WIDESPREAD DISTRIBUTION OF URINARY TRACT INFECTIONS CAUSED BY A MULTIDRUG-RESISTANT ESCHERICHIA COLI CLONAL GROUP

AMEE R. MANGES, M.P.H., JAMES R. JOHNSON, M.D., BETSY FOXMAN, PH.D., TIMOTHY T. O’BRYAN, KATHLEEN E. FULLERTON, M.P.H., AND LEE W. RILEY, M.D.

ABSTRACT

Background The management of urinary tract infections is complicated by the increasing prevalence of antibiotic-resistant strains of Escherichia coli. We studied the clonal composition of E. coli isolates that were resistant to trimethoprim–sulfamethoxazole from women with community-acquired urinary tract infections.

Methods Prospectively collected E. coli isolates from women with urinary tract infections in a university community in California were evaluated for antibiotic susceptibility, O:H serotype, DNA fingerprinting, pulsed-field gel electrophoretic pattern, and virulence factors. The prevalence and characteristics of an antibiotic-resistant clone were evaluated in this group of isolates and in those from comparison cohorts in Michigan and Minnesota.

Results Fifty-five of the 255 E. coli isolates (22 percent) from the California cohort were resistant to trimethoprim–sulfamethoxazole as well as other antibiotics. There was a common pattern of DNA fingerprinting, suggesting that the isolates belonged to the same clonal group (clonal group A), in 28 of 55 isolates with trimethoprim–sulfamethoxazole resistance (51 percent) and in 2 of 50 randomly selected isolates that were susceptible to trimethoprim–sulfamethoxazole (4 percent, P<0.001). In addition, 11 of 29 resistant isolates (38 percent) from the Michigan cohort and 7 of 18 (39 percent) from the Minnesota cohort belonged to clonal group A. Most of the clonal group A isolates were serotype O11:H(n) or O77:H(n), with similar patterns of virulence factors, antibiotic susceptibility, and electrophoretic features.

Conclusions In three geographically diverse communities, a single clonal group accounted for nearly half of community-acquired urinary tract infections in women that were caused by E. coli strains with resistance to trimethoprim–sulfamethoxazole. The widespread distribution and high prevalence of E. coli clonal group A have major public health implications.

Copyright © 2001 Massachusetts Medical Society.
Methods

Study Cohorts

The study subjects included three cohorts of women (defined as those who were at least 17 years old) with urinary tract infections and a comparison group of healthy women whose stool specimens were analyzed to identify the E. coli isolates. The study was approved by the Committee for Protection of Human Subjects of the University of California at Berkeley; informed consent was not obtained, because the study involved neither direct interviews nor chart reviews. The California cohort consisted of women with symptoms of urinary tract infection who were seen at a university health service and were consecutively enrolled in the study between October 11, 1999, and January 31, 2000. A case of E. coli urinary tract infection was defined as symptoms suggestive of infection and a culture of a clean-catch urine specimen with more than 10^5 colony-forming units of E. coli per milliliter. 7

The two comparison cohorts were women with uncomplicated cystitis who were seen at university health services in Minnesota and Michigan. For the Minnesota cohort, we analyzed all E. coli urinary isolates that were resistant to trimethoprim—sulfamethoxazole and 20 isolates that were susceptible to the combined drugs. The isolates were obtained from students with uncomplicated cystitis who were enrolled in a university-based study between September 1996 and May 1999.

Another comparison cohort consisted of 41 healthy residents at the University of California at Berkeley; informed consent was not obtained, because the study involved neither direct interviews nor chart reviews. The New England Journal of Medicine

Pulsed-Field Gel Electrophoresis

The standardized protocol for molecular subtyping of E. coli (O157:H7) by pulsed-field gel electrophoresis (PFGE), as established by the Centers for Disease Control and Prevention, 14 was used to identify a subgroup of the E. coli isolates that were indistinguishable by ERIC2 fingerprinting. XhoI-digested DNA was electrophoresed in the CHEF DR-II apparatus (Bio-Rad, Hercules, Calif.). Isolates that had indistinguishable PFGE patterns with the use of XbaI were reanalyzed with a second enzyme, AscI. The criteria for strain relatedness established by Tenover et al. were used to compare PFGE patterns. 19 The most frequently identified pattern among the California isolates was designated XbaI PFGE pattern A.

Virulence Genotyping

Genotypes for 31 putative virulence factors and the 12 known papA alleles were determined by multiplex PCR assays, supplemented by dot blot hybridization, as previously described. 20-22

Serotyping

Serotyping was performed on E. coli isolates at the E. coli reference center in University Park, Pennsylvania. Strains that were motile but that did not react with O or H antisera were classified as nontypable (nt) — O(nt) and H(nt), respectively.

Conjugation Experiments

Selected wild-type isolates that were resistant to trimethoprim—sulfamethoxazole were conjugated with nalidixic acid—resistant, lactose-negative laboratory strain JM109, 23 according to a standard procedure. 24,25 and plated on Luria—Bertani agar 26 containing trimethoprim—sulfamethoxazole (16 and 350 µg per milliliter, respectively) and nalidixic acid (20 µg per milliliter). The putative transconjugants were tested for susceptibility to 18 antimicrobial agents by a disk-diffusion method to identify markers of resistance to additional antimicrobial agents.

Statistical Analysis

Chi-square analysis with the use of generalized estimating equations based on the PROC GENMOD procedure in SAS (version 8.01, SAS Institute, Cary, N.C.) was used to account for clustered sampling in the California cohort, which included women with multiple urinary tract infections. An exchangeable correlation structure was used in the analysis. The chi-square test or Fisher’s exact test was also used in analyses in which the data were restricted to the first (primary) episode of urinary tract infection during the study period.

Results

Prevalence of Trimethoprim—Sulfamethoxazole Resistance

A total of 228 women (median age, 22 years) seen at the university health service in California had symptoms suggestive of acute urinary tract infection. A total of 505 consecutive urine samples from these women were cultured, 255 of which yielded more than 10^5 colony-forming units of E. coli per milliliter. Twenty-four women had repeated urinary tract infections during the study period: 21 (9 percent) had two infections, and 3 (1 percent) had three infections.

Fifty-five of the 255 E. coli isolates (22 percent) were resistant to trimethoprim—sulfamethoxazole (Table 1). All 55 resistant isolates, which were from 47 women, and 50 susceptible isolates, from 49 other women, were selected for further analysis. In the Minnesota cohort, 18 of 82 E. coli isolates (22 percent) were resistant to trimethoprim—sulfamethoxazole. All

DNA Fingerprinting

For each of the three cohorts of women with urinary tract infections, all isolates that were resistant to trimethoprim—sulfamethoxazole and subgroups of susceptible isolates, selected either randomly (in California and Michigan) or arbitrarily (in Minnesota), were screened with the enterobacterial repetitive intergenic consensus (ERIC2) PCR fingerprinting assay, 11-15 as previously described. 14 Isolates with fingerprints that were indistinguishable on visual inspection were considered to belong to a single clonal group. Pattern A was defined by four predominant bands that were approximately 1145, 1029, 908, and 720 bp; isolates exhibiting this pattern were considered to be members of clonal group A. A pyelonephriticogenic isolate CFT073 (O6:K2:H1), 16 provided by Dr. Harry Mobley at the University of Maryland, was used as a reference strain for each ERIC2 PCR run.

Antibiotic Susceptibility Testing

E. coli isolates were screened for susceptibility to trimethoprim—sulfamethoxazole with the use of E-test strips (AB Biodisk, Solna, Sweden) in California, the MicroScan system (Dade Behring, Sacramento, Calif.) in Michigan, and a standard disk-diffusion assay 9 in Minnesota. E. coli strain 25922 (from the American Type Culture Collection) was used as the reference strain. Susceptibility to 18 additional antimicrobial agents was assessed for selected isolates by the disk-diffusion method to identify markers of resistance to additional antimicrobial agents.

Isolation of E. coli

In California and Minnesota, urine samples were cultured on MacConkey agar. Colonies that were positive for lactose and indole were presumptively identified as E. coli. Culture methods for the Michigan cohort have been described previously. 8 One putative E. coli colony from each urine culture was arbitrarily selected for further analyses. Five E. coli colonies per monthly fecal sample were selected for DNA fingerprinting with a polymerase-chain-reaction (PCR) assay.

DNA Fingerprinting

For each of the three cohorts of women with urinary tract infections, all isolates that were resistant to trimethoprim—sulfamethoxazole and subgroups of susceptible isolates, selected either randomly (in California and Michigan) or arbitrarily (in Minnesota), were screened with the enterobacterial repetitive intergenic consensus (ERIC2) PCR fingerprinting assay, 11-15 as previously described. 14 Isolates with fingerprints that were indistinguishable on visual inspection were considered to belong to a single clonal group. Pattern A was defined by four predominant bands that were approximately 1145, 1029, 908, and 720 bp; isolates exhibiting this pattern were considered to be members of clonal group A. A pyelonephriticogenic isolate CFT073 (O6:K2:H1), 16 provided by Dr. Harry Mobley at the University of Maryland, was used as a reference strain for each ERIC2 PCR run.

Antibiotic Susceptibility Testing

E. coli isolates were screened for susceptibility to trimethoprim—sulfamethoxazole with the use of E-test strips (AB Biodisk, Solna, Sweden) in California, the MicroScan system (Dade Behring, Sacramento, Calif.) in Michigan, and a standard disk-diffusion assay 9 in Minnesota. E. coli strain 25922 (from the American Type Culture Collection) was used as the reference strain. Susceptibility to 18 additional antimicrobial agents was assessed for selected isolates by the disk-diffusion method, 10 with the use of standard interpretive criteria. 7 Intermediate susceptibility was interpreted as full susceptibility.
Clonal pattern A — no./

ERIC2 PCR Fingerprinting (Table 1).

(From 20 other women) were selected for further isolates (from 28 women) and 20 susceptible isolates further analysis. In the Michigan cohort, 29 resistant susceptible isolates (from 20 women), were selected for 18 resistant isolates (from 18 women), plus 20 susceptible isolates (from 20 women), were selected for further analysis. In the Michigan cohort, 29 resistant isolates (from 28 women) and 20 susceptible isolates (from 20 other women) were selected for further analysis (Table 1).

ERIC2 PCR Fingerprinting

In the California cohort, 28 of the 55 E. coli isolates that were resistant to trimethoprim–sulfamethoxazole (51 percent) exhibited the same four-band ERIC2 pattern and were therefore identified as belonging to clonal group A (Fig. 1 and Table 1), as compared with only 2 of 50 randomly selected susceptible isolates (4 percent; P<0.001, with adjustment for clustered sampling). In the Minnesota cohort, 7 of 18 isolates that were resistant to trimethoprim–sulfamethoxazole exhibited clonal pattern A (39 percent), as compared with only 1 of 20 susceptible isolates (5 percent, P=0.02). Likewise, in the Michigan cohort, 11 of 29 isolates that were resistant to trimethoprim–sulfamethoxazole exhibited clonal pattern A (38 percent), as compared with none of the 20 susceptible isolates (P=0.001 by Fisher’s exact test) (Fig. 1 and Table 1).

ERIC2 PCR was also used to evaluate 925 colonies of E. coli isolated from the stool samples obtained from the group of 41 healthy persons. Clonal group A isolates were identified in one or more fecal samples from 5 of the 18 male subjects (28 percent) and 10 of the 23 female subjects (43 percent). Thirteen of the 26 clonal group A isolates (50 percent) were resistant to trimethoprim–sulfamethoxazole (data not shown).

Results of PFGE Analysis

XbaI PFGE analysis was performed on 38 clonal group A isolates (23 from the California cohort and a total of 15 from the other two cohorts). Eleven of the California isolates (48 percent) had indistinguishable PFGE findings (designated XbaI PFGE pattern A); 7 isolates (30 percent) differed from this pattern by only one to three bands, 4 (17 percent) by four to six bands, and l (4 percent) by more than six bands (Fig. 2 and Table 2). ApaII PFGE performed on six of the isolates with XbaI PFGE pattern A showed that four were indistinguishable, and the other two differed by only two bands. Although none of the isolates from Michigan and Minnesota had patterns that were identical to XbaI PFGE pattern A of the isolates from California, two of the eight Michigan isolates and all seven Minnesota isolates had patterns that were moderately or very similar (Table 2).

Antibiotic Susceptibility

Clonal group A isolates from all three cohorts had a significantly higher prevalence of resistance to multiple antibiotics than did the comparison strains (Table 3). A pattern of resistance to six drugs was found in 8 of 19 clonal group A strains (42 percent) but in none of the other strains (P<0.001).

Serotyping

Of 41 representative clonal group A isolates from the three cohorts with urinary tract infections, 32 (78 percent) exhibited one of two distinctive serotypes: O11:H(nt) or O77:H(nt). Serotype O11:H(nt) predominated among the California isolates but was rare or nonexistent among the Michigan and Minnesota isolates, whereas serotype O77:H(nt) accounted for large proportions of the Michigan and Minnesota isolates (Table 2). Three of the remaining clonal group A isolates (7 percent of the total) exhibited a unique O antigen, and 6 (15 percent of the total) could not be typed. Of the 13 clonal group A fecal isolates that were serotyped, 9 (69 percent) were O11:H(nt), 2 were other serotypes, and 2 could not be typed.

Virulence Genotyping

Clonal group A was characterized by a distinctive profile of virulence factors that included a complete copy of pap, the F16 papa allele, paga allele II, intA, kpsMT II, and rvaT, and the absence of sfa/foc, sfa/dra, bly, cnf, iroN, iss, and malX (Table 3). This profile, which corresponds with that previously documented for the O15:K52:H1 clonal group,26,27 was consistent among isolates from all three cohorts, whether or not the strains were resistant to trimethoprim–sulfamethoxazole.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>CALIFORNIA</th>
<th>MICHIGAN</th>
<th>MINNESOTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates</td>
<td>255</td>
<td>NA</td>
<td>82</td>
</tr>
<tr>
<td>No. of women</td>
<td>228</td>
<td>NA</td>
<td>82</td>
</tr>
<tr>
<td>TMP-SMX–resistant isolates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>— no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isolates</td>
<td>55 (22)</td>
<td>67</td>
<td>18 (22)</td>
</tr>
<tr>
<td>Isolates from primary episode</td>
<td>47 (21)</td>
<td>66</td>
<td>—</td>
</tr>
<tr>
<td>Clonal pattern A — no./total no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isolates</td>
<td>28/55 (51)</td>
<td>11/29 (38)‡</td>
<td>7/18 (39)</td>
</tr>
<tr>
<td>Isolates from primary episode</td>
<td>23/47 (49)</td>
<td>10/28 (56)</td>
<td>—</td>
</tr>
</tbody>
</table>

*In California and Michigan, some of the women from whom isolates were obtained had recurrent episodes of urinary tract infection. Information for all isolates and for isolates from the primary episode is presented. In Minnesota, only one isolate was obtained from each woman. Fingerprinting was analyzed with the use of the enterobacterial repetitive intergenic consensus polymerase-chain-reaction assay. NA denotes not available, and TMP-SMX trimethoprim–sulfamethoxazole.

†Clonal pattern A was defined by four predominant bands that were approximately 1145, 1029, 908, and 720 bp.

‡A subgroup of 29 TMP-SMX–resistant isolates was selected for analysis of fingerprinting.
Conjugation

JM109 transconjugants were derived from five clonal group A isolates in California that were resistant to trimethoprim–sulfamethoxazole. The transconjugants acquired resistance not only to trimethoprim–sulfamethoxazole but also to ampicillin, tetracycline, chloramphenicol, and streptomycin (data not shown).

DISCUSSION

We found that 11 percent of uncomplicated, community-acquired urinary tract infections in women seen during a four-month period at a university health service in California were caused by a single, previously unrecognized clonal group of multidrug-resistant E. coli, clonal group A. This clonal group accounted for 51 percent of urinary tract infections caused by E. coli strains that were resistant to trimethoprim–sulfamethoxazole at this health center and for high proportions of such isolates from women seen at university health centers in Michigan and Minnesota (38 and 39 percent, respectively). Although a limited number of isolates and locations were surveyed, these data suggest that a single E. coli clonal group may have contributed to the recently documented increase in antibiotic resistance among E. coli isolates from patients with urinary tract infections in some parts of the United States.\(^3,4\) In the California cohort, if it had not been for this drug-resistant clonal group, the proportion of all primary episodes of urinary tract infections caused by E. coli strains that were resistant to trimethoprim–sulfamethoxazole would have been 11 percent instead of 21 percent.

That the E. coli isolates with resistance to trimethoprim–sulfamethoxazole represent a phylogenetically distinct clonal group was suggested by their similarities to one another and their differences from other strains with respect to five characteristics: the ERIC2 fingerprinting pattern; O:H serotype, with the unusual serotypes O77:H(nt) or O11:H(nt) predominating among members of clonal group A; PFGE profiles; virulence-factor profiles; and patterns of antibiotic susceptibility. Clonal group A isolates accounted for 11 percent of all urinary tract infections in California and for 9 percent in Minnesota, which represents a substantial prevalence for a single E. coli clonal group.\(^20,27\) This finding indicates that clonal group A contributes substantially not only to drug-resistant urinary tract infections but also to urinary tract infections in general.

Clonal group A appears to represent a new lineage of multidrug-resistant, uropathogenic E. coli rather than an established virulent clone that has acquired antibiotic resistance. In previous studies of E. coli iso-
lates from urinary tract infections, O11 and O77 somatic antigens have not been noted, and serogroups O1, O2, O4, O6, O7, O16, O18, O25, and O75 have consistently predominated, accounting for up to 81 percent of isolates.\textsuperscript{27-30}

Although urinary tract infection is usually regarded as a sporadic disease caused by organisms from the host’s own fecal flora, transmission of \textit{E. coli} between sex partners and household members has been reported.\textsuperscript{31,32} Nosocomial outbreaks of \textit{E. coli} pyelonephritis have also been documented.\textsuperscript{33} A community-wide outbreak of urinary tract infection due to a single strain occurred in South London in 1986 and 1987.\textsuperscript{5} In this outbreak, isolates of \textit{E. coli} O15:K52:H1 that exhibited a distinctive multidrug-resistance phenotype similar to that of the clonal group A isolates in our study were recovered from community-acquired cases of pyelonephritis and bacteremia. This serotype was subsequently identified as a cause of endemic urinary tract infection and bacteremia in other European

<table>
<thead>
<tr>
<th>Roteotype and PFGE Pattern</th>
<th>California</th>
<th>Michigan</th>
<th>Minnesota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype</td>
<td>23</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>O11:H(nt)</td>
<td>14 (61)</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>O77:H(nt)</td>
<td>6 (26)</td>
<td>8 (73)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Other or nontypable</td>
<td>3 (13)</td>
<td>3 (27)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>PFGE pattern</td>
<td>23</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Pattern A</td>
<td>11 (48)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pattern that differed from pattern A</td>
<td>7 (30)</td>
<td>2 (25)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>By 1–3 bands</td>
<td>4 (17)</td>
<td>0</td>
<td>2 (29)</td>
</tr>
<tr>
<td>By 4–6 bands</td>
<td>1 (4)</td>
<td>6 (75)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Serotyping was performed on 23 of the 28 isolates in California that were resistant to trimethoprim–sulfamethoxazole and that exhibited pattern A on fingerprinting and on all such isolates in Michigan and Minnesota. Isolates from recurrent episodes of infection are included in the data for California and Michigan. PFGE was performed with XbaI-digested DNA; nt denotes nontypable.
The apparent emergence of clonal group A in three states suggests either the simultaneous expansion in multiple locations of a previously introduced endemic clonal group, possibly as a consequence of increasing antimicrobial selection pressure, or the recent introduction of the clonal group into new environments. The high degree of genetic homogeneity among the California isolates favors the latter hypothesis. One possible explanation for the observed temporal and geographic clustering of a single *E. coli* PFGE type (pattern A) in California is that the strains were spread by one or more contaminated products ingested by community residents, which is similar to the way an enteric pathogen, such as *E. coli* O157:H7, causes community-wide outbreaks after being disseminated by the consumption of contaminated foods.18,37 If a
large proportion of urinary tract infections caused by drug-resistant strains of \textit{E. coli} in a community were due to the ingestion of widely consumed, contaminant foods, this would constitute a serious and novel public health problem.

Clonal group A isolates were resistant to antibiotics that are commonly used in the outpatient setting to treat urinary tract infection and a wide variety of other infections. Resistance to these agents may persist with the use of any of the antibiotics represented in the resistance phenotype. Additional studies are needed to establish the geographic and temporal distribution of this emerging \textit{E. coli} clonal group and to determine whether it is spread by the ingestion of contaminated foods.

Supported by grants from the Alameda County District Attorney General’s Fund (to Dr. Riley); the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (to Dr. Johnson); the National Institutes of Health (DK 47504, to Dr. Johnson, and DK 35368, to Dr. Foxman); and the National Research Initiative Competitive Grants Program and the Department of Agriculture (00-35212-9408, to Dr. Foxman); and the National Research Initiative Competitive Grants Program and the Department of Agriculture (00-35212-9408, to Dr. Foxman); and the National Research Initiative Competitive Grants Program and the Department of Agriculture (00-35212-9408, to Dr. Foxman).

We are indebted to Peter Districh, M.D., M.P.H., and the staff of the clinical laboratory at the University of California, Berkeley, Health Services, for their assistance in enrolling patients and collecting samples; to David Wang, M.D., and the nursing and laboratory staff at the Minnesota clinic; to Sharon Abbott, Jan O’Connell, and Jim Ware at the California Department of Health for technical assistance with PFGE; and to Brent Sugimoto, M.P.H., for assistance in collecting and analyzing the final samples.

REFERENCES

11. Johnson JR, Moseley SL, Roberts PL, Stamm WE. 