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Origin of Class 1 and 2 Integrons and Gene Cassettes in a Population-Based Sample of Uropathogenic *Escherichia coli*

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The prevalence of urinary tract infections (UTI) caused by trimethoprim-sulfamethoxazole (TMP-SMX)-resistant *Escherichia coli* is increasing and varies geographically in the United States. Recent community-based UTI studies have demonstrated geographic clustering of an *Escherichia coli* clonal group, suggesting occurrence of a community outbreak of UTI. A large proportion of this clonal group (designated CgA) isolated from women in a California college community was found to be resistant to TMP-SMX. We wished to determine if the acquisition of TMP-SMX resistance by CgA occurred before or after the CgA strains were introduced into this community. Between October 1999 and January 2000 and between October 2000 and January 2001, 482 *E. coli* isolates were consecutively collected from the urine samples of women with UTI at a student health clinic and analyzed for determinants of TMP-SMX resistance. In particular, the distribution of integrons harboring resistance cassettes for TMP-SMX (*dfr*) was examined. Among 95 TMP-SMX-resistant isolates, 68 and 27 isolates carried class 1 and class 2 integrons, respectively. A class 1 integron was found in 25 (93%) of 27 TMP-SMX-resistant CgA isolates but in only 43 (63%) of 68 TMP-SMX-resistant non-CgA isolates ($P < 0.001$) and in none of 44 TMP-SMX-susceptible *E. coli* isolates ($P < 0.0001$). CgA strains carried only a single arrangement of class 1 gene cassettes (*dfrA17-aadA5*), while the non-clonal group strains carried nine different cassette arrangements. These results support the idea that CgA strains acquired their resistance at a common site prior to their spread to the college community.

Urinary tract infections (UTIs) are the most common bacterial infections in women. Up to a third of women will have an episode of UTI at least once in their lifetime, contributing to an annual health care cost in the United States of about $2.5 billion (9, 15, 23, 31). *Escherichia coli* causes about 80% of community-acquired UTIs. Although there is great regional variation, the proportion of uropathogenic *E. coli* (UPEC) strains resistant to trimethoprim-sulfamethoxazole (TMP-SMX) is over 20% in most regions of the United States and approaches 50% in some localities (21).

Between October 2000 and January 2001, a single clonal group of UPEC was found to be responsible for more than 50% of TMP-SMX-resistant *E. coli* UTI infections in a university community (25). The prevalence of this clonal group, designated CgA as defined by enterobacterial repetitive intergenic consensus 2 (ERIC2) PCR, decreased in this community in the following year (26), but isolates conforming to the CgA ERIC2 PCR pattern have been found in different regions of the United States, as well as abroad (18). CgA *E. coli* isolates tend to be relatively homogenous: most are of sequence type 69 by multilocus sequence type analysis (33); they have a highly homogenous virulence factor profile, including F16 *papA*, *papG* allele II, *iutA*, *kpsM* II, *traT*, and *ompT* (18); they have similar in vitro drug resistance profiles, including resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, and trimethoprim (17, 25); they all belong to ECOR group D (18); and most belong to serogroup O11, O17, O73, or O77 (17, 25).

In the above-mentioned university community study, however, CgA strains that were not resistant to TMP-SMX were also identified from women with UTI (25). Thus, it was possible that the acquisition of resistance by CgA strains occurred due to selective pressures of human antimicrobial drug use after it was introduced into this community. That is, the acquisition of drug resistance by CgA strains could have resulted from multiple independent events; if so, the genetic determinants of resistance should vary among these strains. It is also possible, however, that CgA strains were already multiply resistant when they were introduced into this community, in which case, the resistance determinants are likely to be conserved among CgA strains. We thus analyzed a population-based sample of TMP-SMX-resistant *E. coli* isolates obtained from women with UTI for TMP-SMX resistance genetic determinants.

Integrons are mobile genetic elements thought to play an important role in the dissemination and accumulation of resistance genes in bacteria (reviewed in references 8, 16, and 32). Integrons carry one or more genes in the form of tandem gene cassettes, each of which usually consists of a promoterless open reading frame of about 800 bp. Transcription is initiated by a promoter sequence upstream of the gene cassettes. Each cassette is flanked by conserved sequence, which is recognized by a specialized site-specific recombination enzyme called integrase (*intI*).

The presence of an integron is strongly associated with an-

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timicrobial resistance. In addition to their fungible gene cassettes, which often confer resistance to antimicrobial agents, all class 1 integrons contain an independent *sulI* gene encoding sulfonamide resistance. Because the integron system has the ability to create novel combinations of resistance genes, it may be a dynamic force in the evolution of multidrug-resistant (MDR) bacteria. Furthermore, the entire integron element is often contained within another mobile genetic element such as plasmids and transposons (8), which suggests that entire integron elements, including their gene cassettes, can spread horizontally through bacterial populations.

Recent studies have examined integron distributions in uropathogenic *E. coli* collected in Taiwan (5), Korea (37), southern India (28), and Sweden (14). These studies have established a strong association between the presence of integrons and antimicrobial resistance, both MDR and single-drug resistance, particularly to TMP-SMX. The TMP-SMX resistance cassettes belong to the dihydrofolate reductase (*dfr*) enzyme family, which has over 15 allelic variants (35). Several studies have noted the importance of a single variant from this gene family, *dfrA17*. The *dfrA17* cassette was not described until 2000 (4), and a recent retrospective study suggests that its prevalence has risen dramatically in the last decade (36), in concert with an increase in the overall rate of TMP-SMX resistance. Also, the *dfrA17* cassette has recently been shown to be present in most CgA uropathogenic *E. coli* isolates (11).

This study describes the distribution of class 1 and 2 integron gene cassettes present in a population-based collection of uropathogenic *E. coli*. It compares this distribution within and beyond the CgA group while controlling for geographic and temporal variation.

MATERIALS AND METHODS

Bacterial isolates. Between October 1999 and January 2000, 505 consecutive urine samples were taken from 228 unique women with symptoms of UTI examined at a student health center. From these women, 255 *E. coli* isolates were obtained (25). In a second-phase study between October 2000 and January 2001, 468 consecutive urine samples were taken from 206 unique women at the same health center, yielding 227 *E. coli* isolates (26).

Bacterial isolation, identification, and drug susceptibility tests for these 482 isolates were done as previously described (25, 26). Briefly, urine samples were cultured on MacConkey agar and colonies that were positive for lactose and indole were presumptively identified as *E. coli*. One colony from each urine culture was then selected. These isolates were screened for susceptibility to TMP-SMX with the use of Etest strips (AB Biodisk, Solna, Sweden). Intermediate susceptibility was interpreted as full susceptibility. The isolates were screened with the ERIC2 PCR fingerprinting assay as previously described (19). From this collection, we selected all isolates exhibiting the characteristic CgA ERIC2 fingerprint pattern ($n = 32$, 27 of which were TMP-SMX resistant), all non-CgA isolates with resistance to TMP-SMX ($n = 68$), and 39 randomly selected TMP-SMX-susceptible isolates.

Preparation of bacterial DNA and PCR amplification for integron detection. The bacterial DNA was obtained from a single *E. coli* colony that was picked from LB agar plates, suspended in water, pelleted by centrifugation, resuspended and diluted in water, boiled, and centrifuged. Two microliters of the resulting supernatant was used as template DNA in a 25 μM PCR. For each of the target genes, PCRs contained 1.5 mM $MgCl_2$, 0.4 mM deoxynucleoside triphosphates (dNTPs), 1 U of Invitrogen *Taq* polymerase, 1× Invitrogen PCR buffer, and 2 μM of each of the two primers. Primers and thermocycling conditions were as published: for the 16S gene positive control, 16S8F/16S806R (27); for *intI1*, IntI1F/IntI1R (22); for class 1 gene cassettes, RB317/RB320 (2) or 5′CS/3′CS (24); for *intI2*, RB201/RB202 (2); and for class 2 gene cassettes, Hep51/Hep74 (34). PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

Sequencing of amplified integron gene cassettes. For each PCR product that appeared as a unique size when visualized on the gel, a number of samples of the post-PCR mixture was processed with the QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA) and used for direct sequencing. Sequencing was done on an Applied Biosystems 3730 DNA analyzer (Applied Biosystems, Foster City, CA) at the UC Berkeley DNA Sequencing Facility. Sequence trace files were base called and assembled with the phred-phrap-consed programs (7, 13).

RESULTS

Prevalence of integrons and arrangement of integron gene cassettes. The gene for class 1 integrase (*intI1*) was detected in 68 (49%) of 139 *E. coli* isolates tested. Class 1 gene cassettes were detected in 65 (47%) of 139 isolates; thus, there were three isolates which, while TMP-SMX resistant and possessing *intI1*, failed to yield gene cassettes. There were eight distinct molecular weight classes for the 65 isolates that yielded a class 1 gene cassette PCR product. A subset of the PCR products from each size class was directly sequenced to ascertain the gene cassette arrangements (Table 1). In all but one case, all of the PCR products from a single size class had identical sequences. The one exception was the four isolates which had 1.7-kb amplicons (Table 1).

BLAST alignment of the complete nucleotide sequences against GenBank sequences produced many matches of 99 to 100% identity across a broad range of microbes (Table 1). The most common cassettes observed in the class 1 integrons were those encoding aminoglycoside adenyltransferase A (*aadA*) and dihydrofolate reductase A (*dfrA*), which confer resistance to streptomycin and to TMP-SMX, respectively. *aadA* was present in 59 isolates (91% of those with detectable gene cassettes), predominantly as *aadA5* (38 isolates) and *aadA1* (16 isolates). *dfrA* was present in 52 isolates (80% of those with detectable gene cassettes), predominantly as *dfrA17* (38 isolates). Three other gene cassettes were detected at lower frequencies (Table 1). The gene for class 2 integrase (*intI2*) was detected in 28 (20%) of 139 isolates. One cassette arrangement accounted for 27 (96%) isolates (Table 1). Compared with class 1, class 2 integrons were thus less common and their cassette arrangements were much more homogenous.

Prevalence of integrons in CgA versus non-CgA isolates. The CgA status of an isolate was a significant predictor of class 1 integron status. Class 1 integron was found in 25 (93%) of 27 TMP-SMX-resistant CgA isolates but in only 43 (63%) of 68 TMP-SMX-resistant non-CgA isolates ($P < 0.001$) and in none of 39 TMP-SMX-susceptible non-CgA *E. coli* isolates ($P < 0.0001$). The CgA isolates were also homogenous with regard to their class 1 gene cassette arrangements: a 1.8-kb PCR product was present in 25 (93%) of 27 TMP-SMX-resistant CgA isolates; this was the only size found in CgA isolates. In contrast, only 13 (19%) of 68 TMP-SMX-resistant non-CgA isolates had the 1.8-kb PCR product ($P < 0.001$); 27 others contained seven different PCR products at lesser frequencies. No single cassette arrangement accounted for a majority of the TMP-SMX-resistant non-CgA isolates. Class 2 integrons were detected at nearly the same proportion in CgA and non-CgA samples. Nearly all isolates that were positive for an integron (of either class) were also TMP-SMX resistant; only 1 of the 44 TMP-SMX-susceptible isolates was integron positive (Table 1). Of the 25 CgA isolates containing the *dfrA17-aadA5* cas-

TABLE 1. Prevalence of class 1 and 2 integrase genes and gene cassettes by molecular weight of PCR products

PCR product(s)[a]	Total no. (%) of isolates (n = 139)	No. of sequenced isolates[b]	No. (%) of isolates[c]:		
			TMP-SMX-R		TMP-SMX-S (n = 44)[d]
			CgA (n = 27)	Non-CgA (n = 68)	
intI1 gene	68 (49)[e]		25 (93)[e]	43 (63)[e]	0
Class 1 gene cassette PCR product: GenBank records of ≥99% sequence ID	65	46	25	40	0
0.9 kb (*dfrA5*): AJ419169^{E}, AY827837^{S}, AY968807^{K}, AF512546^{N}	3 (5)[f]	3	0 (0)[f]	3 (8)[f]	0
1.0 kb (*dfrA7*): X58425^{E}, AY245101^{S}, AF139109^{N}	3 (5)	2	0 (0)	3 (8)	0
1.2 kb (*aadA1*): X12870^{E}, AB126601^{S}, AF202976^{N}, AY103457^{N}, AF052459^{P}	10 (15)	5	0 (0)	10 (25)	0
1.7 kb (*dfrA1*, *aadA1*): AJ884723^{E}, AY602405^{S}, AF203818^{S}, AJ879461^{S}, AY309066^{K}	2 (3)	2	0 (0)	2 (5)	0
1.7 kb (*dfrA15*, *aadA1*): DQ322595^{K}	2 (3)	2	0 (0)	2 (5)	0
1.8 kb (*dfrA17*, *aadA5*): AB189264^{E}, AY748452^{E}, AB126604^{S}, AY994155^{K}, AB189979^{N}	38 (58)	28	25 (100)*	13 (32)*	0
2.1 kb (*dfrA12*, orfF, *aadA2*): AB154407^{E}, AY509004^{S}, AY748453^{K}, AF188331^{N}, AB191048^{P}	5 (8)	2	0 (0)	5 (12)	0
2.2 kb (*bla*OXA-30, *aadA1*): AY224185^{E}, AJ009819^{S}, AY574195^{N}	1 (2)	1	0 (0)	1 (2)	0
2.5 kb (*aadB*, *aadA1*, *cmlA6*) DQ018384^{E}	1 (2)	1	0 (0)	1 (2)	0
intI2 gene	28 (20)[e]		4 (15)[e]	23 (34)[e]	1
Class 2 gene cassette PCR product: GenBank records of ≥99% sequence ID	28	3	4	23	1
1.7 kb (*sat2*, *aadA1*): AB161462^{S}, DQ176869^{N}, DQ176451^{N}, AY639870^{N}	1 (4)	1	0	0	1 (100)
2.2 kb (*dfrA1*, *sat1*, *aadA1*): AB161451^{E}, AY140652^{S}, AB161461^{S}, AJ289189^{N}, L36547^{N}	27 (96)	2	4 (100)	23 (100)	0

[a] ID, identity. Superscript abbreviations represent GenBank records from the following organisms: E, *Escherichia coli*; S, *Salmonella* spp.; K, *Klebsiella oxytoca*; N, other gram-negative gammaproteobacteria; P, gram-positive coccus.

[b] The number of the PCR products that were selected for direct sequencing. Within each size class, all PCR products selected for sequencing yielded identical sequences. The unsequenced PCR products are assumed to have the same gene cassette arrangement as those selected for sequencing.

[c] TMP-SMX-R, trimethoprim resistant. *, $P \leq 0.001$.

[d] TMP-SMX-S, trimethoprim susceptible. Of 44 TMP-SMX-S isolates, only 5 were CgA, and only 1 of these carried an integron.

[e] Percentages of integrase-positive isolates are calculated out of the column total.

[f] Percentages of isolates with specific gene cassette assemblies are calculated out of the number of gene cassette-positive isolates.

settes, 3 were serogroup O77, 11 were O11, and 11 were untyped.

DISCUSSION

Our results generally corroborate the integron prevalence observed in uropathogenic *E. coli* studied in Asia and Europe (5, 14, 28, 36, 37). In these studies, class 1 integrons were found in about 70% of TMP-SMX-resistant isolates. The studies that examined class 2 integrons (14, 28, 36) found them in 5 to 15% of clinical isolates, also highly correlated with TMP-SMX resistance. All studies, including the present one, indicate that integrons of either class 1 or 2 are very rare in TMP-SMX-susceptible isolates.

Our results also confirm the high prevalence of the *aadA* and *dfrA* gene cassettes, and particularly the high prevalence of the *dfrA17* gene cassette. All CgA isolates in this study contained the same class 1 integron gene cassette arrangement, composed of *dfrA17* and *aadA5*. The nucleotide sequence of this arrangement, confirmed by sequencing 28 of the 38 1.8-kb PCR products, is identical to GenBank sequences from a variety of gram-negative and gram-positive bacteria (Table 1).

In a recent study from Colorado (3), *dfrA7*, not *dfrA17*, was reported to be present in 9 of 10 UPEC CgA isolates. However, this study used a probe hybridization technique that may not have differentiated between *dfrA7* and *dfrA17*, which share 92% nucleotide sequence identity. We obtained two of those isolates from the authors and found, by sequencing, that they in fact harbor *dfrA17* (personal communication).

In a study from Michigan (10, 11), several different determinants of TMP-SMX resistance were observed within CgA isolates (mostly *dfrA17*, but also including *dfrA12* and an unknown form). In this case, the heterogeneity may relate to differences in the sampling methods used. The present study analyzed a population-based collection of uropathogenic *E. coli* obtained from two discrete time periods from the same community. The difference could also have arisen from differences in the interpretation of ERIC2 PCR electrophoretic banding patterns. In either case, when the authors defined CgA with the *fumC* single-nucleotide polymorphism method (20), their results were quite similar to the present study.

The homogeneity of the class 1 gene cassettes among the CgA isolates and the heterogeneity among the non-CgA *E. coli* strains observed in the present study suggest a recent acquisition and common origin of the TMP-SMX resistance cassette in this clonal group. Since non-CgA isolates contained a variety of class 1 gene cassette arrangements, the observation that genotypically identical or similar *E. coli* strains contained the same integron gene cassette arrangement suggests that the acquisition of this drug resistance determinant by CgA strains did not occur at multiple locales on multiple occasions. Rather, this may have occurred at a common site, and once this occurred, the MDR CgA strains disseminated from there.

Researchers have found class 1 and 2 integrons to be common in *E. coli* and *Salmonella enterica* isolated from livestock (12), in *E. coli* isolated from irrigation water contaminated with farm animal feces (30), and also in gram-positive bacteria isolated from poultry litter. Furthermore, it is noteworthy that the same *dfrA17-aadA5* integron appears to be common in *E. coli* and *Salmonella* spp. isolated from chickens, livestock, and water (1, 6). We have also recently found that one of the six CgA animal isolates (a 1999 cow isolate) reported by Tartof et al. as ST101 (33) contains this *dfrA17-aadA5* integron arrangement, with 100% sequence identity. This finding provides evidence in support of the animal origin of CgA *E. coli* strains (25, 29).

The availability of a large population-based collection of *E. coli* isolates from women with UTI from one geographic site made it possible not only to suggest a possible common source outbreak caused by a single clonal group, but also to suggest that the acquisition of TMP-SMX resistance by the clonal group may have occurred at a point source—in an animal, human, or environmental reservoir. Since we did not compare the sequences of other resistance determinants, we cannot be sure that other drug resistances also originated from a common reservoir. These observations indicate that human clinical use of TMP-SMX may not be playing a major role in the emergence of these resistant uropathogenic *E. coli* strains. Rather, they may play a greater role in maintaining these organisms in the human reservoir, once they are introduced into the human population.

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