Antimicrobial Resistance Markers of Class 1 and Class 2 Integron-bearing Escherichia coli from Irrigation Water and Sediments

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Municipal and agricultural pollution affects the Rio Grande, a river that separates the United States from Mexico. Three hundred and twenty-two Escherichia coli isolates were examined for multiple antibiotic resistance phenotypes and the prevalence of class 1 and class 2 integron sequences. Thirty-two (10%) of the isolates were resistant to multiple antibiotics. Four (13%) of these isolates contained class 1–specific integron sequences; one isolate contained class 2 integron–specific sequences. Sequencing showed that the class 1 integron–bearing strain contained two distinct gene cassettes, sat-1 and aadA. Although three of the four class 1 integron–bearing strains harbored the aadA sequence, none of the strains was phenotypically resistant to streptomycin. These results suggest that integron-bearing E. coli strains can be present in contaminated irrigation canals and that these isolates may not express these resistance markers.

Integron gene sequences contribute to the spread of antimicrobial resistance alleles by lateral gene transfer of gene cassettes in a variety of enteric bacteria, including Campylobacter spp., Escherichia coli, and Salmonella enterica serotype Typhimurium (1–4). The gastrointestinal environment is suspected of serving as a reservoir for integron-bearing strains; when antimicrobial exposure occurs, gene transfer events—which spread cassettes between commensal organisms that are expelled into the environment (2)—would also occur.

The Rio Grande, the river separating the United States from Mexico along the Texas-Mexico region, serves as a source for irrigation water in Texas and Mexico. Previous studies in our laboratory and others have shown that the transboundary region is subject to extensive microbial and chemical contamination. This contamination has been associated with agricultural, municipal, and industrial wastes originating from both sides of the border (5,6). Leaking septic tanks and wastewater effluent discharges result in fecal contamination levels as high as 2,000 CFU/mL of fecal coliforms (7,8).

Because of the strategic importance of the Rio Grande for U.S. agriculture and the potential transmission of antimicrobial resistance determinants by means of food crops, we investigated the prevalence and characteristics of class 1 and class 2 integron–bearing E. coli strains. These strains were previously isolated from a study investigating fecal contaminants in irrigation water and associated sediments at specific locations along the river (9).

Methods

Three hundred and twenty-two E. coli isolates were previously isolated from irrigation water and associated sediments at the El Paso, Presidio, and Weslaco regions of the river (9). After being confirmed as E. coli by MUG (4-methyl umbelliferyl-β-D-glucuronide)–based fluorescence, these isolates were screened for antimicrobial susceptibility by using the agar dilution method (10,11). The isolates were tested against ampicillin, tetracycline, ceftriaxone, cephalothin, gentamicin, kanamycin, streptomycin, chloramphenicol, ciprofloxacin, and trimethoprim/sulfamethoxazole. The antibiotics were tested at concentrations established by the National Antimicrobial Resistance System (12).

Isolates that were multidrug resistant (resistant to two or more antimicrobial agents) were grown overnight in 5 mL of Mueller-Hinton broth (Accumedia, Baltimore, MD) with the appropriate concentration of antimicrobial compound. A 1-mL aliquot of the culture was centrifuged at 10,000 rpm for 2 min. The cell pellet was resuspended in 500 µL of sterile water and boiled for 10 min. The resulting DNA suspension was used as template DNA in polymerase chain reaction (PCR) amplification for the class 1 and class 2 integrase gene and variable regions using the primer sequences shown in the Table (13–15).

The PCR reactions used 10 µL of template DNA, 5 µM of primers, 25 mM MgCl, 10 mM deoxynucleotide triphosphate, and 23 ng bovine serum albumin. Nuclease-free water (Ambion, Austin, TX) was added to achieve a volume of 50 µL. A “hot start” method was used, and 1.25 U of Taq DNA polymerase (Sigma, St. Louis, MO) was

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added after initial template denaturation. The PCR cycle was as follows: initial denaturation for 12 min at 94°C, hot start pause at 80°C followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 5 min at first cycle. An additional 5 s was progressively added to each cycle to reach a final of 7 min, 55 s. PCR products were analyzed on 1% agarose gel.

Amplification products were extracted from the gels with the QIAGEN QIAquick gel extraction kit (Valencia, CA). The amplified products were sequenced at a commercial facility (MWG Biotech Inc., High Point, NC) with the QIAGEN QIAquick gel extraction kit (Valencia, CA). The amplified products were analyzed by using Clustal W version 1.82 (18). Putative gene relationships and sequence data were compared with the Presidio and Weslaco sampling regions.

Results

Of the 322 E. coli isolates from sediment and irrigation water samples analyzed for antimicrobial resistance, 104 (32%) isolates showed resistance to at least one of the antimicrobial compounds (Figure 1). Approximately 10% (32/322) of all the isolates showed a multidrug resistance phenotype. Eighteen percent of the isolates were resistant to ceftriaxone; however, only 5% (2/32) of all isolates were resistant to ceftriaxone, which also belongs to the cephalosporin family. Resistance to ampicillin was prevalent in approximately 35% (11%) of the isolates. Resistance to tetracycline (9%), kanamycin (2%), gentamicin (0.3%), and streptomycin (4%) was also observed. Resistance to the fluoroquinolone ciprofloxacin was seen in one isolate. Three (<1%) of the 322 isolates were resistant to sulfonamide sulfamethoxazole. On the basis of analysis of variance, antimicrobial resistance and the sampling location were correlated. Isolates from the El Paso sampling region had significantly higher (p<0.05) antimicrobial resistance as compared with the Presidio and Weslaco sampling regions (data not shown).

### Table. Oligonucleotide primer sequences used for amplification of class 1 and class 2 integrase and variable regions

| Primer | Primer sequence | Target | Reference |
|--------|----------------|--------|-----------|
| intI-1 | 5′-GGCATCAAGCAGAAG-3′ | 5′-Class 1 integron variable region | Levesque et al. 1995 (13) |
| intI-2 | 5′-AAACAGACTGTGACCTGA-3′ | 3′-Class 1 integron variable region | Levesque et al. 1995 (13) |
| hep51 | 5′-GTAGCCATGCAAGTACGAG-3′ | 5′-Class 2 integron variable region | White et al. 2001 (14) |
| hep74 | 5′-CGGGATCCGACGGCATGCGAACATTGTA-3′ | 3′-Class 2 integron variable region | White et al. 2001 (14) |
| intI1F | 5′-GGGCTAAGATCGATGGGTTTG-3′ | 5′-intI1 gene | Mazel et al. 2000 (15) |
| intI1R | 5′-CATATGGTAAATCATCGTC-3′ | 3′-intI1 gene | Mazel et al. 2000 (15) |
| intI2F | 5′-CAAGGATATCGACAAAGGT-3′ | 5′-intI2 gene | Mazel et al. 2000 (15) |
| intI2R | 5′-GTAGCAACGAGGTACGAAAAATG-3′ | 5′-intI2 gene | Mazel et al. 2000 (15) |
animal wastes regularly harbor multidrug-resistant water. Previous studies have reported that municipal and urbanized sampling locations is not surprising since these isolates, but resistance to the closely related kanamycin was seen. These results are similar to those reported by Zhao et al. (3), who identified that the aadA gene transferred to a strain of Hafnia alvei but did not report resistance to streptomycin or spectinomycin. These researchers attributed their findings to the inefficient expression of the inserted gene cassette by the integron promoter. Previous studies have also shown that the antimicrobial resistance phenotype can be modulated once these strains are exposed to specific environmental conditions (32).

The aadA gene cassette is not novel in class 1 integrons. Earlier work by Zhao et al. (3) and Bass et al. (24) has shown that the aadA gene is highly conserved among Shiga toxin–producing and avian clinical E. coli isolates, respectively. The only class 2 integron-bearing strain isolated in this study also contained the aadA gene in addition to the sat-1 gene, which codes for resistance to kanamycin, a finding in agreement with the phenotypic expression.
The sat-1 gene, which codes for the streptothricin acetyl transferase, was not detected in any other E. coli isolate. The presence of the sat-1 gene cassette, in combination with the aadA gene, suggests that this class 2 integron is likely a derivative of the class 2 integron found on transposon Tn7 (33,34).

The aadA gene was conserved among the class 1 and class 2 integrons, which suggests a possible selective mechanism for this cassette in enteric bacteria from natural waters. The 2-kb integron-specific variable region–containing strain, which was isolated from the Presidio area, harbored the dihydrofolate reductase gene (dhfrXII) instead of the aadA gene (35).

Overall, these results suggest that the irrigation canals and sediments associated with the Rio Grande are contaminated by bacteria of fecal origin that contain antimicrobial resistance genes. Of 322 E. coli isolates, 32 (approximately 10%) were resistant to multiple antimicrobial drugs. Five of these 32 E. coli isolates harbored class 1 and class 2 integron sequences. This study did not investigate the possibility that other integron-bearing nonfecal bacteria were present. The occurrence of integron-bearing E. coli in irrigation water is important since these organisms are known fecal contaminants, and the potential for lateral gene transfer exists. The results also indicate that integron-bearing strains may not always express the antimicrobial phenotype; thus, phenotype-based isolation of resistant organisms can underestimate the levels of resistant organisms. Studies are needed to identify whether integron-mediated antimicrobial resistance transfer does indeed occur within the irrigation canal sediments and on vegetable surfaces, when they are irrigated with contaminated irrigation water.

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