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The changing prevalence of drug-resistant *Escherichia coli* clonal groups in a community: evidence for community outbreaks of urinary tract infections

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SUMMARY

A multidrug-resistant clonal group (CgA) of *Escherichia coli* was shown to cause half of all trimethoprim–sulphamethoxazole (TMP–SMZ)-resistant urinary tract infections (UTIs) in a college community between October 1999 and January 2000. This second study was conducted to determine the fate of CgA. Urine *E. coli* isolates from women with UTI, collected between October 2000 and January 2001, were tested for antibiotic susceptibility, O serogroup, ERIC2 PCR and DNA macrorestriction patterns using pulsed-field gel electrophoresis. The proportion of UTIs caused by CgA declined by 38% ($P<0{\cdot}001$) but the prevalence of resistance to TMP–SMZ did not change. Six additional clonal groups were identified and these were responsible for 32% of TMP–SMZ-resistant UTIs. The temporal decline in the proportion of UTIs caused by CgA provides evidence that CgA caused a community outbreak of UTI. The fluctuation and occurrence of other *E. coli* clonal groups in this community suggest that a proportion of community-acquired UTIs may be caused by *E. coli* disseminated from one or more point sources.

INTRODUCTION

Urinary tract infection (UTI) is not usually thought of as a disease associated with community-wide outbreaks. However, certain *Escherichia coli* lineages have exhibited epidemic behaviour. During 1987–1988, *E. coli* O15:K52:H1 caused an outbreak of community-acquired cystitis, pyelonephritis, and septicaemia in South London [1]. The distinctive antibiotic resistance profile of this clonal group contributed to its recognition in Europe and recent reports have documented its presence in other areas of Europe and the United States [2–4]. In 2001, we reported that a single, multidrug-resistant clonal group (designated CgA) of *E. coli* caused 11% of all *E. coli* UTIs and 49% of all trimethoprim–sulphamethoxazole (TMP–SMZ)-resistant *E. coli* UTIs in a single California community over a 4-month period [5]. Members of this clonal group were also responsible for drug-resistant UTIs in university communities in Michigan and Minnesota, a community in Colorado

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[6], as well as pyelonephritis in several states [7]. CgA was identified in 26% of 495 animal and environmental *E. coli* isolates belonging to serogroups O11, O17, O73, and O77, by enterobacterial repetitive intergenic consensus (ERIC2) PCR genotyping and CgA strains appeared to be over-represented among poultry isolates [8]. Cumulative evidence therefore suggests that increases in drug-resistant uropathogens, and the distinct geographical differences in the prevalence of antimicrobial-resistant UTI may be attributable to a limited number of newly emerging, multidrug-resistant clonal groups of uropathogenic *E. coli*, such as CgA [9, 10].

The degree of genetic homogeneity of certain California CgA isolates suggested that a point source, possibly a contaminated food product, may have been responsible for the dissemination of this clonal group. If so, the prevalence of this group should follow an epidemic pattern and, hence, we would expect to see a decline in the prevalence of CgA-associated UTI over time. This hypothesis led us to conduct a second cross-sectional study, 1 year later. At the same time, we investigated the distribution of other *E. coli* clonal groups causing community-acquired UTI regardless of their drug resistance.

METHODS

Study design

This cross-sectional study was conducted from 11 October 2000 to 31 January 2001 (phase II study) and was designed as a follow-up to the original cross-sectional study conducted from 11 October 1999 to 31 January 2000 (phase I study); both studies were carried out in the same California university community. The study protocol was approved by the University of California at Berkeley, Committee for the Protection of Human Subjects. The study sample consisted of women presenting to the university health service with symptoms of UTI who were consecutively recruited into the study. A case of *E. coli* UTI was defined as any woman with a clinical suspicion of UTI whose clean-catch urine culture yielded $>10^2$ colony-forming units (c.f.u.)/ml of *E. coli* [11].

Identification and antimicrobial susceptibility testing

Urine samples were cultured on MacConkey's agar medium and lactose- and indole-positive colonies were presumptively identified as *E. coli*. One putative *E. coli* colony from each urine culture was arbitrarily selected for further analysis. Isolates were screened for susceptibility to TMP–SMZ, ciprofloxacin, cephalothin and nitrofurantoin by E-test strips (AB Biodisk, Solna, Sweden). *E. coli* strain ATCC 25922 was used as the reference strain. Isolates were defined as resistant, intermediate or susceptible to antimicrobials using NCCLS interpretive criteria [12]. For subsequent analysis isolates exhibiting intermediate resistance were interpreted as susceptible.

Serotyping

O serogroup testing was performed on *E. coli* isolates at the *E. coli* Reference Center at University Park, Pennsylvania, USA.

ERIC2 PCR fingerprinting

All TMP–SMZ-resistant isolates and a randomly sampled subset of susceptible isolates were screened by the ERIC2 PCR fingerprinting assay [13, 14] as previously described [15]. Susceptible isolates were selected with the random number algorithm in S-PLUS 2000 (Insightful, Seattle, WA, USA); sufficient susceptible isolates were sampled to identify at least 4% CgA, as estimated from the phase I study, with a probability of 95%. Isolates exhibiting indistinguishable ERIC2 fingerprints, as assessed by visual inspection, were considered to belong to a single clonal group. The PCR was repeated on selected isolates from the phase I study to ensure that valid patterns were available for comparison with phase II isolates. Isolates exhibiting an ERIC2 PCR pattern indistinguishable from that of the CgA-positive control (ATCC BA-457, included in each PCR reaction and gel) were considered to belong to CgA. A pyelonephritis isolate CFT073 (O6:K2:H1), provided by Dr Harry Mobley (University of Maryland), was also included as a reference standard.

Pulsed field gel electrophoresis (PFGE)

The standardized protocol for subtyping *E. coli* (O157:H7) by PFGE, as established by the Centers for Disease Control and Prevention (CDC) [16], was used to further analyse the *E. coli* isolates that were indistinguishable by ERIC2 fingerprinting. *Xba*I-digested DNA was subjected to electrophoresis in the CHEF DR-II apparatus (Bio-Rad, Hercules, CA,

Table 1. *ERIC2 PCR analysis of* E. coli *UTI isolates from California for study phases I and II*

Assay	Phase I* 11 Oct. 99–31 Jan. 00 *n* (%)	Phase II 11 Oct. 00–31 Jan. 01 *n* (%)	Per cent change (*P* value)
TMP–SMZ-resistant isolates			
Primary isolates†	47 (21)	38 (18)	−2·2% (0·56)
ERIC2 PCR TMP–SMZ-resistant clonal group A			
Primary isolates	23 (49)	4 (11)	−38% (<0·001)

* The results from the phase I study were analysed and published previously [5].

† Primary isolates, refer to the *E. coli* isolates recovered from a woman's first UTI episode during the study period.

USA). Criteria for strain relatedness established by Tenover et al. were used to assess the similarity of the PFGE profiles [17]. A single pulsotype was defined as PFGE patterns that differ by three or fewer bands from a comparison strain belonging to the same ERIC2 PCR genotype.

Statistics

Analyses were restricted to the first UTI episode (primary episode) experienced by a woman during the study period. Two sample tests of proportions, determined by STATA version 7.0 (Stata Corp., College Station, TX, USA), are presented. Two-sided *P* values are shown; $P<0{\cdot}05$ is considered statistically significant.

RESULTS

Study subjects

Between 11 October 2000 and 31 January 2001, 414 women presented to a California university health service with clinically suspected acute UTI. Of the 468 consecutive urine samples collected and cultured from these women, 225 (48%) yielded $>10^2$ c.f.u. *E. coli*/ml of urine. This represented 206 unique women (median age 22 years, range 13–48 years).

Antimicrobial susceptibility

Thirty-eight (18%) of the 206 women were infected with a TMP–SMZ-resistant *E. coli* isolate. The prevalence of TMP–SMZ resistance was 21% during 1999–2000 (percentage change −2·2%, $P=0{\cdot}56$). Changes in resistance for other drugs included (phase I *vs.* phase II), cephalothin [9 (3·9%) *vs.* 2 (1·0%), per cent change −2·9%, $P=0{\cdot}05$] and ciprofloxacin [2 (0·9%) *vs.* 6 (2·9%), per cent change 2·0%, $P=0{\cdot}12$]; there was no change in nitrofurantoin resistance (0% *vs.* 0%). Of the six ciprofloxacin-resistant isolates identified, five were also resistant to TMP–SMZ (ciprofloxacin-TMP–SMZ) and three exhibited intermediate susceptibility to cephalothin.

ERIC2 PCR fingerprinting

ERIC2 PCR screening was performed on all ($n=47$) TMP–SMZ-resistant and 49 randomly selected TMP–SMZ-susceptible *E. coli* in the phase I study, and all ($n=38$) TMP–SMZ-resistant and 104 randomly selected TMP–SMZ-susceptible *E. coli* isolates in the phase II study. A larger number of TMP–SMZ-susceptible isolates were chosen for screening by ERIC2 PCR in the phase II study so that we would be assured of having the power to detect TMP–SMZ-susceptible CgA. In the phase I study, 23 (49%) of the 47 TMP–SMZ-resistant *E. coli* and only two (4%) of the TMP–SMZ-susceptible *E. coli* isolates causing UTI were CgA ($P<0{\cdot}001$). In contrast, in the phase II study only four (11%) of the 38 TMP–SMZ-resistant *E. coli* isolates and three (3%) of the 104 ($P=0{\cdot}06$) randomly sampled TMP–SMZ-susceptible isolates exhibited the CgA ERIC2 PCR pattern (Table 1). A sample of ERIC2 PCR results for CgA isolates from the phase II study is presented in Figure 1. The change in CgA prevalence among TMP–SMZ-resistant UTI episodes across the two time periods was −38% ($P<0{\cdot}001$) (Table 1).

In addition to CgA, six other clonal groups were identified by ERIC2 PCR; they were designated CgB, CgC, CgD, CgE, CgF and CgG (Table 2). Clonal groups CgB, CgD, CgE, and CgG were identified only in the phase II collection and CgC was identified in both study periods. Clonal groups A–G were responsible for 12 (32%) of all UTIs caused by

Table 2. *Summary of ERIC2 PCR clonal groups identified in study phases I and II*

Clonal group	Phase I *n* (%) (*N*=96)	Phase II *n* (%) (*N*=142)	Serogroup (N)	% of total in Phase II† TSR (*N*=38)	TSS (*N*=104)
CgA	25 (11)	7 (3)	O11 (15)‡ O77 (5)	11	3
CgB	0 (0)	8 (4)	O2 (3) O6 (5)	0	8
CgC	6 (3)	6 (3)	O1 (8) O2 (1) O18 (3)	0	6
CgD	0 (0)	9 (4)	O6 (8) O83 (1)	8	6
CgE	0 (0)	2 (1)	O82 (2)	0	2
CgF	2 (1)	2 (1)	O4 (3) Mixed (1)	4	0
CgG	0 (0)	3 (1)	O102, 130 (3)	3	0

† The proportion of this clonal group among all trimethoprim–sulphamethoxazole-resistant (TSR) or trimethoprim–sulphamethoxazole-susceptible (TSS) isolates in the phase II study.
‡ Not all CgA isolates were sent for serotyping.

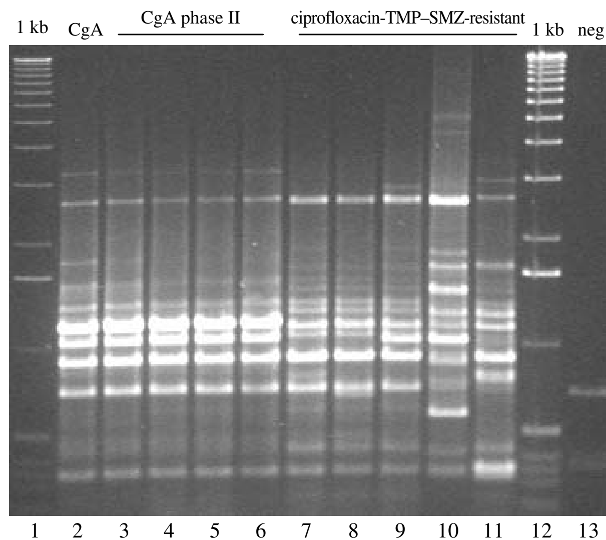

Fig. 1. ERIC2 PCR patterns of TMP–SMZ-resistant, clonal group A (CgA) and ciprofloxacin-TMP–SMZ-resistant *E. coli* UTI isolates. Lanes 1 and 12, 1 kb ladder; lane 2, CgA prototype isolate and positive control; lanes 3–6, putative CgA isolates; lanes 7–11, ciprofloxacin-TMP–SMZ-resistant isolates, including CgG (lanes 7–9); lane 13, negative control. All isolates were recovered from unique women, except 1792 (lane 4) and 2000 (lane 5), which were isolated from two UTI episodes in the same woman, 57 days apart.

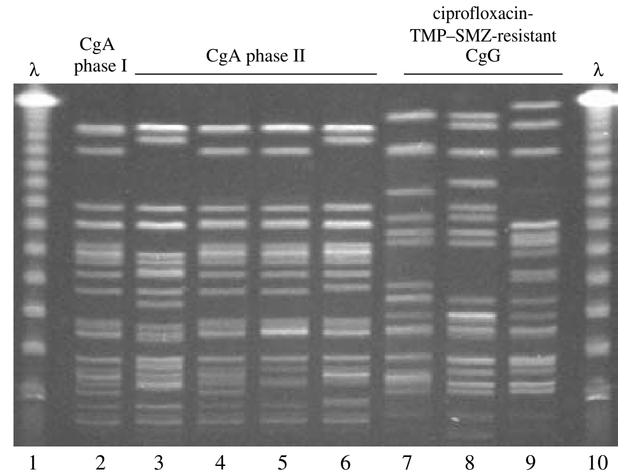

Fig. 2. *Xba*I PFGE patterns of clonal group A (CgA) and ciprofloxacin-TMP–SMZ-resistant CgG isolates from the phase II study. Lanes 1 and 10, lambda ladder; lane 2, prototype CgA isolate; lanes 3–6, putative CgA isolates; lanes 7–9, ciprofloxacin-TMP–SMZ-resistant CgG isolates.

TMP–SMZ-resistant and 25 (24%) of 104 TMP–SMZ-susceptible *E. coli* isolates in the phase II study ($P=0{\cdot}34$).

PFGE

*Xba*I PFGE analysis was performed on all clonal groups identified by ERIC2 PCR. The CgA isolates yielded PFGE profiles indistinguishable or closely related to that of a prototype CgA isolate (ATCC BA-457) collected in the phase I study (Fig. 2).

The eight ERIC2 CgB isolates included five different pulsotypes (data not shown). Nine (75%) out of

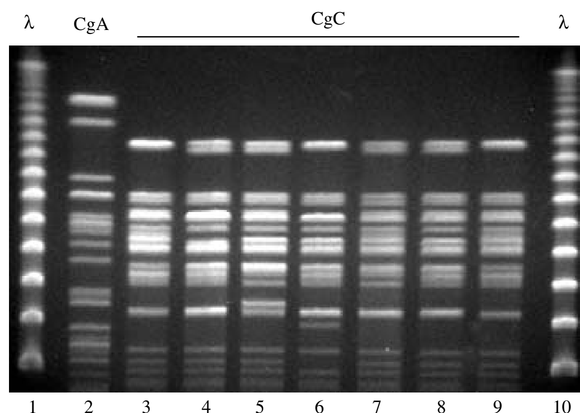

Fig. 3. *Xba*I PFGE patterns of CgC isolates from the phase II study. Lanes 1 and 10, lambda ladder; lanes 2–9, CgC isolates. Lanes 3, 7 and 9 exhibit indistinguishable patterns, as do lanes 4 and 8.

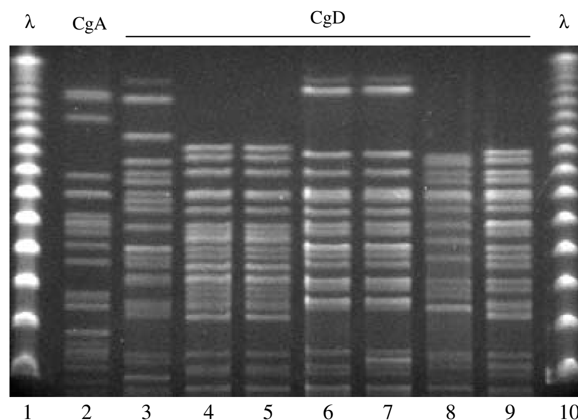

Fig. 4. *Xba*I PFGE patterns of CgD isolates from the phase II study. Lanes 1 and 10, lambda ladder; lanes 2–9, CgD isolates. Two pairs of women were infected by strains with indistinguishable PFGE patterns (see text).

12 CgC members belonged to a single pulsotype. This was the largest cluster identified besides CgA (Fig. 3). CgD included seven pulsotypes; a sample of these patterns is presented in Figure 4. The two CgE isolates were indistinguishable and two of the four CgF *E. coli* isolates were indistinguishable by PFGE (data not shown). Two of the three CgG isolates, characterized by resistance to both TMP–SMZ and ciprofloxacin, were possibly related (differed by fewer than six bands) by PFGE (data not shown).

Serotyping

In the initial study CgA was found to belong to four serogroups including O11, O77, O17 and O73. In the phase II study, three of the four representative CgA isolates were determined to be serogroup O11:H(nt). As with CgA, the other ERIC2 PCR clonal groups identified in this study tended to segregate into specific serogroups. CgB included serogroups O2 and O6 and CgC was primarily O1, but also included serogroups O2 and O18. The remaining four clonal groups consisted of a single serogroup for each clonal group (Table 2).

Temporal distribution of clonal groups

Evidence of temporal clustering is apparent if we consider clustering in time based on closely related PFGE patterns. There were two instances in which women presented to the university health services with UTI caused by *E. coli* exhibiting indistinguishable PFGE patterns on the same day. The first two cases involved CgB strains (data not shown) and occurred on 23 October 2000 and the second two cases involved CgD strains (Fig. 4, lanes 4 and 5) and occurred on 14 November 2000. A second pair of women infected by CgD strains experienced UTIs 13 days apart (Fig. 4, lanes 6 and 7). Although the cases associated with the large CgC cluster were distributed across both study periods, three of the four CgC UTI cases that occurred during the month of January 2000 were caused by isolates with an indistinguishable PFGE pattern. A second CgC pair included two women who experienced infections nearly a year apart (data not shown). Similarly, the two indistinguishable CgE isolates caused infections separated by more than 1 year. Within the CgF group, a pair of indistinguishable PFGE pattern strains caused infections in two women that were separated by 19 days (data not shown).

DISCUSSION

In the previous study, we found that a multidrug-resistant clonal group of uropathogenic *E. coli* (CgA) was responsible for 49% of TMP–SMZ-resistant *E. coli* UTI episodes from unique women at a single student health centre in California. In this follow-up (phase II) study, conducted during the same 4-month period 1 year later, the prevalence of UTI caused by CgA declined significantly (−38%) supporting the initial hypothesis that there was an outbreak of community-acquired UTI during 1999–2000. The four cases of CgA UTI in the phase II study may represent infections resulting from persistent intestinal colonization by this strain in these women. CgA and

other strains of *E. coli* have been shown to colonize the intestines of both female and male subjects for several months [18].

Interestingly, the overall prevalence of TMP–SMZ-resistant *E. coli* isolates causing UTI did not decrease significantly over the two periods. The absence of a decrease in TMP–SMZ resistance prevalence could be attributed to the introduction of other resistant clonal groups. Six additional ERIC2 PCR clonal groups were identified and three of these groups included strains that were resistant to TMP–SMZ. One of these clonal groups (CgG) included strains that were resistant to both TMP–SMZ and ciprofloxacin. In the phase I study, three clonal groups accounted for 55% of all TMP–SMZ-resistant *E. coli* causing UTI (CgA alone accounted for 51%); in phase II CgA plus six additional ERIC2 PCR clonal groups accounted for 32% of all TMP–SMZ-resistant *E. coli* causing UTI.

Results from this study suggest that fluctuation over time in the distribution of *E. coli* clonal groups causing UTI has the potential to influence the prevalence of resistant UTI in a community. It also shows that the prevalence of drug-resistant UTI in a community may be attributable to a small number of *E. coli* clonal groups. This may have clinical implications regarding selection of appropriate empirical treatment of UTI within a given community. A sudden introduction of a clonal group with a new drug-resistance phenotype could rapidly alter the prevalence of drug-resistant UTI in that community. Cost-effective strategies to periodically review sensitivity and resistance information for community UTI should be explored.

In addition to CgA, we observed many instances in which women were infected by *E. coli* with indistinguishable PFGE patterns. This included nine different women infected by closely related CgC strains. The PFGE data show several instances in which women infected with genotypically indistinguishable strains visited the clinic either on the same day or separated by only a few days. However, these analyses were limited by the number of strains sampled and the brief time periods studied. Additionally, since we did not conduct strain typing on all susceptible isolates from both studies it is possible that we missed other examples of clonal groups present during one or both study periods.

Although UTI is usually regarded as a sporadic disease caused by organisms from the host's own intestinal flora, nosocomial and community-wide outbreaks of *E. coli* UTI and pyelonephritis have been described [2, 19, 20]. The degree of genetic homogeneity and the observed temporal and geographical fluctuations of multiple clonal groups of *E. coli* in one community suggests that this is more common than previously thought, and lends support to the hypothesis that uropathogenic clonal groups may be spread by a common source, similar to the way an enteric pathogen, such as *E. coli* O157:H7, causes community-wide outbreaks after being disseminated by contaminated foods [16, 21]. The observation that women may routinely be infected by *E. coli* clonal groups, such as CgA and CgC, also suggests that members of a community may become colonized and infected with uropathogens via exposure to a contaminated source, such as food.

As the prevalence of antimicrobial resistance continues to increase in the United States and globally [9, 10], the hypothesis that epidemic lineages of uropathogenic *E. coli* are spread by widely disseminated contaminated products and contribute substantially to drug-resistant UTI, deserves further examination. The usual recommendation to restrict human use of antimicrobial agents will have limited effect if this mode of spread is responsible for a large proportion of cases of community-acquired resistant UTI.

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DECLARATION OF INTEREST

None.

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