

costs by reducing unnecessary hospitalization and medication for patients with 'false-positive' initial AFB smears.

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Transparency Declaration

All authors declare no potential conflicts of interest.

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Resistance class I integron in clinical methicillin-resistant *Staphylococcus aureus* strains in southern China, 2001–2006

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Abstract

As a novel antibiotic resistance determinant, investigation of the occurrence and characteristics of class I integron was performed on nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) strains sampled during 2001–2006. Seventy-six out of 179 (42.5%) of the tested strains were found to carry class I integrons, with four unique arrays of gene cassettes detected. This is the first report of the comprehensive identification and typing of class I integrons in clinical MRSA isolates over a 6-year period, representing the first evidence for class I integrons as possible antibiotic resistance determinants in clinical MRSA strains.

Keywords: Antibiotic resistance determinant, class I integron, gram-positive bacteria, mobile genetic element, MRSA

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TABLE 1. Phenotypic and genotypic characteristics of 76 integron-bearing MRSA strains

Strain	Isolation year	Class I integron		SCC _{mec}		RAPD-PCR	Resistance profile	
		3'CS	Gene cassette	ccr	mec			
010808	2001	+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
010912		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
011000*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
011001*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	AcChCaCeCiCIELOTs	
011016*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
011024*		-	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTc	
011025		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
011045*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
011052*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
011055*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	AcChCaCeCiCIELOTs	
011058*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	AcChCaCeCiCIELOTs	
011083*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
011098*		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
021138		2002	+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs
021153			+	<i>dfrA1 2-orfF-aadA2</i>	3	A	DAA	AcCaCeCIOTcTs
021206*			+	<i>dfrA1 2-orfF-aadA2</i>	3	A	DAA	AcCaCeCIOTcTs
021207*			+	<i>dfrA1 2-orfF-aadA2</i>	3	A	DAA	AcCaCeCIOTcTs
021238*			+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs
021261*	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	AcChCaCeCiCIELOTs	
021266*	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
021267*	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
021268*	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
021296	-		<i>dfrA1 2-orfF-aadA2</i>	3	A	CBC	AcCeCIEGLOTs	
021542	2003	+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
031788		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	AcChCaCeCiCIELOTs	
032142		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	BBB	ChCaCeCiCIELOTs	
032267		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
032371		+	<i>dfrA1 2-orfF-aadA2</i>	3	A	DAA	AcCaCeCIOTcTs	
032423		+	<i>aacA4-cmlA1</i>	3	A	AAE	AcAmChCaCeCIEGLOTc	
032439		+	<i>dfrA1 7-aadA5</i>	3	A	AAF	AcAmChCaCeCiCIEGLOTs	
032449		+	<i>aacA4-cmlA1</i>	3	A	AAE	AcAmChCaCeCIEGLOTc	
042470		2004	+	<i>aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs
042497			+	<i>aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs
042547	+		<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042564	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042637	+		<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
042649	+		<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
042772	+		<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042848	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
042885	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
042887	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	AAA	AcAmChCaCeCiCIEGLOTcTs	
042898	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042923	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042954	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
042966	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
043000	+		<i>dfrA1 2-orfF-aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053001	2005		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs
053059			+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTc
053147			+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs
053182		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053224		+	<i>aadA2</i>	NT	NT	GEJ	AmChCaCeCiCIGLTcTs	
053332		-	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTc	
053333		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
053401		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053423		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
053443		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTc	
053444		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
053474		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053564		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053610		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053658		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053685		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
053845		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTc	
053899		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
064043	2006	+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
064050		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
064064		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
064100		+	<i>aadA2</i>	3	A	FEI	AcAmCaCeCiCIEGLOTcTs	
064163		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTc	
064221		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
064249		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
064278		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
064375		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
065212		+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs	
065217	+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTc		
065260	+	<i>aadA2</i>	3	A	GEJ	AcAmChCaCeCiCIGLOTcTs		

TABLE 2. Association between integron-bearing and non-integron-bearing MRSA strains

Characteristics	Value for group		p value	OR (95% CI)
	I-MRSA	Non I-MRSA		
Gender				
Female	26 (34.2)	39 (37.9)	0.615	1.172 (0.631–2.176)
Male	50 (65.8)	64 (62.1)		
Age (mean [95% CI])	56 (37–76)	53 (34–78)		
Department				
Internal medicine	39 (51.3)	60 (58.4)	0.356	1.324 (0.729–2.403)
Surgery	11 (14.5)	9 (8.7)	0.229	0.566 (0.222–1.443)
Obstetrics and Gynaecology	4 (5.3)	5 (4.9)	1.000	0.918 (0.238–3.541)
Neurology	7 (9.2)	9 (8.7)	0.913	0.944 (0.335–2.658)
Paediatrics	4 (5.3)	5 (4.9)	1.000	0.918 (0.238–3.541)
Orthopaedics	5 (6.6)	7 (6.8)	0.954	1.035 (0.316–3.396)
Department of Infectious Disease	3 (3.9)	4 (3.9)	1.000	0.983 (0.213–4.528)
General ward	3 (3.9)	4 (3.9)	1.000	0.983 (0.213–4.528)
Infection site				
Bloodstream	7 (9.2)	5 (4.9)	0.249	0.503 (0.153–1.650)
Respiratory tract	19 (25.0)	30 (29.1)	0.541	1.233 (0.630–2.412)
Skin and soft tissue	35 (46.1)	46 (44.6)	0.853	0.945 (0.521–1.714)
Urinary tract	8 (10.5)	11 (10.7)	0.974	1.016 (0.388–2.663)
Other	7 (9.2)	11 (10.7)	0.747	1.179 (0.435–3.196)
Length of hospital stay (days)				
Mean	26.2	23.4		
Median	15.4	13.8		
Range	0–178	0–152		
Resistance to antibiotics				
AMC	74 (97.4)	93 (90.3)	0.061	0.251 (0.053–1.183)
AMK	60 (78.9)	50 (48.5)	<0.001	0.252 (0.128–0.493)
CAZ	70 (92.1)	87 (84.5)	0.124	0.466 (0.173–1.254)
CEF	76 (100)	97 (94.2)	0.085	
CHL	43 (56.6)	47 (45.6)	0.148	0.644 (0.355–1.170)
CIP	70 (92.1)	87 (84.5)	0.124	0.466 (0.173–1.254)
CLI	70 (92.1)	90 (87.4)	0.310	0.593 (0.215–1.640)
ERY	55 (72.4)	52 (50.5)	0.003	0.389 (0.207–0.734)
GEN	66 (86.8)	60 (58.3)	<0.001	0.211 (0.098–0.457)
LEV	72 (94.7)	90 (87.4)	0.097	0.385 (0.120–1.230)
OXA	75 (98.7)	97 (94.2)	0.251	0.216 (0.025–1.829)
SXT	67 (88.2)	40 (38.8)	<0.001	0.085 (0.038–0.190)
TET	63 (82.9)	54 (52.4)	<0.001	0.227 (0.112–0.463)
SCC _{mec} type				
II	0 (0)	3 (2.9)		
III	75 (98.7)	93 (90.3)		
NT	1 (1.3)	7 (6.8)		
RAPD type				
AAA-ST239-WGKAOMQ(†037)-HIJKL	17 (22.4)	0 (0)		
BBB-ST239-WGKAQQ(†030)-HIJKL	6 (7.9)	2 (1.9)		
CBC-ST239-WGKAQQ(†030)-HIJKL	6 (7.9)	3 (2.9)		
BBD-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	18 (17.5)		
BAD-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	2 (1.9)		
CAC-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	3 (2.9)		
DAA-ST239-WGKAOMQ(†037)-HIJKL	4 (5.2)	0 (0)		
AAE-ST239-WGKAOMQ(†037)-HIJKL	2 (2.6)	0 (0)		
AAF-ST239-WGKAOMQ(†037)-HIJKL	1 (1.3)	0 (0)		
BCD-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	6 (5.8)		
EDH-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	27 (26.2)		
FEI-ST239-WGKAOMQ(†037)-HIJKL	23 (30.3)	0 (0)		
EDG-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	38 (36.9)		
GEJ-ST239-WGKAOMQ(†037)-HIJKL	17 (22.4)	0 (0)		
HFK-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	2 (1.9)		
HFL-ST239-WGKAOMQ(†037)-HIJKL	0 (0)	2 (1.9)		
Total	76	103		

Value group: values are number of patients or strains. Values in parentheses are percentages.

Infection sites: blood culture specimens included blood inoculated into aerobic and/or anaerobic blood culture medium. Tracheal secretion specimens included secretions from the trachea and bronchia and bronchoalveolar lavage fluid specimens. Wound specimens included surgical wound specimens but also specimens from ulcers, fistulae, abscesses, drainage fluids, catheter sites, percutaneous endoscopic gastrostomy insertion sites, and tracheostomas. Urine contained native urine from urinary catheters or bladder puncture and inoculated culture systems. Other specimens consisted of skin swab specimens (from the nares, axilla, groin or perianal area), as well as swab specimens of the throat, tonsils, eye, ear and vagina, body liquids, and puncture exudates.

Resistance to antibiotics: AMC, amoxicillin/clavulanic acid; AMK, amikacin; CAZ, ceftazidime; CEF, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; LEV, levofloxacin; OXA, oxacillin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

RAPD type: each RAPD type consisted of RAPD-PCR (the three-letter code summarizes the typing results per primer used; in detail, first digit, primer 1; second digit, primer 7; third digit, primer E2; data represented by a capital letter given in a certain column may be different from the same character in another column), MLST, *spaA* and *coa* types.

Indiscriminate use of existing antibiotics can lead to the proliferation of antibiotic resistance, which poses a dilemma for the future treatment of bacterial infections [1]. Despite a variety of therapeutic options and novel drug discovery efforts, antibiotic resistance in microbes still remains a major public health concern for the treatment of infectious diseases. One such infectious organism is methicillin-resistant *Staphylococcus aureus* (MRSA), a gram-positive coccus that has been recently labelled as a 'super bug' due to its widespread resistance to commonly used antibiotics [2]. China remains one of the worst areas for antibiotic abuse; as a consequence, biosafety and general concerns regarding the threat of unleashing waves of 'Super Bugs' in China have raised the necessity for surveillance and investigation of antibiotic resistance mechanisms involved in clinical MRSA. Since its first discovery in 1961, MRSA has become one of the most prevalent pathogens causing nosocomial infections throughout the world [3,4]. A mobile genetic element, designated staphylococcal cassette chromosome *mec* (SCC*mec*), plays a major role in causing resistance among MRSA [5]. Another novel mobile genetic element, the integron, is potentially the primary agent in dissemination of multidrug resistance among bacteria [6]. However, most studies on integrons were limited to gram-negative microorganisms, and investigation of integrons in gram-positive bacteria, especially MRSA, still remains unclear. In a previous study, we screened the class I integron in 46 gram-positive clinical strains isolated from an outbreak at the First Affiliated Hospital of Jinan University (FAHJU), including 17 *S. aureus* and 20 coagulase-negative staphylococci (CoNS) strains, with a positive identification rate of 100% [7]. Based on this novel observation and previously unnoticed spread of the class I integron in *S. aureus*, this study aimed to investigate the occurrence and prevalence of MRSA-associated class I integron in the same hospital setting during 2001–2006.

A total of 179 MRSA strains isolated from FAHJU were identified as *S. aureus* by the API Staph strip test and Vitek 2 automated system; methicillin resistance was determined by latex agglutination for PBP2a and *mecA* detected by PCR [8,9]. Antimicrobial susceptibility testing and PCR identification of staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*), exfoliative toxins A, B (*eta*, *etb*), TSST-I (*tst*), and Pantone-Valentine leukocidin (*lukS* and *lukF*) genes were performed [8,10,11]. All strains were screened for class I integrons and characterized for variable regions. Briefly, the *intl* gene, variable region and 3'CS were examined using primer sets intlI-U with intlI-D, intlI-K with In-B, and *qacEΔI*-F with *sulI*-B, respectively [1,12]. PCR products of the variable region were characterized by restriction fragment length polymorphism (RFLP) and further confirmed by sequencing. SCC*mec* typing

and random amplified polymorphic DNA (RAPD)-PCR were then performed, with at least one strain representative of each RAPD type selected randomly for further analyses by MLST, *spa* and *coa* typings [5,13–17].

The multidrug resistance rate, which was defined as resistance to at least seven of the tested antimicrobials, was 93.8% (168/179), with negative PCR amplification for exotoxin, exfoliative toxins, TSST-I and PVL. Significant differences to amikacin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole and tetracycline were observed between MRSA strains with and without the class I integron. Genetic analyses demonstrated that class I integron was detected in 76 strains, with 73 carrying the 3'-conserved region of *qacEΔI-sulI*, and all strains contained one of four observed unique types of gene cassette arrays (Table 1). Three and 168 strains belonged to classic nosocomial SCC*mec* type II and III, with eight strains untypable. RAPD-PCR with primers API, AP7 and E2 classified 179 MRSA strains into 8, 6 and 12 distinct groups, respectively, with a total of 16 RAPD types (Table 2). Randomly selected strains fell into the ST239-MRSA-III group (clonal complex, CC239), with *coa* type HIJKL and 2 *spaA* types (WGKAOMQ-t037 and WGKAQQ-t030) (Table 2).

The class I integron was commonly found (42.5%, 76/179), but the incidence rate decreased over the study period, with 65.0% (13/20) in 2001, 55.0% (11/20) in 2002, 46.7% (7/15) in 2003, 44.0% (15/34) in 2004, 36.0% (18/50) in 2005 and 30.0% (12/40) in 2006. This positive identification rate was somewhat lower than that observed in preliminary studies on *S. aureus* (53.3%, 16/30) and CoNS (56.6%, 30/53) in south China [1,18]. Most investigations of class I integrons have been primarily concerned with gram-negative bacteria; however, only four reports regarding staphylococci were currently available, with the limitation of occasional isolation or insufficient samples to comment on the epidemiological significance [1,9,18,19]. To our knowledge, this is the first report of the consistent detection of class I integrons in nosocomial MRSA isolates in a large-scale longitudinal study (6-years), representing the first definite evidence of the class I integron as an unnoticed antimicrobial determinant, possibly involved in the rampant spread of antibiotic resistance mechanisms in clinical MRSA. Furthermore, it is also the first identification of MRSA isolates simultaneously carrying two mobile genetic elements: gene cassettes within a class I integron and SCC*mec*, including four different arrays of gene cassettes and two types of SCC*mec*. Because integrons and SCC*mec* can serve as reservoirs of various resistance genes and function to exchange genes between species, it has been hypothesized that their simultaneous existence may confer increased fitness and better survivability

to bacterial strains harbouring these mobile genetic elements in various environments. Three out of four detected gene cassette arrays were first reported in *S. aureus*, with sequences identical to those in gram-negative and gram-positive bacteria (1, 7, 9, 18). The two most frequently detected resistance genes in class I integrons were of the *aadA* and *dfrA* families, with identification rates of 97.4% (74/76) and 50.0% (38/76), respectively. Arrays *dfrA12-orfF-aadA2* and *aadA2* were the most dominant in the tested strains, making up 48.7% (37/76) and 47.4% (36/76), respectively. Cassette array *dfrA12-orfF-aadA2* was prevalent during 2001–2004 but was undetectable from 2005, while cassette *aadA2* appeared in 2004 and dominated during 2005–2006. It is noteworthy that the similar high prevalence of array *dfrA12-orfF-aadA2* was also observed in CoNS strains during 2001–2004; however, *aadA2* was scarcely found [18]. Similar to our own findings, *aadA2* was the only gene cassette detected in another study from 2005 during an outbreak of *S. aureus* [1]. Because the same combination and temporal pattern of identified gene cassettes had also been characterized in staphylococci and various gram-negative bacteria previously, further studies are required to determine the origins of class I integrons in staphylococci, and whether they can spread via interactions with both gram-positive and gram-negative bacteria. In recent years, community-associated MRSA (CA-MRSA) has emerged and become prevalent in many countries and regions, often outnumbering more traditional hospital-acquired MRSA (HA-MRSA) infections [8,20]. As generally accepted, the indiscriminate and over use of existing antibiotics is the primary cause of the emergence and selection of antibiotic-associated mobile genetic elements, which to some extent, is reflected by the increase in prevalence of integrons and nosocomial SCCmec. Although this study showed a reduction in the prevalence of class I integrons over time, a large proportion of MRSA isolates still harboured these integrons, which may serve as reservoirs of antimicrobial resistance and virulence-associated genes, which can contribute to increased rates of treatment-resistant *S. aureus* infections in both the hospital and community setting.

Transparency Declaration

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