Multidrug-Resistant *Escherichia coli* Clonal Groups Causing Community-Acquired Pyelonephritis

Amee R. Manges,1 Peter S. Dietrich,3 and Lee W. Riley1,2

1Division of Epidemiology, School of Public Health, 2Division of Infectious Diseases and Immunity, and 3University Health Services, Tang Health Center, University of California at Berkeley

From October 1999 through January 2000, an *Escherichia coli* clonal group (designated “CgA”) was isolated from the urine of nearly one-half of all women with urinary tract infections (UTIs) caused by trimethoprim-sulfamethoxazole (TMP-SMZ)–resistant *E. coli* in a California community. This study describes the prevalence of pyelonephritis caused by CgA in the same community. *E. coli* isolates were characterized by enterobacterial repetitive intergenic consensus (ERIC2) polymerase chain reaction (PCR), serogrouping, and pulsed-field gel electrophoresis. Fourteen (11%) of 130 women with UTIs received a diagnosis of pyelonephritis. CgA was associated with 4 (57%) of the 7 pyelonephritis cases caused by TMP-SMZ–resistant *E. coli* and was associated with none of the cases caused by TMP-SMZ–susceptible *E. coli* (P < .02). Six (86%) of these TMP-SMZ–resistant *E. coli* isolates belonged to 2 distinct ERIC2 PCR–defined clonal groups, whereas all of the TMP-SMZ–susceptible *E. coli* strains had unique fingerprints (P < .001). The prevalence of antimicrobial-resistant pyelonephritis in a community may be affected by a limited number of *E. coli* clonal groups.

The incidence of community-acquired acute pyelonephritis is estimated to be 125,000 hospitalized cases and 250,000 ambulatory cases in the United States annually [2]. It has been recognized for some time that the *Escherichia coli* strains that cause community-acquired pyelonephritis belong to a limited number of serogroups and bacterial lineages [3–7]. Recent evidence has reinforced this concept: genotypically related, multidrug-resistant *E. coli* clonal groups causing urinary tract infections (UTIs) have been identified in several different communities [8–10]. From 11 October 1999 to 31 January 2000, a single, multidrug-resistant clonal group (designated “CgA”) of *E. coli* caused 11% of all *E. coli* UTIs and 49% of all trimethoprim-sulfamethoxazole (TMP-SMZ)–resistant *E. coli* UTIs in a single California university community [10]. CgA was also identified from approximately one-third of women with UTIs caused by TMP-SMZ–resistant *E. coli* in university communities in Michigan and Minnesota [10]. The widespread distribution of a single clonal group of uropathogenic *E. coli* suggested that such strains can be spread by contaminated food products. The predominance of CgA strains among patients with UTIs in different communities also suggested that such strains may represent a particularly virulent clonal group and, therefore, may be more frequently associated with a severe manifestation of UTI, such as pyelonephritis. Johnson et al. [11] previously demonstrated that CgA strains were responsible for pyelonephritis cases in 6 different states in the United States. However, the study was not population based, and hence the prevalence of CgA–associated pyelonephritis in any single community is not known [11].

Changes in the prevalence of antimicrobial resistance among uropathogens [12, 13] and the distinct geographic differences in antimicrobial-resistant UTI rates may be attributable to newly emerging, multidrug-resistant clonal groups of uropathogenic *E. coli*, such as
CgA. We report the results of a retrospective cohort study designed to determine the prevalence and distribution of CgA and other E. coli clonal groups associated with pyelonephritis in a single university community.

METHODS

Study population. The study subjects were women presenting to a California university health service with a clinically-suspected UTI who were consecutively recruited into the study between 11 October 1999 and 31 January 2000 [13]. UTI cases were limited to the first episode experienced by the woman during the study period. The Committee for the Protection of Human Subjects, University of California at Berkeley, approved the study protocol.

Medical chart review. Retrospective medical chart review was performed for a sample of women with suspected UTI by the medical director of the clinic (P.S.D.) using a uniform chart abstraction form. A patient with a confirmed UTI was defined as a woman presenting with dysuria, frequency of urination, urgency, pyuria, or hematuria; a clinical laboratory urinalysis result consistent with UTI; and a clean-catch urine culture that yielded \( \geq 10^5 \) colony-forming units of E. coli per mL [14]. The examining clinician caring for the patient at the time of the visit established the clinical definition of pyelonephritis. Symptoms of UTI (e.g., dysuria, frequency of urination, or urgency) or pyelonephritis (e.g., subjective back pain, fever, nausea, or vomiting) were necessary but not sufficient to support the diagnosis of pyelonephritis. Additional documentation of UTI (i.e., positive results of urine analysis and/or urine culture) and costovertebral angle (CVA) tenderness were required signs to establish the clinical diagnosis of pyelonephritis. Fever (temperature, \( \geq 38^\circ\text{C} \)) at the time of the examination was included as being supportive of the diagnosis; however, absence of fever did not preclude the diagnosis of pyelonephritis. Additional information about treatment regimen, previous use of antibiotics, and history of UTI or pyelonephritis was also collected. Women with TMP-SMZ–susceptible UTI episodes were selected by their study identification number using a random number–generating scheme in SPLUS 2000 software (Insightful).

E. coli isolation. Urine samples were cultured on MacConkey agar. Lactose- and indole-positive colonies were presumptively identified as E. coli. One putative E. coli colony grown from each urine culture was arbitrarily selected for further analysis.

Antibiotic susceptibility testing. E. coli isolates were screened for susceptibility to TMP-SMZ by Etest strips (AB Biodisk). E. coli strain 25922 (American Type Culture Collection) was used as the reference strain, and NCCLS interpretive criteria were used for resistance determination [15, 16]. E. coli isolates with intermediate susceptibility were considered to be susceptible to TMP-SMZ.

ERIC2 PCR fingerprinting. All E. coli isolates recovered from patients with clinically confirmed UTIs caused by TMP-SMZ–resistant E. coli and a random sample of E. coli isolates recovered from patients with UTIs caused by TMP-SMZ–susceptible E. coli were screened by the enterobacterial repetitive intergenic consensus (ERIC2) PCR fingerprinting assay [17–21], as described elsewhere [22]. Different isolates exhibiting indistinguishable ERIC2 electrophoretic banding patterns, as assessed by visual inspection, were considered to belong to a single clonal group. ERIC2 pattern A was defined by 4 prominent bands of approximate sizes 1145, 1029, 908, and 720 bp; isolates exhibiting this pattern were considered members of CgA. An isolate obtained from a patient with pyelonephritis (CFT073 [E. coli O6:K2:H1]) [23] (provided by Dr. Harry Mobley, University of Maryland) and a prototype CgA strain (E. coli SEQ102 ATCC BAA-457), identified in the original study [10], were used as reference strains in the ERIC2 PCR assays.

PFGE analysis. The standardized protocol for strain subtyping of E. coli (O157:H7) by PFGE, as established by the Centers for Disease Control and Prevention [24], was used to further genotype the subset of E. coli isolates that were indistinguishable by ERIC2 PCR fingerprinting. XbaI-digested DNA was electrophoresed in the CHEF DR-II apparatus (Bio-Rad). The criteria for strain relatedness established by Tenover et al. [25] were used to compare PFGE profiles.

Serotyping. O:H serotyping was performed on E. coli isolates at the E. coli Reference Center at University Park, Pennsylvania. Strains that were motile but nonreactive with available O or H antisera were designated as nontypeable (O[nt], and H[nt], respectively), and nonmotile strains as were designated as “NM.”

Statistics. ORs and 95% CIs were estimated by Stata, version 7.0 (Stata). Comparison of proportions was performed by Fisher’s exact test or \( \chi^2 \) test.

RESULTS

From 11 October 1999 to 31 January 2000, 228 women (median age, 22 years; range, 17–68 years) presented to a California university health service with 255 clinically suspected acute UTI episodes. From this population, 146 women were selected for detailed medical chart review, including all 49 women infected with TMP-SMZ–resistant E. coli and 97 randomly selected women infected with TMP-SMZ–susceptible E. coli.

Medical chart review. Of 146 women who presented to the health care service with symptoms of UTI, 130 (89%) met the clinical definition of patients with UTI (42 from the TMP-SMZ–resistant group and 88 from the TMP-SMZ–susceptible group). Of these 130 women, 14 (11%) had pyelonephritis; 7
women (17%) were infected with TMP-SMZ–resistant *E. coli*, and 7 women (8%) were infected with TMP-SMZ–susceptible *E. coli*. There were no repeated episodes of pyelonephritis reported in the medical charts of these 14 women during the study period. No patients were pregnant or had a documented medical history of diabetes or other immune disorder.

The mean age of the women with pyelonephritis was 22 years (range, 18–45 years). The mean age did not differ significantly between the TMP-SMZ–resistant group (mean age, 21 years) and the TMP-SMZ–susceptible group (mean age, 23 years), nor did the mean age differ between women with pyelonephritis (mean age, 21 years) and those with cystitis only (mean age, 23 years). Forty-five (35%) of the 130 women with UTI had a documented history of UTI. There was no association between having had a UTI in the previous 6 months and the risk of pyelonephritis (OR, 1.39; 95% CI, 0.47–4.12). Use of antibiotics in the previous 6 months was noted on the medical charts of 33 (26%) of the participants. However, there was no association between the use of antibiotics in the prior 6 months and the development of pyelonephritis (OR, 1.16; 95% CI, 0.39–3.46), nor was there an association between previous use of antibiotics and the development of drug-resistant pyelonephritis.

**ERIC2 PCR.** ERIC2 PCR analyses of the isolates obtained from patients with pyelonephritis identified 2 clonal groups (figure 1). Four isolates (isolates 203, 220, 320, and 486) exhibited ERIC2 PCR patterns indistinguishable from the prototype CgA strain (figure 1). Two isolates, designated CgB (isolates 61 and 423), produced indistinguishable ERIC2 PCR patterns (figure 1). These 2 clonal groups were responsible for 6 (86%) of the 7 TMP-SMZ–resistant *E. coli* isolates associated with cases of pyelonephritis. None of the TMP-SMZ–susceptible isolates produced ERIC2 PCR patterns that were indistinguishable from one another.

*E. coli* strains with the ERIC2 PCR CgA pattern were identified in 23 (49%) of the 47 cases of UTI associated with TMP-SMZ–resistant *E. coli* isolates and were identified in 4 (57%) of the 7 cases of pyelonephritis associated with TMP-SMZ–resistant *E. coli* isolates (*P = .69*). There was no statistically significant association between *E. coli* strains with the CgA pattern and development of pyelonephritis (OR, 1.3; 95% CI, 0.42–4.05). In the original study, only a single putative CgB strain was identified from among 49 TMP-SMZ–susceptible isolates, and none was identified among 47 TMP-SMZ–resistant isolates.

**PFGE analysis.** XbaI PFGE analysis was conducted on isolates producing CgA and CgB ERIC2 PCR patterns (figure 2). Three CgA variants (A1, A2, and A3) were identified by PFGE. These variants differed from the prototype CgA PFGE pattern by 1–4 bands and are therefore considered to be closely related according to Tenover’s criteria (lanes 3–6 in figure 2) [25]. The 2 CgB isolates were indistinguishable from one another by PFGE but differed from the prototype CFT073 strain by ≥6 bands.

![Figure 1](https://academic.oup.com/cid/article-abstract/38/3/329/290666)

**Figure 1.** Enterobacterial repetitive intergenic consensus PCR of *Escherichia coli* isolates associated with pyelonephritis (lanes 3–16). Lane 1, 1 KB marker; lane 2, prototype clonal group A (CgA) strain used as positive control; lanes 5–7 and 9, CgA strains; lanes 3 and 8, clonal group B (CgB) strains.
Figure 2. XbaI PFGE of isolates associated with pyelonephritis that were found to be indistinguishable by enterobacterial repetitive intergenic consensus. Lanes 1 and 10, λ ladder; lane 2, control clonal group A (CgA); lanes 3–6, CgA; lane 7, reference strain CFT073; lanes 8 and 9, clonal group B (CgB) isolates obtained from patients with pyelonephritis.

Serotyping. In our previous study, CgA strains were found to belong to serogroups O11, O77, O17, and O73. The 4 CgA isolates responsible for pyelonephritis during the study period were determined to be serotype O11:H(nt) (isolates 203, 320, and 486) and serotype O77:H(nt) (isolate 220). Both of the CgB isolates were determined to be O4:H5w (isolates 61 and 423) (table 1). The group of TMP-SMZ–susceptible isolates associated with cases of pyelonephritis included 5 different serotypes: O2, O16, O25, O120, and O166 (table 1).

DISCUSSION
The discovery of a multidrug-resistant clonal group (CgA) of uropathogenic E. coli [10] causing a large proportion of community-acquired UTI in a single community led us to investigate whether the same clonal group also contributed to the occurrence of multidrug-resistant community-acquired pyelonephritis. Previously, Johnson et al. [11] found that CgA E. coli was associated with cases of pyelonephritis in several areas of the United States, but they did not determine the prevalence of CgA E. coli in any specific community. In our study, which consecutively examined all UTI episodes diagnosed at a university health service over a period of 4 months, CgA did not produce a proportionately greater number of the pyelonephritis cases than of the cystitis cases. There was no statistically significant association between CgA and the development of pyelonephritis, suggesting that this strain is not necessarily more likely to cause ascending kidney infections than any other infection. However, the small size of the study sample may have limited our power to detect an association.

Of the isolates associated with pyelonephritis, there was considerably less PFGE genotype diversity among those that were TMP-SMZ resistant than among those that were TMP-SMZ susceptible. Among the TMP-SMZ–resistant E. coli strains associated with pyelonephritis, only 3 genotypically distinct strains were detected, whereas all of the strains in the TMP-SMZ–susceptible group produced unique ERIC2 PCR and PFGE patterns. This observation suggests that a limited number of circulating multidrug-resistant E. coli clonal groups contribute substantially to the prevalence of community-acquired drug-resistant cystitis and pyelonephritis. It is possible that drug resistance determinants preferentially enter a limited set of E. coli hosts. It is also possible, however, that the appearance of nearly indistinguishable E. coli strains in multiple women in a single community represents local dissemination of the mul-
Patterns and therefore were not evaluated by XbaI isolates associated with pyelonephritis.

References

Acknowledgment

We thank the staff of the clinical laboratory at the University of California at Berkeley, University Health Services, Tang Health Center, for their assistance in enrolling subjects and collecting samples.

Table 1. Genotypic and serogroup characterization of Escherichia coli isolates associated with pyelonephritis.

<table>
<thead>
<tr>
<th>Resistance to TMP-SMZ, isolate</th>
<th>ERIC2 PCR pattern</th>
<th>Xbal PFGE pattern&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>A</td>
<td>A1</td>
<td>O11:H&lt;nt&gt;</td>
</tr>
<tr>
<td>320</td>
<td>A</td>
<td>A1</td>
<td>O11:H&lt;nt&gt;</td>
</tr>
<tr>
<td>486</td>
<td>A</td>
<td>A2</td>
<td>O11:H&lt;nt&gt;</td>
</tr>
<tr>
<td>220</td>
<td>A</td>
<td>A3</td>
<td>O77:H&lt;nt&gt;</td>
</tr>
<tr>
<td>61</td>
<td>B</td>
<td>B</td>
<td>O4:H5w</td>
</tr>
<tr>
<td>423</td>
<td>B</td>
<td>B</td>
<td>O4:H5w</td>
</tr>
<tr>
<td>141</td>
<td>C</td>
<td>C</td>
<td>OX19:H&lt;nt&gt;</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>D</td>
<td>...</td>
<td>O2</td>
</tr>
<tr>
<td>40</td>
<td>E</td>
<td>...</td>
<td>O2</td>
</tr>
<tr>
<td>51</td>
<td>F</td>
<td>...</td>
<td>O16</td>
</tr>
<tr>
<td>103</td>
<td>G</td>
<td>...</td>
<td>O2</td>
</tr>
<tr>
<td>142</td>
<td>H</td>
<td>...</td>
<td>O25</td>
</tr>
<tr>
<td>153</td>
<td>I</td>
<td>...</td>
<td>O166</td>
</tr>
<tr>
<td>329</td>
<td>J</td>
<td>...</td>
<td>O120</td>
</tr>
</tbody>
</table>

**NOTE.** ERIC2, enterobacterial repetitive intergenic consensus; nt, not typeable; TMP-SMZ, trimethoprim-sulfamethoxazole.

<sup>a</sup> The TMP-SMZ-susceptible strains each produced different ERIC2 PCR patterns and therefore were not evaluated by Xbal PFGE.

tidrug-resistant clonal group by a common vehicle, such as contaminated food items.

An alternative hypothesis that may be important to address is whether certain clonal groups associated with UTI possess biological factors in addition to drug-resistance that contribute to their successful spread in the community [26]. A detailed and thorough investigation of representative drug-susceptible E. coli strains associated with UTI may help determine whether the dissemination of specific clonal groups is mostly due to the selective advantage of drug resistance or whether there are other determinants that independently promote clonal spread and that may be enhanced by the acquisition of drug resistance genes.

Acknowledgment

We thank the staff of the clinical laboratory at the University of California at Berkeley, University Health Services, Tang Health Center, for their assistance in enrolling subjects and collecting samples.

References


