— Regular Article —

Occurrence and Characteristics of Class 1 and 2 Integrons in Clinical Bacterial Isolates from Patients in South China

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Recent studies have shown that integrons play a major role in spreading antibiotic resistance genes in clinical settings, as antibiotic resistance genes are frequently found located at gene cassettes. Polymerase chain reaction (PCR) analyses were carried out for the detection of the integrase genes of the three classes of integrons, and their gene cassettes were characterized in 118 clinical strains, including both gram-positive and gram-negative bacteria, isolated from a hospital in Guangzhou, China during 2004. Class 1 and 2 integrons were detected in 76.3% (90/118) and 0.8% (1/118) of the tested clinical isolates, respectively. Moreover four isolates were positive for both the integrons and no class 3 was detected. Some of them were first reported, such as *Enterococcus faecalis*, *Enterococcus faecium*, *Alcaligenes* sp., and *Flavobacterium* sp. Seven different arrays of gene cassettes of class 1 integron were found, and a high prevalence of *dfrA12-orfF-aadA2* genes was observed. In addition, we identified a new none-open reading frame (ORF) cassette in a class 1 integron positive clinical coagulase-negative *Staphylococcus* strain GH69. The none-ORF cassette, a 700 bp sequence containing a 441 bp ORF and a 59-base element, is first characterized in this study. All class 2 integrons carried an array of gene cassettes *dfrA1-sat2-aadA1*. Moreover bacteria contain more than one integrons were also found in this study. Resistance to trimethoprim-sulfamethoxazole was frequently found to be encoded within integrons. Therefore, it is important that guidelines for the prudent use of antimicrobial agents are adopted and surveillance programs are established.

Key words —— integrons, gene cassette, antibiotic resistance, clinical isolate

INTRODUCTION

Since antibiotics have been found and used for the treatment of infectious diseases, there was an enormously successful period in medical history during the last half century. However, the situation is now threatened by the increasing incidence of antibiotic resistance. Directed by a strong antibiotic selective pressure, microbes (especially bacterial pathogens) eventually develop many different processes to establish resistance.[1)] Now, it is well known that bacterial antibiotic resistance is mostly caused by the acquisition of new genes rather than by mutation, defined horizontal gene transfer.[2)] Dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates of bacteria. Many mobile genetic elements, such as plasmids, transposons, phages, and integrons, are responsible for the horizontal transfer of antibiotic resistance genes.[3–7)] Recent studies have shown that integrons play a major role in spreading antibiotic resistance genes in a clinical setting, as antibiotic resistance genes are frequently found located at gene cassettes, a diverse group of small mobile elements which normally integrated in integrons, in clinical isolates. Integrons are genetic elements that include the component genes and insertion site for a site-specific recombination system that enables them to capture mobile gene cassettes. The signature of an integron is the integrase gene (*intI*), encoding a site-specific recombinase that inserts and removes small DNA cassettes at *attI*, a unique integrase recogni-

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tion site adjacent to the integrase gene.[1,3,6] A gene cassette usually contain only a single gene (mostly antibiotic resistance gene) and a loosely conserved region called *attC* that recognized by the integrase during insertion and excision. The cassettes, in general, lack a promoter but, when integrased, the encoded gene is expressed from a promoter in the integron.[2,4,6] Integrons are found in several major lineages of bacteria and the view of integron diversity is expanding. There are currently thrity-two unique integron integrase genes available in GeneBank and various sequencing projects, an eight-fold increase in the past 3 years.[8] However only 3 classes of integrons are to date the most extensively studied and clinically significant, defined class 1, 2 and 3 integrons. The gene cassettes found in these integrons are much more variable; more than 60 different gene cassettes have been described, including gene cassettes conferring resistance to aminoglycosides, penicillins, cephalosporins, carbapenems, trimethoprim, chloramphenicol, rifampin, erythromycin, and quaternary ammonium compounds. In addition, more than one gene cassettes can be observed in a single integron which are strongly associated with multiple-antibiotic resistance.[4,9]

With the increasing problem of resistance to a wide spectrum of antimicrobial agents in an increasing number of bacterial isolates, the dissemination of antimicrobial resistance genes between organisms and patients is of great concern.[10,11] In order to study the roles of gene cassettes in clinical isolates, we investigated the prevalence of integrons of classes 1, 2 and 3, and characterized their gene cassette arrays in 118 random samples of resistance clinical isolates, collected from a hospital in Guangzhou, China, during the year 2004.

MATERIALS AND METHODS

Clinical Isolates —— During 2004, a total of 118 epidemiologically unrelated isolates with significant drug resistance were obtained from a variety of clinical specimens from diverse units in the clinical microbiology laboratory of a local hospital in Guangzhou City, China. Identification of isolates to the species level was performed by Vitek system (bioMerieux Vitek Systems Inc., Hazelwood, MO, U.S.A.). These organisms were identified as *Klebsiella pneumoniae* (*K. pneumoniae*) (27), *Pseudomonas aeruginosa* (*P. aeruginosa*) (18), *Escherichia coli* (*E. coli*) (11), *Acinetobacter baumannii* (*anitratum*) (*A. baumannii*) (13), *Enterobacter cloacae* (*E. cloacae*) (10), *Stenotrophomonas* (*Xantho.*) *maltophilia* (4), *Proteus mirabilis* (2), *Proteus vulgaris* (1), *Alcaligenes* sp. (2), *Flavobacterium* sp., (1), *Citrobacter freundii* (2), *Salmonella enterica* serovar Typhi (1), *Burkholderia* (*Pseudo.*) *cepacia* (2), *Staphylococcus aureus* (*S. aureus*) (15), *Staphylococcus* (coagulase negative) (3), *Enterococcus faecalis* (*E. faecalis*) (4), and *Enterococcus faecium* (*E. faecium*) (2).

Antimicrobial Susceptibility Test —— The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer disk diffusion test methodology. Antimicrobial drugs tested included ampicillin (AMP), penicillin G (PEN), amoxicillin/clavulanic acid (AMC), piperacillin (PIP), ceftazidime (CAZ), cefotaxime (CTX); cefazolin (FOX), cefuroxime sodium (CXM), ciprofloxacin (CIP), chloramphenicol (CHL), imipenem (IPM), amikacin (AMK), aztreonam (ATM), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT), and vancomycin (VAN).

The results were interpreted according to criteria of Clinical and Laboratory Standards Institute (CLSI).[12] The quality control strains used were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 29213. Strains presenting intermediate resistance values were considered resistant strains.

Multiple Polymerase Chain Reaction (PCR) Amplification of Integrase Genes —— The template DNA for PCR was prepared as described previously.[11] The quantity of the DNA was determined by using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Multiple PCR method was carried out for detection of class 1 and class 2 as well as class 3 integrases, as described previously.[13] While the integrase positive isolates were then sent to other PCR reactions for characterization of gene cassettes captured by integrons. *Vibrio cholerae* O1 strain SK-10 (a gift from Dr. Dalsgaad), and *E. coli* strain having a R483 : Tn*7* plasmid (a gift from Dr. Falbo) or pSMB731 plasmids (a gift from Dr. Hall) were used as positive control for class 1, 2 and 3 integrons, respectively. *E. coli* strain C600 was used as negative control. To ensure reproducibility, PCR was repeated twice with all of the strains.

Amplification and Sequencing of Gene Cassette Regions —— Amplification of the variable region of class 1 and 2 integrons was performed using the primers in-F/in-B and Ti-F/Ti-B, respectively, fol-

Table 1. Association between Antibiotic Susceptibility Profile and Integrons in 118 Clinical Isolates

Antibiotics	Antibiotic susceptibility (n = 118)		Integron-positive isolates (n = 95)		Integron-negative isolates (n = 23)	
	% R (no.)	% S (no.)	% R (no.)	% S (no.)	% R (no.)	% S (no.)
AMP	61.9% (73)	38.1% (45)	60.0% (57)	40.0% (38)	69.6% (16)	30.4% (7)
PEN	15.3% (18)	84.7% (100)	15.8% (15)	84.2% (80)	13.0% (3)	87.0% (20)
AMC	33.9% (40)	66.1% (78)	33.7% (32)	66.3% (63)	34.8% (8)	65.2% (15)
PIP	21.2% (25)	78.8% (93)	22.1% (21)	77.9% (74)	17.4% (4)	82.6% (19)
CAZ	21.2% (25)	78.8% (93)	21.1% (20)	78.9% (75)	21.7% (5)	78.3% (18)
CTX	55.9% (66)	44.1% (52)	61.1% (58)	38.9% (37)	34.8% (8)	65.2% (15)
FOX	36.4% (43)	63.6% (75)	35.8% (34)	64.2% (61)	39.1% (9)	60.9% (14)
CXM	45.8% (54)	54.2% (64)	49.5% (47)	50.5% (48)	30.4% (7)	69.6% (16)
CIP	46.6% (55)	53.4% (63)	48.4% (46)	51.6% (49)	39.1% (9)	60.9% (14)
CHL	13.6% (16)	86.4% (102)	14.7% (14)	85.3% (81)	8.7% (2)	91.3% (21)
IPM	6.8% (8)	93.2% (110)	8.4% (8)	91.6% (87)	0.0% (0)	100.0% (23)
AMK	17.8% (21)	82.2% (97)	18.9% (18)	81.1% (77)	13.0% (3)	87.0% (20)
ATM	44.9% (53)	55.1% (65)	47.4% (45)	52.6% (50)	34.8% (8)	65.2% (15)
GEN	73.7% (87)	26.3% (31)	85.3% (81)	14.7% (14)	26.1% (6)	73.9% (17)
SXT	61.0% (72)	39.0% (46)	73.7% (70)	26.3% (25)	8.7% (2)	91.3% (21)
VAN	0.8% (1)	99.2% (117)	1.1% (1)	98.9% (94)	0.0% (0)	100.0% (23)

lowed by the conditions and procedures described previously.[13] Alternatively, to increase specificity, the annealing temperature was increased to 55°C for the amplification of the variable region of the class 2 integron. Also, primer sets qacEΔ1-F and sul1-B were used to determine whether the class 1 integron contained the 3′-CS segment. For primer sets in-F/in-B and qacEΔ1-F/sul1-B, the conditions for PCR were modified to a primer annealing temperature 52°C and 56°C and an extension time of 2 min and 1 min, respectively as described previously.[13] PCR products of the same size were compared by restriction fragment length polymorphism (RFLP). To determine whether they had the same content, at least two different restriction endonucleases were chosen for each RFLP assay. This experiment was performed twice to ensure reproducibility. Each cassette gene PCR amplicon products with a distinctive size were cloned in pMD20-T using the pMD20-T cloning kit (Takara, Otsu, Japan), and then sequenced at Invitrogen Biotechnology Co., Ltd. (Guangzhou, China). Nucleotide sequences were compared with GenBank and DDBJ, and analyzed with Genetyx_version 7.0 software (Software Development Co. Ltd., Otsu, Japan).

RESULTS

Antibiotics Susceptibility Tests

The isolates were tested to the commonly used antibiotics in the hospital. The results of antibiotic resistance rates for integron-positive and -negative strains and for the whole set of isolates susceptibility to 16 antibiotics are shown in Table 1. Among all 118 clinical isolates, multiple resistance (resistance to ≥ 3 antibiotics) occurred among 84.7% (100/118) isolates. Fifty-five (46.6%) isolates were resistant to more than five antibiotics. Most of the isolates were resistant to GEN (73.7%, n = 87), AMP (61.9%, n = 73), SXT (61.0%, n = 72), CTX (55.9%, n = 66). However, less than 10% of the isolates were resistant to IPM (n = 8) and VAN (n = 1). Resistance to the remaining antimicrobial agents was observed in 10–50% of the isolates. The multidrug resistance (defined as resistance to six or more antibiotics) rates of integron-positive and -negative strains were 50.5% and 30.4%, respectively.

Detection of Three Classes of Integrons

One hundred eighteen isolates (representing seventeen bacterial genera) were screened for the presence of class 1 through 3 integrases. PCR screening results of all isolates detected integrate genes in 95 (80.5%) isolates, consisting of class 1 integrase in 90 (76.3%) isolates, class 2 integrase in 1 (0.8%) strains and only other 4 (3.9%) strains harbored both class 1 and 2 integrases. No class 3 integron was detected (Table 2). The results demonstrate that integrons are generally existed in various species of bacteria, including both gram-negative and gram-positive bacteria.

Table 2. Phenotypic and Antimicrobial Resistance Profiles of 95 Integron-containing Clinical Isolates

Organism & isolate	Isolation Date	Source	Sex & Age	Integrase	Gene cassette	Resistance profiles
Klebsiella pneumoniae						
0435	3/ 5/2004	Sputum	M,74	1	*aadA2*	AMP, AMC, CIP, PIP, GEN
0450	6/27/2004	Sputum	F,76	1	< 200 bp	AMC, CTX, ATM, CXM, AMK
0453	6/30/2004	Sputum	F,63	1	< 200 bp	AMP, AMC, CTX, CXM, PIP, ATM
0455	6/30/2004	Sputum	M,58	1	< 200 bp	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP
0459	7/ 1/2004	Sputum	M,54	1	*dfrA17-aadA5* *dfrA12-orfF-aadA2*	AMP, GEN, SXT
0461	7/ 3/2004	Urine	M,80	1	*dfr17-aadA5*	AMP, AMC, CTX, CHL, GEN, CIP, ATM, CXM, FOX, PIP, SXT
0471	6/26/2004	Sputum	M,58	1	< 200	AMP, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP
0488	6/24/2004	Blood	M,76	1	< 200	AMP, CIP
0499	1/12/2004	Sputum	F,58	1	*dfrA12-orfF-aadA2* *aacA4-catB-dfrA1*	AMP, ATM, CHL, GEN, SXT
04120	1/ 1/2004	Sputum	F,85	1	*dfrA17-aadA5*	AMP, GEN, SXT
04124	7/ 8/2004	Sputum	M,58	1	*aadA2*	AMP, AMC, CTX, GEN, CIP, ATM, CXM, CAZ
04125	7/ 8/2004	Sputum	F,80	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, CIP, CXM, ATM, GEN, SXT
04127	7/ 7/2004	Sputum	M,64	1	*dfrA12-orfF-aadA2*	AMP, CTX, CIP, CXM, , GEN, SXT
04162	7/13/2004	Sputum	M,58	1	*dfrA12-orfF-aadA2*	AMP, AMC, CHL, CTX, CIP, ATM, CXM, FOX, CAZ, AMK, GEN
04173	7/29/2004	Urine	M,2	1	*dfrA12-orfF-aadA2*	AMP, GEN, SXT
04220	8/ 8/2004	Drainage	M,58	1,2	*dfrA17-aadA5* *aadA2* *dfrA1-sat2-aadA1*	AMP, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP, IPM, SXT
04315	9/ 5/2004	Sputum	F,46	1	*dfrA1-orfX*	AMP, CTX, GEN, CXM, SXT
04339	9/14/2004	Sputum	M,63	1	*dfrA1-orfX*	AMP, AMC, GEN, CXM, SXT
04215	8/15/2004	Blood	M,53	1	*dfrA12-orfF-aadA2*	AMP, GEN, SXT
04343	9/ 3/2004	Sputum	F,57	1	< 200	AMP, CIP
Pseudomonas aeruginosa						
04237	8/20/2004	Sputum	M,52	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, FOX, CXM, GEN, SXT
04232	8/ 7/2004	Sputum	F,36	1	*dfrA12-orfF-aadA2*	AMP, ATM, FOX, CXM, CTX, GEN, SXT
0447	5/31/2004	Sputum	M,83	1	*dfrA12-orfF-aadA2*	CTX, CHL, ATM, CIP, GEN, CXM, FOX, CAZ, SXT
0454	6/30/2004	Sputum	M,75	1	< 200	AMC, CHL, CTX, SXT
0463	7/14/2004	Sputum	M,48	1	*dfrA12-orfF-aadA2*	AMC, CXM, GEN, SXT
0476	6/21/2004	Sputum	M,55	1	*aadA2*	AMC, CTX, CXM, FOX, CIP, ATM, GEN
0481	6/17/2004	Sputum	M,15	1	*dfrA12-orfF-aadA2*	CTX, SXT, CHL, GEN
04114	5/11/2004	Sputum	M,83	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, CXM, FOX, CAZ, ATM, SXT
04174	7/29/2004	Sputum	M,48	1	< 200	AMP, AMC, CTX, CIP, ATM, CXM, FOX, SXT, AMK
04217	8/ 8/2004	Sputum	M,64	1	*dfrA12-orfF-aadA2*	AMP, CTX, GEN, CIP, CXM, PIP, SXT
04227	8/23/2004	Secretion	M,51	1	*aadA2*	AMC, CHL, FOX, CTX, GEN, SXT
04273	8/31/2004	Sputum	M,71	1	*dfrA12-orfF-aadA2*	CTX, ATM, PIP, CAZ, CIP, GEN, SXT
04342	9/ 3/2004	Pleural fluid	M,36	1	*dfrA12-orfF-aadA2*	FOX, GEN, SXT
0496	1/12/2004	Sputum	M,68	1	*dfrA12-orfF-aadA2*	CTX, CIP, IPM, ATM, GEN, SXT
04113	3/ 9/2004	Urine	F,22	1	*dfrA12-orfF-aadA2*	CTX, ATM, GEN, SXT
Escherichia coli						
04182	7/27/2004	Sputum	F,86	1,2	*dfrA17-aadA5* *dfrA1-sat2-aadA1*	AMP, CTX, CIP, CXM, ATM, FOX, PIP, GEN, SXT
04204	7/30/2004	Nose	M,19	1	*dfrA17-aadA5*	ATM, CAZ, PIP, CTX, CIP

Table 2. Continued

Organism & isolate	Isolation Date	Source	Sex & Age	Integrase	Gene cassette	Resistance profiles
04213	8/19/2004	Sputum	F,69	1	*dfrA12-orfF-aadA2*	AMP
04240	8/17/2004	Sputum	M,56	1	*aadA2* *dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, CXM, SXT
04272	9/ 1/2004	Sputum	F,45	1	*dfrA12-orfF-aadA2*	AMP, GEN, SXT
04287	9/ 2/2004	Pus	M,39	1	*dfrA17-aadA5*	AMP, CTX, GEN, CIP, CXM, SXT
04289	9/ 2/2004	Sputum	F,58	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, SXT
04312	9/ 5/2004	Urine	F,37	1	*dfrA17-aadA5*	AMP, GEN, CIP, CXM, SXT
0426	1/14/2004	Secretion	M,2	1	*dfrA12-orfF-aadA2*	AMP, CTX, PIP, GEN, SXT
0431	2/19/2004	Sputum	F,14	1	*dfrA12-orfF-aadA2*	AMP, ATM, CTX, GEN, SXT
0434	2/27/2004	Sputum	M,40	1	*dfrA12-orfF-aadA2*	ATM, CXM, CAZ, CTX, GEN, SXT
0460	7/ 2/2004	Sputum	F,67	1	*dfrA12-orfF-aadA2*	AMP, AMC, ATM, FOX, CTX, CXM, GEN, SXT
0474	1/31/2004	Blood	F,68	1	*aadA2* *dfrA12-orfF-aadA2*	AMP, ATM, CTX, CXM, GEN, SXT
04140	7/26/2004	Sputum	M,16	1	*aacA4-cmlA1* *aacA4-catB3-dfrA1*	AMP, CTX, ATM, CXM, CHL, SXT
04147	7/16/2004	Sputum	M,82	1	*dfrA12-orfF-aadA2*	AMP, CTX, CXM, GEN, SXT
04161	7/13/2004	Sputum	M,60	1	*dfrA12-orfF-aadA2*	AMP, CXM, CTX, GEN, SXT
04201	7/29/2004	Urine	M,60	1	*dfrA12-orfF-aadA2*	AMP, CIP, ATM, FOX, CTX, GEN, SXT
04231	8/ 7/2004	Sputum	M,59	1	*dfrA12-orfF-aadA2*	AMC, ATM, FOX, CTX, CXM, PIP, GEN
04239	8/15/2004	Sputum	F,57	1	*dfrA12-orfF-aadA2*	AMP, FOX, CTX, ATM, CXM, PIP, GEN, SXT
Enterobacter cloacae						
0475	7/24/2004	Sputum	F,67	1	*aadA2*	AMP, AMC, FOX, CAZ, GEN, SXT
04119	2/22/2004	Sputum	M,95	1	*dfrA17-aadA5* *dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, ATM, CXM, CAZ, AMK, PIP, SXT
04177	7/29/2004	Cerebrospinal fluid	F,42	1	*dfrA17-aadA5* *dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP, SXT
04180	7/27/2004	Blood	F,10	1	*dfrA17-aadA5* *dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP, SXT
04236	8/20/2004	Sputum	F,81	1	< 200	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK, PIP
04314	9/ 6/2004	Sputum	M,67	1	< 200	AMP, AMC, CTX, GEN, CIP, ATM, CXM, FOX, CAZ, AMK
04332	9/14/2004	Urine	F,38	1	< 200	CTX, GEN, CIP, ATM, CXM, FOX, CAZ
0430	2/ 5/2004	Sputum	F,46	1	*dfrA17-aadA5*	CTX, IPM, SXT, AMK, ATM, GEN
0497	2/ 6/2004	Secretion	M,36	1	*dfrA12-orfF-aadA2*	AMP, CTX, GEN, ATM, IPM, PIP, SXT
04112	4/14/2004	Blood	M,61	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, GEN, ATM, FOX, IPM, CAZ, AMK, PIP, CIP, SXT
04126	7/ 7/2004	Sputum	F,75	1	*dfrA12-orfF-aadA2*	AMP, CTX, GEN, ATM, CXM, FOX
Proteus mirabilis						
0470	6/14/2004	Secretion	M,62	1	*dfrA17-aadA5*	AMP, AMC, CXM, FOX, GEN, SXT
04221	8/ 8/2004	Urine	M,18	1,2	*dfrA17-aadA5* *dfrA1-sat2-aadA1*	CHL, GEN, CTX, SXT
Proteus vulgaris						
04223	8/23/2004	Secretion	F,65	2	*dfrA1-sat2-aadA1*	AMP, CTX, CXM, FOX
Alcaligenes sp.						
0479	7/ 8/2004	Sputum	F,31	1	*dfrA12-orfF-aadA2*	CHL, GEN, CIP, SXT

Table 2. Continued

Organism & isolate	Isolation Date	Source	Sex & Age	Integrase	Gene cassette	Resistance profiles
Flavobacterium sp.						
0443	5/11/2004	Sputum	M,61	1	*dfrA12-orfF-aadA2*	AMC, CTX, CAZ, GEN, ATM, CXM, FOX, IPM, AMK
Citrobacter freundii						
0446	5/14/2004	Sputum	F,81	1	*dfrA17-aadA5*	AMP, AMC, CTX, ATM, CIP, GEN, CXM, FOX, SXT
04175	7/29/2004	Urine	M,83	1	*dfrA12-orfF-aadA2*	AMP, CTX, CIP, FOX, GEN, SXT
Salmonella typhi						
04181	7/27/2004	Blood	F,33	1	*dfrA12-orfF-aadA2*	AMP, CTX, CIP, ATM, FOX, CXM, PIP, GEN, SXT
Burkholderia (*Pseudo.*) *cepacia*						
04222	8/23/2004	Sputum	M,19	1	*dfrA12-orfF-aadA2*	AMC, CHL, GEN, AMK, ATM, IPM
04235	8/20/2000	Sputum	M,63	1	*dfrA12-orfF-aadA2*	AMP, AMC, CTX, IPM, AMK, ATM, FOX, CXM, PIP, GEN
Staphylococcus aureus						
0439	4/ 2/2004	Sputum	M,23	1	*dfrA12-orfF-aadA2*	PEN, GEN, SXT
0490	4/15/2004	Sputum	M,73	1	*dfrA12-orfF-aadA2*	PEN
04109	2/ 1/2004	Secretion	M,72	1	*dfrA12-orfF-aadA2*	CHL, PEN, GEN, SXT
04115	2/22/2004	Sputum	F,32	1	*dfrA12-orfF-aadA2*	PEN, CIP, GEN
04121	7/ 9/2004	Sputum	M,25	1	*dfrA12-orfF-aadA2*	PEN, GEN, SXT
04178	7/28/2004	Semen	M,46	1	*dfrA12-orfF-aadA2*	CHL, PEN, , CIP, GEN, SXT
04200	7/29/2004	Urine	M,82	1	*dfrA12-orfF-aadA2*	ATM, AMK, CTX, CXM, SXT
04206	7/30/2004	Pleural fluid	M,64	1	*dfrA12-orfF-aadA2*	AMP, PEN, SXT
04212	8/19/2004	Pus	M,30	1	*dfrA12-orfF-aadA2*	PEN, GEN
04229	8/21/2004	Drainage	M,19	1	*dfrA12-orfF-aadA2*	GEN, PEN, CIP, SXT
04233	8/ 8/2004	Drainage	M,33	1	*dfrA12-orfF-aadA2*	PEN, GEN, SXT
04270	8/31/2004	Sputum	M,85	1	*dfrA12-orfF-aadA2*	PEN, CHL, CIP, GEN, SXT
Staphylococcus (coagulase negative)						
0469	1/11/2004	Sputum	M,75	1	none-ORF (441 bp) +attc	PEN, CIP, SXT, GEN
0480	7/ 4/2004	Urine	F,31	1	*dfrA12-orfF-aadA2*	PEN, CIP, GEN, SXT
04146	7/16/2004	Pus	M,27	1	*dfrA12-orfF-aadA2*	PEN, GEN, SXT
Enterococcus faecium						
0441	4/12/2004	Sputum	M,51	1	*dfrA12-orfF-aadA2*	GEN, SXT
Enterococcus faecalis						
0424	1/ 9/2004	Pleural fluid	F,62	1	*dfrA12-orfF-aadA2*	SXT, CIP, VAN
0457	7/ 1/2004	Sputum	M,25	1	*dfrA12-orfF-aadA2*	CIP, GEN, SXT
04218	8/ 8/2004	Drainage	M,58	1,2	*dfrA17-aadA5* *dfrA1-sat2-aadA1*	AMP, PEN, CIP, GEN
04241	8/18/2004	Urine	F,5	1	*dfrA12-orfF-aadA2* *dfrA17-aadA5*	CIP, GEN, SXT

Characterization of Antibiotic Resistance Gene Cassettes in the Class 1 and Class 2 Integron Positive Isolates

Isolates identified as having class 1 or class 2 integrase genes were further characterized through PCR amplification of class 1 and 2 variable regions.[13] Among the 95 integrase positive isolates, gene cassette regions were amplified with the following sizes 635, 1009, 1179, 1913, 1664, 2360, 2385 and 2655 bp, which constructed fourteen different arrays of electrophoresis mapping (data not shown).

Thirteen different genes, including genes encoding resistance to aminoglycosides (*aadA1*, *aadA2*, *aadA5* and *aacA4*), chloramphenicol (*catB3* and *cmlA1*), trimethoprim (*dfrA1*, *dfrA12* and *dfrA17*), or streptothricin (*sat2*), and one unknown genes were detected (Table 2). The genes most com-

monly found among class 1 integron were aminoglycoside and trimethoprim resistance genes. The five isolates containing *intI2* were amplified with the primers Ti-F/Ti-B. All of them produced a 2386 bp amplicon harboring the same arrays of gene cassettes *dfrA1-sat2-aadA1*, conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively. Eleven isolates showed the existence of amplicons of less than 200 bp, contained 'empty' integrons that lacked resistance cassettes.

In this study, integron-associated antibiotic resistance genes were detected in mostly reported bacteria such as *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Salmonella* sp., and *Acinetobacter* sp. However, other pathogenic bacteria, which few have been researched, were found harbored integrons as well. Some of them were first reported, such as *E. faecalis*, *E. faecium*, *B. pseudomallei*, *Alcaligenes* sp., and *Flavobacterium* sp. In addition, it is interested to find a novel gene in an integron from coagulase-negative *Staphylococcus* strain 0469. An amplicon about 0.7 kb was detected by amplification of the integron variable region from strain 0469. Complete sequencing revealed the presence of one ORF of 441 bp. Additional features included the *attC* (59-base element) associated with the terminal 3′-ends of an ORF required for the recombination of cassettes with corresponding *att1* sites on the integrons. BLAST searches with the ORF identified no matching sequences.

DISCUSSION

The prevalence of antimicrobial-resistant bacterial pathogens has become a major public health concern. Horizontal gene transfer has already been proved a significant mechanism for disseminating antimicrobial resistance in bacterial populations. Antimicrobial drug resistance can also be facilitated by integrons in case of many other bacteria.[1, 3, 13–15)]

This work was initiated with the detection of 3 classes of integrons. Our results indicate that among 118 clinical isolates, 95 (80.5%) were detected to harbor integrons, which is an uncommonly high rate. In this study, the most bacteria with multiple-resistance from hospitalized patients were selected.

The results from what we studied indicated that integrons are widespread among clinical isolates. It seems there are no significant difference between gram-negative and gram-positive bacteria, for integrase gene was detected in 20 out of 24 (83.3%) gram-positive isolates, while 79.8% (75/94) in gram-negative isolates. Nevertheless, integrons are most likely to harbor in intestinal bacteria, as 100% of bacteria isolated from human intestine contained integrons in this study. Other reports have also revealed the identical phenomenon.[13, 17, 18)]

Thirteen different gene cassettes were detected. Cassette genes encoding resistance to aminoglycosides were found to be predominant in the class 1 integron. These results are in accordance with reports from other laboratories.[5, 9, 10)] The *aadA*-type genes that encode resistance to aminoglycosides were most commonly found. Aminoglycosides, such as streptomycin and spectinomycin, were widely used for treating urinary tract infections in the early years. Therapeutic uses of streptomycin and spectinomycin were excluded for a long period, but they continue to be used in agriculture and food animals, particularly in livestock. At the same time, *aadA1* gene has been found in clinical isolates. It offered the evidence for horizontal transfer of resistance genes from bacteria that infect animals to those that infect humans. Therefore, *aadA* gene cassette may remains prevalent.

The *dfr* cassettes (*dfrA1*, *-A12*, and *-A17*) that confer resistance to trimethoprim were also detected frequently. Interestingly, they were found strongly combined with the *aadA* gene cassettes. All the *dfr* cassettes found in this study were followed by an *aadA* gene cassette downstream, except in three isolates. Moreover, all the *dfr* cassettes, except for one, were located directly behind the 5′conserved segment, which is closest to the promoter, thereby providing high-level expression and conditional resistance. This could be explained by which trimethoprim-sulfamethoxazole were preferred for treating *E. coli* infections or urinary tract infections in last 10 years. Other individual cassettes, *cmlA1* and *catB3* (resistance to chloramphenicol), were found only in an *A. baumannii* isolates. In addition, in this study the *S. aureus* isolates were containing mostly the same integrons and some integrons were present frequently in certain strains, *e.g.*, PCR mapping of variable regions which showed 2 fragments simultaneously (product sizes 1664 and 1913 bp) were detected in *E. cloacae* isolates from different origins (Table 3). In the current study, eleven isolates showed variable region of the integron less than 0.2 kb. This implies these isolates carry an 'empty' integrons that lacked re-

Table 3. Results of PCR Amplification of Integron-containing Gene Cassette in Bacterial Isolates

Gene cassette (PCR product, bp)	No. of isolates	Genus categories represented (no. of isolates)
dfrA12-orfF-aadA2[a] (1913)	54	*Staphylococcus aureus* (12), *Acinetobacter baumannii* (*anitratum*) (9), *Pseudomonas aeruginosa* (11), *Klebsiella pneumoniae* (5), *Staphylococcus* (coagulase negative) (2), *Burkholderia* (*Pseudo.*) *cepacia* (2), *Escherichia coli* (3), *Stenotrophomonas* (*Xantho.*) *maltophilia* (3), *Alcaligenes* sp. (1), *Acinetobacter calcoaceticus* (9), *Citrobacter freundii* (1), *Enterococcus faecalis* (2), *Enterococcus faecium* (1), *Flavobacterium* sp. (1), *Salmonella typhi* (1)
dfrA17-aadA5[a] (1664)	8	*Escherichia coli* (3), *Citrobacter freundii* (1), *Klebsiella pneumoniae* (2), *Proteus mirabilis* (1), *Stenotrophomonas* (*Xantho.*) *maltophilia* (1)
dfrA1-orfX[a] (1179)	2	*Klebsiella pneumoniae* (2)
aadA2[a] (1009)	5	*Klebsiella pneumoniae* (2), *Enterobacter cloacae* (1), *Pseudomonas aeruginosa* (2)
unknown[b] (635)	1	*Staphylococcus*, coagulase negative (1)
empty integron[c] (< 200)	11	*Klebsiella pneumoniae* (6), *Enterobacter cloacae* (3), *Pseudomonas aeruginosa* (2)
aadA2 & *dfrA12-orfF-aadA2*[a] (1009 & 1913)	2	*Acinetobacter baumannii* (*anitratum*) (1), *Escherichia coli* (1)
dfrA17-aadA5 & *dfrA12-orfF-aadA2*[a] (1664 & 1913)	5	*Enterobacter cloacae* (3), *Enterococcus faecalis* (1), *Klebsiella pneumoniae* (1)
aacA4-cmlA1 & *aacA4-catB3-dfrA1*[a] (2360 & 2655)	1	*Acinetobacter baumannii* (*anitratum*) (1)
dfrA12-orfF-aadA2 & *aacA4-catB3-dfrA1*[a] (1913 & 2655)	1	*Klebsiella pneumoniae* (1)
dfrA1-sat2-aadA1[d] (2385)	1	*Proteus vulgaris* (1)
dfrA17-aadA5 & *dfrA1-sat2-aadA1*[e] (1664 & 2385)	3	*Escherichia coli* (1), *Enterococcus faecalis* (1), *Proteus mirabilis* (1)
aadA2 & *dfrA17-aadA5* & *dfrA1-sat2-aadA1*[e] (1009, 1664 & 2385)	1	*Klebsiella pneumoniae* (1)

a) Contain class 1 integron only, *b*) no homologous sequence was found in NCBI, *c*) not detected Gene cassette, *d*) contain class 2 integron only, *e*) contain both class 1 & class 2 integrons.

sistance cassettes, which have been reported previously.[19] All the class 2 integron carried the same gene cassettes as those found in Tn*7*, *dfrA1-sat2-aadA1* genes.[9]

Furthermore, in the present study, integrons with identical cassette array were identified in most isolates of different species, suggesting that these different species may have the same mechanisms for acquisition of multiresistance. It revealed that gene cassettes are most likely to transfer between strains with the whole integron elements, rather than move individually.[18]

Multiple-resistance phenotypes were observed among both class 1 and class 2 integron-containing isolates. We found a significant association between multiresistance and integrons. A high correlation between the presence of integrons carrying dihydrofolate reductase and the phenotypic expression of resistance to trimethoprim-sulfamethoxazole was observed. Many isolates also showed resistance to antibiotics but did not possess the corresponding antibiotic resistance gene cassettes within the integrons characterized from such isolates. It is probable that such resistances are encoded by nonintegron elements.

In conclusion, antibiotic resistance in bacteria has been surveyed by the government since 1985 in accordance with the suggestion of the World Health Organization, while the situation of antibiotic resistance in bacteria is still serious at present in China. Increasing antimicrobial resistance is a serious clinical problem worldwide. Integrons may provide an innovative and effective device for surveying antimicrobial resistance in bacteria. Our data demonstrated that the high prevalence of integrons found clinical isolates, which will continue to threaten the usefulness of antibiotics as therapeutic agents. Consequently, the study of integrons and their associ-

ated gene cassettes can provide important information on the mechanisms of acquisition of multiple antibiotic resistance genes in clinical isolates, as well as on the selection of antibiotic therapy.

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