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Antimicrobial Resistance and Integrons Found in Commensal *Escherichia coli* Isolates from Healthy Humans

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The emergence and spread of antimicrobial resistance among the pathogenic and commensal *Enterobacteriaceae* are of great concern worldwide. We characterized the antimicrobial resistance and integrons found in commensal *Escherichia coli* from healthy humans in the community. Class 1 integrase (*intl1*) and class 2 integrase (*intl2*) genes were identified in 22 (13.3%) and 2 (1.2%) of 165 *E. coli* isolates, respectively. *dfrA17-aadA5* and *dfrA1-aadA2* were the most common class 1 integrons. The prevalence of each type of class 1 integron among commensal *E. coli* isolates during 2001~2003 was similar to that of clinical *E. coli* isolates from hospital-acquired infections during 1994~1999. The resistant rates of commensal *E. coli* isolates carrying *intl1* to ampicillin, streptomycin, gentamicin, sulfamethoxazole, trimethoprim, chloramphenicol, and tetracycline were significantly higher than those of *intl1*-negative *E. coli* isolates ($p<0.05$). Integrons were directly associated with multidrug resistance in commensal *E. coli* isolates. It is hypothesized that multidrug-resistant *Enterobacteriaceae* from hospital-acquired infections are a potential reservoir for integrons associated with resistance genes found in commensal *E. coli* isolates in the community.

Key Words: Integron, Commensal fecal flora, Multiresistance, *Escherichia coli*

INTRODUCTION

Multidrug resistance to antimicrobial agents among *Enterobacteriaceae* is of great concern in clinical settings and is a major public health issue. The use of antimicrobial agents is the main selective force in the emergence and maintenance of antimicrobial resistance (5,12,14). In addition, the acquisition of resistance genes by horizontal transfer through plasmids and transposons plays an important role in the dissemination of resistance genes and in the development of multidrug resistance in bacterial populations (11).

A substantial portion of commensal fecal flora in humans is resistant to antimicrobial agents commonly used to treat bacterial infections. Commensal fecal floras are considered to be a potential reservoir for antimicrobial resistance genes and play an important role in the ecology of antimicrobial resistance of bacterial populations (8,16). Commensal *Escherichia coli* have been exploited as sensitive indicators in the surveillance of antimicrobial resistance (17,19). However, the acquisition of antimicrobial resistance in commensal fecal flora is still not fully understood.

Integrons are genetic elements capable of integrating or mobilizing gene cassettes by site-specific recombination. Many resistance genes such as *dfr* genes, aad genes, and β-lactamase genes present as gene cassettes and, therefore, these resistance genes can be moved to other genetic sites or transferred horizontally to other bacteria (2,3,6,18). Of the three classes of integrons associated with antimicrobial resistance, class 1 integrons are the most commonly found in clinical isolates of Gram-negative bacteria (4,18). Among

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Enterobacteriaceae isolates obtained from hospitalized patients in Korea, half of the clinical isolates carried integrons and 21 different gene cassettes including resistance genes were detected (10,22,23). Apart from being carried by clinical pathogens, few studies have reported integrons and gene cassettes in commensal fecal flora. The current study characterized the integrons found in commensal *E. coli* isolates from healthy humans in the community, and evaluated the association of integrons and multidrug resistance.

MATERIALS AND METHODS

1. Bacterial strains

A total of 165 *E. coli* strains were isolated from the stools of healthy students at Kyungpook National University, Daegu, Korea from 2001 to 2003 after written informed consent for study participation was obtained. Only students who were not admitted to any hospital during the previous year were included.

2. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) were determined by the agar dilution method in Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) with a Steer's multiple inoculator according to the National Committee for Clinical Laboratory Standards (15). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The antimicrobial agents included were β-lactams (ampicillin, piperacillin, cephalothin, cefamandole, and ceftazidime), aminoglycosides (streptomycin, kanamycin, gentamicin, and tobramycin), fluoroquinolone (ciprofloxacin and norprofloxacin), antifolates (sulfamethoxazole and trimethoprim), chloramphenicol, and tetracycline.

3. Conjugal transfer of plasmids

E. coli RG488 Rif^r and RG176 Nal^r were used as the recipients for the conjugation experiment (9). The donor and recipient strains in the logarithmic phase were grown in a trypticase soy broth (TSB, Difco Laboratories), mixed, and incubated at 37℃ for 20 h. The transconjugants were then selected on Mueller-Hinton agar supplemented with trimethoprim (50 μg/ml) or streptomycin (32 μg/ml) and rifampin (50 μg/ml) or nalidixic acid (50 μg/ml).

4. Plasmid DNA isolation and Southern hybridization

Plasmid DNA was isolated using the alkaline extraction method (1). The extracted DNA was separated by electrophoresis on 0.7% agarose gel. After agarose gel electrophoresis of plasmids, the denatured DNAs were transferred onto a positively charged nylon membrane (Boehringer Mannheim, Manheim, Germany) using a capillary method (20). For the hybridization assays, a DIG DNA labeling and detection kit (Roche, Manheim, Germany) was used according to the manufacturer's instructions. The purified integrase gene from the PCR products was used as the probe and was labeled with digoxigenin-11-dUTP according to random labeling methods.

5. PCR amplification of integrase genes

The conserved regions of integrase gene were amplified with the degenerate primer pair, hep35 and hep36, and the PCR products were further digested using either *Rsa*I or *Hin*fI to determine the class of integrons as previously described (21). The template was prepared by suspending a loopful of each isolate, which had been growing on a trypticase soy agar (Difco Laboratories) plate, in 200 μL of sterile water, followed by boiling for 10 min and centrifuging for 5 min.

6. Amplification and sequencing of gene cassette regions

The gene cassette regions for the class 1 and class 2 integrons were amplified using primer pairs hep58-hep59 and hep74-hep51, respectively, as previously described (21). To determine identical arrays of cassette genes, same-sized amplicons were restricted with *Rsa*I and *Hin*fI. Each PCR product of cassette regions that showed different restriction patterns were ligated with a pGEM T-easy vector (Promega, Madison, WI, USA) and transformed to *E. coli* DH5α cells. Sequencing reactions were then performed with a double-stranded plasmid preparation using dideoxy chain termination with T7 and Sp6 primers.

7. Statistical analyses

Statistical analyses were performed using the χ^2 test or Fisher's exact test. A P value of <0.05 was considered to be statistically significant. Statistical analyses were performed

Table 1. Characterization of integrons found in commensal *E. coli* isolates

Cassette array	No. of isolates	No. of isolates that carry integrons at		
		Conjugative plasmids	Non-conjugative plasmids	Chromosome
Class 1 integrons				
dfrA17-aadA5	6	6	0	0
dfrA1-aadA2	5	3	2	0
dfrA12-orfF-aadA2	1	1	0	0
dfrA5	1	1	0	0
dfrA7	2	0	2	0
aadA1	2	0	0	2
No cassettes	5	1	3	1
Class 2 integrons				
dfrA1-sat-aadA1	2	0	0	2
Total	24	12	7	5

using SPSS, version 12 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Prevalence of integrase genes among commensal *E. coli* isolates

Integrase genes were amplified and PCR products were digested with either *Rsa*I or *Hin*fI to determine the classes of integrons. Integrase genes were detected in 14.5% (24/165) of commensal *E. coli* isolates (Table 1). Of the commensal 24 *E. coli* isolates carrying integrase genes, *intl1* and *intl2* were amplified in 22 and 2 isolates, respectively. No class 3 integrons were detected. Leverstein-van Hall et al (13) reported that 19% of commensal *E. coli* isolates in their community sample carried class 1 integrons. However, the prevalence of integrons was up to 59% in *Enterobacteriaceae* isolated from clinical specimens (23). This suggests that integrons are more prevalent in *Enterobacteriaceae* isolated from hospital-acquired infections than from commensal fecal flora in the community.

2. Characterization of integrons among commensal *E. coli* isolates

To characterize the gene cassettes of integrons, the gene cassette regions were amplified and subsequently sequenced. Of the 24 integrase-positive *E. coli* isolates, 19 (79.2%) isolates carried the gene cassettes that encoded resistance to trimethoprim and aminoglycosides, whereas five *E. coli* isolates carried *intl1* gene, but not carried gene cassettes in the class 1 integrons (Table 1). Eight different genes cassettes encoding resistance to trimethoprim (*dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, and *dfrA17*), and aminoglycosides (*aadA1*, *aadA2*, and *aadA5*) were detected in the class 1 integrons. Six different cassette arrays of class 1 integrons were found in commensal *E. coli* isolates. *dfrA17-aadA5* was the most prevalent, being found in six isolates, followed by *dfrA1-aadA2* in five, *dfrA7* in two, *aadA1* in two, *dfrA5* in one, and *dfrA12-orfF-aadA2* in one (Table 1 and Fig. 1). Two *intl2*-positive *E. coli* isolates carried the *dfrA1-sat-aadA1*

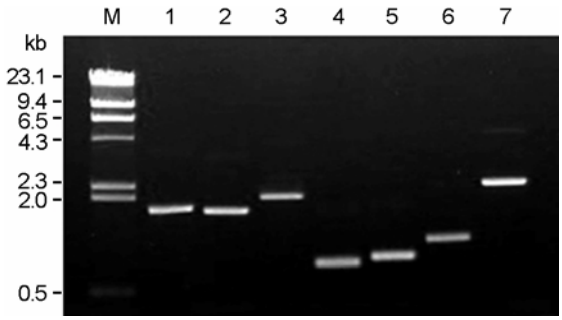

Figure 1. PCR amplification of gene cassette regions of class 1 and 2 integrons. Lane 1-6, class 1 integron carrying *dfrA17-aadA5*, *dfrA1-aadA2*, *dfrA12-orfF-aadA2*, *dfrA5*, *dfrA7*, and *aadA1*, respectively. Lane 7, class 2 integron carrying *dfrA1-sat-aadA1*. Lane M, lambda DNA fragments digested with *Hin*dIII.

cassette array. All cassette arrays of class 1 integrons found in the current study were previously detected in *Enterobacteriaceae* isolated from hospital-acquired infections in Korea (9,10,22,23).

The prevalence of each type of gene cassette found in commensal *E. coli* isolates in the current study was similar to that of the clinical *E. coli* isolates from Kyungpook National University Hospital, Daegu, Korea in the 1990s. *dfrA17-aadA5* and *dfrA12-orfF-aadA*2 were the most prevalent in *E. coli* isolates from hospital-acquired infections since 1994 in Korea (23). *dfrA1-aadA2* was the third most common in nosocomial *E. coli* isolates from the 1990s, but this type of class 1 integron was rarely detected in nosocomial *E. coli* isolates from the 2000s. *aadA1* was the most common in nosocomial *E. coli* isolates from the 1980s, but the incidence of this type of class 1 integron sharply decreased in nosocomial *E. coli* isolates from the 1990s and finally was not detected in nosocomial *E. coli* isolates from the 2000s. *dfrA7* was only detected in one clinical *E. coli* isolate from the 1990s.

Commensal fecal flora from humans can acquire resistance genes or integrons from food-producing animals. However, the prevalence of class 1 integrons is totally different between commensal *E. coli* isolates from humans and food-producing animals (8). *dfrA12-orfF-aadA2* and *aadA1* were the most prevalent in commensal *E. coli* from poultry and swine in the 2000s, respectively. Furthermore, our previous study demonstrated that pulsed-field gel electrophoresis patterns and restriction fragment length polymorphism patterns of the plasmids carrying identical cassette arrays were different between *E. coli* isolates from hospitalized patients and food-producing animals (8). This finding suggests that class 1 integrons found in commensal *E. coli* isolates from humans are not originated from food-producing animals.

3. Genetic localization of integrons among commensal *E. coli* isolates

To determine the transferability of integrons in *E. coli* isolates, a conjugation experiment was performed. Resistance to trimethoprim and/or streptomycin was conjugally transferred to a recipient *E. coli* strain in 12 (50.0%) of 24 isolates. The *intl1* gene was detected in all transconjugants (Table 1). To differentiate the genetic location of integrons in the 12 remaining *E. coli* isolates that did not transfer antimicrobial resistance, Southern hybridization with integrase gene probe was performed, which revealed that seven and three *E. coli* isolates carried class 1 integrons in the nonconjugative plasmids and chromosome, respectively. Class 2 integrons found in two commensal *E. coli* isolates were located in the chromosome (Table 1). R-plasmids from transconjugants carrying the identical cassette array showed the different RFLP patterns. The *intl1* gene probe was hybridized with different fragments of DNA (data not shown). These results suggest that R-plasmids carrying class 1 integrons originate from different sources. Class 1 integrons harbored by commensal *E. coli* isolates may be acquired from other pathogenic or commensal bacteria by horizontal transfer. Although the present study did not directly characterize the transfer of class 1 integrons between commensal *E. coli* isolates and *Enterobacteriaceae* from hospital-acquired infections, the influx of class 1 integrons from *E. coli* isolates from hospital-acquired infections was partly responsible for antimicrobial resistance in commensal *E. coli* isolates in the community. Our further investigations will examine whether class 1 integrons are transferred horizontally between commensal *E. coli* isolates and *Enterobacteriaceae* from hospital-acquired infections.

4. Antimicrobial susceptibility of commensal *E. coli* isolates

We examined the antimicrobial susceptibilities of commensal *E. coli* isolates to 15 antimicrobial agents. Resistance to ampicillin (43.6%) was the highest among the commensal *E. coli* isolates tested, whereas no isolates were resistant to third-generation cephalosporins such as ceftazidime (Table 2). Commensal *E. coli* isolates were moderately resistant to tetracycline (38.8%), streptomycin (35.2%), and sulfamethoxazole (25.4%). Fifty-five (33.3%) commensal *E. coli* isolates were susceptible to the antimicrobial agents tested. Our previous study investigated the differences in antimicrobial susceptibility between commensal *E. coli* isolates and clinical *E. coli* isolates from hospital-acquired infections (8). The rates of resistance to antimicrobial agents were higher in clinical *E. coli* isolates from hospital-acquired infections than those of commensal *E. coli* isolates. Only 1.5% of the clinical *E. coli* isolates from hospital-acquired infections were susceptible to the antimicrobial agents tested.

Table 2. Antimicrobial susceptibility of commensal *E. coli* isolates according to the presence of integrons

Antimicrobial agent	No. (%) of isolates			P
	Total (n=165)	*intl*-positive (n=24)	*intl*-negative (n=141)	
β-lactams				
Ampicillin	72 (43.6)	18 (75.0)	54 (38.3)	0.001*
Piperacillin	28 (17.0)	6 (25.0)	22 (15.6)	0.257
Cephalothin	9 (5.5)	1 (4.2)	8 (5.7)	0.764
Cefamandole	6 (3.6)	0	6 (4.3)	0.303
Ceftazidime	0	0	0	
Aminoglycosides				
Streptomycin	58 (35.2)	17 (70.8)	41 (29.1)	< 0.001*
Kanamycin	6 (3.6)	2 (8.3)	4 (2.8)	0.184
Gentamicin	3 (1.8)	2 (8.3)	1 (0.7)	0.010*
Tobramycin	3 (1.8)	1 (4.2)	2 (1.4)	0.352
Fluoroquinolones				
Ciprofloxacin	2 (1.2)	0	2 (1.4)	0.557
Norfloxacin	3 (1.8)	2 (8.3)	1 (0.7)	0.184
Antifolates				
Sulfamethoxazole	42 (25.4)	18 (75.0)	24 (17.0)	< 0.001*
Trimethoprim	23 (13.9)	16 (66.7)	7 (5.0)	< 0.001*
Chloramphenicol	16 (9.70)	12 (50.0)	4 (2.8)	< 0.001*
Tetracycline	64 (38.8)	18 (75.0)	46 (32.6)	< 0.001*

* Asterisk indicates statistical significance

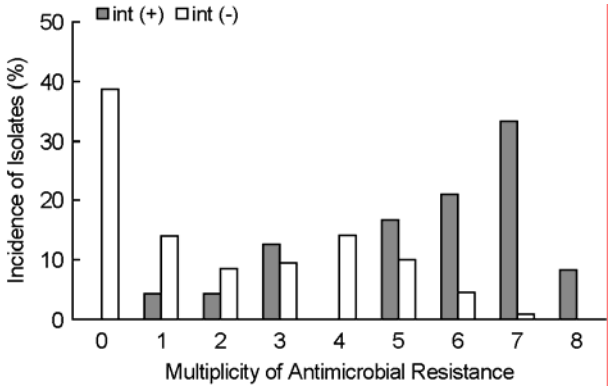

Figure 2. Multiplicity of antimicrobial resistance and its association with the presence of integrons in commensal *E. coli* isolates. *E. coli* isolates were examined for antimicrobial susceptibility to 15 agents.

These data suggest that the use of antimicrobial agents is an important selective force in the maintenance of antimicrobial resistance in bacterial populations.

5. Multidrug resistance of integron-carrying commensal *E. coli* isolates

All commensal *E. coli* isolates carrying integrons were resistant to two or more antimicrobial agents, except for one isolate that was resistant to tetracycline (Fig. 2). This exceptional tetracycline-resistant *E. coli* isolate carried the *intl1* gene, but harbored empty gene cassettes. Integrons were not found in antimicrobial-susceptible *E. coli* isolates. The presence of an integron was significantly associated with multidrug resistance in commensal *E. coli* isolates ($p<0.05$), indicating that integrons are directly responsible for multidrug resistance.

In conclusion, we suggest that class 1 integrons found in nosocomial pathogens can spread to and circulate in commensal fecal flora in the community. Integrons are directly responsible for resistance to trimethoprim and aminoglycosides and the presence of integrons is significantly associa-

ted with multidrug resistance in commensal *E. coli* isolates. Therefore, careful monitoring is necessary for the prevention of a wide dissemination of integrons and an increase of community-acquired infections by multidrug-resistant pathogens.

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