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Identification and Characterization of Integron-Mediated Antibiotic Resistance among Shiga Toxin-Producing *Escherichia coli* Isolates

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A total of 50 isolates of Shiga toxin-producing *Escherichia coli* (STEC), including 29 O157:H7 and 21 non-O157 STEC strains, were analyzed for antimicrobial susceptibilities and the presence of class 1 integrons. Seventy-eight ($n = 39$) percent of the isolates exhibited resistance to two or more antimicrobial classes. Multiple resistance to streptomycin, sulfamethoxazole, and tetracycline was most often observed. Class 1 integrons were identified among nine STEC isolates, including serotypes O157:H7, O111:H11, O111:H8, O111:NM, O103:H2, O45:H2, O26:H11, and O5:NM. The majority of the amplified integron fragments were 1 kb in size with the exception of one *E. coli* O111:H8 isolate which possessed a 2-kb amplicon. DNA sequence analysis revealed that the integrons identified within the O111:H11, O111:NM, O45:H2, and O26:H11 isolates contained the *aadA* gene encoding resistance to streptomycin and spectinomycin. Integrons identified among the O157:H7 and O103:H2 isolates also possessed a similar *aadA* gene. However, DNA sequencing revealed only 86 and 88% homology, respectively. The 2-kb integron of the *E. coli* O111:H8 isolate contained three genes, *dfr*XII, *aadA*2, and a gene of unknown function, *orfF*, which were 86, 100, and 100% homologous, respectively, to previously reported gene cassettes identified in integrons found in *Citrobacter freundii* and *Klebsiella pneumoniae*. Furthermore, integrons identified among the O157:H7 and O111:NM strains were transferable via conjugation to another strain of *E. coli* O157:H7 and to several strains of *Hafnia alvei*. To our knowledge, this is the first report of integrons and antibiotic resistance gene cassettes in STEC, in particular *E. coli* O157:H7.

Shiga toxin-producing *Escherichia coli* (STEC) have been an important cause of foodborne illness worldwide. Human infection with STEC is potentially fatal and may be associated with serious complications such as hemolytic -uremic syndrome (HUS) and hemorrhagic colitis (7). Among STEC strains, O157:H7 is the classical serotype that was first associated with enterohemorrhagic diseases in the early 1980s as a cause of serious foodborne disease outbreaks. Since then, over 100 STEC serotypes, other than O157:H7, have been associated with human illness (17). In the United States approximately 73,000 cases of infections annually have been attributed to O157:H7 strains and more than 36,000 to non-O157:H7 STEC strains (16). There are more than 100 deaths each year due to STEC infections. Several studies in Japan have suggested that initiation of antibiotic therapy early in the stages of STEC infection was able to prevent the disease progression to HUS (5, 11, 30). However, antimicrobial treatment for STEC infections is still regarded as controversial (10, 23, 35). Currently, no specific therapy for HUS is available, and most patients require prolonged clinical and outpatient treatment (31).

Initially, *E. coli* O157:H7 was found to be susceptible to many antibiotics (3, 24). However, several recent studies have documented antibiotic resistance among *E. coli* O157:H7 isolates (4, 13, 19, 29). Non-O157 STEC strains isolated from humans and animals also have developed antibiotic resistance, and many are resistant to multiple antimicrobials commonly used in human and veterinary medicine (4, 6, 29). There is currently a great deal of speculation regarding the role that the therapeutic and subtherapeutic use of antimicrobials in animals has played in accelerating the development and dissemination of antimicrobial-resistant bacterial pathogens (1, 33, 34). Research is urgently needed to determine the potential effect of antimicrobials used in animal production environments on the emergence and spread of bacterial antibiotic resistance in both veterinary and human medicine.

A novel system has been identified in multiple resistant bacteria and is postulated to play an important role in the acquisition and dissemination of antibiotic resistance genes (8). This system has been referred to as bacterial integrons. Integrons are mobile DNA elements with a specific structure consisting of two conserved segments flanking a central region containing "cassettes" that usually code for resistance to specific antimicrobials (9). Four classes of integrons have been identified to date (15). The majority of integrons identified among clinical isolates belong to class 1 type (12, 27). In class 1 integrons, the 5′ conserved region encodes a site-specific recombinase (integrase) and a strong promoter or promoters that ensure expression of the integrated cassettes. The 3′ conserved segment carries *qac*Δ*E*, which specifies resistance to antiseptics and disinfectants; the *sul-1* gene, which confers sulfonamide resistance; and an open reading frame of unknown function (9). Multiple cassette insertions and more than 40 distinct cassettes have been identified among integrons (25). Currently, there is a paucity of data regarding the mechanisms of acquisition and dissemination of antibiotic resistance in *E. coli* O157:H7 and other STEC. This study was initiated to

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TABLE 1. Characteristics of O157:H7 and non-O157 STEC isolates used in the study

Isolate	Serotype	*stx* gene[a]	Presence (+) or absence (−) of:		Source	Yr
			eae gene	*hly*A gene		
CVM65	O157:H7	1, 2	+	+	Cattle	1991
CVM68	O157:H7	1, 2	+	+	Cattle	1991
CVM990	O157:H7	1, 2	+	+	Cattle	1991
CVM996	O157:H7	1, 2	+	+	Cattle	1991
CVM1001	O157:H7	1, 2	+	+	Cattle	1991
CVM69	O157:H7	1, 2	+	+	Cattle	1992
CVM73	O157:H7	1, 2	+	+	Cattle	1992
CVM998	O157:H7	1, 2	+	+	Cattle	1992
CVM1002	O157:H7	1, 2	+	+	Cattle	1992
CVM1000	O157:H7	1, 2	+	+	Cattle	1992
CVM1010	O157:H7	1, 2	+	+	Cattle	1992
CVM35	O157:H7	1, 2	+	+	Cattle	1993
CVM37	O157:H7	2	+	+	Cattle	1993
CVM44	O157:H7	1, 2	+	+	Cattle	1993
CVM978	O157:H7	2	+	+	Cattle	1993
CVM981	O157:H7	1, 2	+	+	Cattle	1993
CVM988	O157:H7	1, 2	+	+	Cattle	1993
CVM1006	O157:H7	2	+	+	Cattle	1993
CVM1007	O157:H7	1, 2	+	+	Cattle	1993
CVM79	O157:H7	1, 2	+	+	Ground beef	1988
CVM33	O157:H7	1, 2	+	+	Ground beef	1993
CVM1004	O157:H7	1, 2	+	+	Ground beef	1993
CVM46	O157:H7	1, 2	+	+	Ground beef	1994
CVM47	O157:H7	1, 2	+	+	Ground beef	1994
CVM1009	O157:H7	1, 2	+	+	Ground beef	1994
CVM48	O157:H7	1, 2	+	+	Ground beef	1995
CVM90	O157:H7	1, 2	+	+	Human	1983
CVM51	O157:H7	1, 2	+	+	Human	1992
CVM1008	O157:H7	1, 2	+	+	Human	1992
CVM1871	O26:H11	1	+	+	Cattle	1991
CVM1888	O26:H11	1	+	+	Cattle	1993
CVM1875	O103:H2	1, 2	+	+	Cattle	1993
CVM1876	O103:H2	1	+	+	Cattle	1993
CVM1877	O111:H8	1	+	+	Cattle	1993
CVM1884	O111:NM	1	+	+	Cattle	1993
CVM1878	O111:H11	1, 2	+	+	Cattle	1994
CVM1879	O111:H11	1	+	+	Cattle	1994
CVM1880	O111:NM	1, 2	+	+	Cattle	1994
CVM1881	O118:H16	1	+	+	Cattle	1993
CVM1887	O126:H8	1	+	−	Cattle	1991
CVM1874	O91:H21	1, 2	−	+	Cattle	1993
CVM1889	O45:H2	1	+	+	Cattle	1992
CVM1873	O88:H49	1,	+	+	Ground beef	1994
CVM1872	O46:H38	1, 2	−	+	Ground beef	1991
CVM1870	O22:H8	2	−	+	Ground beef	1993
CVM1882	O125:NM	1	+	+	Human	1990
CVM1883	O113:K75:H21	2	+	+	Human	1988
CVM1885	O103:H2	1, 2	+	+	Human	1987
CVM1890	O5:NM	1	+	+	Human	1996
CVM1891	O_{rough}:H9	2	+	+	Human	1996

[a] 1, *stx-1* positive; 2, *stx-2* positive.

characterize antimicrobial susceptibility patterns among STEC strains, including *E. coli* O157:H7, isolated from cattle, ground beef, and humans and to determine if resistance phenotypes observed could be attributed to the acquisition of integron-mediated resistance gene cassettes.

MATERIALS AND METHODS

Bacterial strains. A total of 50 STEC isolates including 29 O157:H7 strains and 21 non-O157 STEC strains from humans, animals, and foods were used in the study (Table 1). The STEC isolates originated from humans ($n = 8$), cattle ($n = 32$), and ground beef ($n = 10$). The 29 *E. coli* O157:H7 strains were selected among 118 isolates from a previous study (19) and were resistant to at least one antibiotic based on disk diffusion in vitro susceptibility testing (Difco, Detroit, Mich). *Salmonella enterica* serovar Typhimurium DT104 CVM786 was used as a positive control for integron PCR reactions. Three *Hafnia alvei* strains isolated from ground beef and one strain of *E. coli* O157:H7 originating from human stools were used as recipient strains in conjugation experiments. Bacteria were grown on MacConkey agar (Difco) and stored in Trypticase soy broth (Difco) containing 50% glycerol at −80°C until use.

Quantitative antimicrobial susceptibility determination. Antimicrobial MICs of *E. coli* isolates were determined via the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for broth microdilution methods (20, 21). Sensititre susceptibility testing was performed according to the manufacturer's instructions. The following antimicrobials were tested: amikacin, amoxicillin-clavulanic acid, ampicillin, apramycin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. *E. coli* ATCC 25922 and 35218, *Enterococcus faecalis* ATCC 29212, *Staphyloccoccus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls in antimicrobial MIC determinations.

Bacterial DNA preparation, PCR, and DNA sequencing. Bacterial DNA used for PCR was prepared by boiling a bacterial culture in 500 μl of distilled water for 10 min, followed by centrifugation. Isolates were initially characterized for STEC-associated virulence genes, *stx-1* and *stx-2* coding for Shiga toxins, *eae* for intimin that is associated with attaching and effacing lesion, and *hlyA* for hemolysin (18). The presence of class 1 integrons and the associated sulfonamide resistance gene (*sul-1*) and integrase gene (*int*) were detected using the PCR method described by Levesque et al. (14) and Sandvang et al. (28). Class 1 integrons were amplified using the following PCR primers: 5′-CS (5′-GGCAT CCAAGCACAAGC-3′) and 3′-CS (5′-AAGCAGACTTGACTGAT-3′). The class 1 integrase gene and sulfonamide resistance gene were also amplified as described above, but the following primers were used: *int*-F (5′-CCTCCCGCA CGATGATC-3′), *int*-R (5′-TCCACGCATCGTCAGGC-3′), *sul1*-F (5′-CTTC GATGAGAGCCGGCGGC-3′), and *sul1*-R (5′-GCAAGGCGGAAACCCGC GCC-3′). All primers were synthesized by Life Technologies (Gaithersburg, Md.). Amplification reactions were carried out with 10 μl of boiled bacterial suspensions, 250 μM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 50 pmol of primers, and 1 U of Gold *Taq* polymerase (Perkin-Elmer, Foster City, Calif.). Distilled water was added to bring the final volume to 50 μl. The PCR cycle for class 1 integron and integrase gene included an initial denaturation at 94°C for 10 min, followed by 30 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 54°C, and extension for 2 min at 72°C, and a final extension at 72°C for 10 min. The PCR cycle for amplification of the *sul-1* gene was identical to that described above, except for the annealing temperature, which was 59°C. The reaction products were then analyzed by electrophoresis in 1.0% agarose gels stained with ethidium bromide, visualized under UV light, and recorded by using a gel documentation system (Bio-Rad, Hercules, Calif.). For each set of PCR reactions, serovar Typhimurium DT104 CVM786 was included as a positive control. The PCR-generated DNA fragments were then purified using a PCR purification kit (Boehringer Mannheim, Indianapolis, Ind.) and sequenced in an ABI automatic DNA sequencer (Model 377; Perkin-Elmer) at the University of Maryland Biotechnology Institute by using the above-described forward and reverse primers. DNA sequences were analyzed by searching the GenBank database of the National Center for Biotechnology Information via the BLAST network service. The *E. coli* O157:H7 integron sequence has been assigned the GenBank accession number AF234167.

Southern blot hybridization. Southern blot hybridizations were performed to determine the locations of identified integrons. Genomic and plasmid DNA were isolated from integron-positive STEC isolates using the High Pure Plasmid Isolation Kit (Boehringer Mannheim). Genomic and plasmid DNA were both digested with *Bam*HI. The class 1 integrase gene was used as a DNA probe and was labeled with digoxigenin using the PCR DIG Probe Synthesis Kit (Boehringer Mannheim). Digested DNA was run in 1% agarose gels and transferred to nylon membranes. Hybridization procedures and conditions were performed as specified with a nonradioactive labeling and detection kit (Boehringer Mannheim).

Conjugation experiments. *E. coli* O157:H7 strain CVM990 and *E. coli* O111:NM strain CVM1884 that possessed integrons were used as donor strains. *E. coli* O157:H7 strain JM263 and *H. alvei* strains CVM1202, CVM1203, and CVM1208 were used as recipient strains (see Table 4). Streptomycin and tetracycline resistance were used as selective markers for *E. coli* O157:H7 strains CVM990 and CVM1884, respectively, based on their antibiotic resistance phenotypes (see Table 4). Nalidixic acid and ampicillin resistance were used as selective markers for *E. coli* O157:H7 JM263 and *H. alvei* strains CVM1202, CVM1203, and CVM1208, respectively. Conjugation was performed by mating a

donor strain with a recipient strain on Trypticase soy agar (TSA; Difco) plates (36). After incubation at 37°C overnight, the mating mixture was streaked onto a TSA plate containing a combination of selective antibiotics dependent upon the donor and recipient strains used (100 μg of streptomycin and 100 μg of nalidixic acid per ml, 100 μg of streptomycin and 100 μg of ampicillin per ml, 30 μg of tetracycline and 100 μg of nalidixic acid per ml, or 30 μg of tetracycline and 100 μg of ampicillin per ml). Transconjugants were examined for antibiotic susceptibility profiles, and integron transfer was confirmed via PCR assays and/or DNA sequencing.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed to determine DNA fingerprinting profiles of donors, recipients, and transconjugates and to verify the gene transfer from donor to recipient strains. The PFGE procedure of the Centers for Disease Control and Prevention was used. Briefly, bacteria were grown on TSA plates supplemented with 5% defibrinated sheep blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.) at 37°C for 18 h. Bacterial colonies were suspended in cell suspension buffer (100 mM Tris HCl, 100 mM EDTA; pH 8.0) and adjusted to an optical density of 0.50 to 0.54 using Dade MicroScan Turbidity Meter (Dade Behring, Inc., West Sacramento, Calif.). The cell suspension (200 μl) was mixed with 10 μl of proteinase K (10 mg/ml) and an equal volume of melted 1% SeaKem Gold agarose (FMC BioProducts, Rockville, Maine) containing 1% sodium dodecyl sulfate. The mixture was carefully dispensed into a sample mold (Bio-Rad). After solidification, the plugs were transferred to a tube containing 5 ml of lysis buffer (50 mM Tris HCl; 50 mM EDTA, pH 8.0; 1% sarcosyl) and 0.1 mg of proteinase K per ml. Cells were lysed overnight in a water bath at 54°C with agitation at 180 rpm. After lysis, the plugs were washed twice with distilled water and four times with TE buffer (10 mM Tris HCl, 1 mM EDTA; pH 8.0) for 15 min per wash at 50°C with agitation at 180 rpm. Agarose-embedded DNA was digested with 50 U of *Xba*I (Boehringer Mannheim) overnight in a water bath at 37°C. The plugs were placed in a 1% SeaKem Gold agarose gel. Restriction fragments were separated by electrophoresis in 0.5× TBE (Tris-borate-EDTA) buffer at 14°C for 18 h using a Chef Mapper (Bio-Rad) with pulse times of 2.16 to 54.17 s. The gel was stained with ethidium bromide, and DNA bands were visualized with a UV transilluminator.

RESULTS

Antimicrobial susceptibility profiles. The antimicrobial susceptibilities of 21 non-O157 and 29 O157:H7 STEC isolates were determined using microbroth dilution (Table 2). All *E. coli* O157:H7 isolates tested were resistant to at least one antibiotic, which agreed with results from our previous study using disk diffusion in vitro susceptibility testing (19). The majority of the *E. coli* O157:H7 isolates were resistant to several antimicrobials tested, particularly sulfamethoxazole (93%), tetracycline (93%), and streptomycin (76%). *E. coli* O157:H7 isolates were also resistant to kanamycin (21%) and ampicillin (14%). Several isolates were resistant to multiple antimicrobial agents, and the most common pattern was resistance to streptomycin, sulfamethoxazole, and tetracycline. Four *E. coli* O157:H7 isolates were multiple resistant to five antibiotics: ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. Two of these isolates (CVM73 and CVM1001) were isolated from cattle in 1991 and 1992, respectively, one (CVM90) was from a human sample, and the fourth (CVM79) was obtained from ground beef in 1988.

The majority of the non-O157:H7 isolates displayed resistance to multiple antimicrobials compared to the *E. coli* O157:H7 isolates (Table 2). Resistance to chloramphenicol (29%), trimethoprim-sulfamethoxazole (19%), cephalothin (19%), florfenicol (10%), and amoxicillin-clavulanic acid (5%) was only observed among non-O157:H7 isolates (Table 2). CVM1877 (O111:H8) and CVM1889 (O45:H2) were resistant to seven antibiotics: ampicillin, chloramphenicol, kanamycin, streptomycin, sulfamethoxazole, tetracycline, and either cephalothin or trimethoprim-sulfamethoxazole. CVM1879 (O111:H11) was multiply resistant to nine antibiotics, including ampicillin, cephalothin, chloramphenicol, florfenicol, kanamycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. Four non-O157:H7 isolates (CVM1877, CVN1878, CVM1879, and CVM1889) were resistant to kanamycin and chloramphenicol.

TABLE 2. Antimicrobial resistance of *E. coli* O157:H7 and non-O157 STEC isolates

Class and/or antimicrobial	% Resistant isolates[a]	
	O157:H7	Other STEC
β-Lactams		
Ampicillin	14	33
Amoxicillin-clavulanic acid	0	5
Cephalosporins		
Cephalothin	0	19
Ceftiofur	0	0
Ceftriaxone	0	0
Phenicols		
Chloramphenicol	0	29
Florfenicol[b]	0	10
Tetracycline	93	43
Aminoglycosides		
Amikacin	0	0
Apramycin	0	0
Kanamycin	21	19
Gentamicin	0	0
Streptomycin	76	43
Sulfonamides and/or trimethoprim		
Sulfamethoxazole	93	48
Trimethoprim-sulfamethoxazole	0	19
Quinolones (including fluoroquinolones)		
Nalidixic acid	0	0
Ciprofloxacin	0	0

[a] MICs were determined via microdilution methods according to NCCLS guidelines (20, 21). *E. coli* O157, 29 isolates; non-O157 STEC, 21 isolates.
[b] The resistant breakpoint used was ≥16 μg/ml.

Presence of class one integrons and integron-associated genes. Class 1 integrons were identified among nine STEC isolates representing multiple serotypes: O157:H7, O111:H11, O111:H8, O111:NM, O103:H2, O45:H2, and O26:H11 (Table 3). The majority of integron amplified fragments were 1 kb in size, except for one *E. coli* O111:H8 isolate which contained a 2-kb amplicon. DNA sequence analysis revealed that the integrons identified within the O111:H11, O111:H8, O111:NM, O45:H2, and O26:H11 isolates contained the *aadA* gene encoding resistance to streptomycin-spectinomycin. Integrons identified among the O157:H7 and O103:H2 isolates also possessed a similar *aadA* gene; however, DNA sequencing revealed only 86 and 88% homology, respectively. The 2-kb integron of the *E. coli* O111:H8 isolate contained three genes, i.e., *dfrXII* encoding trimethoprim resistance, *aadA*2 encoding resistance to streptomycin and spectinomycin, and a gene of unknown function, *orfF*, which were 86, 100, and 100% homologous to previously reported gene cassettes identified in integrons found in *Citrobacter freundii* and *Klebsiella pneumoniae*, respectively. All integron-positive STEC strains were positive for *int-1* and *sul-1*. Figure 1 shows the PCR amplicons of *sul-1* (417 bp) and *int-1* (280 bp) genes that are present in class 1

TABLE 3. Characterization of class 1 integron-associated genes among STEC isolates

Isolate	Serotype	Size of integron amplicon (kb)	Inserted gene cassettes[a]	Presence (+) of	
				sul gene	*int* gene
CVM990	O157:H7	1	*aadA6*	+	+
CVM1878	O111:11	1	*aadA1*	+	+
CVM1879	O111:11	1	*aadA1*	+	+
CVM1877	O111:H8	2	*aadA2-dfr12*	+	+
CVM1880	O111:NM	1	*aadA1*	+	+
CVM1884	O111:NM	1	*aadA1*	+	+
CVM1875	O103:H2	1	*aadA1*	+	+
CVM1889	O45:H2	1	*aadA1*	+	+
CVM1871	O26:H11	1	*aadA1*	+	+

[a] *aadA*, aminoglycoside adenyltransferase; *dfr*, dihydrofolate reductase.

integrons. Additionally, Southern blot hybridizations indicated that class 1 integrons were located on plasmids among the STEC isolates (data not shown).

Conjugal transfer of plasmids harboring class 1 integrons. Conjugal transfer of plasmids carrying class 1 integrons occurred from donor strains (O157:H7 CVM990 and O111:NM CVM1884) to recipient strains (O157:H7 JM263 and *H. alvei* CVM1202, CVM1203, and CVM1208). Class 1 integrons were detected among transconjugants of the recipient strains using integron-specific PCR assays (data not shown). DNA fingerprinting by PFGE revealed that several transconjugants, i.e., CVM990-JM263, CVM990-CVM1202, and CVM990-CVM1208, acquired a 90-kb band when CVM990 was used as the donor strain, whereas transconjugants CVM1884-JM263, CVM1884-CVM1202, and CVM1884-CVM1208 gained a 127-kb band when CVM1884 was used as the donor strain (Fig. 2)

Antimicrobial susceptibility testing of donor, recipient, and transconjugant isolates revealed that all *Hafnia* transconjugants acquired resistance to streptomycin, sulfamethoxazole, and tetracycline regardless of the donor strain (Table 4). Interestingly, in contrast to CVM1884-JM263, transconjugants CVM1884-CVM1202, CVM1884-CVM1203, and CVM1884-CVM1208 acquired streptomycin resistance even though the donor strain (CVM1884) was susceptible. DNA sequencing of the integrons amplified from both CVM1884 and transconjugant CVM1884-CVM1208 identified the identical *aadA1* gene that encodes resistance to streptomycin and spectinomycin. This most likely indicates that although the *aadA1* gene was present in the donor strain (CVM1884), it was not being efficiently expressed from the integron promoter. None of the gene cassettes identified within the integrons from the STEC isolates conferred resistance to tetracycline, despite the fact that all of the transconjugants acquired tetracycline resistance. This may be due to the presence of the tetracycline resistance gene on the same plasmid which contained the integrons or on a different plasmid that was cotransferred by conjugation with the integron-containing plasmid into the recipient cells.

DISCUSSION

The emergence and dissemination of antimicrobial resistance among STEC strains may have potential negative clinical implications, although the diarrheal phase of illnesses associated with STEC strains is usually self-limiting and the role of early antimicrobial therapy in the prevention of HUS is still unclear (7). Trimethoprim-sulfamethoxazole and β-lactam antibiotics are commonly used for the treatment of gastroenteritis. In the case of STEC infections, antibiotic treatment is not recommended. However, such antibiotics are often administrated before the disease is diagnosed. For children with acute bloody diarrhea, the most widely accepted recommendation is to obtain a stool culture and initiate empirical antibiotic treatment, because appropriate treatment shortens the duration of the diarrhea, decreases the incidence of complications, and reduces the risk of transmission by shortening the duration of bacterial shedding (22).

Unfortunately, several studies, including the present study, have revealed that many STEC strains have developed resistance to trimethoprim-sulfamethoxazole, β-lactams, and other antibiotics. Additionally, the present study also demonstrated that STEC strains possess integrons which encode antibiotic resistance genes conferring resistance to trimethoprim and sul-

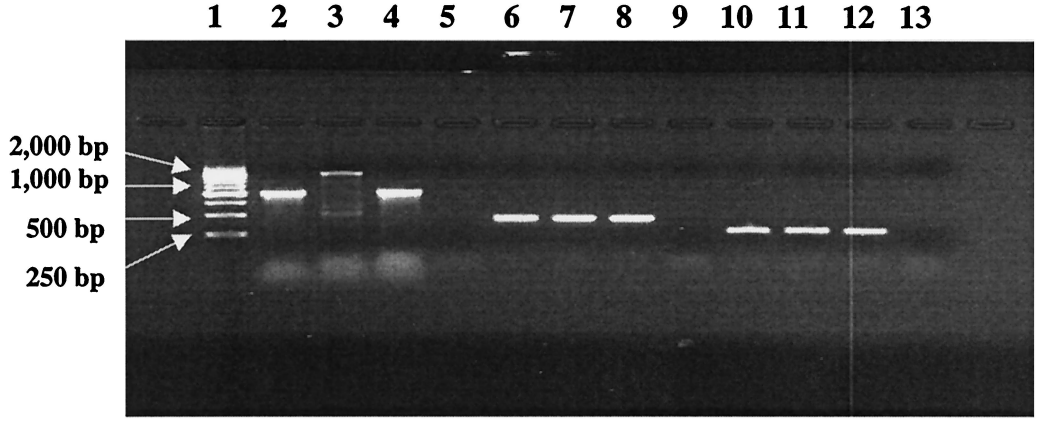

FIG. 1. PCR amplicons of STEC integrons. Lanes 2 to 5 were generated with the CS primers specific for class 1 integron. Lanes 6 to 9 were obtained using the *sul* primers specific for the *sul* gene that confers sulfonamide resistance. Lanes 10 to 13 were obtained using the primers specific for integrase. Specifically, lanes 2, 6, and 10 show positive controls (serovar Typhimurium DT104); lanes 3, 7, and 11 show *E. coli* O111:H8 strain CVM1877; lanes 4, 8, and 12 show *E. coli* O157:H7 strain CVM990; and lanes 5, 9, and 13 show negative controls (*E. coli* K-12).

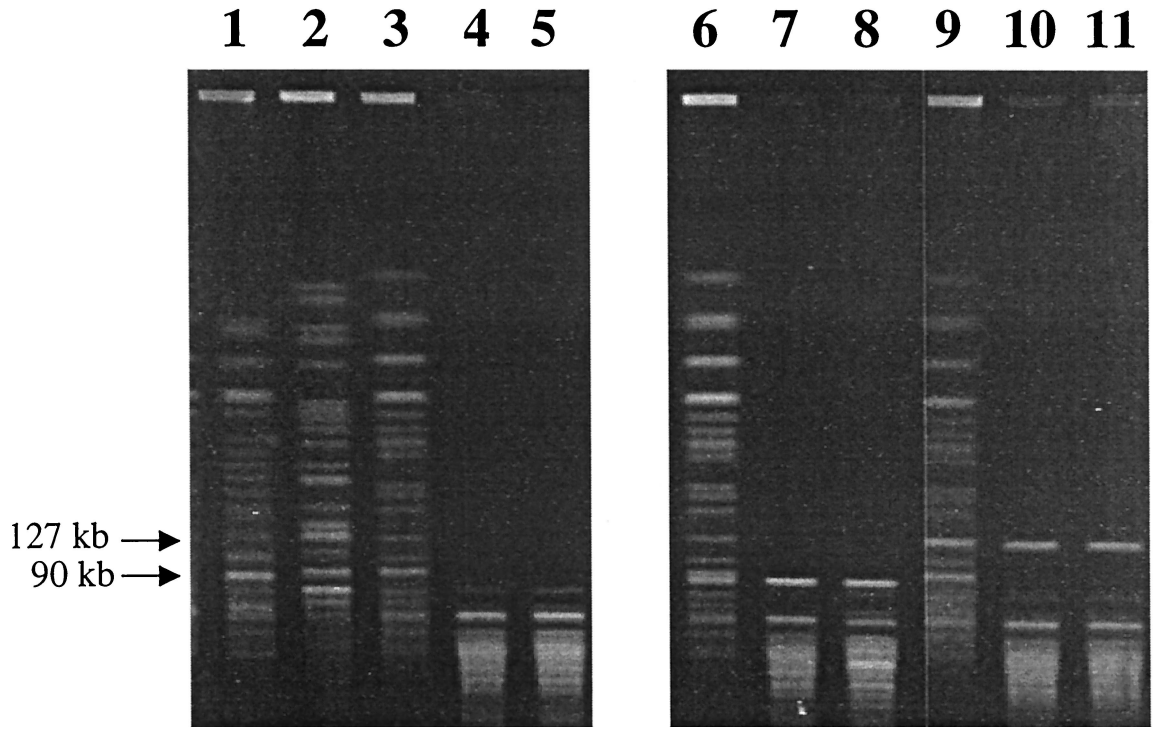

FIG. 2. PFGE profiles with *Xba*I digestion of donor, recipient, and transconjugant strains. Lanes 1 to 5 show, respectively, results obtained with donor strains CVM990 (*E. coli* O157:H7) and CVM1884, (*E. coli* O111:NM) and recipient strains JM263 (*E. coli* O157:H7), *H. alvei* CVM1202, and *H. alvei* CVM1203 before the conjugations. Lanes 6 to 8 show results with the transconjugants CVM990-JM263, CVM990-CVM1202, and CVM990-CVM1203, respectively, that have gained a 90-kb band. Lanes 9 to 11 show results with the transconjugants CVM1884-JM263, CVM1884-CVM1202, and CVM1884-CVM1203, respectively, that have gained a 127-kb band.

famethoxazole. Antibiotic resistance and resistance integrons in STEC would not only complicate future antibiotic therapy but could also potentially stimulate the transfer of resistance genes. Also, antibiotic-resistant STEC strains could possibly possess selective advantages over other bacteria colonizing the gastrointestinal tracts of animals that are treated with antibiotics (therapeutically or subtherapeutically). Resistant STEC strains could then become the predominant *E. coli* present

TABLE 4. Antimicrobial resistance profiles and MICs for donor, recipient, and transconjugant isolates

Bacterial strain(s)	MIC[a] (μg/ml) of:								
	AMC	AMP	CEF	CHL	KAN	NAL	STR	SUL	TET
Donors									
CVM990 (*E. coli* O157:H7)	4	4	4	≤4	**>64**	≤4	**>256**	**>512**	**32**
CVM1884 (*E. coli* O111:NM)	4	4	8	≤4	≤16	≤4	<32	**>512**	**>32**
Recipients									
JM263 (*E. coli* O157:H7)	4	4	**32**	**32**	≤16	**>256**	≤32	≤128	8
CVM1202 (*H. alvei*)	**>32**	**>32**	**>32**	≤4	≤16	≤4	≤32	≤128	≤4
CVM1203 (*H. alvei*)	**>32**	**>32**	**>32**	≤4	≤16	≤4	≤32	≤128	≤4
CVM1208 (*H. alvei*)	**>32**	**>32**	**>32**	≤4	≤16	≤4	≤32	≤128	≤4
Transconjugants									
CVM990-JM263	4	8	**32**	**32**	≤16	**>256**	**>256**	**>512**	**>32**
CVM990-CVM1202	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**
CVM990-CVM1203	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**
CVM990-CVM1208	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**
CVM1884-JM263	4	8	**32**	**32**	≤16	**>256**	≤32	**>512**	**>32**
CVM1884-CVM1202	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**
CVM1884-CVM1203	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**
CVM1884-CVM1208	**>32**	**>32**	**>32**	≤4	≤16	≤4	**128**	**>512**	**>32**

[a] MICs were determined via microdilution methods according to NCCLS standards (20, 21). Abbreviations: AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CEF, cephalothin; CHL, chloramphenicol; KAN, kanamycin; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline. Numbers in boldface represent resistant phenotypes.

under antibiotic selective pressures. This could result in STEC population increases and perhaps greater shedding, which could lead to greater contamination of animal food products with STEC.

Integrons and gene cassettes have been found primarily in gram-negative bacterial species belonging to the family *Enterobacteriaceae* and to the *Pseudomonas* genus (8, 27). Several studies have reported the presence of class 1 integrons among clinical isolates of *K. pneumoniae, K. oxytoca, P. aeruginosa, E. coli*, and *C. freundii* (12). Numerous studies have also revealed that integron-borne gene cassettes are present among *S. enterica* serotype Typhimurium isolates. A recent study (26) reported that integrons were found in all 45 human and 21 animal isolates of *Salmonella* serovar Typhimurium DT104 isolated between 1984 and 1997, including 58 isolates from the United Kingdom and 8 isolates from other European countries, the United States, Trinidad, and South Africa. All strains had the pentamer R-type of ampicillin-chloramphenicol-streptomycin-sulfonamides-tetracycline (ACSSuT) and contained two integrons of approximately 1 and 1.2 kb. These isolates also contained the same inserted gene cassettes, irrespective of source and country of origin, suggesting the spread of an epidemic clone. Integrons have also been identified among veterinary *E. coli* that were isolated from the normal intestinal flora of swine (32) and diseased poultry (2). The ant(3″)-Ia (*aadA*) gene responsible for resistance to streptomycin-spectinomycin was identified in all of the integrons present in the swine *E. coli*. Other integron-associated genes identified among the swine *E. coli* isolates included *dfr-1* encoding resistance to trimethoprim and the β-lactamase gene *oxa-1*. Integrons from 8 isolates were determined to be located on plasmids and were transferred to an *E. coli* DH5 laboratory strain. Additionally, Bass et al. (2) reported that 63% of 100 clinical isolates of avian *E. coli* exhibited class 1 integrons of approximately 1 kb. These integrons were determined to contain the *aadA1* gene cassette conferring resistance to streptomycin-spectinomycin. This wide distribution has presumably been achieved by the transposition of integrons to broad-host-range plasmids. However, the role of integrons in the acquisition and spread of antibiotic resistance has not yet been fully investigated.

The present study reports the presence of class 1 integrons conferring multiple resistance phenotypes among STEC strains isolated from cattle, ground beef, and humans, which is, to our knowledge, the first report on the presence of integrons in O157:H7 and non-O157 STEC strains. More significantly, the antibiotic resistance phenotypes observed in STEC isolates were also transferable, by conjugal plasmids, within species and between STEC and *Hafnia* sp. (horizontal transfer). The integron-borne *aadA* gene was silent in *E. coli* O111:NM, whereas it was fully expressed when it was transferred to *H. alvei*, suggesting that horizontal transfer may enhance the expression of a resistance gene. As this and other studies have shown, many integron-associated gene cassettes are identical, indicating that they may originate from a common source and are readily disseminated among bacteria. It is also evident that integrons conferring the same resistance phenotype can be genetically different since more than one gene cassette can encode resistance to one particular antibiotic. Some STEC strains in our study contain an *aadA* cassette that confers resistance to streptomycin and spectinomycin, but the gene cassettes were only 86% identical, suggesting that they may have been evolving independently for some time before their emergence in antibiotic-resistant STEC.

E. coli O157:H7 is considered a newly emerged pathogen, which may explain in part why integrons were only found in one strain among the 29 antibiotic-resistant strains tested. It is likely that integrons will be identified among additional STEC isolates in the future since this and other studies have demonstrated that integrons are readily transferable through conjugation. However, integrons and their associated gene cassettes did not always account for the total phenotypic resistance exhibited by the STEC isolates. Several STEC isolates displayed resistance to multiple antibiotics but did not contain any gene cassettes conferring resistance. Clearly, other mechanisms also contribute to the STEC antibiotic resistance phenotypes.

In summary, STEC strains have developed resistance to multiple classes of antimicrobials. Integrons not only are associated with multiple antibiotic resistance but also may play a significant role in the dissemination of resistance genes. Although the findings of this study strongly support the hypothesis that integrons provide a very efficient strategy for the acquisition and dissemination of new antibiotic resistance genes, a prospective long-term investigation of the natural evolution of gene transfer among bacterial pathogens inhabiting food animals is required.

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