JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 5860–5864 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.5860–5864.2005

Analysis of a Uropathogenic *Escherichia coli* Clonal Group by Multilocus Sequence Typing

Sara Y. Tartof,[1,4] Owen D. Solberg,[2] Amee R. Manges,[3] and Lee W. Riley[1,4]*

Division of Infectious Diseases and Immunity, University of California at Berkeley, Berkeley, California[1]; Department of Integrative Biology, University of California at Berkeley, Berkeley, California[2]; Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, Quebec, Canada[3]; and Division of Epidemiology, School of Public Health, University of California at Berkeley, Berkeley, California[4]

Received 11 May 2005/Returned for modification 8 August 2005/Accepted 26 September 2005

Although many strain typing methods exist for pathogenic *Escherichia coli*, most have drawbacks in terms of resolving power, interpretability, or scalability. For this reason, multilocus sequence typing (MLST) is an appealing alternative. However, its applicability to different pathogens in specific epidemiologic contexts is not well understood. Here, we applied a previously established MLST method based on housekeeping genes to a well-characterized collection of uropathogenic *E. coli* isolates to compare the discriminatory ability of this procedure with that of enterobacterial repeat intergenic consensus (ERIC2) PCR, serogrouping, and pulsed-field gel electrophoresis (PFGE). Among 45 *E. coli* isolates studied, 17 different multilocus sequence types (ST) were identified. One MLST group (designated ST69 complex) was comprised of 22 isolates, all belonging to uropathogenic and bacteremic *E. coli* strains previously defined as clonal group A (CgA) by ERIC2 PCR. The ST69 strains contained five different serogroups and 14 PFGE types. ERIC2 PCR CgA strains belonging to different MLST groups were also identified. Interestingly, one cow *E. coli* isolate, previously shown by PFGE to be closely related to a human uropathogenic CgA strain, was found to cluster with the ST69 strains. All of the other animal and environmental CgA isolates had different MLST profiles. The discriminatory power of this MLST method based on housekeeping genes appears to be higher than that of ERIC2 PCR but lower than that of PFGE for epidemiologic study of uropathogenic *E. coli*.

The morbidity and financial burden associated with urinary tract infections (UTIs) is substantial: almost half of all women experience at least one UTI in their lifetime, and overall expenditures for UTI treatment in women in the United States was approximately $2.47 billion in 2000 (3, 5). The primary etiologic agent of UTI is *Escherichia coli*, accounting for as much as 90% of all UTIs seen among ambulatory care patients (23). Antimicrobial resistance among uropathogenic *E. coli* continues to rise in the United States, contributing to greater difficulty in the management of UTI (6, 7).

The genotypic characterization of pathogens has become an important objective in epidemiologic investigations of infectious agents. Genotyping tools are amenable to validation when the diseases in question occur as recognizable outbreaks, such as those caused by *E. coli* O157:H7 or *Salmonella* spp. The problem arises in characterizing organisms that cause diseases that are not usually thought of as causing outbreaks, such as *E. coli* associated with community-acquired UTI. Recent studies that applied genotyping methods to differentiate uropathogenic *E. coli* suggest that community-acquired UTIs could occur as outbreaks.

Manges et al. reported in 2001 that a single clonal group of *E. coli*, designated clonal group A (CgA), based on a characteristic enterobacterial repeat intergenic consensus (ERIC2) PCR fingerprint pattern, was responsible for nearly half of trimethoprim-sulfamethoxazole-resistant *E. coli* isolates from 47 women with UTIs diagnosed at a California university health clinic between October 1999 and January 2000 (13). Human UTI isolates belonging to CgA were also found to share a random amplified polymorphic DNA PCR fingerprint, virulence factor profile, similar antimicrobial resistance phenotype, O antigen groups (O11, O17, O73, and O77), and indistinguishable or closely related pulsed-field gel electrophoresis (PFGE) patterns (9), suggesting that they caused an outbreak in this California community. Most recently, a gene-specific PCR was devised to identify CgA based on a single-nucleotide polymorphism (SNP) (C288T) identified within the *fumC* housekeeping gene (11).

Certain genotyping methods (PCR-based methods and PFGE) rely on comparison of banding patterns generated by gel electrophoresis. Such techniques are condition dependent and therefore can suffer from interlaboratory variability (20). Furthermore, visual inspection remains integral to their interpretation, introducing subjectivity and greater potential for error (22). While PFGE does offer superior reproducibility and discriminating power and remains the method most commonly used to identify food-borne outbreaks of *E. coli* O157:H7 at reference laboratories, it is labor intensive and technically demanding (8, 13, 21). Although gene-specific PCR is highly reproducible, a recent study evaluating 45 isolates exhibiting an ERIC2 PCR CgA pattern found that only 16 isolates contained the C288T SNP (4). Thus, a more reliable, relevant, and consistent typing method for investigating the clonal distribution of uropathogenic *E. coli* strains, such as CgA isolates, is needed for epidemiologic investigations. The availability of a large, well-characterized, and population-based collection of uropathogenic *E. coli* isolates provided us with an opportunity to evaluate another genotyping method.

Multilocus sequence typing (MLST) uses nucleotide sequences of internal fragments of selected genes as the unit of

* Corresponding author. Mailing address: Divisions of Epidemiology and Infectious Diseases and Immunity, 140 Warren Hall, School of Public Health, University of California at Berkeley, Berkeley, CA 94720. Phone: (510) 642-9200. Fax: (510) 642-6350. E-mail: lwriley@berkeley.edu.

TABLE 1. Forward and reverse sequences of primers[c]

Gene (function)	Primer sequence (5′–3′)[a]	Annealing temp (°C)	Amplicon size (bp)[b]
adk (adenylate kinase)	ATTCTGCTTGGCGCTCCGGG (F) CCGTCAACTTTCGCGTATTT (R)	52	583
fumC (fumarate hydratase)	TCACAGGTCGCCAGCGCTTC (F) GTACGCAGCGAAAAAGATTC (R)	52	806
icd (isocitrate/isopropylmalate dehydrogenase)	ATGGAAAGTAAAGTAGTTGTTCCGGCACA (F) GGACGCAGCAGGATCTGTT (R)	52	878
purA (adenylosuccinate dehydrogenase)	CGCGCTGATGAAAGAGATGA (F) CATACGGTAAGCCACGCAGA (R)	54	816
gyrB (DNA gyrase)	TCGGCGACACGGATGACGGC (F) ATCAGGCCTTCACGCGCATC (R)	58	911
recA (ATP/GTP binding motif)	CGCATTCGCTTTACCCTGACC (F) TCGTCGAAATCTACGGACCGGA (R)	58	780
mdh (malate dehydrogenase)	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG (F) TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT (R)	58	932

[a] F, forward; R, reverse.
[b] The sequenced DNA strands were shorter than the original PCR amplicon.
[c] Primers are maintained at http://web.mpiib-berlin.mpg.de and http://www.mlst.net.

comparison and, therefore, does not suffer from the drawbacks of gel-based fingerprinting methods. Sequence data are easily comparable and transferable between laboratories and are highly reproducible (2, 12). Furthermore, the digital format of MLST data has facilitated the establishment of global, web-accessible databases for a variety of organisms and is rapidly contributing to our understanding of the clonal distribution of infectious disease agents. The most commonly used MLST schemes index the neutral genetic variation in housekeeping genes, which are believed to evolve slowly because they are under stabilizing, and not directional, selective pressure. The SNPs observed across several different MLST loci represent neutral genetic variation and presumably exhibit minimal autocorrelation, which could confound epidemiologic conclusions. MLST is thus a powerful tool for global and long-term surveillance.

In this study, we evaluated an MLST protocol standardized for *E. coli* maintained at the Max-Planck Institut fuer Infektionsbiologie website (http://web.mpiib-berlin.mpg.de) using a well-characterized population-based collection of CgA and other *E. coli* strains and compared its discriminatory power with that based on PFGE, ERIC2, and serogroup analyses.

MATERIALS AND METHODS

Bacterial isolates. A total of 45 *E. coli* isolates were studied: 29 were previously identified as CgA (23 human and 6 animal and environmental) and 16 were identified as human non-CgA strains, defined by ERIC2 PCR (13, 19). Isolates were selected to include groups of strains from various geographic and host sources to investigate potential groupings by these factors. Dates of collection of isolates ranged from 1988 to 2003.

Isolates included strains from the following sources: (i) UTI isolates from California ($n = 15$) women with symptoms of UTI who were seen at a university health service (they were consecutively enrolled into a study between 11 October 1999 and 31 January 2000) and UTI-causing, trimethoprim-sulfamethoxazole-resistant *E. coli* isolates ($n = 6$) from Minnesota (obtained from students who were seen at university health services with uncomplicated cystitis and were enrolled in a study between June 1998 and August 1999); (ii) animal and environmental isolates ($n = 6$) provided by the Gastroenteric Disease Center at Pennsylvania State University (University Park, PA); (iii) human bacteremia isolates ($n = 12$) from San Francisco General Hospital provided by Francoise Perdreau-Remington; and (iv) other geographic comparison isolates recovered from humans ($n = 6$) provided by James R. Johnson of the Minneapolis VA Medical Center, University of Minnesota.

Serotyping. Serotyping was performed on *E. coli* isolates at the *E. coli* Reference Center in University Park, Pennsylvania.

Molecular strain typing analyses. All *E. coli* isolates were initially typed by the ERIC2 PCR fingerprinting assay, as described elsewhere (10, 13). The CgA ERIC2 PCR electrophoretic pattern was defined by 4 predominant bands of approximately 1,145, 1,029, 908, and 720 bp; isolates exhibiting this pattern were considered to be members of CgA. A pyelonephritogenic isolate, CFT073 (provided by Harry Mobley, University of Maryland, Baltimore), was used as a reference strain for each ERIC2 PCR run.

PFGE. The standardized protocol for subtyping *E. coli* O157:H7 by PFGE, as established by the Centers for Disease Control and Prevention (Atlanta, GA), was used (1). XbaI-digested DNA was electrophoresed in the CHEF DR-II apparatus (Bio-Rad, Hercules, CA). Images of PFGE and ERIC2 PCR electrophoretic patterns were imported and analyzed with GelCompar II, version 2.0 (Applied Maths, Kortrijk, Belgium), with the pattern of the prototype CgA strain (SEQ102, deposited in the American Type Culture Collection as ATCC BAA-457) used as a reference, as previously described (19). To minimize gel-to-gel banding pattern variation, we used the autosearch band calling function of GelCompar with the following parameters: minimum profiling, 5%; gray zone, 0%; minimum area, 1%; shoulder sensitivity, 1. A distance matrix was calculated by GelCompar's Dice algorithm with a band position tolerance of 1%. A dendrogram was generated from the distance matrix by the neighbor-joining method.

MLST. (i) Allele templates selected. Housekeeping genes for typing were selected from the *E. coli* database at the MLST website maintained at the Max-Planck Institut fuer Infektionsbiologie (http://web.mpiib-berlin.mpg.de) (Table 1). The genes were shown to be unlinked on an *E. coli* K-12 genome map. Product lengths varied from 583 to 932 bp (Table 1). Allele templates, included on the website, were based on the genome sequence of *E. coli* strain MG1655.

(ii) DNA isolation. *E. coli* isolates were obtained from a collection of strains stored in 15% glycerol at −80°C. Isolates were incubated at 37°C on LB agar plates overnight. Single colonies were picked and inoculated into 2 ml LB media and further incubated in a shaking incubator for 12 to 15 h at 37°C. A 1-ml suspension of bacteria was centrifuged, and DNA was extracted from the bacterial pellet with the QIAGEN DNeasy tissue kit (QIAGEN, Valencia, CA).

PCR. Amplifications were carried out in a total volume of 50 μl with 2 μl of template DNA, 4 μl of each 10 mM primer (Sigma, Genosys, The Woodlands, TX), 5 μl 10× buffer, 15 mM $MgCl_2$ (Applied Biosystems, Foster City, CA), 5 μl 2 mM deoxynucleoside triphosphate mix (Invitrogen, Carlsbad, CA), and 0.5 U Ampli*Taq* Gold (Applied Biosystems). Reaction conditions used were: 2 min of denaturation at 95°C; 30 cycles of 1 min of denaturation at 95°C, 1 min of annealing at specific temperature (Table 1), 2 min of extension at 72°C; and a final additional 5 min at 72°C in an Applied Biosystems GeneAmp PCR System 2400 thermocycler.

Sequencing. PCR products were purified for sequencing with the QIAGEN QIAquick PCR purification kit. Both the forward and reverse strands were sequenced with the PCR primer set, with the exception of *mdh*, which was sequenced by the forward primer 5′-TCTGGTGAAGATGCGACTCC-3′ and reverse primer 5′-CCCAGGGCGATATCTTTCTT-3′. Sequencing was per-

formed at the University of California at Berkeley Sequencing Facility, which is equipped with an Applied Biosystems 48 capillary 3730 and two Applied Biosystems 16 capillary 3100 genetic analyzer systems. A Perkin Elmer (Wellesley, MA) 9600, an Eppendorf (Hamburg, Germany) Mastercycler, and three MJ Research (Bio-Rad, Hercules, CA) PTC-200s were used for cycle sequencing. The facility runs a 25-cycle sequencing reaction with the following program: 96°C for 10 s plus 50°C for 5 s plus 60°C for 4 min.

Sequence analysis. Raw sequences were reviewed by visual inspection with Chromas, version 1.45 (32-bit) (Technelysium Pty. Ltd., Tewantin Qld 4565, Australia). DNA sequences were aligned by the neighbor-joining method with 1,000 bootstrap iterations in ClustalW (Hinxton, England). Forward and reverse sequences were aligned for each strain for comparison and editing purposes. Alignments were edited to equally sized fragments and aligned against allele templates based on *E. coli* strain MG1655, provided at http://www.mlst.net. Sequence editing was conducted with BioEdit (Carlsbad, CA), version 7.0.1. Sequences for the seven genes of each strain were concatenated to produce an alignment sequence of 3,423 bp. A dendrogram of SNP groupings based on these alignments was then constructed by the Phylip programs (Seattle, WA) DNA DIST and NEIGHBOR, and the final dendrogram was visualized with the software TreeView (Win32) (Glasgow, Scotland), version 1.6.6.

ST designation. Gene sequences were submitted to the curator of the MLST *E. coli* submission page maintained at http://web.mpiib-berlin.mpg.de. Sequence types (STs) and sequence complexes were designated by the curator of the MLST database for sequence submissions that contained tracings found to be acceptable by the curator (Table 2). ST complexes were defined as STs that are linked by distances of one to two allelic differences.

RESULTS

Of 45 *E. coli* isolates analyzed by MLST, 41 generated sequence tracings acceptable for an ST number assignment. The 41 isolates were grouped into 17 distinct STs (Table 2). Of the 17 STs, 12 were already included in the *E. coli* MLST website database. Of the 23 human isolates defined by ERIC2 PCR as CgA, one MLST cluster comprised of 21 human isolates, designated ST69 complex, was observed (Table 2; Fig. 1). Of these, 19 showed identical sequences in all seven genes regardless of geographic or clinical sources. One isolate from Maryland (A1707) and one isolate from Minnesota (G) differed by one single-nucleotide polymorphism each, in the *fumC* gene and the *gyrB* gene, respectively. Of the 8 human bacteremia CgA isolates, two isolates (H42937 and M32569) fell outside cluster ST69 and shared exact sequence identity. The non-CgA bacteremia isolates ($n = 4$) each belonged to a distinct MLST type (Fig. 1). Five of the six animal/environmental CgA isolates (AN300, AN298, AN45, AN431, and AN437) fell outside the ST69 grouping. The 21 human strains in the ST69 complex were composed of five serogroups (O11, O77, O17, O73, and O86). The non-ST69 complex CgA isolates that belonged to serogroups O77, O11, and O15 fell into three clusters in which serogroup and sequence type were shared among the isolates in that branch.

One CgA animal (cow) isolate (AN559) was found to belong to the MLST complex ST69, which included only human UTI and bacteremia CgA isolates (Fig. 1). None of the strains previously identified as non-CgA fell into this ST69 complex. Of the non-CgA strains, the three UTI isolates from Minnesota all belonged to another MLST complex (Fig. 1). These isolates were indistinguishable by ERIC2 PCR and were the only clustered non-CgA isolates.

Of 22 ST69 complex isolates, 19 had identical sequences in all 7 gene templates. These 19 ST69 strains were further separated into 14 subtypes by PFGE (Fig. 1, inset).

TABLE 2. STs and ST complexes

Isolate	Source (location)	Serotype(s)	CgA	ST	ST complex
102	UTI (California)	011	Yes	ST69	ST69
160	UTI (California)	011	Yes	ST69	ST69
403	UTI (California)	011	Yes	ST69	ST69
431	UTI (California)	011	Yes	ST69	ST69
477	UTI (California)	011	Yes	ST69	ST69
220	UTI (California)	077	Yes	ST69	ST69
283	UTI (California)	077	Yes	ST69	ST69
44	UTI (California)	077	Yes	ST69	ST69
A1707	UTI (Maryland)	017, 077	Yes	ST407	ST69
A556	UTI (Munich, Germany)	017, 077	Yes	ST69	ST69
A925	UTI (Barcelona, Spain)	077	Yes	ST69	ST69
PY3	UTI (Los Angeles, Calif.)	017, 077	Yes	ST69	ST69
B	UTI (Minnesota)	NT[a]	Yes	ST69	ST69
G	UTI (Minnesota)	017	Yes	****[c]	****
Y	UTI (Minnesota)	077	Yes	ST69	ST69
AN559	Cow	017	Yes	ST408	ST69
AN298	Cow	011	Yes	ST101	ST101
AN45	Pig	011	Yes	ST101	ST101
AN300	Cow	011	Yes	ST101	ST101
AN431	Pig	077	Yes	ST394	ST69
AN437	Water	077	Yes	ST394	ST69
H42937	Blood	015	Yes	ST393	ST31
M32569	Blood	015	Yes	ST393	ST31
F21065	Blood	073	Yes	ST69	ST69
H3708	Blood	017	Yes	ST69	ST69
S48427	Blood	077	Yes	ST69	ST69
W55291	Blood	077	Yes	ST69	ST69
X19714	Blood	086	Yes	ST69	ST69
X47726	Blood	011	Yes	ST69	ST69
SEQ 111	UTI (California)	†[b]	No	****	****
SEQ 020	UTI (California)	†	No	****	****
SEQ 329	UTI (California)	†	No	ST355	ST73
SEQ 338	UTI (California)	†	No	ST88	ST29
SEQ 240	UTI (California)	†	No	ST127	None
SEQ 336	UTI (California)	†	No	****	****
1702	UTI (California)	†	No	ST402	ST405
A219	UTI (Toulouse, France)	†	No	ST23	ST23
A563	UTI (Munich, Germany)	†	No	ST10	ST10
Jo J	UTI (Minnesota)	†	No	ST73	ST73
Jo K	UTI (Minnesota)	†	No	ST73	ST73
Jo L	UTI (Minnesota)	†	No	ST73	ST73
W38035	Blood	†	No	ST144	None
F591	Blood	†	No	ST131	None
X42574	Blood	†	No	ST372	None
W18993	Blood	†	No	ST12	None

[a] NT, nontypeable.
[b] †, serotype not available.
[c] ****, MLST designation not yet available.

DISCUSSION

The evaluation of new molecular strain typing techniques with well-characterized isolates and previously established typing methods is an important component of infectious disease epidemiologic research. Although the limitations of ERIC2 PCR have been reported (14), it was demonstrated here that an MLST scheme based on housekeeping genes revealed highly similar clustering of human isolates previously defined as CgA by ERIC2 PCR (13). However, this MLST method was able to further discriminate human and animal/environmental CgA strains. Five of six animal/environmental isolates defined as CgA did not cluster with human CgA isolates, whereas one did. Thus, while ERIC2 PCR is not as discriminating as the MLST based on housekeeping genes, the observations made in this study suggest that it is still a reliable screening tool that can be used to rapidly separate a large collection of uropathogenic *E. coli* isolates into groups that have possible epidemiologic relationships.

Interestingly, strain AN559, isolated from a cow in 1988 that was previously shown to be 94% similar by PFGE to a human UTI-causing CgA *E. coli* isolate (19), was found to occur within

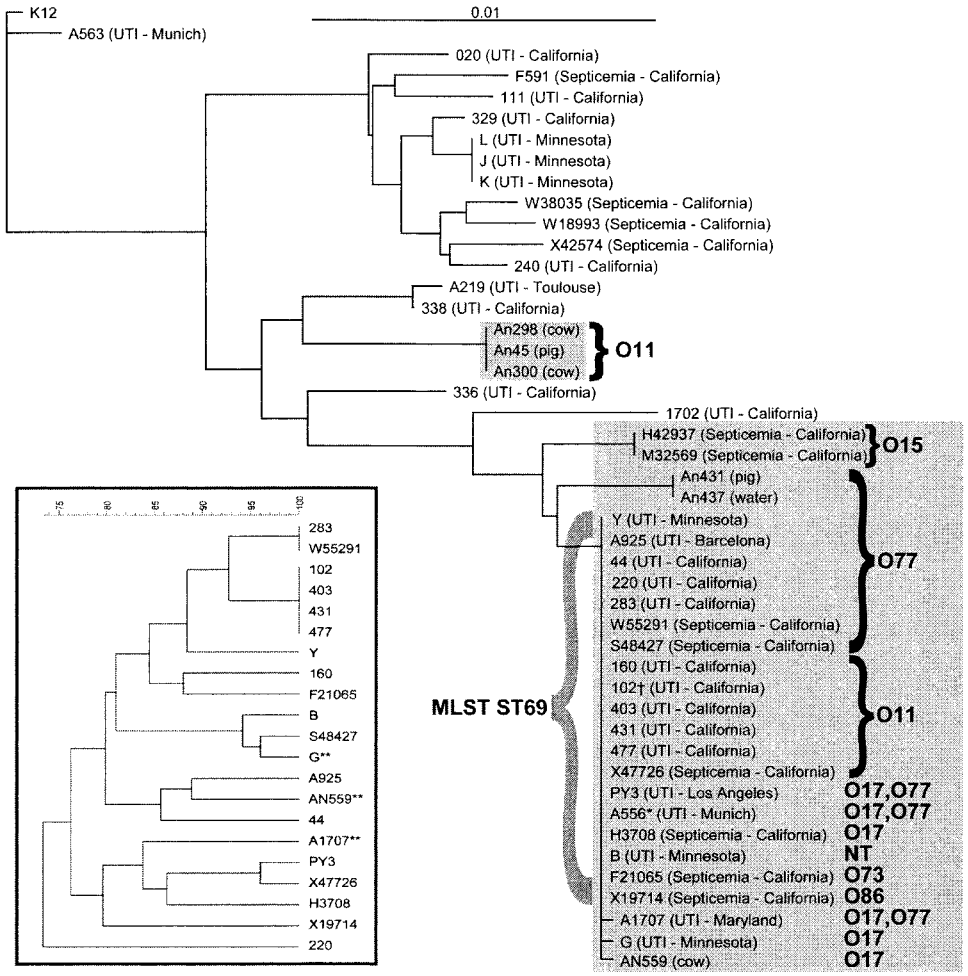

FIG. 1. Neighbor-joining tree constructed from the concatenated sequences of the 7 MLST genes described in the text. The 29 previously identified CgA isolates are shown with shaded backgrounds. Serogroups are indicated for the CgA isolates. The inset shows a dendrogram based on Dice distance coefficient measurements of PFGE banding patterns among the 21 isolates belonging to the ST69 complex. *, no PFGE data available; **, ST69 complex members, not ST69 (see Table 2); †, prototype human uropathogenic CgA strain ATCC BAA-457.

the ST69 complex. This was the only animal strain to cluster within this group, differing by a single nucleotide in the *adk* gene.

Recently, CgA isolates shown to be highly similar by multiple techniques, including C288T SNP analysis, drug susceptibility testing, presence of the 1.8-kb class I integron bearing the *dfrA17* gene, and virulence gene profile analysis, were each shown to be distinct by PFGE profile (4). Our investigation of the 21 CgA strains evaluated by PFGE showed that there was no difference in clustering by MLST among the strains that shared PFGE types and the strains that were unique by PFGE. Thus, PFGE still has superior discriminatory ability for uropathogenic *E. coli* than any of the other methods, including this MLST scheme. A similar observation was made by Noller et al., who compared PFGE and MLST for their discriminatory ability using a collection of diarrheagenic *E. coli* serotype O157:H7 (16). In one study, MLST was found to be more discriminatory than PFGE only if gene templates in addition to the 7 housekeeping genes were included (15).

ST69 belongs to ECOR D group. A previous study of CgA strains by virulence factor profile analysis found them to have a profile similar to that of the O15:K52:H1 clonal group that was reported to be responsible for community outbreaks of UTI and bacteremia in Europe (8, 17, 18).

Ultimately, the appropriate level of discriminatory power of any strain typing test is determined by the epidemiologic question of interest. Just as highly discriminatory typing methods can obscure important epidemiologic relationships between related strains, typing schemes with low discriminatory power can obscure important epidemiologic associations. Uropathogenic CgA *E. coli* strains clearly include multiple subtypes, as was shown by the present MLST technique, PFGE analysis, and analysis by France et al. (4). Here, a housekeeping gene-based MLST applied to a well-characterized CgA collection demonstrated discriminatory power that fell between ERIC2 PCR and PFGE. Therefore, for an MLST scheme to be applicable for community-based microepidemiologic studies of uropathogenic *E. coli*, it will have to be based on allelic templates that show a greater degree of variation.

ACKNOWLEDGMENTS

We thank the staff of Tang Health Center, James R. Johnson, Francoise Perdreau-Remington, and Chitrita DebRoy for providing

isolates; Sherry Smith for editorial input; Amy White for logistical support; Mark Achtman for advice in executing this MLST analysis; and Linda and David Tartof for general support.

This work was supported by NIH grant RO1 AI059523.

REFERENCES

1. **Bender, J. B., C. W. Hedberg, J. M. Besser, D. J. Boxrud, K. L. MacDonald, and M. T. Osterholm.** 1997. Surveillance by molecular subtype for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. N. Engl. J. Med. **337:**388–394.
2. **Enright, M. C., and B. G. Spratt.** 1999. Multilocus sequence typing. Trends Microbiol. **7:**482–487.
3. **Foxman, B.** 2003. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis. Mon. **49:**53–70.
4. **France, A. M., K. M. Kugeler, A. Freeman, C. A. Zalewski, M. Blahna, L. Zhang, C. F. Marrs, and B. Foxman.** 2005. Clonal groups and the spread of resistance to trimethoprim-sulfamethoxazole in uropathogenic *Escherichia coli*. Clin. Infect. Dis. **40:**1101–1107.
5. **Griebling, T. L.** 2005. Urologic diseases in America project: trends in resource use for urinary tract infections in women. J. Urol. **173:**1281–1287.
6. **Gupta, K.** 2003. Addressing antibiotic resistance. Dis. Mon. **49:**99–110.
7. **Gupta, K., D. F. Sahm, D. Mayfield, and W. E. Stamm.** 2001. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clin. Infect. Dis. **33:**89–94.
8. **Johnson, J. R., A. R. Manges, T. T. O'Bryan, and L. W. Riley.** 2002. A disseminated multidrug-resistant clonal group of uropathogenic *Escherichia coli* in pyelonephritis. Lancet **359:**2249–2251.
9. **Johnson, J. R., A. C. Murray, M. A. Kuskowski, S. Schubert, M. F. Prere, B. Picard, R. Colodner, and R. Raz.** 2005. Distribution and characteristics of *Escherichia coli* clonal group A. Emerg. Infect. Dis. **11:**141–145.
10. **Johnson, J. R., and T. T. O'Bryan.** 2000. Improved repetitive-element PCR fingerprinting for resolving pathogenic and nonpathogenic phylogenetic groups within *Escherichia coli*. Clin. Diagn. Lab. Immunol. **7:**265–273.
11. **Johnson, J. R., K. Owens, A. R. Manges, and L. W. Riley.** 2004. Rapid and specific detection of *Escherichia coli* clonal group A by gene-specific PCR. J. Clin. Microbiol. **42:**2618–2622.
12. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
13. **Manges, A. R., J. R. Johnson, B. Foxman, T. T. O'Bryan, K. E. Fullerton, and L. W. Riley.** 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N. Engl. J. Med. **345:**1007–1013.
14. **Meacham, K. J., L. Zhang, B. Foxman, R. J. Bauer, and C. F. Marrs.** 2003. Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J. Clin. Microbiol. **41:**5224–5226.
15. **Nemoy, L. L., M. Kotetishvili, J. Tigno, A. Keefer-Norris, A. D. Harris, E. N. Perencevich, J. A. Johnson, D. Torpey, A. Sulakvelidze, J. G. Morris, Jr., and O. C. Stine.** 2005. Multilocus sequence typing versus pulsed-field gel electrophoresis for characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates. J. Clin. Microbiol. **43:**1776–1781.
16. **Noller, A. C., M. C. McEllistrem, O. C. Stine, J. G. Morris, Jr., D. J. Boxrud, B. Dixon, and L. H. Harrison.** 2003. Multilocus sequence typing reveals a lack of diversity among *Escherichia coli* O157:H7 isolates that are distinct by pulsed-field gel electrophoresis. J. Clin. Microbiol. **41:**675–679.
17. **Phillips, I., S. Eykyn, A. King, W. R. Gransden, B. Rowe, J. A. Frost, and R. J. Gross.** 1988. Epidemic multiresistant *Escherichia coli* infection in West Lambeth Health District. Lancet **i:**1038–1041.
18. **Prats, G., F. Navarro, B. Mirelis, D. Dalmau, N. Margall, P. Coll, A. Stell, and J. R. Johnson.** 2000. *Escherichia coli* serotype O15:K52:H1 as a uropathogenic clone. J. Clin. Microbiol. **38:**201–209.
19. **Ramchandani, M., A. R. Manges, C. DebRoy, S. P. Smith, J. R. Johnson, and L. W. Riley.** 2005. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli*. Clin. Infect. Dis. **40:**251–257.
20. **Riley, L. W.** 2004. Molecular epidemiology of infectious diseases: principles and practices. ASM Press, Washington, D.C.
21. **Swaminathan, B., T. J. Barrett, S. B. Hunter, and R. V. Tauxe.** 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. **7:**382–389.
22. **van Belkum, A., W. van Leeuwen, M. E. Kaufmann, B. Cookson, F. Forey, J. Etienne, R. Goering, F. Tenover, C. Steward, F. O'Brien, W. Grubb, P. Tassios, N. Legakis, A. Morvan, N. El Solh, R. de Ryck, M. Struelens, S. Salmenlinna, J. Vuopio-Varkila, M. Kooistra, A. Talens, W. Witte, and H. Verbrugh.** 1998. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of SmaI macrorestriction fragments: a multicenter study. J. Clin. Microbiol. **36:**1653–1659.
23. **Zhang, L., and B. Foxman.** 2003. Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. Front. Biosci. **8:**e235–e244.