primary samples. After sample collection, swabs were
placed immediately in culture tubes containing 10 ml tryptic soy broth sup-
plemented with 20 mg/ml vancomycin and 0.35 mg/ml 4-methyl-umbel-
liliferyl-β-n-glucuronide (MUG), a substrate hydrolyzed by β-glucuronidase
(an enzyme characteristic of E. coli) to produce a fluorescent metabolite
(16). Samples were kept at ambient temperature during transport to the
laboratory (2 h maximum) and then were incubated at 37°C overnight.
After incubation, turbid broths were checked with UV light, and 10 µl from
each tube that fluoresced was streaked onto eosin-methylene blue
agar plates, which were incubated at 37°C overnight. For plates that
yielded presumptive E. coli (i.e., lactose- and indole-positive, citrate-neg-
ative Gram-negative bacilli with a characteristic E. coli colonial morphol-
y), one such colony per sample, plus mixed Gram-negative growth
harvested from the inoculum area of the plate, was saved for PCR-based
genotyping.

Virulence genotyping. To assess the extraintestinal virulence poten-
tial of any E. coli strains present, lysates were tested for 38 virulence genes
of ExPEC, using established multiplex PCR-based assays (17, 18). The
presence of ExPEC was inferred from detection of ≥2 of the following:
apa/ah and/or papa/P (Fimbriae), sfap/ocsDE (S and FIC fimbriae), sfap/
drabC (Dr family adhesins), iutA (aerobactin receptor), and kpsMT II
(group 2 capsule synthesis) (19–21). The virulence score was the total
number of virulence genes detected, adjusted for multiple detection of the
pap, sfap, and kps operons.

Phylogenetic analysis. For individual E. coli isolates, extended viru-
genotype and ExPEC status were determined as described above for
lyses. Phylogenetic background (for groups A, B1, B2, C, D, E, and F and
Escherichia cryptic clade I) was determined by multiplex PCR (22). Clonal
lineage, as indicated by sequence type (ST), was determined using a com-
bination of established ST-specific PCR assays (23), sequence analysis of
fimC and fimH (i.e., CH typing) (24), and multilocus sequence typing
(MLST) according to the Achtman system (http://mlst.warwick.ac.uk
/mlst/dbs/Ecoli).

Antimicrobial susceptibility testing. Susceptibility to 19 antimicro-
bial agents was determined by disk diffusion, using Clinical and Lab-
oratory Standards Institute-specified methods, reference strains, and
interpretive criteria (25). The agents tested included amikacin, amoxicil-
in-clavulanate, ampicillin, aztreonam, cefepime, cefoxitin, ceftazidime,
chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin, imipenem, nali-
dixic acid, nitrofurantoin, pipercillin-tazobactam, streptomycin, tetra-
cycline, trimethoprim, and trimethoprim-sulfamethaxozide. Intermedi-
ate interpretations were analyzed as resistant. The resistance score was the
number of agents to which an isolate exhibited resistance. Multidrug re-
istance status was defined as resistance to at least one representative each of
≥3 drug classes, counting penicillins and cephalosporins separately
(26).

PFGE analysis. XbaI pulsed-field gel electrophoresis (PFGE) analysis
was used to assign isolates to pulstotypes based on 94% profile similarity to
reference strains (27). Dendrograms were inferred within BioNumerics,
version 6.6 (Applied Maths, Austin, TX) according to the unweighted-
pair group method based on Dice coefficients. Profiles also were com-
pared with a large private PFGE profile reference library containing 1,925
pulsotypes and representing 4,916 pulstotypes as collected from diverse
locales, specimen types, hosts, clinical syndromes, and time periods
(28).

Statistical analysis. Comparisons of proportions were assessed using a
chi-square test [with (N–1)/N correction] (29). When multiple sub-
groups were present (i.e., restroom category and gender), pairwise com-
parisons between subgroups were tested statistically only if the overall
comparison was significant. Comparisons of scores were assessed using the
Mann-Whitney U test (two-tailed). The significance criterion was P <
0.05.

RESULTS

Screening for E. coli and ExPEC. In total, 1,120 environmental
surveillance samples were collected systematically during 2003
from 56 diverse public restrooms in the greater Minneapolis-St.
Paul metropolitan area. Of the 1,120 corresponding MUG-sup-
plemented broth cultures, 168 (15%) from 50 different restrooms
(89% of 56) fluoresced, suggesting the presence of E. coli (Fig. 1). Of
these, 25 (2.2% overall; 14.9% of fluorescent cultures) from 18
different restrooms (32% of 56) yielded a confirmed *E. coli* isolate. Of these, 10 (40% of 25; 0.9% overall) from 9 different restrooms (16% of 56) presumptively contained ExPEC according to population DNA PCR, whereas 8 yielded a confirmed ExPEC isolate.

**Epidemiological associations.** The characteristics of the restrooms and the sites sampled were compared statistically with culture results to identify epidemiological correlates of the microbiological outcomes of interest: i.e., fluorescence, *E. coli*, and ExPEC. At the restroom level, restroom category was significantly associated only with the prevalence of fluorescence, which ranged from 24% (public parks) to 10% (supermarkets) (6-group comparison, *P* < 0.001) (Fig. 2). In pairwise comparisons by restroom category, fluorescence was significantly more prevalent for public parks than gas stations (*P* = 0.005), the MVAMC (*P* < 0.001), or supermarkets (*P* < 0.001) and for fast food outlets than the MVAMC (*P* = 0.04) or supermarkets (*P* = 0.03) (Fig. 2).

Restroom gender also was associated significantly with culture results, for both fluorescence and *E. coli* but not for ExPEC (Fig. 3). For fluorescence, female and unisex restrooms exhibited a significantly higher prevalence than male restrooms (*P* = 0.02 and 0.01, respectively) but did not differ from one another. For *E. coli*, female restrooms exhibited a significantly higher prevalence than unisex restrooms, with male restrooms not significantly different from the others (Fig. 3).

Restroom cleanliness level, which spanned the full range from
1 to 4 (median, 2), was associated weakly with fluorescence (P = 0.04) but not with E. coli or ExPEC (data not shown). In contrast, restroom traffic score, which spanned the full range from 0 to 6 (median, 2), was not associated with any microbiological endpoint (data not shown).

Regarding characteristics of individual samples, the presence of visible feces-like material was significantly associated with all three endpoints, i.e., fluorescence (50% versus 14.6%; P < 0.001), E. coli (33% versus 2%; P < 0.001), and ExPEC (8% versus 0.8%; P = 0.006) (Fig. 4). Toilet-associated sites likewise were significantly associated with all three endpoints: i.e., fluorescence (18.2% versus 12%; P = 0.004), E. coli (4% versus 0.5%; P < 0.001), and ExPEC (1.6% versus 0.2%; P = 0.01) (Fig. 5). Nonetheless, E. coli and/or ExPEC strains were recovered from multiple non-toilet-associated sites and from sites without visible fecal material, including some likely to be touched by bare hands, such as a sink drain, stall lock, and cold water tap (see Table S2 in the supplemental material).

**Phylogenetic group distribution.** To infer their likely origins and pathogenic potential, the 25 individual E. coli isolates were characterized further as to phylogenetic group, extended virulence genotype, antimicrobial susceptibility profile, and sequence type. Of the eight recognized E. coli phylogenetic groups, five (A, B1, B2, D, and F) were represented, accounting for from 4% to 32% of isolates each, with groups A (commensal-associated, 32%) and B2 (virulence-associated, 28%) predominating (Table 1). Phylogenetic group distribution varied in relation to ExPEC status, with ExPEC isolates being predominantly from group B2 (50% versus 18% for non-ExPEC) and never from group B1 (0% versus 35% for non-ExPEC; P = 0.03).

**Extended virulence genotypes.** Of the 38 studied virulence genes, 32 (84%) were detected in ≥1 of the 25 E. coli isolates, in prevalences ranging from 4% to 100% (Table 2). Multiple virulence genes were detected within each functional category. Compared with 17 non-ExPEC isolates, the 8 ExPEC isolates had a significantly higher prevalence of 8 virulence genes apart from those used to determine ExPEC status and a significantly lower prevalence of none (Fig. 6). Genes encoding adhesins or toxins were found only among ExPEC isolates (Fig. 6). Similarly, multiple virulence genes were significantly more prevalent among group B2 isolates (i.e., 14 to 100%) than non-group B2 isolates (i.e., 0 to 34%), whereas none were significantly more prevalent among non-B2 isolates (Fig. 6). These patterns mirror those commonly seen among human-source isolates (19).

**Overall,** virulence gene scores ranged from 1 to 13 (median, 5). Collectively, the 8 ExPEC isolates exhibited much higher virulence gene scores (median, 9.5; range, 6 to 13) than the 17 non-ExPEC isolates (median, 2; range, 1 to 10; P < 0.001). Virulence scores also varied significantly by phylogenetic group, along a descending gradient by group median as follows: group B2, 11 (range, 7 to 13); group F, 7 (no range [1 isolate]); group D, 4 (range, 1 to 10); group A, 3 (range, 1 to 8); and group B1, 2 (range, 1 to 5) (P < 0.001). Group B2 isolates had significantly higher scores (median, 45) than group F isolates (median, 5).

**Table 1 Phylogenetic group distribution by ExPEC status among 25 Escherichia coli isolates from public restrooms**

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>Total (n = 25)</th>
<th>ExPEC (n = 8)</th>
<th>Non-ExPEC (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8 (32)</td>
<td>2 (25)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>B1</td>
<td>6 (24)</td>
<td>0 (0)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>B2</td>
<td>7 (28)</td>
<td>4 (50)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>D</td>
<td>3 (12)</td>
<td>1 (12.5)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>F</td>
<td>1 (4)</td>
<td>1 (12.5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*For each phylogenetic group, for extraintestinal pathogenic E. coli (ExPEC) versus non-ExPEC, P > 0.10 [by chi-square test, with (N−1)/N correction]. For prevalence of ExPEC among group B2 versus B1, P = 0.03.*
TABLE 2 Virulence-associated traits of 25 restroom-source Escherichia coli isolates

<table>
<thead>
<tr>
<th>Trait§</th>
<th>Description</th>
<th>Total (%) of 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>papA, -EF, C, or -G</td>
<td>Pili associated with pyelonephritis (P fimbriae)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>sfa</td>
<td>sfaCDE§</td>
<td>S and F1C fimbriae</td>
</tr>
<tr>
<td>sfaS</td>
<td>S fimbriae (sialic acid specific)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>afaldradBC§</td>
<td>Dr antigen-specific adhesin operons</td>
<td>3 (12)</td>
</tr>
<tr>
<td>iha</td>
<td>Iron-regulated gene homologue adhesin</td>
<td>6 (24)</td>
</tr>
<tr>
<td>fimH</td>
<td>v-Mannose-specific adhesion, type 1 fimbriae</td>
<td>25 (100)</td>
</tr>
<tr>
<td>hlyD</td>
<td>Alpha-hemolysin</td>
<td>1 (4)</td>
</tr>
<tr>
<td>cnf1</td>
<td>Cytotox necrotizing factor 1</td>
<td>1 (4)</td>
</tr>
<tr>
<td>cdT8</td>
<td>Cytotox distending toxin</td>
<td>2 (8)</td>
</tr>
<tr>
<td>ireA</td>
<td>Iron-regulated element</td>
<td>2 (8)</td>
</tr>
<tr>
<td>sat</td>
<td>Secreted autotransporter toxin</td>
<td>5 (20)</td>
</tr>
<tr>
<td>astA</td>
<td>Enterohaemorrhagic E. coli heat-stable cytotoxin</td>
<td>1 (4)</td>
</tr>
<tr>
<td>iroN</td>
<td>Catecholate siderophore receptor</td>
<td>5 (20)</td>
</tr>
<tr>
<td>fyuA</td>
<td>Veronina siderophore receptor</td>
<td>14 (56)</td>
</tr>
<tr>
<td>iutA§</td>
<td>Ferric aerobactin receptor (iron uptake: transport)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>kpsMT II§</td>
<td>Group II capsule polysaccharide synthesis</td>
<td>12 (48)</td>
</tr>
<tr>
<td>kpsMT K1§</td>
<td>K1 capsule</td>
<td>5 (20)</td>
</tr>
<tr>
<td>kpsMT K2/K100§</td>
<td>K2 or K100 capsule</td>
<td>3 (12)</td>
</tr>
<tr>
<td>eaeA</td>
<td>Microcin (colicin) V</td>
<td>4 (16)</td>
</tr>
<tr>
<td>traT</td>
<td>Surface exclusion, serum survival associated</td>
<td>14 (56)</td>
</tr>
<tr>
<td>ibeA</td>
<td>Invasion of brain endothelium</td>
<td>4 (16)</td>
</tr>
<tr>
<td>ompT</td>
<td>Outer membrane protein T ( protease)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>iss</td>
<td>Increased serum survival</td>
<td>3 (12)</td>
</tr>
<tr>
<td>usp</td>
<td>Uropathogenic-specific protein (bacteiriocin)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>malX</td>
<td>Pathogenicity-associated island marker</td>
<td>7 (28)</td>
</tr>
<tr>
<td>H7 FIC</td>
<td>H7 flagellin variant</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

§ Six additional traits were sought but were not found, including focG (F1C fimbriae), bmaE, (M fimbrissae), gagD (G and F17c fimbrissae), cpgK (K88-related CS31A adhesin), adaD8 (variant Dr-binding afimbrial adhesin), and cfr (O4 lipopolysaccharide synthesis).

§§ Traits contributing to molecular definition of ExPEC.

PFGE analysis. Of the 25 PFGE profiles, 21 (84%) were ≤80% similar to another profile within this study, indicating the independence of most isolates (Fig. 8). The four exceptions were two pairs of indistinguishable profiles, each of which comprised two isolates from the same or adjacent (men’s versus women’s) restrooms, suggesting local cross-contamination. Comparison of the present PFGE profiles with those in a large private PFGE database (4,916 total profiles, 1,925 total pulsotypes) identified a close match between the ST95 study isolate (FF5-2) and multiple reference ST95 human clinical isolates from diverse locales and clinical syndromes (Fig. 9).

DISCUSSION

This point-prevalence survey of 56 diverse public restrooms from 10 municipalities in three counties in the greater Minneapolis-St. Paul metropolitan area (2003) yielded four main findings, which have potentially significant public health implications. First, public restrooms were sporadically contaminated with E. coli and ExPEC, many strains of which were antimicrobial resistant. Second, this contamination occurred in somewhat predictable ways in relation to the characteristics of the restrooms, providing some guidance for avoidance and remediation. Third, although the presence of E. coli and ExPEC corresponded significantly with toilet-associated sites and gross fecal material, consistent with a human fecal source, several other sites also yielded E. coli and/or ExPEC, indicating more widespread contamination and, therefore, risk areas. Fourth, some of the restroom-derived ExPEC strains exhibited extensive virulence gene profiles, were multiply antimicrobial resistant, represented classic human-associated STs, and/or resembled known human pathogens by PFGE, implying a potential human health threat. Collectively, these findings provide novel evidence that public restroom-source E. coli strains, many of which represent ExPEC and are antimicrobial resistant, likely reflect human fecal contamination and conceivably could contribute to the population-wide dissemination of such strains.

Regarding the overall prevalence of contamination, although 15% of samples (including at least one sample from 89% of restrooms) fluoresced, suggesting the presence of (E. coli-specific) β-glucuronidase, barely 14% of fluorescing samples (i.e., 2.2% of all samples) yielded confirmed E. coli colonies. This could represent either false-positive fluorescence screens (e.g., from non-E. coli organisms producing β-glucuronidase) or false-negative plate cultures (e.g., from a small E. coli subpopulation dominated on the plates by a majority population of other Gram-negative bacilli). Nonetheless, the confirmed E. coli-positive samples represented 32% of the screened restrooms, indicating fairly widespread contamination at the by-restroom level.

Notably, this is the first reported study to assess restroom-source E. coli for virulence-related traits. We anticipated that we would find E. coli in public restrooms, as humans are known carriers of E. coli and feces are a likely source of environmental E. coli in restrooms. However, a study was needed to empirically verify this hypothesis. Although a fairly small proportion of the present E. coli isolates, as well as the corresponding mixed Gram-negative growth, fulfilled the molecular criteria for ExPEC, the resulting prevalence estimates (0.9% of samples, 18% of restrooms) doubtless underestimate the true prevalence of ExPEC. This is because we screened only a small fraction of the total surface area of each restroom, albeit focusing on areas that we considered most likely to be contaminated with E. coli, and did not screen for ExPEC in...
samples that yielded fluorescent broths but no visible *E. coli* colonies. As such, our findings provide at best a minimum estimate of the true prevalence of *E. coli* and ExPEC contamination.

Most of the studied restroom characteristics predicted contamination. For example, samples from restrooms in public parks and fast food outlets, as well as visibly unclean restrooms, were more likely to yield fluorescence (although not *E. coli* isolates or ExPEC) than those from the MVAMC or supermarkets or that appeared clean. Thus, a restroom’s location and appearance may predict its likelihood of low-level *E. coli* contamination, if “fluorescence-only” cultures signify this. Likewise, unisex and female restrooms were significantly associated with fluorescence, and female restrooms were significantly associated with *E. coli*. Since females are especially vulnerable to urinary tract infections (30), this finding suggests that females might benefit from using extra caution when frequenting public restrooms, e.g., by practicing fastidious hand hygiene and perhaps using barriers on toilet seats.

### FIG 6 Virulence genotypes of 25 *Escherichia coli* isolates from public restrooms. (Left) Extrainestinal pathogenic *E. coli* (ExPEC) versus non-ExPEC; right, phylogenetic group B2 versus non-B2. The traits shown are those (among 38 total) that yielded P values of <0.05 for the comparisons of ExPEC isolates (pink bars) versus non-ExPEC isolates (blue bars) and/or for group B2 isolates (pink bars) versus non-B2 isolates (blue bars). Traits are arranged from top to bottom in order of descending prevalence among ExPEC isolates. P value symbols are shown adjacent to the higher-prevalence group when P is <0.05 as follows: *, P < 0.05, **, P < 0.01, and ***, P < 0.001, as determined by chi-square test [with (N-1)/N correction]. Rectangles enclose traits contributing to molecular definition of ExPEC. Trait definitions: *afa/draBC*, Dr antigen-specific adhesion operons; *cdtB*, cytolethal distending toxin; *fyuA*, *Yersinia* siderophore receptor; *ibeA*, invasion of brain endothelium; *sha*, iron-regulated gene homologue adhesin; *iroA*, iron-regulated element; *iroN*, catecholate siderophore receptor; *iutA*, ferric aerobactin receptor; *kpsM II*, group 2 capsule polysaccharide synthesis (e.g., K1, K5, and K12); *kpsM K1 and K2/K100*, group 2 capsule variants; *malX*, pathogenicity-associated island marker; *ompT*, outer membrane protein T (protease); *papA*, P fimbrial structural subunit (with *papC*, *papE*, *papG*, and *papG* allele II giving the same result as *papA*); *sat*, secreted autotransporter toxin; *traT*, surface exclusion, serum survival-associated; *usp*, uropathogenic-specific protein (bacteriocin).

### FIG 7 Virulence scores among 25 *Escherichia coli* isolates from public restrooms. (Left) Extrainestinal pathogenic *E. coli* (ExPEC) (solid squares) versus non-ExPEC (squares) isolates. (Right) Phylogenetic group B2 (solid circles) versus non-B2 (circles) isolates. Horizontal lines, group medians. P values, as determined by the Mann-Whitney U test (two tailed), are for ExPEC versus non-ExPEC and group B2 versus non-B2.

### TABLE 3 Prevalence of antimicrobial resistance among 25 *Escherichia coli* isolates from public restrooms

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>Prevalence of resistance, no. of isolates (% of 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12 (48)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>6 (24)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Multidrug resistance (≥3 classes)</td>
<td>6 (24)</td>
</tr>
</tbody>
</table>

* No resistance was detected to amikacin, aztreonam, cephalotin, ceftriaxone, chloramphenicol, ciprofloxacin, ertapenem, gentamicin, imipenem, nitrofurantoin, and piperacillin-tazobactam.

* Extrainestinal pathogenic *Escherichia coli* (ExPEC) and non-ExPEC isolates did not differ significantly for the prevalence of any single antimicrobial agent or multidrug resistance status [P > 0.10, by chi-square test, with (N-1)/N correction].
The finding that the highest-risk areas for contamination were toilet-associated sites and those with feces-like material suggests that human feces are the main source for the recovered *E. coli* and ExPEC strains, as opposed to the organisms being free-living environmental strains (31, 32). In turn, this suggests that these organisms likely are well adapted for colonization of new human hosts, if acquired by restroom users. Notably, however, *E. coli* and ExPEC were recovered from certain other areas likely to be touched by hands (sink drain, stall lock, and cold water tap), possibly even after hand washing. Thus, the risk for transmission is not confined to toilet-associated sites or those with visible fecal contamination, so it could not be fully eliminated by careful hand washing or avoidance of fecal-appearing debris. Individuals conceivably could reduce their risk of acquiring *E. coli* from touchable sites by using hand sanitizers after exiting the restroom. Likewise, the community conceivably could benefit if disinfectant products were used to clean public restrooms for greater bio-burden reduction; however, personal hand hygiene remains crucial (33).
Regarding the isolates’ characteristics, many of these environmental E. coli strains were from phylogenetic group B2, contained multiple virulence genes, qualified molecularly as ExPEC, and/or represented classic human-associated lineages, such as STs 10, 14, 69, 95, 127, and 405 (34). Some of these lineages have recently spread widely, via as-yet-undefined mechanisms, to emerge as prominent antibiotic-resistant human pathogens (35–40). Moreover, the ST95 study isolate closely resembled multiple known human clinical ST95 isolates according to PFGE, a more discriminating genomic typing method than MLST (28). These findings further support that the isolates likely originated from human feces, have human virulence potential, and so presumably pose a threat of transmission to restroom users and the possibility of a subsequent infection.

Since several of the restroom-source ExPEC isolates were antimicrobial resistant, transmission to a restroom user conceivably could result in an antimicrobial-resistant infection (41). Additionally, to the extent that non-ExPEC antimicrobial-resistant isolates contain transmissible resistance elements, they also may pose a threat, since if they were to be acquired during restroom use, they conceivably could transfer their resistance elements to a restroom user’s (antibiotic-susceptible) endogenous intestinal ExPEC strains (42, 43).

Our study can be contrasted with prior work in the field, which includes three studies that surveyed public restrooms (10–12) and one that surveyed household restrooms (13). For geographic diversity and number of restrooms surveyed, only one prior study exceeded ours (Mendes et al.), and it did not report on E. coli distribution in relation to restroom category (10). Mkrtychyan et al. and Flores et al. surveyed 18 and 12 public restrooms, respectively (11, 12). They reported data mainly for Staphylococcus species and for general gut- and skin-associated taxa, without providing data specifically for E. coli (or did not even study E. coli). For prevalence of contamination in relation to site within restroom, these two studies identified toilet-related sites as highest risk and identified contamination also of some non-toilet sites (hand dryer systems, inner door surfaces, taps, and soap dispensers) but provided no specific details for E. coli. Thus, our study provides novel data relevant to dissemination specifically of pathogenic and antimicrobial-resistant E. coli.

The study limitations include the limited number of restrooms per category, the single-region study design (which may reduce generalizability), reliance on molecular typing to infer virulence potential, and the remote sampling period (2003). Repetition of such a study today to screen for current epidemic clonal groups such as ST131 (39) would be of interest. The study strengths in such a study today to screen for current epidemic clonal groups potential, and the remote sampling period (2003). Repetition of generalizability), reliance on molecular typing to infer virulence

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