Multiplex PCR-Based Reverse Line Blot Assay for Simultaneous Detection of 22 Virulence Genes in Uropathogenic *Escherichia coli*

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Urinary tract infections (UTIs) are among the most common bacterial infections and are responsible for significant morbidity and health care costs worldwide. The main bacterial cause of uncomplicated UTI is *Escherichia coli*, which possesses numerous virulence factors (VFs). Many studies of the pathogenesis of *E. coli* UTI have centered on VF genes. Hence, the development of better molecular assays to study VF genes would facilitate these studies. We developed a highly sensitive and specific multiplex PCR-based reverse line blot (mPCR/RLB) assay to simultaneously detect 22 VF genes of uropathogenic *E. coli* and then used it to characterize 180 isolates from nonpregnant women of child-bearing age with cystitis and 153 fecal isolates from similar-age healthy women, in regional New South Wales, Australia. The assay accurately identified all VF genes (of the 22 under study) known to be present in 30 previously characterized control strains. The detection limits were 28 ng of DNA from *E. coli* isolates and 50 CFU/ml in mock-infected urine specimens containing known concentrations of *E. coli*. Cystitis isolates (compared to the fecal isolates) showed a significantly higher prevalence of 18 individual VF genes and contained significantly more VF genes per isolate (median number, 18.5 versus 6.5 [\(P = 0.001\)]. Discordance between paired probes for a given VF gene occurred in several clinical test isolates but no reference strains and among the test isolates was associated with fecal source (10% of VF genes versus 2% for cystitis isolates [\(P < 0.001\)]. This novel mPCR/RLB method is a potentially powerful tool for investigating the prevalence and distribution of VFs in *E. coli*.

Potential nonantimicrobial methods for UTI prevention include immunization, with VFs being logical vaccine targets. Therefore, knowledge of which VFs are prevalent in specific clinical syndromes and host populations is needed to inform the selection of VFs to be targeted. Because of the large number of uropathogenic *E. coli* VF genes and isolates to be studied, rapid and inexpensive molecular methods, with high throughput, are needed. Existing multiplex PCR (mPCR) assays for studying UPEC genes detect \(\leq 7\) genes per PCR (12, 15). Primers must be sorted into multiple pools according to compatibility and amplicon length, for resolution of PCR products by size in gel electrophoresis. Therefore, to study \(\geq 30\) VF genes, one must run more than five different mPCRs and then perform gel electrophoresis on the products, which is labor-intensive, expensive, and time-consuming. Recently, capillary electrophoresis-based multiplex PCR assays have been described for the detection of several pathogens but have not been applied to UPEC VF genes or to such large numbers of targets (31).

Multiplex PCR-based reverse line blot (mPCR/RLB) assay is a well-established method for the simultaneous detection and genotyping of bacteria, fungi, and viruses (19, 20, 36). Reverse line blotting involves the covalent coupling of amine-labeled oligonucleotide probes to a carboxyl-activated nylon membrane, followed by hybridization with biotin-labeled mPCR products from the test sample and then detection of the bound PCR products using an avidin-horseradish peroxidase complex and a chemiluminescent substrate; this is somewhat analogous to an immunoblot. The aim of the present study was to develop and evaluate a mPCR/RLB assay for the detection of 22 UPEC VF genes, using a combination of previously characterized *E. coli* control strains, urine isolates from women with cystitis, and fecal isolates from similar-age healthy women in a regional area of New South Wales (NSW), Australia.
MATERIALS AND METHODS

Setting. This study was performed in the diagnostic microbiology laboratory of the Central West Pathology Service, Orange, NSW, Australia, which serves the Orange Base Hospital and other health facilities and general practices in the region.

E. coli reference strains. The reference strains used for assay development and validation included 30 UPEC isolates for which VF gene content had been documented previously using five separate mPCRs (J. R. Johnson, unpublished data). Here, these isolates were initially tested blindly using single PCR (sPCR), which detected all 22 VF genes included in the present study in ≥1 of the isolates, in precise agreement with previous testing results (Johnson, unpublished). Strains were stored in 5% glycerol in Trypticase soy broth at −70°C until further use.

Cystitis isolates. E. coli isolates were selected from urine specimens submitted to the microbiology department for culture from nonpregnant women of child-bearing age with uncomplicated cystitis. This diagnosis was based on clinical information on the request form, as confirmed by the treating medical practitioner, and required one or more of dysuria, frequent urination, and suprapubic tenderness, without fever or loin pain, and a clean-catch, mid-stream urine sample yielding ≥10⁵ CFU of E. coli per liter of urine. Quantitative urine culture was done using blood agar, MacConkey agar, and chromogenic agars, followed by conventional identification. E. coli isolates were stored in 5% glycerol in Trypticase soy broth at −70°C. Relevant information recorded included clinical symptoms, urine microscopy results, and age. Since patient identifiers were removed, patient consent was not sought. The research ethics committees of the Sydney West Area Health Service and Charles Sturt University approved the study protocol.

Fecal isolates. Volunteers were recruited by local medical practitioners from among nonpregnant women of childbearing age (18 to 45 years old) who had no UTI-associated symptoms and were receiving health care for other conditions. Each participant provided written consent. A rectal swab was collected from each consenting volunteer and processed within 10 h for isolation of E. coli, which was identified by conventional biochemical tests. One arbitrarily chosen E. coli colony per sample was analyzed.

DNA extraction. Approximately five colonies from a pure culture of E. coli on horse blood or MacConkey agar were suspended in 100 μl of digestion buffer (10 mM Tris-HCl [pH 8.0], 0.45% [vol/vol] Triton X-100, 0.45% [vol/vol] Tween 20). This suspension was boiled for 10 min, followed by rapid cooling on ice. The tubes were then centrifuged at 16,000 × g for 2 min and 400 μl of 0.5× TBE (1× TBE is composed of 45 mM Tris, 45 mM boric acid, and 1 mM EDTA [pH 8.0]) was added. After recentrifugation, the supernatants were removed and stored at −20°C for future use as template DNA.

Targets. Twenty-two VF genes were chosen as PCR targets, based on their presumed importance in UTI pathogenesis (11, 14, 17). These genes included the following: papA, F fimbria structural subunit; papC, F fimbria assembly; papEF, F fimbria tip pilins; papG, F fimbria adhesin (and alleles I, II, and III); sfaS, S fimbriae; focG, F1C fimbriae; afal/draABC, afimbrial adhesin (Dr antigen-specific fimbriae); fimH, type 1 fimbriae; hlyA, alpha hemolysin; crf1, cytotoxic necrotizing factor type 1; fyua, ferric ye- rsiniabactin receptor; intA, aerobactin receptor; ironC, catecholate sidero- phore receptor; kpsMIII, group 2 capsule; kpsMTIII, group 3 capsule; traT, serum resistance associated; ompT, outer membrane protein T (protease); bmaE, M fimbriae; gafJ, G fimbriae; and usp, uropathogenic-specific protein.

Primer and probe design. Primers used to detect VF genes were as described previously (12, 16). In addition, two sets of E. coli-specific primers were designed using the 16S-23S rRNA intergenic spacer region, which harbors both conserved and variable domains within species (see Table S1 in the supplemental material). Pairs of probes (sense and antisense) for each VF and E. coli primer were then designed based on the sequences immediately downstream or upstream of the sense and antisense primers (18). Probes were designed to have similar physical characteristics (namely, melting temperature 58 to 65°C, length, 18 to 30 bases; moderate, weak, or no secondary structure; and no dimer formation) to allow simultaneous hybridization without loss of sensitivity (18). Primers and probes sequences were checked for specificity against all sequences in GenBank using Sequencesearch in the Australian National Genomic Information Services (ANGIS) programs (www.angis.org.au).

Primers were 5′ labeled with biotin to enable detection by hybridization with a streptavidin-peroxidase substrate. Probes were 5′ labeled with an amine group to allow covalent binding to nylon membranes and to enable membrane stripping and reuse without probe loss. Primers and probes were synthesized by AuGCT Biotechnology Synthesis Laboratory, Beijing, China.

PCR amplification. sPCR and mPCR amplifications used a 30-μl reaction mixture comprising 2 μl of template DNA, 0.075 μl of each of 24 primer pairs (100 pmol μl⁻¹), 1.25 μl of deoxynucleoside triphosphate (0.125 mM each), 2.5 μl of 10× buffer (Qiagen), 3.0 μl of 25 mM MgCl₂, 0.2 μl of HotStart Taq polymerase (5 U μl⁻¹) (Qiagen), and molecular-grade water (Eppendorf) to 30 μl. Amplification was performed on a MasterCycler gradient thermocycler under cycling conditions of 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final 10-min extension at 72°C and a hold at 22°C. The amplified PCR products were processed immediately or within 1 week of storage at 4°C. For sPCR, amplified products were stained with SYBR DNA gel stain, separated electrophoretically in 2% agarose gels, and imaged using an UV transilluminator and digital capture system. To confirm the presence of previously identified VF genes, the 30 reference strains were processed in duplicate using DNA lysates prepared from separate colonies. Reference strains and test isolates were tested in parallel by both sPCR and mPCR/RLB.

RLB hybridization assay. RLB hybridization assay was performed as previously described (18). Briefly, 5′-labeled probes were covalently bound to a nylon membrane. For this, a Bio dye C membrane was cut to 15 cm², labeled, and placed in a 45-lane (slot) minibliterator (MN45; Immunetics), and 150 μl of each diluted oligonucleotide probe was added to a separate lane. After room temperature incubation and several washing steps, the membrane was ready for hybridization.

Amplified PCR products were denatured by boiling for 10 min and then cooled on ice. The probe-labeled membrane was placed in the blower with the slots perpendicular to the probe lanes. Denatured PCR products (150 μl) were added to the slots, one sample per slot, such that each sample contacted each of the probes. After hybridization for 1 h at 60°C, the membrane was washed twice at 60°C for 10 min and incubated in peroxidase-labeled streptavidin conjugate (Roche Diagnostics, Germany) at 42°C for 1 h. It was then washed at 42°C and again at 25°C. Detection involved 2 min of incubation at 21°C in a chemiluminescence blotting substrate (Amersham/GE Healthcare), and then the samples were covered with X-ray chemiluminescence film (Amersham Hyperfilm ECL; GE Healthcare) and exposed for 5 min. A clearly visible black hybridization signal was considered a positive result.

Sensitivity of the mPCR/RLB assay. The sensitivity of the mPCR/RLB assay was assessed by first preparing a series of 5× dilutions containing from 5 to 100 ng of total genomic DNA in Tris-EDTA buffer from the 30 reference E. coli strains and testing them in the mPCR/RLB assay. Second, mock-infected urine specimens were prepared by diluting suspensions of each of the 30 reference strains by from 10⁻¹ to 10⁻⁵ in fresh urine samples obtained from healthy people. Bacterial concentrations were determined by quantitative plating. Template DNA was extracted from each dilution using the Roche COBAS Amplicor extraction kit (23). In addition, to simulate field conditions, 10 clinical urine specimens that contained ≥10⁵ CFU of E. coli/liter were tested in parallel by sPCR and mPCR/RLB. Template DNA was extracted as for the mock-infected urine samples. Finally, to assess the species specificity of the mPCR/RLB assay, single isolates of each of three bacterial species closely related to E. coli (Klebsiella pneumoniae, Enterobacter aerogenes, and Shigella sonneti) were tested in duplicate.
Quality control of mPCR/RLB results. The positive control for each membrane was the mPCR product pool from a reference strain template DNA sample containing all 22 targeted VF genes. The negative control contained only master mix. Isolates with weak hybridization signals or discordant signals between the probes for a given target were retested. A test isolate’s hybridization signal was considered positive if of similar or stronger intensity than the corresponding positive control. The results for a given probe pair were considered positive if either probe yielded a positive hybridization signal.

Data analysis. Comparisons of proportions between cystitis and fecal isolates were tested using a chi-square test or the Fisher exact test. P values of <0.05 were considered statistically significant. The VF score for an isolate was the sum of pap (counted only once regardless of the number of pap genes detected) plus all other VF genes detected in the isolate. VF scores were compared by using the Mann-Whitney U test.

RESULTS
sPCR versus mPCR/RLB. Overall, in comparison to sPCR, the new mPCR/RLB assay was extremely accurate in detecting specific UPEC VF genes. The 30 reference UPEC strains were tested in duplicate (using separate colonies) by both sPCR and mPCR/RLB. One strain produced a discrepant result, for a single VF gene, between the duplicate lysates. This was resolved by reculturing the isolate from a single colony and retesting. The mPCR/RLB assay correlated precisely with sPCR, detecting 100% of the VFs known to be present in the reference strains, with no false detections (i.e., the sensitivity, specificity, and accuracy were all 100%). For each target, both probes gave concordant results. In addition, there were no cross-reactions with sPCR or mPCR/RLB, for any of the gene targets, with representatives of three non-E. coli enteric species, i.e., Klebsiella pneumoniae, Enterobacter aerogenes, and Shigella sonnei.

Among the 333 test E. coli isolates, sPCR and mPCR/RLB again exhibited 100% correspondence for gene presence or absence. However, in the mPCR/RLB assay, 7 (4%) of 180 cystitis isolates and 8 (5%) of 153 fecal isolates showed within-probe-pair discordance for ≥1 VF genes. Within-probe-pair discordance varied in frequency by both gene and source group, i.e., fecal versus cystitis (Table 1). That is, such discrepancies occurred in up to 4% of isolates for each of the adhesion genes papAH, papEF, papG, papGII-papGIII, afu/dra, fimH, sfaS, and focG. For each VF gene with such discrepancies, the proportion of isolates with discordant results was generally higher among fecal isolates (≥9% for all VFs except fimH) than cystitis isolates (≤4% for all genes except cnf1). This was most evident with the pap operon genes, each of which exhibited within-probe-pair discordance significantly more frequently among the fecal isolates. Using detected VF genes as the unit of analysis, for all VF genes combined, discordance was significantly more common among fecal isolates (10% of VF genes) than cystitis isolates (2% of VF genes) (P < 0.001).

For each isolate exhibiting within-probe-pair discordance, repeat testing by both mPCR/RLB and sPCR was done for all 22 VF genes. All previously noted mPCR/RLB within-probe-pair discrepancies were confirmed. In addition, for isolates with a single probe-positive result for a given VF gene, sPCR uniformly yielded an amplicon of the expected size for that VF gene, confirming the presence of an intact VF gene despite the mPCR/RLB within-probe-pair discordance.

Analytical sensitivity of the mPCR/RLB assay. Based on testing of serial dilutions of total DNA from the 30 reference UPEC strains, the mPCR/RLB assay’s limit of detection for each targeted VF gene was 28 ng of DNA (total amount). In mock-infected urine specimens containing ≥50 × 10^6 CFU of E. coli/liter, and in 10 naturally infected urine specimens containing ≥10^6 CFU of E. coli/liter, the assay also detected all targeted VF genes present in the sample, as defined by sPCR testing of the respective urine isolates.

Molecular epidemiology of cystitis and fecal isolates. To assess the mPCR/RLB assay’s functionality in a molecular epidemiological application, 180 E. coli urine isolates from 180 women with cystitis, and 153 E. coli rectal isolates from 153 healthy women volunteers were tested. Subject ages ranged from 18 to 45 years (median per group, 32 years). All subjects resided in the Central West region of NSW (population, ~180,000; area, 63,262 km²).

All VF genes sought were found in ≥1 cystitis isolate, ranging in prevalence from 1% (gafD and bmaE) to 96% (fimH) (Table 2), whereas four VF genes were not found in any fecal isolate. Compared to fecal isolates, cystitis isolates had a higher prevalence of 18 of the 22 studied genes, including 8 adhesin genes, 2 toxin genes, 3 siderophore genes, and 5 genes for protectins, plus a numerically higher prevalence of the remaining 4 genes. Accordingly, cystitis isolates had significantly higher VF scores than did fecal isolates (median, 18.5 versus 6.5 [P = 0.001]).

DISCUSSION
Current PCR-based assays for detection of UPEC VF genes are limited by the number of genes that can be detected in each PCR (12, 15), thereby creating a need for ongoing improvement in these assays. We successfully developed a novel mPCR/RLB assay

### Table 1: Distribution of discordant mPCR results among 180 and 153 E. coli cystitis and fecal isolates, respectively, from nonpregnant women of childbearing age

<table>
<thead>
<tr>
<th>Category</th>
<th>Virulence gene(s)</th>
<th>Cystitis isolates (n = 180)</th>
<th>Fecal isolates (n = 153)</th>
<th>Discordance, proportion of detected genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesins</td>
<td>papA/papB, papEF, papG, papGII-papGIII, afu/dra, fimH, sfaS, and focG.</td>
<td>7/68 (10)</td>
<td>2/23 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Toxins</td>
<td>cnf1</td>
<td>7/68 (10)</td>
<td>2/23 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>0/1,521 (0)</td>
<td>0/296 (0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* P values (Fisher exact test) are shown where P < 0.05 comparing cystitis with fecal isolates. NS, difference not significant. The 22 virulence factors analyzed were as follows: papA, P fimbria structural subunit; papC, P fimbria assembly; papEF, fimbria tip pilins; papG, P fimbria adhesin (and alleles I, II, and III); sfaS, S fimbiaeae; focG, F1C fimbiaeae; afu/dra, afimbrial adhesin (and Dr-binding adhesins); fimH, type 1 fimbiaeae; hlyA, hemolysin; cnf1, cytotoxic necrotizing factor type 1; fyuA, ferric yersiniabactin receptor; iutA, aerobactin receptor; iroN, catecholate siderophore receptor; papA, papB, papC, papD, papEF, papG, papGII-papGIII, afu/dra, fimH, sfaS, focG. For each VF gene with such discrepancies, the proportion of isolates with discordant results was generally higher among fecal isolates (≥9% for all VFs except fimH) than cystitis isolates (≤4% for all genes except cnf1). This was most evident with the pap operon genes, each of which exhibited within-probe-pair discordance significantly more frequently among the fecal isolates. Using detected VF genes as the unit of analysis, for all VF genes combined, discordance was significantly more common among fecal isolates (10% of VF genes) than cystitis isolates (2% of VF genes) (P < 0.001).

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DISCUSSION
Current PCR-based assays for detection of UPEC VF genes are limited by the number of genes that can be detected in each PCR (12, 15), thereby creating a need for ongoing improvement in these assays. We successfully developed a novel mPCR/RLB assay
that detects, simultaneously, 22 UPEC VF genes using a single mPCR reaction. The assay exhibited 100% sensitivity and specificity compared to sPCR in detecting the 22 targeted VF genes among 30 previously characterized reference strains and 333 clinical test isolates. It also exhibited no cross-reactivity with representatives of three bacterial species closely related to E. coli.

We tested the new assay’s practical utility by comparing the distribution of VF genes among cystitis and fecal isolates from nonpregnant women of child-bearing age in a region of NSW, Australia. Cystitis isolates contained significantly more VF genes than fecal isolates, which in turn tend to contain sequence variants of VF genes. In addition, we showed that the mPCR/RLB assay could detect VF genes directly, in naturally or mock-infected urine specimens, as demonstrated by its performance with 10 archetypal UPEC VF genes, which our novel mPCR/RLB assay should facilitate. These findings demonstrate the need for additional studies of this sort, which our novel mPCR/RLB assay should facilitate.

From a technical perspective, the mPCR/RLB assay allows the reliable, simultaneous detection of 22 known UPEC VF genes, with the potential to screen up to 43 samples for up to 43 genes simultaneously (the MiniBlotter has 43 usable lanes), using one mPCR reaction per isolate. Once pure cultures are obtained, the assay’s turnaround time is ≤24 h, including DNA extraction, mPCR setup and performance, and RLB hybridization. The RLB membrane can be prepared in ≤2 h and can be reused at least 20 times, with interval stripping (18). Although several DNA microarrays and probe hybridization techniques have been described for studying E. coli VF genes (1, 4, 9), the mPCR/RLB assay is cheaper, simpler to perform, and more flexible than DNA microarrays (8, 33) and can be used to evaluate candidate primers and probes for microarray use.

This assay is sufficiently sensitive to detect VF genes directly in urine specimens, as demonstrated by its performance with 10 artificially or naturally infected urine samples. However, its main value will be for high-throughput analysis of pure cultures of E. coli in research laboratories. It can be further developed to detect most of the currently recognized VF genes of UPEC in one mPCR reaction, thereby facilitating the study of UPEC.

**ACKNOWLEDGMENTS**

We thank staff of the Department of Microbiology at Central West Pathology services for help with the culturing and identification of E. coli isolates. We thank Brian O’Toole for help with the statistical analysis.

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**TABLE 2** Distribution of virulence-associated genes by source among 333 E. coli cystitis and fecal isolates from nonpregnant women of child-bearing age

<table>
<thead>
<tr>
<th>Category</th>
<th>Virulence gene(s)</th>
<th>Cystitis, no. (column %)</th>
<th>Fecal, no. (column %)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesins</td>
<td>afu/draBC</td>
<td>27 (15)</td>
<td>3 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>bmaE</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>sfaS</td>
<td>47 (26)</td>
<td>17 (11)</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>fimH</td>
<td>173 (96)</td>
<td>136 (89)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>focG</td>
<td>90 (50)</td>
<td>20 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>papG1</td>
<td>4 (2)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>papG11</td>
<td>59 (33)</td>
<td>17 (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>papG111</td>
<td>47 (26)</td>
<td>12 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>papA</td>
<td>108 (59)</td>
<td>54 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>papC</td>
<td>144 (80)</td>
<td>88 (57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>papEF</td>
<td>139 (77)</td>
<td>57 (37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>gfdD</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Toxins</td>
<td>cnf1</td>
<td>68 (38)</td>
<td>23 (15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>hlyA</td>
<td>122 (68)</td>
<td>29 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Siderophores</td>
<td>iutA</td>
<td>121 (67)</td>
<td>18 (12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>fyuA</td>
<td>140 (78)</td>
<td>24 (16)</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>iroN</td>
<td>122 (68)</td>
<td>44 (29)</td>
<td>&lt;0.01</td>
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<tr>
<td>Protectins</td>
<td>kpsMII</td>
<td>108 (60)</td>
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<td>kpsMTIII</td>
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<td>&lt;0.001</td>
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<tr>
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<td>ompT</td>
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<td>73 (48)</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>usp</td>
<td>131 (73)</td>
<td>38 (25)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*P values (Fisher exact test) are shown where P < 0.05 comparing cystitis with fecal isolates. NS, difference not significant. The 22 virulence factors analyzed were as follows: papA, P fimbria structural subunit; papG, P fimbria assembly; papEF, P fimbria tip pilins; papG, P fimbria adhesin (and alleles I, II, and III); sfaS, S fimbriae; focG, FIC fimbriae; afu/draBC, afimbrial adhesin (Dr-binding adhesin); fimH, type 1 fimbriae; hlyA, hemolysin; cnf1, cytotoxic necrotizing factor type I; fyuA, ferric yersiniabactin receptor; iutA, aerobactin receptor; iroN, catecholate siderophore receptor; kpsMII, group 2 capsule (with K1 and K2 variants); kpsMTIII, group 3 capsule; traT, serum resistance associated; ompT, outer membrane protein T (protease); bmaE, M fimbriae; and gfdD, (G) fimbria adhesin.

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Preceding pages follow:
REFERENCES


