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Molecular Characterization of Multidrug-Resistant *Proteus mirabilis* Isolates from Retail Meat Products

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ABSTRACT

Sixty-four multidrug-resistant isolates of *Proteus mirabilis* were collected from retail meat products in Oklahoma. The isolates showed four different patterns of antibiotic resistance based on their resistant phenotype and genotypes. Most of these isolates were resistant to ampicillin, tetracycline, gentamycin, and kanamycin. Class 1 integrons were detected as a common carrier of the antibiotic-resistant genes, such as *aadA1, aadB,* and *aadA2.* A few isolates (9%) contained class 2 integrons with three gene cassettes included: *dhfr1, sat1,* and *aadA1.* These isolates were even resistant to nalidixic acid due to mutations in *gyrA* and *parC.* All ampicillin-resistant isolates contained $bla_{\text{TEM-1}}$. Plasmids that contained class 1 or 2 integrons and $bla_{\text{TEM-1}}$ were able to be transferred from *P. mirabilis* isolates into *Escherichia coli* by conjugation, indicating that conjugal transfer could contribute to the dissemination of antibiotic resistance genes between the *Enterobacteriaceae* species.

Increased antibiotic resistance in human bacterial pathogens continues to be a major public health concern. Routine use of antibiotics in medical and agricultural practices has resulted in widespread horizontal transfer of mobile genetic elements that carry one or more resistance genes *(4, 33, 39)*. Plasmids, transposons, and integrons play major roles in the dissemination of antibiotic gene cassettes among bacterial species *(13)*.

Many antibiotic resistance genes in gram-negative bacteria are found as a part of gene cassettes in integrons *(29)*. Integrons consist of two conserved segments of DNA separated by a variable region that contains one or more integrated antibiotic resistance gene cassettes *(17)*. The structure of the integrons are as follows: (i) the stationary integron platform, including the integrase gene (*intI*), a strong promoter (p_C), and a recombination site (*attI*); and (ii) the mobile gene cassettes with a recombination site (*attC*) or a 59-base element *(2)*. The integrase is responsible for a site-specific recombination between the *attI* and *attC* sites and, thus, integrates or excises antibiotic resistance gene cassettes. Four classes of integrons (classes 1, 2, 3, and 4) are distinguished by their class-specific integrase gene. A 45 to 58% homology among the integrase of class 1, 2, and 3 integrons suggests that their evolutionary divergence has been extended *(31)*. The class 1 integrons are mostly associated with the Tn21 transposon family, whereas the class 2 integrons are associated with the Tn7 transposon family *(2)*.

Class 1 integrons are of particular relevance to the development of multidrug resistance in gram-negative bacteria *(20, 24)*. Class 1 integrons possess the genes *intI1* and *attI* in the 5′ conserved sequence and genes that encode resistance to sulfonamides (*sulI*) and disinfectants (*qacEΔ1*) and a gene of unknown function (ORF5) in the 3′ conserved sequence *(18)*. The conserved 5′ and 3′ regions flank gene cassettes, which contain single or multiple antibiotic resistance gene(s) and a recombination site (the 59-base element) at the downstream end of each resistance gene cassette *(17)*.

The mobility of the gene cassette contributes to the dissemination of antibiotic resistance genes *(29)*. Furthermore, the genetic flexibility allows numerous cassettes rearrangement under antibiotic selective pressure *(27)*. It is known that integrons are most widely disseminated in the family of *Enterobacteriaceae,* and a large number of gene cassettes, each containing a different antibiotic-resistant gene, have been identified in many species of *Enterobacteriaceae (15, 20, 28)*. Identified gene cassettes in the integrons confer resistance to aminoglycosides, β-lactams, chloramphenicol, trimethoprim, erythromycin, and rifampin *(25)*.

In this study, antibiotic-resistant *Enterobacteriaceae* isolates were collected from retail meat products. Among the tested strains, *Proteus mirabilis* was prevalently detected as a multidrug-resistant species. *P. mirabilis* frequently causes urinary tract infections *(7, 36)*. It also infects wounds, burns, and the respiratory tract as an opportunistic pathogen. The prevalence of multidrug-resistant *P. mirabilis* in hospitals has resulted in difficulty in treating patients *(5, 14)*. Since *P. mirabilis* is psychrotrophic and is often isolated from retail meat and seafood products *(11)*, the presence of antibiotic-resistant strains in retail meat products, which show similar genotypes and phenotype as the antibiotic-resistant clinical isolates, may play an important role in disseminating the resistant genes among the *Enterobacteriaceae* in the meat products. Therefore, diver-

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sity of integron-mediated antibiotic resistance genes in *P. mirabilis* isolates was evaluated for a better understanding of dissemination of the resistance genes among species in meat products. For nalidixic acid–resistant isolates, the quinolone resistance–determining regions of *gyrA* and *parC* were sequenced to verify the occurrence of point mutations in quinolone resistance–determining regions of the isolates.

MATERIALS AND METHODS

Bacterial isolation. Meat products, including ground beef ($n = 15$), ground turkey ($n = 15$), ground chicken ($n = 15$), and ground pork ($n = 15$), were obtained from a local grocery store from February to June 2004 (Stillwater, Okla.). The products were transported on ice to the laboratory and used immediately for bacterial isolation using the conventional culture methods listed in the U.S. Food and Drug Administration *Bacteriological Analytical Manual (35)*. Each sample (25 g) was blended in 225 ml of lactose broth (Difco, Becton Dickinson, Sparks, Md.) and enriched at 35°C for 24 h. After one loopful of broth from each tube was streaked onto MacConkey agar (Difco, Becton Dickinson), the plates were incubated at 35°C for 24 h. Colonies (approximately 30 to 40) on each plate were picked based on their morphological characteristics, such as color, size, opacity, and form, and inoculated onto tryptic soy agar (Difco, Becton Dickinson) slants for antibiotic susceptibility test.

Screening and identification of antibiotic-resistant isolates. Antibiotic-resistant isolates were screened with ampicillin, chloramphenicol, streptomycin, tetracycline, and sulfamethoxazole at 32 μg/ml. Bacterial isolates were inoculated into 2 ml of Luria-Bertani (LB) broth (Difco, Becton Dickinson) and incubated overnight at 37°C. The bacterial strains were diluted with phosphate-buffered saline (pH 7.4) and inoculated at 10^5 CFU/ml into 100 μl of Mueller-Hinton (MH) broth that contained each antibiotic on 96-well plates. The plates were incubated overnight at 37°C. Bacterial growth was determined by checking absorbance at 570 nm using an enzyme-linked immunosorbent assay reader (Bio-Rad Laboratories, Hercules, Calif.). Multidrug-resistant isolates were then identified with an API 20E test kit following the manufacturer's instruction (bioMérieux, Inc., Hazelwood, Mo.).

Antibiotic-resistant profiles of the isolates. Antibiotic-resistant profiles of the bacterial isolates were examined by the Kirby-Bauer disc diffusion test on MH agar according to the standard procedures outlined by the NCCLS *(22)*. Overnight cultures prepared in LB broth were spread evenly on MH agar (Difco, Becton Dickinson). Antibiotic susceptibility of the isolates was determined by testing with various antimicrobial agents, such as β-lactams (ampicillin, 10 μg; amoxicillin–clavulanic acid, 30 μg; cefoxitin, 30 μg; ceftiofur, 30 μg; ceftriaxone, 30 μg; and cephalothin, 30 μg), chloramphenicol (30 μg), tetracycline (30 μg), aminoglycosides (amikacin, 30 μg; gentamicin, 10 μg; kanamycin, 30 μg; and streptomycin, 10 μg), quinolone and fluoroquinolone (ciprofloxacin, 5 μg; and nalidixic acid, 30 μg), and sulfamethoxazole-trimethoprim (10 μg). *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as the quality control organisms. Following incubation at 37°C for 24 h, the zones of inhibition were measured and interpreted as resistant or sensitive according to the NCCLS guidelines *(22)*.

PCR analysis of antibiotic-resistant genes. PCR assay was performed to determine the presence of different types of integrons and specific antibiotic-resistant genes in the tested isolates. Primers for the amplification of the resistance genes were synthesized following the published sequences: class 1 *(17)*, *intl2 (33)* and *intl3 (33)*; and class 2 *(20)*, bla_{TEM} *(16)*, bla_{AmpC} *(16)*, bla_{Carb2} *(16)*, and *parC (36)*. The primers for *gyrA* were designed in this study (5′-ACTGAAGCCAGTACA CCG-3′ and 5′-CAA ATCCGCCAGCAGTTC-3′). Each 50-μl PCR mixture contained 1 μl of overnight bacterial culture, 250 μM deoxynucleoside triphosphate mix, 1.5 mM $MgCl_2$, 50 pmol of primers, 5 μl of 10× *Taq* polymerase buffer, and 1 U of *Taq* polymerase (Promega, Madison, Wis.) *(6)*. PCR amplification was performed with a thermal cycler (Bio-Rad) using the following conditions: 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 a specific annealing temperature for the corresponding primers, extension for 1 min at 72°C, and a final extension at 72°C for 7 min. The amplicons were analyzed by electrophoresis in 1.0% agarose gels staining with ethidium bromide and photographed by using a gel documentation system (Bio-Rad).

Plasmids were isolated from the overnight culture of the isolates that contained the integrons. The plasmids were prepared with a Qiaprep Spin Miniprep Kit (Qiagen, Valencia, Calif.) and subjected to PCR assay to determine whether the integrons were located in chromosome or plasmid. *E. coli* stain that contained a chromosomally encoded integron was used as a negative control.

Sequencing of antibiotic-resistant genes. The PCR amplification products were purified from the gel with a gel extraction kit (Qiagen) and cloned by using a TA cloning kit (Invitrogen, Carlsbad, Calif.) as recommended by the manufacturer. The plasmid DNA from the overnight culture was prepared by using a Qiaprep Spin Miniprep Kit. The DNA was sequenced in an ABI automatic DNA sequencer. The sequences were analyzed with the BLAST (National Center for Biotechnology Information) for similarity search and Vector NTI 8.0 program (InforMax Inc., Bethesda, Md.) for sequence alignments.

Plasmid conjugation. Representative bacterial isolates were subjected to conjugation to determine if horizontal conjugal transfer of antibiotic-resistant genes would occur. Candidate donor strains that possessed the antibiotic-resistant genes were mated with a rifampin-resistant derivative of *E. coli* K-12 strain *(9)*. Overnight cultures of the donor and recipient cells in LB broth were diluted with fresh LB broth (100-fold) and then incubated for 3 h. They were then mixed in a ratio of 1:3 (donor:recipient) and incubated at 37°C for 16 h. Transconjugants were selected on tryptic soy agar that contained rifampin (50 μg/ml), streptomycin (16 μg/ml), and ampicillin (16 μg/ml). Presumptive *E. coli* transconjugants were confirmed by using the API 20E test kit. Plasmids were prepared from the overnight cultures of the transconjugants by using a Qiaprep Spin Miniprep Kit. The presence of class 1 or 2 integrons and bla_{TEM-1} were confirmed by the PCR analysis.

The MIC of various antibiotics for the transconjugants and donor bacteria was determined by microdilution with MH broth (Difco, Becton Dickinson) according to the NCCLS *(22)*. Antibiotics, such as ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline, were serially 2-fold diluted with MH broth on 96-well plates. The overnight bacterial cultures were diluted with phosphate-buffered saline and tested as described above.

Nucleotide sequence accession numbers. The nucleotide sequences of *aadA1*, *aadB-aadA2*, *dhfr1-sat1-aadA1*, *gyrA*, *parC*, and bla_{TEM-1} from *P. mirabilis* were submitted to the GenBank under accession nos. AY677093, AY681136, AY736324, AY729026, AY736325, and AY729027, respectively.

TABLE 1. *Profiles of antibiotic resistance and integrons of* Proteus mirabilis *isolated from meat products*

Type	Integron (cassettes)		Antibiotic resistance[a]	No. of isolates
	Class 1	Class 2		
1	*aadA1*		AMP, TET, GEN, KAN, STR, SUL/TRI	28/64
2	*aaB-aadA2*		AMP, TET, GEN, KAN, STR	17/64
			TET, GEN, KAN, STR	11/64
3	*aadA1, aadB-aadA2*		AMP, TET, GEN, KAN, STR	2/64
4		*dhfr1-sat1-aadA*	AMP, CHL, TET, GEN, KAN, STR, NAL, SUL/TRI	6/64

[a] Antibiotic-resistant profiles of the isolates were examined by Kirby-Baner disc diffusion tests on MH agar. AMP, ampicillin; TET, tetracycline; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; NAL, nalidixic acid; and SUL/TRI, sulfamethoxazole/trimethoprim.

RESULTS

Isolation of multidrug-resistant *P. mirabilis* from retail meat products. Sixty-four multidrug-resistant isolates of *P. mirabilis* were collected from retail meat products in Stillwater, Okla. *P. mirabilis* was most frequently screened as the multidrug-resistant species among the enteric bacterial species isolated from the meat samples such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *E. coli.* Multidrug-resistant *P. mirabilis* was most commonly isolated from ground turkey (14 of 15) followed by ground chicken (5 of 15) and ground pork (3 of 15) (data not shown). No antibiotic-resistant *P. mirabilis* was found in any of the tested ground beef samples. The species was resistant to at least 4 different antibiotics. All isolates were resistant to tetracycline and two aminoglycosides: gentamicin and streptomycin (Table 1). Most of the bacterial isolates (83%) were resistant to ampicillin. A few isolates were also found to be resistant to nalidixic acid. However, no isolate was resistant to amikacin or ciprofloxacin.

Antibiotic-resistant genes identified in *P. mirabilis.* The multidrug-resistant *P. mirabilis* found in this study possessed either class 1 or 2 integrons but no class 3 integrons (Table 1). Class 1 integrons were found in 91% of the isolates, and they were located in plasmids. The size of the PCR amplicons was either 1.0 or 1.6 kb. The antibiotic-resistant gene found in the 1.0-kb amplicons was *aadA1* encoding aminoglycoside transferase, which confers resistance to streptomycin and spectinomycin (type 1). The deduced peptide sequence of *aadA1* of *P. mirabilis* isolates showed 100% identity with those of *E. coli* (NCBI accession no. X12870), *Enterobacter cloacae* (AF202976), and *E. faecalis* (AF052459). The genes identified in the 1.6-kb amplicons were *aadB* and *aadA2* encoding aminoglycoside 2″ adenyltransferase and aminoglycoside transferase, respectively (type 2). The *aadB* confers resistance to gentamicin, kanamycin, and tobramycin. This gene structure was also found in *Citrobacter freundii* (AF458082). The *aadB* sequence of the isolates showed 100% identity with those of *E. cloacae* (AY173047), *Shigella sonnei* (M86913), *Salmonella* Typhimurium (AJ310778), *Serratia marcescens* (AF453998), and *K. pneumoniae* (X12618). The *aadA2* sequence of the isolates showed 100% identity with those of *E. coli* (AY259085), *Salmonella* Typhimurium DT104 (AF071555), and *C. freundii* (AF550415). Interestingly, two isolates had two class 1 integrons (type 3), with the 1.0-kb *aadA1* located in the plasmid and the 1.6-kb *aadB* and *aadA2* located in the chromosome.

Although six of the *P. mirabilis* isolates were resistant to eight antibiotics, including nalidixic acid, none of them contained class 1 integrons (Table 1). The presence of other integrase genes, such as *intI2* and *intI3,* in these strains was further tested. All four of these isolates contained a typical *intl2,* which contained an early stop codon. The gene cassettes located in the class 2 integrons were amplified with primers and hep74 binding to *attl2* and hep51 binding to *orfX* at the right end of the cassette region in transposon Tn7. The size of the obtained PCR product was 2.2 kb. The class 2 integrons contained three gene cassettes, *dhfr1, sat1,* and *aadA1,* encoding antibiotic resistance to trimethoprim, streptothricin, and spectinomycin-streptomycin, respectively (type 4). The quinolone resistance–determining regions of chromosomal *gyrA* and *parC* of the isolates were sequenced to identify the specific mutations associated with the resistance to nalidixic acid. Their deduced amino acid sequences of the isolates were compared with those of *E. coli* and *P. mirabilis* (Fig. 1). In the quinolone resistance–determining regions of the GyrA, the codons for Ser-83 and Asp-87 were changed to Ile and Glu, respectively. In the ParC, the codons for both Ser-80 and Val-100 were changed to Ile.

Since many different types of β-lactamase have been identified in *Enterobacteriaceae*, the type of the responsible β-lactamase gene in the *P. mirabilis* isolates was determined by PCR assay. The size of the PCR amplicon was 1.1 kb. All the ampicillin-resistant isolates contained $bla_{\text{TEM-1}}$ (861 bp), which was controlled by promoters *Pa* and *Pb.* Sequencing comparison of the genes showed 100% identity with those of *E. coli* (AY30700), *K. pneumoniae* (AY293072), *C. freundii* (AF550415), *S. marcescens* (AB103506), and *Shigella flexneri* (U48775). These genes were all located in the plasmids.

Conjugational transfer of antibiotic-resistant genes. Integrons located in the plasmids of the representative *P. mirabilis* strains of types 1, 2, 3, and 4 were transferred

GyrA

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                                    83  87
E. coli AF052254        SARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG
P. mirabilis 62*        ................I...E....L.....M.............V..
P. mirabilis AF397169   ....................E....L.....M.............V..
P. mirabilis AJ515017   ....................E....L.....M.............V..
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ParC

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                                        80
E. coli NC004431        SAKFKKSARTVGDVLGKYHPHGDSACYEAMVLMAQPFSYRYPLVDGQGN
P. mirabilis 62*        ......................I...................I.....
P. mirabilis AJ363611   ......................S...................I.....
P. mirabilis AF515019   ......................S...................I.....
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FIGURE 1. *Amino acid sequence alignments of GyrA and ParC of* E. coli *and* P. mirabilis. *Amino acid positions are based on* E. coli GyrA *and* ParC *sequences. The quinolone resistance–determining regions were underlined. The* gyrA *and* parC *of* P. mirabilis *62 were sequenced in this study. Other sequences were obtained from the GenBank.*

into *E. coli* by transconjugation. The presence of integron-borne antibiotic resistance genes was confirmed by PCR assay of class 1 or 2 integrons in the plasmids of the transconjugants (data not shown). All the resulting *E. coli* transconjugants expressed resistance to ampicillin, streptomycin, and kanamycin (Table 2). *E. coli* transconjugants 15 and 62 had the same MICs of streptomycin as the donor strains *P. mirabilis* 15 and 62. However, *E. coli* transconjugants 11, 43, and 62 had much lower MICs of ampicillin than those of the donor strains (>1,024 μg/ml) although they contained the gene $bla_{\text{TEM-1}}$. Similarly, the MICs of kanamycin for *E. coli* transconjugants 11 and 15 were lower than those of the donor bacteria. Only *E. coli* transconjugant 62, which acquired class 2 integrons, had the same MICs of kanamycin as the donor strain. *E. coli* transconjugants showed variable MICs of chloramphenicol and tetracycline when compared with the donor isolates.

DISCUSSION

Antibiotic-resistant bacteria are detected in retail meat products. Routine use of antimicrobial agents for domestic livestock to prevent and treat diseases, as well as to promote growth, contributes to the emergence of antibiotic-resistant bacteria that can be subsequently transferred to humans through the food chain *(24, 38)*. Multidrug-resistant bacteria, such as *Salmonella enterica* serotype Typhimurium DT104 and *E. coli* O157:H7, were isolated from ground beef and turkey *(38, 40)*. These pathogens were typically resistant to ampicillin, streptomycin, chloramphenicol, tetracycline, and sulfamethoxazole.

In this study, we demonstrated that multidrug-resistant *P. mirabilis* was prevalent in poultry products. The resistance profiles of this bacterial species to antibiotics were associated with those drugs that are frequently used in the poultry industry (Table 1). Antibiotics approved for treatment of poultry infections are erythromycin, fluoroquinolone, gentamicin, neomycin, penicillin, spectinomycin, tetracycline, and virginiamycin *(19)*. The results of our study therefore indicate the potential possibility that the commonly used antibiotics, such as tetracycline, streptomycin, kanamycin, and gentamicin, may become ineffective in treating bacterial infections that are caused by multidrug-resistant *P. mirabilis*. Similarly, our results demonstrate that a few strains of *P. mirabilis* are also resistant to nalidixic acid. This is of particular importance, since nalidixic acid–resistant enteric bacteria had rarely been detected in retail meat products previously *(21, 32, 39)*. In fact, the emergence and prevalence of nalidixic acid–resistant bacteria have been more problematic in hospitals than in any other environments *(34)*. The most frequently involved mechanism for nalidixic acid resistance is related to mutations in the *gyr* encoding for DNA gyrase or *parC* for topoisomerase IV *(37)*. Single mutations, usually in *gyrA,* have been associated with a high level of resistance to nalidixic acid and decreased susceptibility to fluoroquinolones. Additional mutations in *parC* contribute to higher levels of resistance to fluoroquinolones. Weigel et al. *(36)* described double mutations involving both *gyrA* (S83-R or -I) and *parC* (S80-R or -I) in clinical isolates of *P. mirabilis*. For the first time to our knowledge, our results demonstrate the

TABLE 2. *MICs of antibiotics for donor strains of* Proteus mirabilis *and their transconjugants*[a]

Type	Strain	MIC (μg/ml)				
		AMP	STR	CHL	TET	KAN
1	*P. mirabilis* 11 (D)	1,024	256	32	64	64
	E. coli 11 (T)[b]	128	128	—	32	32
2	*P. mirabilis* 15 (D)	128	256	16	64	1,024
	E. coli 15 (T)	32	256	—	—	64
3	*P. mirabilis* 43 (D)	1,024	256	16	64	1,024
	E. coli 43 (T)	256	128	—	—	—
4	*P. mirabilis* 62 (D)	1,024	256	256	32	512
	E. coli 62 (T)	256	256	128	16	512

[a] MICs of antibiotics for the donor bacteria and their transconjugants were determined by broth microdilution with MH broth. AMP, ampicillin; STR, streptomycin; CHL, chloramphenicol; TET, tetracycline; KAN, kanamycin; D, donor strain; and T, transconjugant.
[b] The recipient *E. coli* was originally susceptible to the tested antibiotics (<1 μg/ml).

occurrence of double mutations in the *gyrA* and *parC* of *P. mirabilis* in retail turkey products. This finding indicates the potential for spread of this nalidixic acid- and fluoroquinolone-resistant bacterial species to humans through the consumption of improperly cooked meat products or contamination during the handling of the meat products.

Dissemination of antibiotic-resistant genes among enteric bacteria in food animals and the environment is mainly mediated by integrons *(10)*. The class 1 and 2 integrons were respectively present in approximately 46 and 14% of the *Enterobacteriaceae* isolates from the poultry and cattle. These integrons were also found in bacterial species isolated from the poultry carcasses, the broiler house, and the poultry-processing environments *(30)*. The prevalence rates for class 1 and class 2 integrons in on-farm carcass samples were 75 and 21%, respectively. Based on the findings that class 1 integrons were more prevalent in the processing environments and they were the more common carriers of antibiotic-resistant genes in *P. mirabilis* isolates from retail meat products, we concluded that class 1 integrons are the most important genetic elements in mediating drug resistance among *Enterobacteriaceae* strains in the food chains and the processing environments.

Different types and sizes of antibiotic-resistant gene cassettes have been found in the variable regions of integrons. The most common types of gene cassettes carried in class 1 integrons in enteric bacteria were those that conferred resistance to streptomycin and spectinomycin *(39)*. Currently, the use of streptomycin in clinical medicine is limited. However, this antibiotic remains important for therapy and growth promotion in animals and for bacterial disease control in plants *(26)*. Since all class 1 integrons tested in this study contained *aadA1* or *aadA2*, it is possible that streptomycin can serve as a valuable marker in screening multidrug-resistant bacteria. The class 2 integrons identified in most enteric bacteria generally carried three gene cassettes, *dfrA1*, *sat1*, and *aadA1 (13, 20)*. Recently, an unusual class 2 integron that carried four cassettes, *sat*, *ere*(A), *aadA1*, and *orfX*, was found in clinical isolates of *E. coli (2)*. Only the typical class 2 integrons were found in *P. mirabilis* isolates in this study. The sequences of the antibiotic-resistant genes and the 59-base elements in the integrons of the *P. mirabilis* isolates were identical to those of enteric bacterial species regardless of their origin. Therefore, we suggest that horizontal transfer of antibiotic-resistant genes mediated by integrons is relevant among species and/or strains of *Enterobacteriaceae*.

The ease with which the bacteria acquire new resistance genes by self-transmissible and mobile plasmids and conjugative transposons may significantly contribute to the increasing incidence of antibiotic-resistant bacterial strains. The acquired antimicrobial resistance phenotypes most often develop via conjugative plasmids *(26)*. Conjugative plasmids encoding resistance to multiple antibiotics have been identified in many enteric bacteria such as *S. sonnei (8)*. In addition, conjugative plasmid-mediated β-lactamase genes are located within or near the mobile elements, such as integrons or transposons, which enhance their rapid dissemination *(1)*. In this study, we demonstrated conjugal transfer of integrons and $bla_{\text{TEM-1}}$ from *P. mirabilis* isolates into *E. coli* (Table 2). TEM-1 is the most common enzyme and is produced by 58.6% of penicillinase-producing *P. mirabilis (3)*. The gene found in this study was controlled by the Pa/Pb promoter, which leads to a 10-fold increase in transcriptional levels compared with a weak promoter such as P3 *(12)*. However, the MICs of ampicillin for the conjugants were generally low. Therefore, we suggest that multiple factors are involved in contributing to the increased resistance to the antibiotic in the bacteria. Neuwirth et al. *(23)* reported that the presence of penicillin-binding protein greatly affected the resistance to β-lactams for the species.

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