INTRODUCTION

The rapid dissemination of drug-resistant bacteria is now an increasing global concern, as it seriously complicates the treatment of infections (1,2). Until now, most reports surveying antimicrobial resistance have been focused on clinical isolates (3-5). In recent years, several studies have examined normal commensal flora, which could act as a reservoir for drug resistance genes recruited by pathogens under antibiotic pressure (6,7). Surveillance of normal flora from apparently healthy persons could potentially become the preferred method to assess antimicrobial resistance. Indeed, high resistance rates to several antimicrobial agents have been observed in healthy persons could potentially become the preferred method to assess antimicrobial resistance. Indeed, high resistance rates to several antimicrobial agents have been observed in normal flora from apparently healthy persons could potentially become the preferred method to assess antimicrobial resistance. Indeed, high resistance rates to several antimicrobial agents have been observed in normal flora remain unclear. The aim of this study was to determine the susceptibility of Escherichia coli isolates from human fecal samples in Taiwan to currently available antimicrobial agents and to determine the presence of class 1 and/or class 2 integrons.

SUMMARY: Two hundred and twenty-five fecal strains of Escherichia coli isolated from 109 non-hospitalized adults in 2006 were investigated for susceptibility to antibiotics and for the presence of integrons. High resistance rates in fecal strains of E. coli were observed for streptomycin (52.0%), ampicillin (50.2%), piperacillin (50.2%), trimethoprim/sulfamethoxazole (47.6%) and chloramphenicol (33.8%). Integrons were found in 31.5% (71/225) of the strains using an integrase gene PCR assay. Among 71 integrase-positive strains, 65 strains belonged to class 1 integrons, while the remainder belonged to class 2. Gene cassette patterns of class 1 integrons were further characterized by PCR and direct sequencing. Among those class 1 integrase-containing isolates, the integron cassette region was amplified by PCR in 40.0% (26 of 65) of isolates. Five different antimicrobial resistance gene cassette arrays were found in those isolates. These gene cassettes included those encoding resistance to trimethoprim (dfrF, dfrA7, dfrA12, dfrA17) and streptomycin (aadA1, aadA2, aadA5). Among those gene cassette arrays, dfrA12-orfF-aadA2 was found in 53.8% (14/26) of the isolates. These findings indicate that multidrug resistance of fecal flora is common in Taiwan and that integrons play an important role in resistance to trimethoprim and streptomycin in humans.

MATERIALS AND METHODS

Samples from non-hospitalized adults: Fecal samples were obtained from adults during their outpatient physical examinations in 2006 and were stored at 4°C. All samples were sent to the clinical microbiology laboratory at Yuanpei University for processing within 6 h of collection. Samples were diluted with normal saline and were streaked on eosin
methylenedi blue (EMB) agar plates (Difco Laboratories, Det-
roit, Mich., USA) for differential culture. Three individual colonies from each differential EMB plate were selected and the species level was identified using established bio-
chemical procedures (19). E. coli strains were then subjected to standardized antimicrobial susceptibility testing. The 19 antimicrobial disks included gentamicin (GM) (10 μg), amikacin (AN) (30 μg), kanamycin (K) (30 μg), streptomycin (S) (10 μg), neomycin (N) (30 μg), ofloxacin (OFX) (5 μg), ciprofloxacin (CIP) (5 μg), lomefloxacin (LOM) (10 μg), ampicillin/sulbactam (SAM) (10/10 μg), ampicillin (AM) (10 μg), piperacillin (PIP) (100 μg), cefotaxime (CTX) (30 μg), cefturoxime (CMX) (30 μg), cefazolin (CZ) (30 μg), cepofine (FEP) (30 μg), cephaplatin (CF) (30 μg), trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg), chloramphenicol (C) (30 μg), and imipenem (IPM) (10 μg). Those strains with different antimicrobial spectrums within one specimen were collected and the antimicrobial resistance rates were determined. The isolates were stored at −70°C in trypticase soy broth (TSB) (Difco Laboratories) supplemented with 20% glycerol until they were tested.

**Disk diffusion antimicrobial susceptibility testing:** The antimicrobial susceptibilities of the bacterial isolates were determined using the standard Kirby-Bauer disk diffusion method for 19 antibiotics, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20). These antimicrobials were chosen on the basis of their importance in treating human or animal infections and their use as animal feed additives to promote growth (17,21).

Each 150-mm Muller-Hinton medium plate (Difco Laboratories) was swabbed with TSB inoculated with E. coli incubated to the turbidity of the 0.5 McFarland standard. Nineteen commercially prepared antimicrobial disks were placed on the inoculated plates, which were then incubated at 35°C for 16 to 18 h. Inhibition zone diameters were measured in millimeters using a ruler. The breakpoints used to categorize isolates as resistant, intermediate or susceptible to each anti-
microbial agent followed guidelines issued by the CLSI (20). E. coli ATCC 25922 (American Type Culture Collection) was used as the standard for quality control.

**PCR amplification:** Colonies of isolates were picked and suspended in PCR Master Mix solution (iNtRON Bio-
technology, Taipei, Taiwan). PCR amplification was performed using a GeneAmp 2720 thermal cycler (PE Applied Biosystems, Foster City, Calif., USA). Amplification products were resolved by electrophoresis at 100 V for 1 h on 2% agarose gels with 0.5× Tris-acetate-EDTA buffer. After staining with ethidium bromide, each gel was visualized under ultraviolet (UV) light.

PCR amplification for the detection of class 1 integron cassettes (integron PCR) was performed with primer 5′CS and 3′CS, as described previously (22). The sulI gene was amplified with primer sequences described previously (23). For PCR detection of intI1 and intI2 integrase genes, two sets of primers were used, intIF and intIR, and int2F and int2R, as described previously (24). Primers IntIF and IntIR were used to amplify a 160-bp fragment of the intI1 gene. The combination of Int2F and Int2R primers amplified a 288-bp fragment, specific to the intI2 gene. The simultaneous PCR amplification of integrase gene types 1 and 2 was performed for 35 cycles: 30 s of denaturation at 94°C, 30 s of annealing at 55°C, and 30 s of extension at 72°C.

**DNA sequencing of PCR products:** Template PCR products were purified using the Gel-M Gel Extraction system (Viogene, Taipei, Taiwan). Purified PCR products were se-
quenced with dye terminators on an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, Calif., USA). DNA sequences were compared with those registered in the National Center for Biotechnology Information (NCBI) database.

**RESULTS**

**Antimicrobial susceptibility of E. coli isolates from non-
hospitalized adults:** Of 299 E. coli isolates obtained from the fecal samples of 109 non-hospitalized adults in 2006, 225 isolates were selected for determination of antimicrobial resistance patterns (Fig. 1). At least 2 isolates with different antibiotype were found in 82.6% (90/109) of specimens. Except for the high susceptibility to cefepine (resistance rate 0.0%) and imipenem (0.9%), high resistance to ampicillin (50.2%), piperacillin (50.2%), streptomycin (52.0%), trimethoprim/sulfamethoxazole (47.6%), and chloramphenicol (33.8%) were found. The resistance rates to fluoroquinolones (ciprofloxacin, lomefloxacin, and ofloxacin) ranged from 8.4
to 20.9% in those isolates. Except for streptomycin, lower resistance rates (4.9–22.2%) were observed for the other aminoglycosides. Lower resistance rates (0–24.0%) were also observed for the cephalosporins.

**Integron carriage and the association of resistance to antimicrobials**: PCR detection of the intI1 and intI2 genes demonstrated the presence of an integrase gene in those E. coli strains. Of the 225 isolates tested, 71 (31.5%) carried an integrase gene. Those integrase gene-containing strains were obtained from 23.6% (45/190) of specimens. Among those integrase-positive strains, 65 carried the class 1 integrase gene (160-bp fragment) and 6 possessed the class 2 integrase gene (288-bp fragment) (Fig. 2). To confirm those PCR products, amplification products were sequenced and found to be 100% identical to previously published sequences of the intI1 and intI2 genes (25).

To investigate the relationship between gene cassettes and phenotypic resistance among those class 1 integrase gene-containing isolates, the detection of antimicrobial gene cassettes was performed by PCR amplification with a primer for the 5′- and 3′-conserved segments. Integrons with inserted gene cassettes were found in 40% (26 of 65) of class 1 integrase-containing isolates. Bacteria with gene cassette-containing integrons were found in 12.8% (14 of 109) of specimens. Five different gene cassette arrays with sizes ranging from 700 to 1,900 bp were found in those isolates (Fig. 3). Sequence analysis of the obtained gene fragments were identical with those of known sequences: dfrV (0.72 kb) (26), dfrA7 (0.77 kb) (27), aadA1 (1.01 kb) (28), dfrA17-aadA5 (1.66 kb) (accession no. Eu650403), and dfrA12-orfF-aadA2 (1.91 kb) (28) as shown in Fig. 4. The association between a gene cassette and the corresponding antibiotic-resistant phenotype was also investigated (Table 1). These gene cassettes included those encoding resistance to trimethoprim (dfrV, dfrA7, dfrA12, dfrA17) and streptomycin and spectinomycin (aadA1, aadA2, aadA5). The most common type of cassette carried by class 1 integrons, aadA, confers resistance to TMP.
streptomycin and spectinomycin, and is present in 88.5% (23/26) of all integrons. The second most prevalent cassette, dfrA, conferring resistance to trimethoprim, was found in 80.8% (21/26) of the integrons. The dfrA12-orfF-aadA2 (53.8%, 14/26) and dfrA17-aadA5 (23.1%, 6/26) gene arrays conferring resistance to streptomycin/spectinomycin and to trimethoprim were frequently observed in those gene cassette-containing isolates (Table 1). Our study revealed that the integron-carrying genes did not account for all the phenotypic resistance of the E. coli isolates. For example, among 14 of the dfrA12-orfF-aadA2 gene array-containing isolates, only 8 isolates were resistant to streptomycin and 9 isolates were resistant to trimethoprim/sulfamethoxazole. Among 17 trimethoprim/sulfamethoxazole-resistant strains, all 17 isolates contained dfrA or dfrv genes and only 3 isolates possessed the sulI gene. However, those isolates were also resistant to antimicrobial drugs other than streptomycin and trimethoprim/sulfamethoxazole, to which no integron-associated resistance determinants were found.

**DISCUSSION**

Fecal samples were obtained from non-hospitalized adults during their physical examinations. Although we did not obtain preliminary data about whether those people had received any antibiotics in the sampling period, the surveillance data could account for the resistance status of intestinal E. coli isolates. The different antibiotypes of E. coli from one specimen implied the heterogeneity of microbes in the human intestine.

The resistances to streptomycin (52.0%), ampicillin (50.2%), piperacillin (50.2%), trimethoprim/sulfamethoxazole (47.6%), and chloramphenicol (33.8%) were higher than those to the other antimicrobials (0-24.0%) tested in our study. With regard to the first-line antimicrobials used in Taiwan, such as ampicillin, kanamycin, neomycin, cephalothin, streptomycin, and chloramphenicol, 19.6-52.0% of the E. coli isolates from humans were resistant. Of these, kanamycin, chloramphenicol, and streptomycin have been widely used as growth promoters or prophylactic agents in animal husbandry in Taiwan during the past 2 or 3 decades. The high resistance of commensal flora may be due to the extensive and long-term use of these antimicrobials in humans and in livestock. These results agree with those previously reported in Taiwan (29) and other countries (30).

Our study found that 50.2% of E. coli isolates were resistant to ampicillin. However, previous studies dealing with commensal E. coli resistance have had variable results. Comparing the results of the resistance to ampicillin with those previously reported for commensal isolates in Germany (31), Spain (32), Mexico (33), Korea (15), and the Netherlands (34), the resistance rates for our isolates were higher (50.2%) than those for Germany (16.7%), the Netherlands (21%), and Korea (43.7%), but were lower than those for Spain (58.5%) and Mexico (100%). Inappropriate use of antimicrobial agents and the spread of resistance determinants might be the reasons for the higher resistance rates.

Fluoroquinolone resistance in Gram-negative bacteria has been noted all over the world. Ofloxacin, ciprofloxacin, and lomefloxacin have been the three most commonly prescribed fluoroquinolones in Taiwan since their launch in 1985. Compared with other countries (35,36), the resistance rate (10.7%) in our human isolates to fluoroquinolones such as ciprofloxacin was higher. Importantly, an emerging resistance in our isolates to third-generation cephalosporins such as cefotaxime (4.9%) and cefuroxime (5.3%) was observed. The resistance rate of human fecal isolates to third-generation cephalosporins was higher than that in Japan (2.4%) (34), while such resistance was not found in Korea and Spain (15,30). Also, resistance of E. coli isolates to imipenem (0.9% in this study) was not found in Japan (37); such a result is alarming.

The prevalence of the integrase gene (31.5%) in our isolates was much higher than that in commensal E. coli isolates reported in Korea (13%) (15), but was lower than that in another report from Taiwan (70.9%) (30). Blake et al. demonstrated that the persistence of resistant genes within enteric microflora could be influenced by host antimicrobial exposure history (38). Thus, the higher prevalence of the integrase gene in fecal E. coli isolates in Taiwan may reflect the wide usage of antimicrobials.

Currently, several gene cassette arrays with approximate lengths from 0.7- to 3.0-kb amplicons have been found in E. coli isolates (15,31,39). Hsu et al. divided the gene cassette arrays into 10 integron groups according to amplicon size among human and swine isolates (29). However, we found only five integron groups. Among the inserted gene cassettes, dfrA12-orfF-aadA2, dfrA17-aadA5, and dfrA1-aadA2 are commonly found in E. coli isolates from human and animal origins (15). The gene cassette array dfrA12-orfF-aadA2 was also found among Salmonella enterica serovar Choleraesuis isolated from swine (15). Those gene cassettes were related to resistance to aminoglycosides, sulfonamides and trimethoprim, which is consistent with our findings. These results indicate that the integron genes might be transmitted from animals to humans through commensal isolates.

Although high resistance to several antibiotics was observed in the fecal isolates in our study, only 31.5% of isolates carried the integrase gene. Among 14 of dfrA12-orfF-aadA2 gene array-containing isolates, only 8 isolates were resistant to streptomycin and 9 isolates were resistant to trimethoprim/sulfamethoxazole. These results imply that the presence of strictly genetic determinants for resistance may not be satisfied in all cases. Resistance to some antimicrobial agents could be due to chromosome mutation, lack of drug penetration or presence of other resistance mechanisms (40).

In conclusion, the present study reveals that the multidrug resistance phenotype and presence of class 1 integrons are widespread in fecal E. coli in Taiwan. The integrons contain antibiotic-resistant gene cassettes and may contribute to the horizontal dissemination of antibiotic resistance in the bowel. Those data provide useful information regarding the dissemination of antibiotic resistance. Continued surveillance of normal flora in apparently healthy persons may be useful for predicting the antimicrobial resistance trends of E. coli.

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**REFERENCES**


