

Class 1 integron in staphylococci

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Abstract As a major concern in public health, methicillin-resistant staphylococci (MRS) still remains one of the most prevalent pathogens that cause nosocomial infections throughout the world and has been recently labeled as a “super bug” in antibiotic resistance. Thus, surveillance and investigation on antibiotic resistance mechanisms involved in clinical MRS strains may raise urgent necessity and utmost significance. As a novel antibiotic resistance mechanism, class 1 integron has been identified as a primary source of antimicrobial resistance genes in Gram-negative organisms. However, most available studies on integrons had been limited within Gram-negative microbes, little is known for clinical Gram-positive bacteria. Based on series studies of systematic integrons investigation in hundreds of staphylococci strains during 2001–2006, this review concentrated on the latest development of class 1 integron in MRS isolates, including summary of prevalence and occurrence of class 1 integron, analysis of correlation

between integron and antibiotic resistance, further demonstration of the role integrons play as antibiotic determinants, as well as origin and evolution of integron-associated gene cassettes during this study period.

Keywords Class 1 integron · Methicillin-resistance staphylococci (MRS) · Antibiotic resistance · Mobile genetic element · *SCCmec*

Staphylococci are a group of Gram-positive, facultative aerobic and usually unencapsulated organisms, which are responsible for various tissues infection and a multitude of diseases. These bacterium, are carried, mostly transiently, by approximately 20 and 30% of healthy adults on the skin and anterior nares, respectively. Over 30 different types of staphylococci are infectious for humans, and its related illness can range from mild to severe, from no treatment required to even potentially fatal. Most of these infections are caused by *Staphylococcus aureus*, which has been regarded as leading issues both in medicine and food safety, and can typically causes a wide variety of infections, including skin infections and sometimes pneumonia, endocarditis, osteomyelitis, gastroenteritis, scalded skin syndrome and toxic shock syndrome [103]. Coagulase-negative staphylococci (CoNS) are regarded as a frequent cause of nosocomial infection and bacteremia, especially in patients with indwelling medical devices [5]. CoNS have also become the most frequently isolated pathogens in intravascular catheter related infections (CRI), accounting for an estimated 28% of all nosocomial bloodstream infections [63]. Since the first discovery in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most prevalent pathogens that cause nosocomial infections throughout the world. As this pathogen can

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spread easily by either direct or indirect contact between patients and environment, or via patients and medical personnel, it is considered to be an important risk factor for nosocomial infection, which continues to be a challenge for clinicians, hospital epidemiologists and administrators [11]. Methicillin resistance in staphylococci is caused by PBP2a protein encoded by the *mecA* gene. The *mecA* gene is located on a mobile genetic element, designated as staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mec* gene complex (the *mecA* gene and its regulators) and the *ccr* gene complex encoding site-specific recombinases responsible for the mobility of SCC*mec* [39]. Methicillin-resistant coagulase-negative staphylococci (MRCNS), which are more frequent carriers of SCC*mec* than MRSA, have been postulated to be the reservoir for the transfer of methicillin resistance to *S. aureus*. One assumes that the *ccr* and *mec* genes were bought together in CoNS from an unknown source, where deletion in the *mec* regulatory genes occurred, before the genes were transferred into *S. aureus* [34]. As one example of the leading “Super Bugs”, methicillin-resistant staphylococci (MRS) strains show resistance to practically all β -lactam antibiotics and usually other multiple drugs due to the *mecA* and associated resistance genes carried by SCC*mec*, respectively [77]. China remains one of the worst areas for antibiotics abuse, with an estimate annual consumption of 140 g per person, which is ten times higher than that in the United Kingdom and the United States. General concerns for the threaten of unleashing waves of “Super Bugs” in China raised necessity for surveillance and investigation on antibiotic resistance mechanisms involved in clinical MRSA and MRCNS strains.

Introduction of integrons

Indiscriminate use of existing antibiotics leads to proliferation of antibiotic resistance and poses a dilemma for the future treatment of bacterial infection. Antibiotic resistance in microbes still remains one of the leading concerns in global public health, and several mechanisms involving mobile genetic elements such as plasmids and transposons, have been shown to contribute to the wide spread and distribution of antibiotic resistant genes among bacteria. In recent years, the role of integrons as a mobile genetic mechanism in horizontal transfer of antibiotic resistance has been well established [29, 31, 32, 84]. A complete functional integron platform comprises three elements (Fig. 1): the integrase gene (*intI*) encoding an integrase, a proximal primary recombination site *attI* and a promoter gene (Pc) which had been functionally demonstrated for all integrons [43]. *IntI* encodes a tyrosine-recombinase family integrase, which is characterized by the presence of

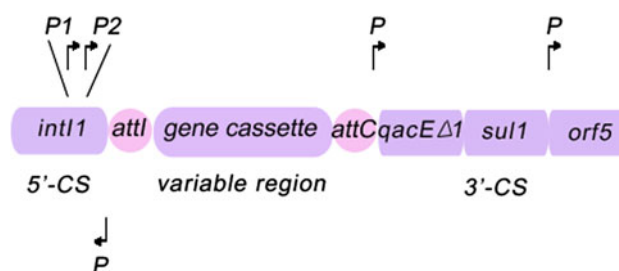


Fig. 1 Structure of class 1 integron

invariant RHRY amino-acids in the conserved motifs (termed as box 1 and box 2), and mediates recombination between the *attI* site and a secondary target called an *attC* site (also known as 59 base elements or 59-be sites [59be]). The simple *attI* site consists of two inverted sequences that bind the integrase, and two additional integrase-binding sites termed strong (DR1) and weak (DR2) binding sites which are located 24–37 and 41–55 bp to the left of the cross-over point, respectively [22–24, 33, 78]. The *attC* sites comprise a family of diverse sequences which are not highly conserved and vary considerably in size from 57 to 141 bp [15, 16, 50, 69]. The *attC* region consists of four essential sites called 1R, 2R, 1L and 2L, with 1R and 2R as part of the RH consensus sequence, and 1L and 2L as part of the LH consensus sequence [23, 24]. The similarities of the *attC* sites are primarily restricted to their boundaries, which correspond to the inverse core site (ICS) as RYYAAC and the core site (CS) as GTTRRRY [15, 85]. The *attC* sites are generally associated with a single ORF in a structure termed gene cassettes, which are not necessarily observed in integrons, but once integrated they become part of the integron [22]. These smallest known mobile genetic elements can exist in one of two forms, including the independent circular DNA molecule which is unable for stably maintain during cell division, and the linear form which is created by a highly orientation-specific insertion of the free circular element into the integron [43]. They contain a coding sequence, but are usually lack of promoters to constitute the mobile component of the system [31, 69, 72, 85, 87]. Mostly gene cassettes encode resistance against antibiotics cover a wide range of antibiotics, and up to date, more than 100 different antibiotic resistance gene cassettes have been characterized, with mostly unique *attC* sites. The position of a cassette in the integron, including both order and distance, is strictly related to the level of antibiotic resistance. Insertion of the gene cassette at the *attI* site, which is located downstream of a resident promoter internal to the *intI* gene, drives expression of the encoded proteins. In class 1 integron, gene cassettes are expressed from a common promoter located in the 5'-conserved segment (5'-CS) region, where two potential promoter sites Pc and P2 locate. Pc, also known as P_{ANT},

locates around 200 bp upstream of the integration site; and P2 is inactive for the replacement of the optimal 17 nucleotides between the -35 and -10 boxes to only 14 nucleotides [17]. Though not a part of site-specific recombination platform, Pc plays key role in the functioning of integron as it ensures the correct expression of gene cassette [43]. The 3'-conserved segment (3'-CS) of class 1 integrons possesses the genes *qacE Δ 1* and *sull1*, encoding resistance to quaternary ammonium salts and sulfonamide, respectively [70].

Several classes of integrons have been identified and distinguished by differences and divergence in the *intI* sequences, and integron classes 1–3 are so-called multi-resistant integron (RIs) which appear to be able to acquire same gene cassettes [30]. Class 4 integron is considered to be a distinct type of integron and termed super integron (SI), which was found on the small chromosome of *Vibrio cholerae* and known to be an integral component of many γ -proteobacterial genomes [3, 49, 74]. As approximately 9% of the sequenced bacterial genomes containing integrons, of these, class 1 integron platform is the most ubiquitous among clinical microbes and remains the focus of numerous studies [4, 43, 98]. As a direct result of the linkage of class 1 integrons with Tn402-like transposons, this integron had been reported to be associated with Tn3 transposon family (Tn21 or Tn1696) [43]. Class 1 integron has been reported in a large variety of clinical Gram-negative organisms, including *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Burkholderia*, *Campylobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Mycobacterium*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella* and *Vibrio*, as well as in a few Gram-positive bacteria [9, 12, 19, 21, 25, 27, 40, 42, 44, 46, 57, 59, 61, 62, 71, 89, 91, 94–96, 98–100]. Class 2 integron has an organization similar to that of class 1 but is associated with the Tn7 transposon family [31, 79]. The typical *intI2* gene, with the amino-acid sequences less than 50% homologous to the *IntI1* integrase, is not functional due to the replacement of the internal termination codon with a codon for glutamic acid (amino acid 179). *IntI2* may be a pseudogene, however, the reason for the stop codon still remains unclear. The two possible explanations for the truncated *intI2* may be the regulatory function, and the presence of another type of integrase, such as *intI1*. The latter hypothesis is supported by the frequently detection of class 1 integrons in isolates simultaneously with class 2 integrons and the small number of different gene cassettes observed in class 2 integrons comparing with class 1 integrons. This mutation has been attributed to the low diversity of integrated gene cassettes and most reported class 2 integron carry three specific gene cassettes, *dfrA1*, *sat1* and *aadA1*, which confer resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively [35, 43]. Thus, class 2 integron has been regarded as a contributor to the antibiotic resistance issue,

and commonly observed in some species of Gram-negative organisms such as *Acinetobacter*, *Enterobacteriaceae*, *Salmonella* and *Pseudomonas* [1, 51, 58, 67, 68, 92, 97]. *IntI2* is capable of site specific excision and integration of gene cassettes precisely into *attI2*. However, this integrase is unable to recognize gene cassettes from class 1 integrons, despite the identical gene cassettes found in both class 2 and class 1 integrons. Recombination site *attI2* and promoter Pc are found within transposons such as Tn7, and the 3'-CS contains five *tns* genes involved in the movements of the transposon, which mediates the mobility of class 2 integron via a preferential insertion into a unique site within bacterial chromosomes [35, 43]. Class 3 integron contains a comparable structure to that of class 2 integron, and up to date has only been described in *Pseudomonas*, *Alcaligenes*, *Serratia marcescens* and *Klebsiella pneumoniae* [2, 18, 58, 79]. Both *IntI1* and *IntI3* are part of the soil/freshwater Proteobacteria group, as class 2 integrases within the marine γ -Proteobacteria group. Functionally similar to *IntI1*, *IntI3* is able to recognize different *attC* sites and integrate the cassettes into the *attI3* site. Class 4 integron harbors hundreds of gene cassettes encoding adaptations that extend beyond antibiotic resistance and pathogenicity, and has been detected in isolates from the last century and indicates its existence pre-dating the antibiotic era [73]. The two key features that define class 4 integron and distinguish it from other RIs includes: (1) A large number of cassettes that are incorporated, which in the case of *V. cholerae*, the cluster of VCR-associated ORFs represents at least 216 unidentified genes in an array of 179 cassettes and occupies about 3% of the genome; (2) The high homology between the *attC* sites of those gathered cassettes [72]. Class 4 integron has been identified and characterized among the Vibrionaceae, *Shewanella*, *Xanthomonas*, *Pseudomonas*, as well as other proteobacteria [13, 36, 60, 72, 74]. The remaining classes of integrons may also contain antibiotic resistance gene cassettes, but their worldwide prevalence remains low [31, 60].

The integron platforms are defective for self-transposition, however, the transposons and conjugative plasmids associated can serve as vehicles for the intra- and inter-species transmission of genetic material [72]. This site-specific recombination reaction can be mediated by either the Tn21 integrase or the integron integrase *IntI1* when the integration sites conform to the consensus sequence GWTMW or GNT (Fig. 2), respectively [22]. *IntI1* recognises three types of recombination sites including *attI1*, *attC* and secondary sites, and via this site-specific recombination event, class 1 integron is capable of capturing gene cassettes. Recombination event between *attI1* site and *attC* is slightly more efficient than recombination between two *attC* sites, but those between two *attI1* sites is far less efficient. For secondary sites, recombination with *attC* is more efficient than *attI1*.

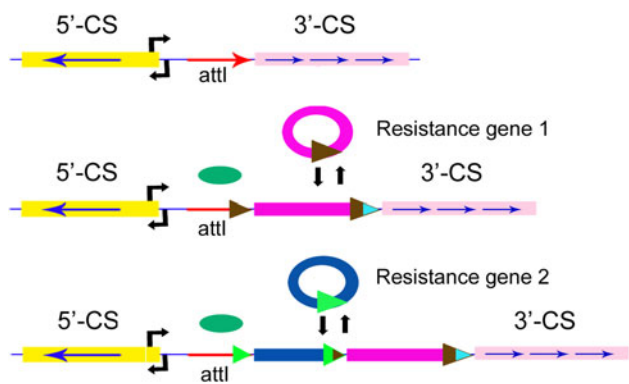


Fig. 2 Mechanism of class 1 integron-mediated excision and integration

Development of class 1 integron

Since class 1 integron has been identified as a primary source of antimicrobial resistance genes and suspected to serve as reservoirs and exchanging platforms of resistance genes within microbial populations, its role in spread and dissemination of antibiotic resistance genes in a variety of Gram-negative bacteria had been well investigated and documented, with a broad distribution of 22–59% among Gram-negative organisms [43, 45, 47, 75]. Nevertheless, little is known about the prevalence of class 1 integron in Gram-positive bacteria. In 1998, a typical class 1 integron associated streptomycin/spectinomycin resistance determinant has been detected on a 29-kb plasmid pCG4 from *Corynebacterium glutamicum*, which showed even higher expression ability comparing to integron found in *Escherichia coli*, representing the first identification of class 1 integron in Gram-positive bacterial [59]. In 2002, an *intI1*-like gene together with a novel aminoglycoside adenylyltransferase gene cassette *aadA9* had been observed on a 27.8-kb R-plasmid pTET3 from *C. glutamicum*, which encodes streptomycin, spectinomycin, and tetracycline resistance [89]. Nandi et al. screened class 1 integron in Gram-positive organisms isolated from poultry litter, and found class 1 integron in several species of Gram-positive bacteria as *Corynebacterium* sp. (including *C. ammoniagenes*, *C. casei* and *C. glutamicum*), *Aerococcus* sp., *Brevibacterium thiogenitalis* and *Staphylococcus* sp. (including *S. lentus*, *S. nepalensis* and *S. xylosum*) [57]. Enterococci bacterium are another genus with frequent detection of integrons, with the first observation of class 1 integron-related gene, *aadA*, was found in *E. faecalis* strain W4470 [12]. In our novel report, class 1 integron was detected in 11 *E. faecalis* and two *E. faecium* strains, with two *E. faecalis* also positive for class 2 integron [98]. This is the first time to report class 2 integron in *E. faecalis* and class 1 integron in *E. faecium*, which is the first evidence of class 2 integron outside the Gram-negative organisms. Nevertheless,

illustration and demonstration of the correlation between genetic integrons and clinical antibiotic resistance requires large scale number of clinical microbes and long-term track surveillance, which were limitations of the studies aforementioned. Based on series studies of systematic integrons investigation in hundreds of staphylococci strains during 2001–2006 [94–96, 99], this review concentrated on the latest development of class 1 integron in MRS isolates, including summary of prevalence and occurrence of class 1 integron, analysis of correlation between integron and antibiotic resistance, further demonstration of the role integrons play as antibiotic determinants, as well as origin and evolution of integron-associated gene cassettes during this study period.

Epidemiologic study of MRS isolates

During 2001–2006, a total of 262 MRS (209 MRSA and 53 MRCNS) strains isolated from various clinical samples were studied, including 20 MRSA and 13 MRCNS strains in 2001, 20 MRSA and 20 MRCNS strains in 2002, 15 MRSA and nine MRCNS strains in 2003, 34 MRSA and 11 MRCNS strains in 2004, 80 and 40 MRSA strains in 2005 and 2006, respectively. Two hundred and sixty-two MRS strains were isolated from First Affiliated Hospital of Jinan University (FAHJU), with 30 MRSA isolates sampled from Guangdong Provincial People's Hospital (GDPPH). Both of the medical settings are located in Guangzhou, China. During this period, the hospital staff conducted an active surveillance and data collection for staphylococci-colonized or -infected patients, with a tracking log used to identify epidemiologic relatedness between patients in order to select isolates for testing and thus document cluster. Each isolate was from an individual subject, and no repeat isolates were included, using the CDC definitions for nosocomial infections [7]. Only first patient isolates obtained from cultures performed more than 48 h after admission were included in the analysis. All MRS strains were identified to the species level using standard procedures: colony morphology, Gram staining, catalase test, the Vitek 2 automated system and the API-Staph commercial kit. Methicillin resistance was determined by susceptibility testing on oxacillin-screening agar, confirmed by latex agglutination for PBP2a and *mecA* detection by PCR as described previously [56, 96]. Detection of five exotoxin genes, encoding for staphylococcal enterotoxins SEA (*sea*), SEB (*seb*), SEC (*sec*), SED (*sed*), SEE (*see*); exfoliative toxin A, B (ETA; *eta*, ETB; *etb*); TSST-1 (*tst*); Panton-Valentine leukocidin (*lukS* and *lukF*) genes were performed as described previously [38, 52], and none of the tested strains were found to carry any of toxin genes. SCC*mec* types were assigned by PCR analysis of the

cassette chromosome recombinase (*ccr*) and *mec* gene complexes (*ccr-mec* genes), using the primers of Ito et al. and Hisata et al. to identify the *ccr* and *mec* genes, followed by a further multiplex PCR confirmation [37, 38, 65]. The distribution of SCC*mec* type in 262 MRS strains showed that the classic nosocomial SCC*mec* type (I, II and III) dominated among the tested strains. For MRSA strains, three and 198 strains belonged to SCC*mec* type II and III, with eight strains untypable; for MRCNS strains, nine, 24 and 12 strains were classified as SCC*mec* type I, II and III, respectively, with eight strains untypable. None of the tested strain carried type IV or V. Genotyping were performed by distinctly individual RAPD assays, in brief, RAPD typing for 179 MRSA from FAHJU and 30 MRSA from GDPPH were performed as described by Van Belkum et al. and Obayashi et al., respectively [64, 90]; and 23 I-MRCNS isolates of three *Staphylococcal* species carrying a highly prevalent array of *dfrA12-orfF-aadA2* gene cassettes were subjected to separate RAPD fingerprinting using the assay of Obayashi et al. as aforementioned [64]. For MRSA isolates, At least one strain representative of each RAPD type was selected randomly for further analyses by multilocus sequence typing (MLST), *spa* and *coa* typings [20, 82, 83]. MLST was performed by amplification of the internal region of seven housekeeping genes, including *arc*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*. The sequences of PCR products were compared with the existing sequences available in the MLST website (<http://www.mlst.net>) for *S. aureus*, and the allelic number was determined for each sequence. The sequence type (ST) was determined according to the pattern of the combination of the seven alleles, and the clonal complex (CC) was defined by the BURST (based upon related sequence types) program by accessing the MLST website. Typing of the polymorphic repeat region of protein A (*spaA* typing) and coagulase gene (*coa* typing) were performed by analyzing the *spaA* and *coa* repeats with the FINDPATTERNS program from Genetics Computer Group Wisconsin Package 9.1 and comparing the *spaA* repeat sequences as well as their distribution through <http://www.ridom.de/spaserver>. Thirty MRSA strains from GDPPH were classified into six distinct genotypes by RAPD-PCR, with 16 integron-positive and 14 integron-negative strains belonging to two and four genotypes, respectively. RAPD-PCR with primers AP1, AP7 and E2 classified 179 MRSA strains from FAHJU into eight, six and 12 distinct groups, respectively, with a total of 16 RAPD types detected. All MRSA strains fell into ST239-MRSA-III group (clonal complex, CC239), with the same *coa* type HIJKL. *SpaA* type of most MRSA isolates was WGKAOMQ (t037), with 12 I-MRSA and five non I-MRSA strains while belonged to WGKAQQ (t030). For 23 I-MRCNS isolates harbouring *dfrA12-orfF-aadA2* array, all tested strains were phylogenetically unrelated and

exhibited distinct RAPD patterns with low Dice coefficients.

Investigation of class 1 integron

Integron characterization was performed by PCR amplification for the integrase gene, variable region and 3'-conserved region. In brief, 262 MRS strains were screened by multiple PCR amplification for three classes of integrase genes. One hundred and twenty-two strains yielded a 565-bp PCR product, suggesting the existence of class 1 integrase (*intI1*), with none of class 2 and 3 integrase gene obtained (Table 1). *IntI1*-positive strains were further characterized for the variable region and 3'-conserved region. Most reported class 1 integron has classic 3'-CS including a $\Delta qacE$ and a *sull* gene and ORF5 [54, 66], and in this series of studies, one hundred and fifteen strains yielded an 800-bp amplicons in the PCR amplification of 3'-CS of *qacE Δ 1-sull*, with a rate of 94.3% (115/122). Variable region was determined by PCR with primers in-F and in-B, and PCR products were characterized by restriction fragment length polymorphism (RFLP) and at least three strains representative of each cassette type was selected randomly for further confirmation by sequencing. The PCR products of variable region were cut out from the agarose gel, purified by the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) and ligated with the pGEM-T easy vector (Promega, Madison, WI, USA). The ligation mixture was transformed into *E. coli* DH5 α strain and the recombinants were selected on Luria–Bertani agar containing ampicillin (100 μ g/ml). Recombinant plasmid DNA was purified by standard method and subjected for DNA sequencing for further analyses. The nucleotide sequences of gene cassette were determined by BigDye Terminator Cycle Sequencing FS Ready Reaction Kit on ABI PRISM 310 Genetic Analyzer (Perkin-Elmer Japan Applied Biosystems, Tokyo, Japan). Nucleotide sequence homology searches were performed against all sequences in the GenBank database by using the BLAST algorithm, which is available through the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>). A total of four different arrays of gene cassette arrays were found in tested staphylococci strains, with fragments varied in length between 975 and 2360-bp. A 975-bp amplicon was obtained from 53 strains, and the sequence demonstrated an *aadA2* gene cassette encoding resistance to aminoglycoside. Sixty-one strains gave a 1913-bp PCR product, the sequence of which demonstrated a *dfrA12-orfF-aadA2* array of gene cassettes. The *dfrA12* and *aadA2* conferred resistance to trimethoprim and aminoglycoside respectively, and the second ORF encodes an unknown function protein. A 1664-bp product was found from three

Table 1 Phenotypic and genotypic characteristics of I-MRS strains

Strain	Species	Date	Infection site	Dept	Antibiotic resistance	SCC _{mec}		Integron	
						<i>ccr</i>	<i>mec</i>	3'-CS	Gene cassettes
010808	<i>S. aureus</i>	2001	O	O	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
010912	<i>S. aureus</i>	2001	O	S	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011000	<i>S. aureus</i>	2001	P	D	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011001	<i>S. aureus</i>	2001	B	G	AcChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011016	<i>S. aureus</i>	2001	U	S	AcCiEGLOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011024	<i>S. aureus</i>	2001	S	I	AcChCiCIEGLOTc	3	A	-	<i>dfrA12-orfF-aadA2</i>
011025	<i>S. aureus</i>	2001	S	S	AcCiEGLOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011045	<i>S. aureus</i>	2001	S	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011052	<i>S. aureus</i>	2001	S	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011055	<i>S. aureus</i>	2001	P	O	AcChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011058	<i>S. aureus</i>	2001	P	N	AcChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011083	<i>S. aureus</i>	2001	P	N	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
011098	<i>S. aureus</i>	2001	S	I	AcCiEGLOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021138	<i>S. aureus</i>	2002	S	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021153	<i>S. aureus</i>	2002	O	S	AcCIOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021206	<i>S. aureus</i>	2002	S	I	AcCIOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021207	<i>S. aureus</i>	2002	S	I	AcCIOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021238	<i>S. aureus</i>	2002	S	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021261	<i>S. aureus</i>	2002	U	N	AcChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021266	<i>S. aureus</i>	2002	U	S	AcCiEGLOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021267	<i>S. aureus</i>	2002	S	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021268	<i>S. aureus</i>	2002	P	S	AcCiEGLOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
021296	<i>S. aureus</i>	2002	B	O	AcCiEGLOTs	3	A	-	<i>dfrA12-orfF-aadA2</i>
021542	<i>S. aureus</i>	2002	P	S	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
031788	<i>S. aureus</i>	2003	S	I	AcChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
032142	<i>S. aureus</i>	2003	S	I	ChCiCIELOTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
032267	<i>S. aureus</i>	2003	O	S	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
032371	<i>S. aureus</i>	2003	S	I	AcCIOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
032415	<i>S. aureus</i>	2003	S	S	AcChCiCIGLO	3	A	+	<i>aacA4-cmlA1</i>
032423	<i>S. aureus</i>	2003	S	I	AcChCIEGLOTc	3	A	+	<i>dfrA17-aadA5</i>
032439	<i>S. aureus</i>	2003	S	I	AcChCiCIEGLOTs	3	A	+	<i>aacA4-cmlA1</i>
042457	<i>S. aureus</i>	2004	S	I	AcChCiCIGLO	3	A	+	<i>aadA2</i>
042497	<i>S. aureus</i>	2004	U	I	AcChCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
042547	<i>S. aureus</i>	2004	S	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
042564	<i>S. aureus</i>	2004	S	I	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042637	<i>S. aureus</i>	2004	S	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
042649	<i>S. aureus</i>	2004	S	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
042772	<i>S. aureus</i>	2004	B	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
042848	<i>S. aureus</i>	2004	S	D	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042885	<i>S. aureus</i>	2004	O	I	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042887	<i>S. aureus</i>	2004	U	G	AcChCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042898	<i>S. aureus</i>	2004	P	OG	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042923	<i>S. aureus</i>	2004	O	I	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042954	<i>S. aureus</i>	2004	P	P	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
042966	<i>S. aureus</i>	2004	O	I	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
043000	<i>S. aureus</i>	2004	S	I	AcCiCIEGLOTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
032147	<i>S. aureus</i>	2005	O	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>

Table 1 continued

Strain	Species	Date	Infection site	Dept	Antibiotic resistance	SCC <i>mec</i>		Integron	
						<i>ccr</i>	<i>mec</i>	3'-CS	Gene cassettes
032148	<i>S. aureus</i>	2005	O	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050511	<i>S. aureus</i>	2005	O	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050512	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050513	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050518	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050557	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050558	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050559	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050560	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050561	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050562	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050581	<i>S. aureus</i>	2005	O	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050582	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050583	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
050585	<i>S. aureus</i>	2005	R	I	AcChCiCIEGOTcTs	3	A	+	<i>aadA2</i>
053001	<i>S. aureus</i>	2005	O	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053059	<i>S. aureus</i>	2005	R	I	AcCiCIEGLOTc	3	A	+	<i>aadA2</i>
053147	<i>S. aureus</i>	2005	B	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053182	<i>S. aureus</i>	2005	R	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053224	<i>S. aureus</i>	2005	R	I	ChCiCIGLTcTs	1	N	+	<i>aadA2</i>
053332	<i>S. aureus</i>	2005	B	I	AcChCiCIGLOTcTs	3	A	-	<i>aadA2</i>
053333	<i>S. aureus</i>	2005	O	I	AcChCiCIGLOTc	3	A	+	<i>aadA2</i>
053401	<i>S. aureus</i>	2005	O	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053423	<i>S. aureus</i>	2005	U	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
053443	<i>S. aureus</i>	2005	B	I	AcChCiCIGLOTc	3	A	+	<i>aadA2</i>
053474	<i>S. aureus</i>	2005	B	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053564	<i>S. aureus</i>	2005	O	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053610	<i>S. aureus</i>	2005	O	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053658	<i>S. aureus</i>	2005	R	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053685	<i>S. aureus</i>	2005	R	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
053845	<i>S. aureus</i>	2005	R	I	AcCiCIEGLOTc	3	A	+	<i>aadA2</i>
053899	<i>S. aureus</i>	2005	O	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
064043	<i>S. aureus</i>	2006	R	I	AcCiCIEGLOTcTs	3	N	+	<i>aadA2</i>
064050	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
064064	<i>S. aureus</i>	2006	R	I	AcCiCIEGLOTcTs	N	-	+	<i>aadA2</i>
064100	<i>S. aureus</i>	2006	R	I	AcCiCIEGLOTcTs	3	A	+	<i>aadA2</i>
064163	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTc	3	A	+	<i>aadA2</i>
064221	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
064249	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
064278	<i>S. aureus</i>	2006	O	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
064375	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
065212	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
065217	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTc	3	A	+	<i>aadA2</i>
065260	<i>S. aureus</i>	2006	R	I	AcChCiCIGLOTcTs	3	A	+	<i>aadA2</i>
012216	<i>S. epidermidis</i>	2001	R	I	AcChCiELTcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
012219	<i>S. epidermidis</i>	2001	R	I	AcCiCIGLTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
012228	<i>S. epidermidis</i>	2001	U	I	AcCiCILTcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>

Table 1 continued

Strain	Species	Date	Infection site	Dept	Antibiotic resistance	SCCmec		Integron	
						<i>ccr</i>	<i>mec</i>	3'-CS	Gene cassettes
022212	<i>S. epidermidis</i>	2002	B	S	AcChEGLTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
022218	<i>S. epidermidis</i>	2002	R	I	AcCiCIELTcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022225	<i>S. epidermidis</i>	2002	B	I	AcChCiTs	1	B	+	<i>dfrA12-orfF-aadA2</i>
022230	<i>S. epidermidis</i>	2002	R	I	AcCIEGLTc	2	A	–	<i>aadA2</i>
022237	<i>S. epidermidis</i>	2002	B	I	AcCIEGLTcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022244	<i>S. epidermidis</i>	2002	R	O	AcChCiCIELTcTs	3	A	+	<i>dfrA12-orfF-aadA2</i>
022256	<i>S. epidermidis</i>	2002	R	I	AcCIEGLTs	N	–	+	<i>dfrA12-orfF-aadA2</i>
022258	<i>S. epidermidis</i>	2002	U	S	AcChEGLTcTs	N	–	+	<i>dfrA12-orfF-aadA2</i>
032237	<i>S. epidermidis</i>	2003	R	I	AcChCiCIEGTc	2	A	–	<i>aacA4-cmlA1</i>
032211	<i>S. epidermidis</i>	2003	R	S	AcChEGLTcTs	N	–	+	<i>dfrA12-orfF-aadA2</i>
032224	<i>S. epidermidis</i>	2003	R	I	ChCiTs	1	B	+	<i>dfrA12-orfF-aadA2</i>
042219	<i>S. epidermidis</i>	2004	U	S	AcChCiCIEGL	2	A	–	<i>aacA4-cmlA1</i>
042237	<i>S. epidermidis</i>	2004	U	S	AcCiTcTs	3	A	+	<i>dfrA17-aadA5</i>
012303	<i>S. hominis</i>	2001	R	I	AcEGTcTs	1	B	–	<i>dfrA12-orfF-aadA2</i>
012305	<i>S. hominis</i>	2001	R	O	AcChELTs	1	B	+	<i>dfrA12-orfF-aadA2</i>
012306	<i>S. hominis</i>	2001	U	S	EGTcTs	1	B	+	<i>dfrA12-orfF-aadA2</i>
022303	<i>S. hominis</i>	2002	B	I	AcChCIEGLTcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
032309	<i>S. hominis</i>	2003	R	I	AcCIETcTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
032315	<i>S. hominis</i>	2003	R	S	AcChCiLTs	3	A	+	<i>dfrA17-aadA5</i>
042306	<i>S. hominis</i>	2004	U	I	AcChCiCIEGLTcTs	2	A	+	<i>aacA4-cmlA1</i>
042315	<i>S. hominis</i>	2004	U	S	AcCiETs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022405	<i>S. haemolyticus</i>	2002	R	I	AcCiCIETs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022407	<i>S. haemolyticus</i>	2002	B	S	AcChEGLTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022411	<i>S. haemolyticus</i>	2002	R	S	AcEGLTs	2	A	+	<i>dfrA12-orfF-aadA2</i>
022413	<i>S. haemolyticus</i>	2002	B	I	AcCiTcTs	N	–	+	<i>dfrA12-orfF-aadA2</i>
042403	<i>S. haemolyticus</i>	2004	U	I	AcCiTcTs	N	–	+	<i>dfrA12-orfF-aadA2</i>
012501	<i>S. warneri</i>	2001	B	I	AcCiCIEGLTs	2	A	+	<i>dfrA12-orfF-aadA2</i>

Resistance profile, antibiotics used in the current study included: amoxicillin/clavulanic acid (*Ac*), chloramphenicol (*Ch*), ciprofloxacin (*Ci*), clindamycin (*Cl*), erythromycin (*E*), gentamicin (*G*), levofloxacin (*L*), oxacillin (*O*), tetracycline (*Tc*), trimethoprim-sulfamethoxazole (*Ts*)

Infection site: *B* bloodstream, *R* respiratory tract, *S* skin and soft tissue, *U* urinary tract, *O* others

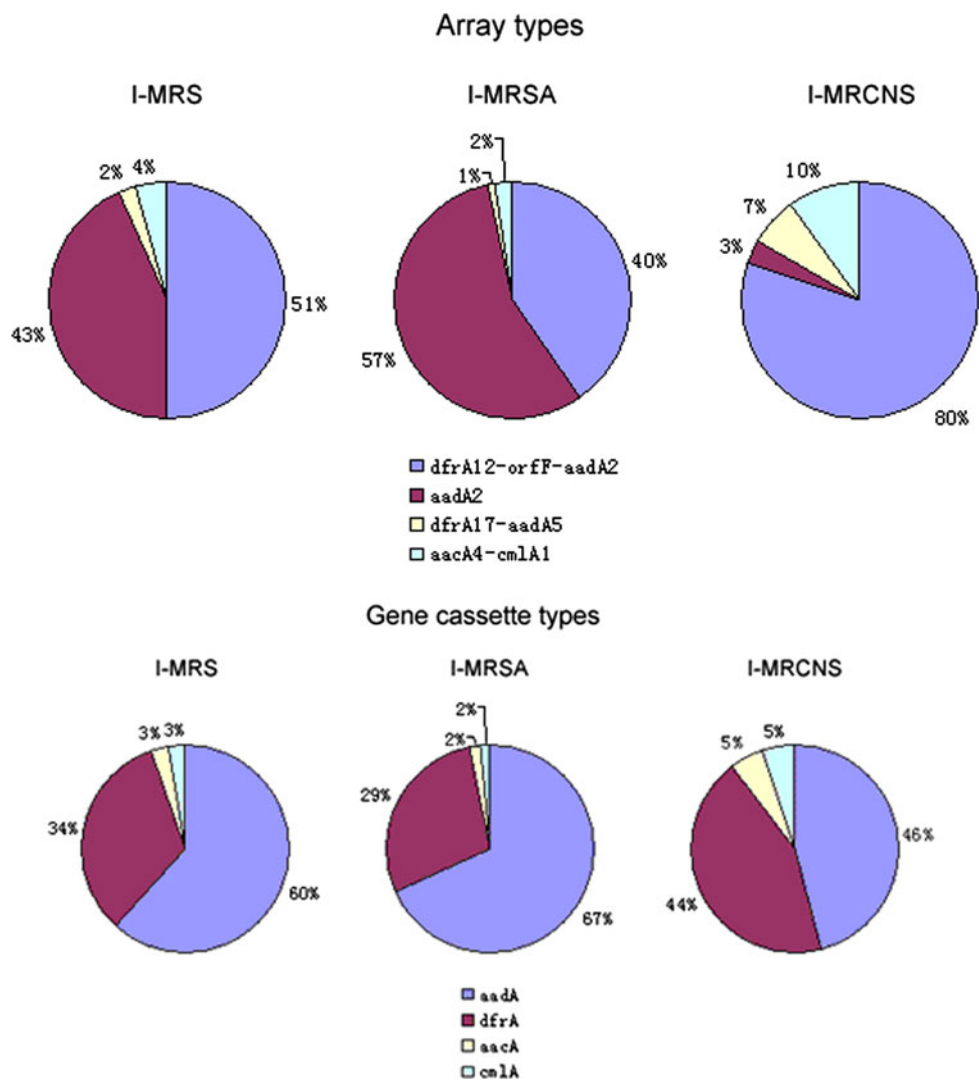
Dept: *D* dept. infectious disease, *G* general ward, *I* internal medicine, *N* neurology, *O* orthopedics, *OG* obstetrics and gynecology, *P* pediatrics, *S* surgery

+, carrying a 3'-CS of *qacEΔ1-sulI*; –, not carrying 3'-CS; *N* not typeable

strains and the sequence confirmed the presence of gene cassettes *dfrA17* and *aadA5*, which were resistant to trimethoprim and aminoglycoside, respectively. Five strains yielded an amplicon of 2360-bp, harboring *aacA4* and *cmlA1* genes which conferred resistant to aminoglycoside and chloramphenicol, respectively. To further determine the location of class 1 integron, *intI1* and gene cassettes of 28 MRSA and 30 MRCNS were investigated by Southern blot hybridization. According to the result of Southern hybridization, no signal had been observed on plasmid DNA, whereas the PCR-generated *intI* and cassette probes hybridized with genomic DNA, demonstrating the class 1 integrons were located on chromosomes, not plasmid.

Class 1 integron was commonly found in the tested staphylococci isolates (46.6%, 122/262), and the proportion had been decreasing during this study time span. During 2001–2004, the detection rate of class 1 integron for MRSA and MRCNS was 51.7% (46/89) and 56.6% (30/53), respectively. Nevertheless, only 38.3% (46/120) of MRSA isolates carried class 1 integron. Concerning cassette types, *dfrA12-orfF-aadA2* and *aadA2* remained prevalent, taking up 50.0% (61/122) and 43.4% (53/122) among all I-MRS strains (Fig. 3). For I-MRSA, major types as *dfrA12-orfF-aadA2* and *aadA2* consisted of 40.2% (37/92) and 56.5% (52/92), respectively. However, for I-MRCNS, *dfrA12-orfF-aadA2* dominated during the study

Fig. 3 Proportion of array types of gene cassettes in I-MRS strains



period, with only one *aadA2* case observed. The most frequently detected resistance genes in class 1 integron were *aadA* and *dfrA* family, with the rate 95.9% (117/122) and 52.5% (64/122), respectively, which was similar to previous studies [28, 97] (Figs. 3, 4). It was noticed that most of the known gene cassettes encoded resistance to the oldest groups of antibiotics which have been used for more than 20 years, in spite of an increasing number of new gene cassettes defining resistance against newer groups of antibiotics. It should also be noted that a large proportion of gene cassettes encoding resistance against streptomycin and spectinomycin, despite the fact that use of these antibiotics (at least in a clinical setting) has long ago been discontinued. Nevertheless, since it has been hypothesized that the presence of an integron may lead to a more extensive exchange of resistance determinants than gene cassettes alone, the consistent detection of class 1 integron in MRS isolates strongly suggest class 1 integron may serve as reservoirs of antimicrobial resistance and contribute to increased rates of treatment-resistant

staphylococci infections in both the hospital and community setting.

Antibiotic resistance determinant

In order to investigate the role of class 1 integron played as antibiotic resistance determinant in MRS strains, antimicrobial susceptibility testing was performed by standard disk diffusion method, and minimum inhibitory concentration (MIC) were determined for amoxicillin/clavulanic acid, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, oxacillin, teicoplanin, tetracycline, trimethoprim-sulfamethoxazole and vancomycin according to Clinical Laboratory and Standards Institute (CLSI) methods [14]. Furthermore, associations between integron-bearing and non integron-bearing MRS were analyzed by the χ^2 test or analysis of variance. A *P* value of <0.05 was considered statistically significant. All analyses were performed with SPSS 12.0G for

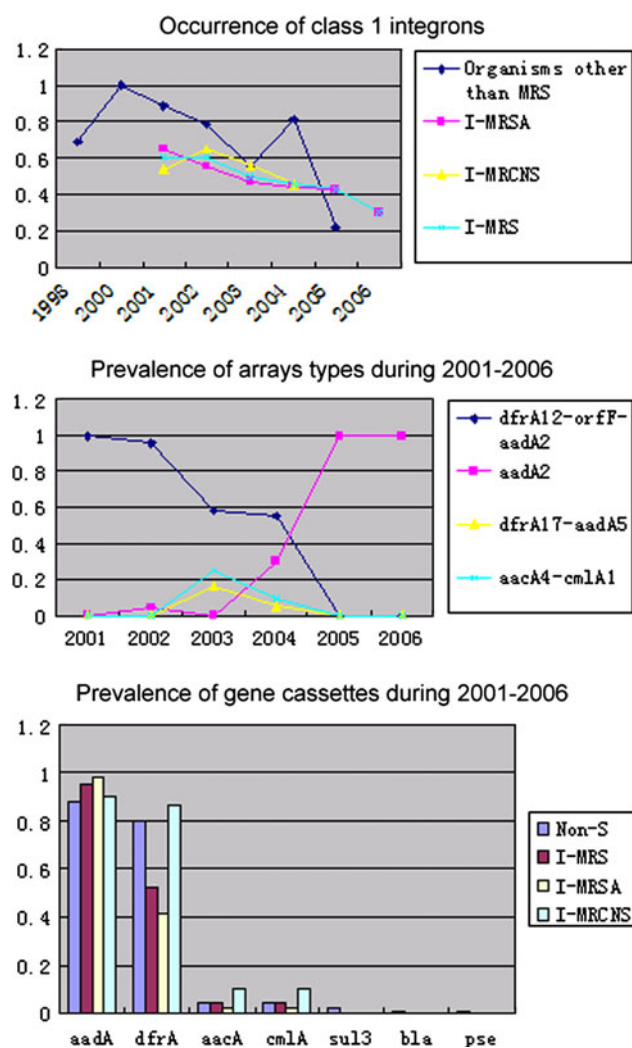


Fig. 4 Occurrence and prevalence of integrons and array types during 2001–2006

Windows. Antimicrobial susceptibility testing showed the multidrug resistance (defined as resistance to six or more antibiotics) rates of integron-positive and -negative strains were 85.2% (104/122) and 79.3% (111/140), with the highest number of amoxicillin/clavulanic acid resistance (93.5%, $n = 245$), followed by ciprofloxacin (84.0%, $n = 220$) and clindamycin (81.0%, $n = 214$). The percentage of resistance to chloramphenicol, erythromycin, gentamicin, levofloxacin, oxacillin, tetracycline and trimethoprim-sulfamethoxazole ranged from 51.5 to 76.7% (Table 2). None of the tested isolates showed resistance to vancomycin and teicoplanin. The MIC of oxacillin ranged from 16 to 512 $\mu\text{g/ml}$. The antibiotic resistance data between I-MRS and non I-MRS isolates was compared and the χ^2 test was used to calculate the P value in terms of resistant and susceptible numbers. Class 1 integron was significantly associated with resistance to certain antibiotics in both types of strains, including erythromycin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole. Regular treatment of MRS infection is usually with penicillinase-resistant β -lactams, however, since antibiotic resistance becomes common, vancomycin or other newer antibiotics may be required. Some strains had been reported partially or totally resistant to all but the newest antibiotics, including linezolid, quinupristin/dalfopristin, daptomycin, telavancin, dalbavancin, and tigecycline. In the current investigation, erythromycin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole had been found correlated strongly with the presence of class 1 integron. Therapeutic options for deep-seated infection due to MRS strains are limited. In treating complicated MRS infections, gentamicin has been often used by many clinicians as combination with vancomycin in the

Table 2 Correlation of antibiotic resistance between I-MRS and non I-MRS strains

Antibiotics	All MRS strains		I-MRS strains		Non I-MRS strains		P value	OR (95% CI)
	R	S	R	S	R	S		
Amoxicillin/clavulanic acid	245 (93.5%)	17 (6.5%)	117 (44.7%)	5 (1.9%)	128 (48.9%)	12 (4.6%)	0.053	0.107–1.061
Chloramphenicol	135 (51.5%)	127 (48.5%)	67 (25.6%)	55 (20.9%)	68 (26.0%)	72 (27.6%)	0.249	0.461–1.222
Ciprofloxacin	220 (84.0%)	42 (16.0%)	102 (38.9%)	20 (7.7%)	118 (45.0%)	22 (8.4%)	0.893	0.493–1.854
Clindamycin	214 (81.7%)	48 (18.3%)	97 (37.0%)	25 (9.6%)	117 (44.7%)	23 (8.8%)	0.557	0.654–2.257
Erythromycin	118 (64.1%)	94 (35.9%)	88 (33.6%)	34 (12.9%)	85 (30.5%)	60 (23.0%)	0.007	0.292–0.828
Gentamicin	170 (64.9%)	92 (35.1%)	89 (34.0%)	33 (12.5%)	81 (30.9%)	59 (22.6%)	0.006	0.287–0.820
Levofloxacin	194 (74.0%)	68 (26.0%)	89 (34.0%)	33 (12.5%)	105 (40.1%)	35 (13.4%)	0.866	0.603–1.824
Oxacillin	201 (76.7%)	61 (23.3%)	90 (34.4%)	32 (12.1%)	111 (42.4%)	29 (11.2%)	0.464	0.718–2.262
Tetracycline	171 (65.3%)	91 (34.7%)	90 (34.4%)	32 (12.1%)	81 (30.9%)	59 (22.6%)	0.004	0.274–0.788
Trimethoprim-sulfamethoxazole	164 (62.6%)	98 (37.4%)	107 (40.8%)	15 (5.6%)	57 (21.8%)	83 (31.8%)	0.000	0.046–0.170

expectation of a more rapid bacteriologic response based on a synergistic interaction between the two antibiotics [53]. Erythromycin has been commonly used for *penicillin-allergic* patients. Trimethoprim/sulfamethoxazole is often the first choice to cure community-acquired cutaneous infections likely to be due to MRSA strains, and its combination use with rifampin has been useful in treating MRSA in carriers, though the organism recurs in up to 50% and frequently becomes resistant.

Mobility and evolution

As a mobile genetic element, the mobility of integrons is defined as being associated with mobile DNA elements (transposons or plasmids) and antibiotic-resistance genes in addition to having a small array size and substantial heterogeneity in the sequence of *attC* sites [6, 48]. It has been generally accepted most existent class 1 integrons were located on plasmids as facilitation of conjugative-mediated transfer, which was also supported by the frequently detected plasmid-integrons in non-staphylococci in this study. In previous studies, Southern hybridization analysis was used to determine the location and the copy numbers of integron in 58 staphylococci isolates (data of 28 MRSA unpublished) [95]. Strikingly, all strains were detected to carry one copy of class 1 integron on chromosomes, not plasmid. Integron is not self-movable, but it contains gene cassettes that can be mobilized to other integrons or to secondary sites in the bacterial genome. As a natural capture system and assembly platform, the class 1 integron system allows bacteria to incorporate gene cassettes and convert them to functional genes by ensuring their correct expression, which has been regarded as key player in the dissemination and spread of resistance genes, responsible for the facile spread of resistance genes and the rapid evolution of resistance to a wide range of unrelated antibiotics among diverse bacteria [48, 58]. It is conceivable that any ORF can be structured as a gene cassette and vital to decipher the mechanism governing cassette genesis. Through the recombination platform (*IntI1* and *attI*), integron has the potentially limitless capacity to exchange and stockpile functional gene cassettes which permits rapid adaptation to selective pressure and may ultimately endow increased fitness and advantage to the host. Altogether, hundreds of gene cassettes, all types of other mobile DNA elements such as conjugative plasmids, transposons, insertion sequences, even entire chromosome, would probably be the vast reservoirs of integron, lending support to the longstanding concept of a single massive genetic pool that is available and shared among bacteria [72]. The common observation of integrons in microorganisms from

general environment and its enormous sequence diversity detected from such microbes, as well as various products unrelated to antibiotic resistance encoded by integron-associated cassette genes, strongly suggests that integrons are ancient genetic element in structure of genomes and have played a general role in evolution and adaptation for a considerable period of time [43]. For the staphylococci strains, on the other side, *SCCmec* is a genomic island (G island) inserted at the 3' end of *orfX* and located near the replication origin, which is defined as a basic mobile genetic element demarcated by a pair of direct repeats and inverted repeats, having a set of site-specific recombinase genes (*ccrA* and *ccrB*) required for its movement and carrying the *mecA* gene complex [41]. *SCCmec* may have evolved from a primordial mobile element, SCC, into which the *mec* complex was inserted, with the staphylococci chromosome. But there is no reason to limit the putative SCC to being only the conveyer of methicillin resistance (mediated by *mecA* gene) alone, while it might be serving as a vehicle for exchange of useful genes for the better survival for staphylococci in various environments, which means that *SCCmec* is a general genetic information exchange system of staphylococci with *ccrA* and *ccrB* involved in the recombination events (integration and excision) [38]. For both Integron and *SCCmec* serve as the reservoir of all kinds of genes and possess the function to exchange genes between species, so whether the simultaneous existence of Integron system and *SCCmec* would speed up the gene exchange and genome evolution in staphylococci require further investigation. As a novel antibiotic resistance determinant, general concerns for integrons in staphylococci may raise necessity for surveillance and investigation of its occurrence, mechanism and evolution.

Origins and dissemination

In a long-term integron investigation of MRSA, 16 genotypes were identified from 179 MRSA strains, and I-MRSA and non I-MRSA representative of 14 specific clones, with two genotypes shared. It was noteworthy that some I-MRSA strains containing the same organization of gene cassettes were found in various RAPD-PCR genotypes (*aadA2* and *dfrA12-orfF-aadA2* shared three and five genotypes, respectively), suggesting a horizontal transfer of integrons, which had been suspected in other studies [75, 80, 88]. In contrast, certain strains grouped in the same genotype were found to carry different arrays of gene cassette, indicating the acquisition and exchange of different cassettes by site-specific recombination of *IntI1*. In a preliminary investigation on an outbreak of 30 MRSA isolates,

six genotypes were obtained, with I-MRSA representative of two specific clones. Despite the 3-month outbreak, large proportion of I-MRSA strains were isolated during a short time span of 12 days (May 19–30), with non I-MRSA strains prevalent for more than 2 months. Remarkably, strains with the same genotype were obtained from clinical specimens on consecutive days or patients in adjacent beds, as well as both from environment and patients, strongly suggesting pathogen contamination and transmission may occur by direct or indirect contact between patients, medical personnel and environment. Many potential reservoirs of *S. aureus* in the hospital environment, including medical equipment such as parenteral solution and rinse solution and ward stuff such as doorknob and console are probably important risk factors and serve as vehicle for pathogen spreading. Strikingly, high diversity in genetic background was observed in the I-MRCNS investigation. Twenty-three CoNS strains with array of *dfrA12-orfF-aadA2* exhibited distinct RAPD patterns and were divided into different genetic groups, demonstrating that they were phylogenetically unrelated. The results strongly suggested that the wide spread and distribution of class 1 integron in diverse clones of CoNS strains may be mainly due to the horizontal transfer of integrons, not by specific clone. Remarkably, the arrays of gene cassettes detected in staphylococci, had been previously reported in clinical isolates of various negative bacteria [59, 89, 101, 102], with identical sequences. For example, cassettes *dfrA12-orfF-aadA2* within class 1 integron was also present in the Gram-negative enteroinvasive *E. coli* O164 strain RIMD05091045 isolated from a

patient in Japan [1]. When the cassette arrays *dfrA12-orfF-aadA2*, *aadA2*, *dfrA17-aadA5* and *aacA4-cmlA1* were compared to those deposited in GenBank, homology of nucleotides ranged from 95 to 100%, including array *dfrA12-orfF-aadA2* in *E. coli* (AB154407, DQ157751), *Salmonella enterica* (DQ238105), *K. pneumoniae* (AF180731), *Acinetobacter baumannii* (DQ141318) and *P. aeruginosa* (AB191047), array *dfrA17-aadA5* in *E. coli* (AB189264, AB194702, DQ663488, DQ322597, AY828551, AY748452) and *K. pneumoniae* (AF220757, AY994155), array *aacA4-cmlA1* in *E. coli* (AB212941). The integron sequences identified in staphylococci were at least 99.5% homologous to those from isolates sampled in the same hospital setting (Table 3). It is noteworthy that in staphylococci strains, prevalent array cassettes such as *dfrA12-orfF-aadA2* and *aadA2* also dominated in other species. The observance of identical integrons in genomes of phylogenetically distant bacteria and the similarity of prevalent arrays in diverse clinical organisms strongly suggest intergeneric horizontal transfer of genetic cassettes in the hospital setting. Several classes of integrons had been reported capable of spreading among Gram-negative bacteria, with one example being the transfer of class 1 integron via plasmid from *E. faecalis* strain W4770 to a recipient strain of *E. faecalis* [12]. However, issues concerning horizontal transfer and dissemination of integrons between Gram-positive and Gram-negative organisms, as well as the origins of class 1 integron in Gram-positive bacteria, still remained unclear and required further investigation.

Table 3 Comparison of integron sequences between MRS and other species isolated from FAHJU

<i>S. aureus</i>	Other organisms	Homology	GenBank no.	Reference
Class 1 integron				
<i>dfrA12-orfF-aadA2</i>	<i>S. epidermidis</i>	100% homology	AB297447	95
	<i>S. hominis</i>	100% homology	AB297448	95
	<i>S. haemolyticus</i>	99.9% homology with 860 T–C	AB297449	95
	<i>S. warneri</i>	100% homology	AB297450	95
	<i>E. faecalis</i>	99.8% homology with 398 A–G, 458 A–G, 984 T–G and 1603 G–A	Not deposited	98
	<i>E. faecium</i>	99.8% homology with 398 A–G, 458 A–G, 984 T–G and 1603 G–A	Not deposited	98
<i>dfrA17-aadA5</i>	<i>S. epidermidis</i>	99.9% homology with 1289 T–C	AB291061	95
	<i>S. hominis</i>	100% homology	AB291062	95
	<i>E. faecalis</i>	99.9% homology with 1289 T–C	Not deposited	98
	<i>E. coli</i>	99.9% homology with 1289 T–C	AB189264	86
<i>aadA2</i>	<i>S. epidermidis</i>	100% homology	AB481131	95
	<i>E. faecalis</i>	99.5% homology with 217 C–T, 322 C–A, 586 G–A, 717 G–T and 857 A–G	Not deposited	98
<i>dfrA17-aadA5</i>	<i>S. epidermidis</i>	99.9% homology with 1291 T–C	AB291061	95
	<i>S. hominis</i>	99.9% homology with 1291 T–C	AB291062	95

Development of I-MRS

Contemporary integron investigation had been conducted on a large scale of clinical organisms isolated from FAHJU, involving 254 Gram-negative and 163 Gram-positive strains, with a total detection rate of 73.4% (248/338) (Table 4). Class 1 integrons were found in 72.8% (185/254) of the Gram-negative strains, of which 91.3% (21/23) were in *Acinetobacter* spp., 86.7% (13/15) in *Enterobacter cloacae*, 81.2% (56/69) in *E. coli* [86], 87.5% (28/32) in *K. pneumoniae*, 47.4% (49/95) in *P. aeruginosa* [97] and 90% (18/20) in other organisms. For Gram-positive isolates, class 1 integrons were detected in 86.7% (13/15) of enterococci and 83.3% (5/6) of streptococci strains [86], and for MRS the detection rate was 46.6% (122/262), with 42.5% (76/179) for I-MRSA and 56.6% (30/53) for MRCNS. Remarkably, a significant decrease in class 1 integron positive rate was found during this studying time period, with 68.3% (28/41) in 1998, 100% (14/14) in 2000, 88.9% (40/45) in 2001, 78.4% (29/37) in 2002, 55.3% (26/47) in 2003, 80.9% (106/131) in 2004 and 21.7% (5/23) in 2005. This tendency had also been observed in I-MRS, with 60.6% (20/33) in 2001, 60.0% (24/40) in 2002, 50.0% (12/24) in 2003, 45.5% (20/44) in 2004, 42.5% (34/80) in 2005 and 30.0% (12/40) in 2006. It was noteworthy that class 2 integron was commonly found in 9.8% (33/338) of tested strains, including *E. coli*, *P. aeruginosa* and *E. faecalis*, which represented the first evidence of class 2 integron in Gram-positive bacteria and indicated the potential risk of spread of class 2 integron from Gram-negative to -positive bacteria, as staphylococci, though none of class 2 integron were found in staphylococci strains. Since integron is best known for its role in contributing to clinical antibiotic resistance and a large variety of different gene cassettes containing genes that confer resistance to antibiotics had been found, gene cassettes-associated multiple insertion events can lead to the accumulation of many cassettes within an integron, thus contributing to multidrug resistance [43]. Multiple copies of class 1 integron had been observed in 9.8% (33/338) isolates and 22 of them showed different arrays of gene cassettes, however, this had not been observed in staphylococci isolates as aforementioned. The prevalent gene cassette arrays were *dfrA12-orfF-aadA2* and *aadA2*, taking up 50.0% (61/122) and 42.6% (52/122) of the integron-positive strains. Nevertheless, a significant tendency of changing of gene cassettes was observed, cassette array *dfrA12-orfF-aadA2* dominated during 2001–2004, with 100% in 2001 (20/20) and 2002 (23/23), 58.3% (7/12) in 2003, 55.0% (11/20) in 2004, and disappeared afterwards. Nevertheless, gene cassette *aadA2* appeared in 2004 (6/20, 30.0%) and became prevalent during 2005–2006 (46/46, 100%),

however, it was noticed that one MRCNS strain isolated in 2002 also carried *aadA2*. Gene cassettes carried by isolates from 2003 and 2004 revealed the most diversity, with four different arrays of gene cassettes. A striking good concordance was observed between staphylococci and non-staphylococci isolates. As far as those cassette types identified in staphylococci were concerned, *dfrA12-orfF-aadA2* first emerged in 2000 (42.9%, 6/14) and had been frequently obtained in 2001 (80.0%, 32/40) and 2002 (69.0%, 20/29), however, its proportion decreased in 2003 (65.4%, 17/26) and 2004 (57.5%, 61/106), with none of this cassette observed afterwards. Another prevalent cassette *aadA2* was first acquired in 2002 (6.9%, 2/29), and had been slightly increased in 2003 (19.2%, 5/26) and 2004 (14.2%, 15/106). Cassette array *dfrA17-aadA5* dominated in 1998 (46.4%, 13/28) and 2000 (64.3%, 9/14), but the detection rate decreased from then on, with 15.0% (6/40) in 2001, 6.9% (2/29) in 2002, 11.5% (3/26) in 2003 and 21.7% (23/104) in 2004. Detection rate of another infrequently detected array *aacA4-cmlA1* ranged from 1 to 21% during 2001–2004. For non-staphylococci isolates, a total of six cassette genes had been found, including *aadA* (217/248), *dfrA* (200/248), *aacA* (11/248), *cmlA* (10/248), *sul3* (5/248), *bla* (2/248) and *pse* (2/248). Family *aadA* and *dfrA* remained the most frequently detected, which was similar in staphylococci strains, with 95.9% (117/122) and 52.5% (64/122), respectively. In the recent decade, as regulations on the appropriate use of antibiotics have been effectively enforced in developed countries, community-associated methicillin-resistant staphylococci (CA-MRS) emerged and infections caused by CA-MRS have been observed with increasing frequency in a variety of countries and geographic regions [8, 10, 26, 93, 102]. CA-MRS, characterized by several distinctive properties such as: more susceptible antimicrobial phenotype, the presence of different exotoxin gene profiles and a much smaller SCCmec (type IV or V), is becoming more and more prevalent and has the tendency to replace traditional hospital-associated methicillin-resistant staphylococci (HA-MRS) worldwide [55, 76]. While in South China, according to our studies, classic nosocomia SCCmec still dominated, with no strain of type IV or V SCCmec acquired. As generally accepted, indiscriminate use of existing antibiotics resulted in antibiotic selective pressure and proliferation of antibiotic resistance, which was the rudimentary and intrinsic cause of the emergence and development of mobile genetic resistance mechanism as integron and gene cassettes, and was reflected by the domination of nosocomial SCCmec and prevalence of integron in the series studies. In South China, as trained practitioners were unavailable in many areas, regulations on the clinical use of antibiotics had been poorly enforced or absent and the surveillance

Table 4 Contemporary integron investigation in FAHJU during 2001–2004

Strains	Class 1 integron-positive rate	Year	Prevalent gene cassettes	No.
<i>Acinetobacter</i> spp.	91.3% (21/23)	2001	<i>dfrA12-orfF-aadA2</i>	4
			<i>dfrA17-aadA5</i>	1
	1/2 11/12	2002	<i>dfrA12-orfF-aadA2</i>	2
		2003	<i>dfrA12-orfF-aadA2</i>	1
		2004	<i>dfrA12-orfF-aadA2</i>	11
			<i>aacA4-catB3-dfrA1-noncoding</i>	1
		<i>dfrA12-orfF-aadA2; aadA2</i>	1	
<i>Alcaligenes</i> spp.	100% (2/2)	2001	<i>dfrA12-orfF-aadA2</i>	1
		2004	<i>dfrA12-orfF-aadA2</i>	1
<i>Burkholderia pseudomallei</i>	75.0% (3/4) 2/3	2002	<i>dfrA12-orfF-aadA2</i>	1
		2004	<i>dfrA12-orfF-aadA2</i>	2
<i>Citrobacter freundii</i>	100% (2/2)	2004	<i>dfrA12-orfF-aadA2</i> <i>dfrA17-aadA5</i>	1 1
<i>Enterobacter cloacae</i>	86.7% (13/15)	2001	<i>dfrA12-orfF-aadA2</i>	3
		2002	<i>aadA2</i>	1
	1/3	2003	<i>dfrA17-aadA5</i>	1
		2004	<i>aadA2</i>	1
			<i>dfrA12-orfF-aadA2; dfrA17-aadA5</i>	4
		Others	3	
<i>Enterococcus faecalis</i>	83.3% (10/12)	2001	<i>dfrA12-orfF-aadA2</i>	3
		2002	<i>dfrA12-orfF-aadA2</i>	2
	1/3	2003	<i>dfrA12-orfF-aadA2</i>	1
		2004	<i>dfrA12-orfF-aadA2</i>	2
			<i>dfrA17-aadA5</i>	1
		<i>dfrA12-orfF-aadA2; aadA2</i>	1	
<i>Enterococcus faecium</i>	100% (1/1)	2004	<i>dfrA12-orfF-aadA2</i>	1
<i>E. coli</i>	88.3% (98/111)	1998	<i>aadA1-dfrA12</i>	10
			<i>dfrA17-aadA5</i>	13
			Others	5
	2000	<i>dfrA17-aadA5</i>	7	
		<i>dfrA12-orfF-aadA2</i>	4	
		<i>dfrA12-orfF-aadA2; dfrA17-aadA5</i>	2	
		Others		
		<i>dfrA12-orfF-aadA2</i>	7	
		<i>dfrA17-aadA5</i>	3	
		<i>dfrA12-orfF-aadA2</i>	5	
	2002	<i>dfrA17-aadA5</i>	2	
		<i>dfrA12-orfF-aadA2</i>	3	
	2003	<i>dfrA17-aadA5</i>	1	
<i>aacA4-cmlA1</i>		1		
2004	<i>dfrA12-orfF-aadA2</i>	17		
	<i>dfrA17-aadA5</i>	11		
	<i>dfrA12-orfF-aadA2; dfrA17-aadA5</i>	3		
	<i>aacA4-cmlA1</i>	1		
	Others	2		
<i>Flavobacterium</i>	100% (1/1)	2004	<i>dfrA12-orfF-aadA2</i>	1
<i>Hemophilus influenzae</i>	100% (1/1)	2003	<i>dfrA12-orfF-aadA2</i>	1

Table 4 continued

Strains	Class 1 integron-positive rate	Year	Prevalent gene cassettes	No.	
<i>Klebsiella pneumoniae</i>	87.5% (28/32)	2001	<i>dfrA12-orfF-aadA2</i>	3	
		2002	<i>dfrA12-orfF-aadA2</i>	2	
		2003	<i>dfrA12-orfF-aadA2</i>	2	
	3/6	2003	<i>dfrA17-aadA5</i>	1	
			2004	<i>dfrA12-orfF-aadA2</i>	6
		20/21		<i>dfrA17-aadA5</i>	1
				<i>drfA1-orfX</i>	3
				<i>aadA2</i>	2
				<i>dfrA17-aadA5, aadA2</i>	1
				Others	7
<i>Pseudomonas aeruginosa</i>	45.8% (54/118)	2001	<i>dfrA12-orfF-aadA2</i>	6	
			<i>aacA4-cmlA1</i>	2	
			<i>dfrA17-aadA5</i>	2	
		2002	<i>dfrA12-orfF-aadA2</i>	6	
			<i>aacA4-cmlA1</i>	6	
		2003	<i>dfrA12-orfF-aadA2</i>	8	
			<i>aadA2</i>	5	
		2004	<i>aadA2</i>	7	
			<i>dfrA12-orfF-aadA2</i>	3	
			<i>dfrA12-orfF-aadA2; aadA2</i>	2	
		2005	Others	2	
			<i>Sul3</i>	3	
			<i>bla_{VIM4}-pse1</i>	2	
<i>Proteus</i> spp.	80.0% (4/5)	2001	<i>dfrA12-orfF-aadA2</i>	2	
		2004	<i>dfrA17-aadA5</i>	2	
<i>Salmonella typhi</i>	100% (2/2)	2001	<i>dfrA12-orfF-aadA2</i>	1	
		2004	<i>dfrA12-orfF-aadA2</i>	1	
<i>Streptococcus</i> spp.	83.3% (5/6)	2001	<i>dfrA12-orfF-aadA2</i>	2	
			<i>dfrA12-orfF-aadA2</i>	1	
		2002	<i>dfrA12-orfF-aadA2; aadA2</i>	1	
			<i>dfrA12-orfF-aadA2</i>	1	
<i>Xanthomonas maltophilia</i>	100% (3/3)	2003	<i>dfrA12-orfF-aadA2</i>	1	
		2004	<i>dfrA12-orfF-aadA2</i>	2	
			<i>dfrA17-aadA5</i>	1	

Data of *E. faecalis*, *E. faecium*, *E. coli* and *P. aeruginosa* were from previous studies [81, 86, 97, 98]

system was not as effective, there may be more opportunity for the inappropriate use of antibiotics, resulting in heavier antibiotic selective pressure. Since the present existence and distribution of integron is due to multiple losses and gene transfer events and the ability of excision and integration may be selectively advantageous with the selective impact of integron on genomes [58] it is reasonable to presume in this region, MRS strains developed into a different direction, which inclined to carry genetic elements that would endow more fitness and advantage, resulting in their rapid adoption to selective pressure and survival.

Concluding remarks

As a common genetic element existed in 9% of bacteria and representatives from a broad range of phyla and environments, integron plays core role in antibiotic resistance among clinical organisms and contributes to the evolution and adaptation of bacteria. However, integrons in Gram-positive bacteria has barely been touched upon, which may be an unnoticed and neglected antibiotic resistance determinant. Many question have been arisen and posed regarding the number and types of cassettes, rate of integration and excision, diversity of cassette genes pool

and its distinct access, the functional role and genetic regulation of cassettes, as well as the extent to which it facilitates adaptation and evolution to environmental selection pressure. The review offers important information for the epidemiology of class 1 integron in staphylococci strains, which will aid in the investigation of integrons in Gram-positive organisms.

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References

- Ahmed AM, Nakano H, Shimamoto T (2005) Molecular characterization of integrons in non-typhoid *Salmonella serovars* isolated in Japan: description of an unusual class 2 integron. *J Antimicrob Chemother* 55:371–374
- Arakawa Y, Murakami M, Suzuki K, Ito H, Wacharotayankun R, Ohsuka S, Kato N, Ohta M (1995) A novel integron-like element carrying the metallo- β -lactamase gene *bla*IMP. *Antimicrob Agents Chemother* 39:1612–1615
- Barker A, Clark CA, Manning PA (1004) Identification of VCR, a repeated sequence associated with a locus encoding a hemagglutinin in *Vibrio cholerae* O1. *J Bacteriol* 176:5450–5458
- Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS (2004) Isolation and characterization of integron-containing bacteria without antibiotic selection. *Antimicrob Agents Chemother* 48:838–842
- Ben-Ami R, Navon-Venezia S, Chwartz D, Carmeli Y (2003) Infection of a ventriculoatrial shunt with phenotypically variable *Staphylococcus epidermidis* masquerading as polymicrobial bacteremia due to various coagulase-negative staphylococci and *Kocuria varians*. *J Clin Microbiol* 41:2444–2447
- Boucher Y, Labbate M, Koenig JE, Stokes HW (2007) Integrons: mobilizable platforms that promote genetic diversity in bacteria. *Trends Microbiol* 15:301–309
- Centers for Disease Control and Prevention (1972) Outline for surveillance and control of nosocomial infections, revised. U.S. Department of Health, Education and Welfare, Public Health Service
- Centers for Disease Control and Prevention (2003) Methicillin-resistant *Staphylococcus aureus* infections among competitive sports participants—Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000–2003. *MMWR Morb Mortal Wkly Rep* 52:793–795
- Centron D, Roy PH (2002) Presence of a group II intron in a multiresistant *Serratia marcescens* strain that harbors three integrons and a novel gene fusion. *Antimicrob Agents Chemother* 46:1402–1409
- Chambers HF (2001) The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* 7:178–182
- Chang S, Sun C, Yang L, Luh K, Hsieh W (1997) Increasing nosocomial infections of methicillin-resistant *Staphylococcus aureus* at a teaching hospital in Taiwan. *Int J Antimicrob Agents* 8:109–114
- Clark NC, Olsvik Ø, Swenson JM, Spiegel CA, Tenover FC (1999) Detection of a streptomycin/spectinomycin adenyltransferase gene (*aadA*) in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 43:157–160
- Clark CA, Purins L, Kaewrakon P, Focareta T, Manning PA (2000) The *Vibrio cholerae* O1 chromosomal integron. *Microbiology* 146:2605–2612
- Clinical and Laboratory Standards Institute (2005) Performance standards for antimicrobial susceptibility testing: 15th informational supplement CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute, Wayne
- Collis CM, Hall RM (1995) Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrob Agents Chemother* 39:155–162
- Collis CM, Grammaticopoulos G, Briton J, Stokes HW, Hall RM (1993) Site-specific insertion of gene cassettes into integrons. *Mol Microbiol* 9:41–52
- Collis CM, Kim MJ, Stokes HW, Hall RM (1998) Binding of the purified integron DNA integrase *IntI1* to integron- and cassette-associated recombination sites. *Mol Microbiol* 29:477–490
- Correia M, Boavida F, Grosso F, Salgado MJ, Lito ML, Cristino JM, Mendo S, Duarte A (2003) Molecular characterization of a new class 3 integron in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 47:2838–2843
- Crowley D, Daly M, Lucey B (2002) Molecular epidemiology of cystic fibrosis-linked *Burkholderia cepacia* complex isolates from three national referral centres in Ireland. *J Appl Microbiol* 92:992–1004
- Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 38:1008–1015
- Falbo V, Carattoli A, Tosini F, Pezzella C, Dionisi AM, Luzzi I (1999) Antibiotic resistance conferred by a conjugative plasmid and a class I integron in *Vibrio cholerae* O1 El or strains isolated in Albania and Italy. *Antimicrob Agents Chemother* 43:693–696
- Fluit AC, Schmitz FJ (1999) Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 18:761–770
- Fluit AC, Schmitz FJ (2004) Resistance integrons and super-integrons. *Clin Microbiol Infect* 10:272–288
- Francia MV, Zabala JC, de la Cruz F, Garcia Lobo JM (1999) The *IntI1* integron integrase preferentially binds singlestranded DNA of the *attC* site. *J Bacteriol* 181:6844–6849
- Gibrel A, Skold O (2000) An integron cassette carrying *df*r1 with 90-bp repeat sequences located on the chromosome of trimethoprim-resistant isolates of *Campylobacter jejuni*. *Microb Drug Resist* 6:91–98
- Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M (2002) Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 359:753–759
- Girlich D, Karim A, Poirel L, Cavin MH, Verny C, Nordmann P (2000) Molecular epidemiology of an outbreak due to IRT-2 beta-lactamase-producing strains of *Klebsiella pneumoniae* in a geriatric department. *J Antimicrob Chemother* 45:467–473
- Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, Zhao W (2007) Prevalence and characterization of class 1 integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. *J Clin Microbiol* 45:241–243
- Hall RM, Collis CM (1995) Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol* 15:593–600
- Hall RM, Collis CM (1998) Antibiotic resistance in gram-negative bacteria: the role of gene cassettes and integrons. *Drug Res Updates* 1:109–119

31. Hall RM, Stokes HW (1993) Integrons: novel DNA elements which capture genes by site-specific recombination. *Genetica* 90:115–132
32. Hall RM, Brown HJ, Brookes DE, Stokes HW (1994) Integrons found in different locations have identical 5b ends but variable 3b ends. *J Bacteriol* 176:6286–6294
33. Hall RM, Collis CM, Kim MJ, Partridge SR, Recchia GD, Stokes HW (1999) Mobile gene cassettes and integrons in evolution. *Ann N Y Acad Sci* 870:68–80
34. Hanssen A, Kjeldsen G, Sollid JUE (2004) Local variants of staphylococcal cassette chromosome *mec* in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci: evidence of horizontal gene transfer? *Antimicrob Agents Chemother* 48:285–296
35. Hansson K, Sundstrom L, Pelletier A, Roy PH (2002) IntI2 integron integrase in Tn7. *J Bacteriol* 184:1712–1721
36. Heidelberg JF, Eisen JA, Nelson WC, Clayton RA, Gwinn ML, Dodson RJ (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406:477–483
37. Hisata K, Kuwahara-Arai K, Yamanoto M, Ito T, Nakatomi Y, Cui L (2005) Dissemination of methicillin-resistant staphylococci among healthy Japanese children. *J Clin Microbiol* 43:3364–3372
38. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramatsu K (2001) Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 45:1323–1336
39. Katayama Y, Ito T, Hiramatsu K (2000) A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 44:1549–1555
40. Koелеman JGM, Stoof J, Van Der Bijl MW, Vandenbroucke-Grauls CMJE, Savelkoul PHM (2001) Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. *J Clin Microbiol* 39:8–13
41. Kuroda MT, Ohta I, Uchiyama T, Baba H, Yuzawa I, Kobayashi L, Cui A, Oguchi K, Aoki YN, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K (2001) Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 357:1225–1240
42. L’Abee-Lund TM, Sorum H (2001) Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. *Microb Drug Resist* 7:263–272
43. Labbate M, Case RJ, Stokes HW (2009) The integron/gene cassette system: an active player in bacterial adaptation. In: Gogarten M, Gogarten P, Olendzenski L (eds) Horizontal gene transfer. *Genomes in flux*, vol 532, chap 6. Humana Press, New York
44. Liebert CA, Hall RM, Summers AO (1999) Transposon Tn21, flagship of the floating genome. *Microbiol Mol Biol Rev* 63:507–522
45. Maguire AJ, Brown DFJ, Gray JJ, Desselberger U (2001) Rapid screening technique for class 1 integrons in *Enterobacteriaceae* and nonfermenting gram-negative bacteria and its use in molecular epidemiology. *Antimicrob Agents Chemother* 45:1022–1029
46. Martin C, Timm J, Rauzier J, Gomez-Lus R, Davies J, Giequel B (1990) Transposition of an antibiotic resistance element in mycobacteria. *Nature* 345:739–743
47. Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VSC, Verhoef J, Jones ME (1998) Class 1 integrons in gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *J Antimicrob Chemother* 42:689–696
48. Mazel D (2006) Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 4:608–620
49. Mazel D, Dychinco B, Webb VA, Davies J (1998) A distinctive class of integron in the *Vibrio cholerae* genome. *Science* 280:605–608
50. Mazel D, Dychinco B, Webb VA, Davies J (2000) Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. *Antimicrob Agents Chemother* 44:1568–1574
51. McIver CJ, White PA, Jones LA (2002) Epidemic strains of *Shigella sonnei* biotype g carrying integrons. *J Clin Microbiol* 40:1538–1540
52. Mehrotra M, Wang G, Johnson W (2000) Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* 38:1032–1035
53. Mulazimoglu L, Drenning SD, Muder BR (1996) Vancomycin-gentamicin synergism revisited: effect of gentamicin susceptibility of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 40:1534–1535
54. Naas T, Sougakoff W, Casetta A, Nordmann P (1998) Molecular characterization of OXA-20, a novel class D beta-lactamase, and its integron from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 42:2074–2083
55. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J (2003) Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 290:2976–2984
56. Nakatomi Y, Sugiyama J (1998) A rapid latex agglutination assay for the detection of penicillin-binding protein 2'. *Microbiol Immunol* 42:739–743
57. Nandi S, Maurer JJ, Hofacre C, Summers AO (2004) Gram-positive bacteria are a major reservoir of class 1 antibiotic resistance integrons in poultry litter. *Proc Natl Acad Sci USA* 101:7118–7122
58. Nemergut DR, Robeson MS, Kysela RF, Martin AP, Schmidt SK, Knight R (2008) Insights and inferences about integron evolution from genomic data. *BMC Genomics* 9:1–12
59. Nesvera J, Hochmannova J, Patek M (1998) An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium *Corynebacterium glutamicum*. *FEMS Microbiol Lett* 169:391–395
60. Nield BS, Holmes AJ, Gillings MR, Recchia GD, Mabbutt BC, Nevalainen KM, Stokes HW (2001) Recovery of new integron classes from environmental DNA. *FEMS Microbiol Lett* 195:59–65
61. Nordmann P, Poirel L (2002) Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect* 8:321–331
62. Nørskov-Lauritsen N, Sandvang D, Hedegaard J (2001) Clonal origin of aminoglycoside-resistant *Citrobacter freundii* isolates in a Danish county. *J Med Microbiol* 50:636–641
63. Nouwen JL, Van Belkum A, De Marie S, Sluijs J, Wielenga JJ, Kluytmans JW, Verbrugh HA (1998) Clonal expansion of *Staphylococcus epidermidis* strains causing hickman catheter-related infections in a hemato-oncologic department. *J Clin Microbiol* 36:2696–2702
64. Obayashi Y, Fujita J, Ichiyama S, Hojo S, Negayama K, Takashima C, Miyawaki H, Tanabe T, Yamaji Y, Kawanishi K, Takahara J (1997) Investigation of nosocomial infection caused by arbekacin-resistant, methicillin-resistant. *Diagn Microbiol Infect Dis* 28:53–59
65. Oliveira DC, Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the *mec*

- element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 46:2155–2161
66. Paulsen IT, Littlejohn TG, Radstrom P, Sundstrom L, Skold O, Swedberg G, Skurray RA (1993) The 3b conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. Antimicrob Agents Chemother 37:761–768
 67. Ramirez MS, Vargas LJ, Cagnoni V, Tokumoto M, Centron D (2005) Class 2 integron with a novel cassette array in a *Burkholderia cenocepacia* isolate. Antimicrob Agents Chemother 49:4418–4420
 68. Ramirez MS, Quiroga C, Centron D (2005) Novel rearrangement of a class 2 integron in two non-epidemiologically related isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 49:5179–5181
 69. Recchia GD, Hall RM (1995) Gene cassettes, a new class of mobile element. Microbiology 141:3015–3027
 70. Recchia GD, Hall RM (1997) Origins of the mobile gene cassettes found in integrons. Trends Microbiol 5:389–394
 71. Ridley A, Threlfall EJ (1998) Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT 104. Microb Drug Resist 4:113–118
 72. Rowe-Magnus DA, Mazel D (2001) Integrons: natural tools for bacterial genome evolution. Curr Opin Microbiol 4:565–569
 73. Rowe-Magnus DA, Guerout AM, Mazel D (1999) Super-integrons. Res Microbiol 150:641–651
 74. Rowe-Magnus DA, Guerout AM, Ploncard P, Dychinco B, Davies J, Mazel D (2001) The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc Natl Acad Sci USA 98:652–657
 75. Sallen B, Rajoharison A, Desvarenne S, Mabilat C (1995) Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of Enterobacteriaceae. Microb Drug Resist 1:91–98
 76. Salmenlinna S, Lyytikäinen O, Vuopio-Varkila J (2002) Community acquired methicillin resistant *Staphylococcus aureus*, Finland. Emerg Infect Dis 8:602–607
 77. Schito GC (2006) The importance of the development of antibiotic resistance in *Staphylococcus aureus*. Clin Microbiol Infect 12(Suppl 1):3–8
 78. Segal H, Francia MV, Lobo JM, Elisha G (1999) Reconstruction of an active integron recombination site after integration of a gene cassette at a secondary site. Antimicrob Agents Chemother 43:2538–2541
 79. Senda K, Arakawa Y, Ichiyama S (1996) PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. J Clin Microbiol 34:2909–2913
 80. Seward RJ, Towner KJ (1999) Detection of integrons in worldwide nosocomial isolates of *Acinetobacter* spp. Clin Microbiol Infect 5:308–318
 81. Shi L, Zheng M, Xiao Z, Asakura M, Su J, Lin L (2006) Unnoticed spread of class 1 integrons in Gram-positive strains isolated in Guangzhou, China. Microbiol Immunol 50:463–467
 82. Shopsis B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol 37:3556–3563
 83. Shopsis B, Gomez M, Waddington M, Riehman M, Kreiswirth BN (2000) Use of coagulase gene (*coa*) repeat region nucleotide sequences for typing of methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol 38:3453–3456
 84. Stokes HW, Hall RM (1989) A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1669–1683
 85. Stokes HW, O’Gorman DB, Recchia GD, Parsekhian M, Hall RM (1997) Structure and function of 59-base element recombination sites associated with mobile gene cassettes. Mol Microbiol 26:731–745
 86. Su J, Shi L, Yang L, Xiao Z, Li X, Li L, Yamasaki S (2006) Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. FEMS Microbiol Lett 254:75–80
 87. Sundstrom L (1998) The potential of integrons and connected programmed rearrangements for mediating horizontal gene transfer. APMIS Suppl 84:37–42
 88. Sundstrom L, Roy PH, Skold O (1991) Site-specific insertion of three structural gene cassettes in transposon Tn7. J Bacteriol 173:3025–3028
 89. Tauch A, Gotker S, Puhler A, Kalinowski J, Thierbach G (2002) The 27.8-kb R-plasmid pTET3 from *Corynebacterium glutamicum* encodes the aminoglycoside adenylyltransferase gene cassette *aadA9* and the regulated tetracycline efflux system Tet 33 flanked by active copies of the widespread insertion sequence *IS6100*. Plasmid 48:117–129
 90. Van Belkum A, Kluytmans J, Van Leeuwen W, Bax R, Quint W, Peters E, Fluit A, Vandenbroucke GC, Vanden BA, Koel-eman H, Melchers W, Meis J, Elaichouni A, Vaneechoutte M, Moonens F, Maes N, Struelens M, Tenover F, Verbrugh H (1995) Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. J Clin Microbiol 33:1537–1547
 91. Van Belkum A, Goessens W, Van Der Schee C (2001) Rapid emergence of ciprofloxacin-resistant Enterobacteriaceae containing multiple gentamicin resistance-associated integrons in a Dutch hospital. Emerg Infect Dis 7:862–871
 92. Van Essen-Zandbergen A, Smith H, Veldman K, Mevius D (2007) Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. J Antimicrob Chemother 59:746–750
 93. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984
 94. Xu Z, Shi L, Zhang C, Zhang L, Li X, Cao Y, Li L, Yamasaki S (2007) Nosocomial infection caused by class 1 integron-carrying *Staphylococcus aureus* in a hospital in South China. Clin Microbiol Infect 13:980–984
 95. Xu Z, Shi L, Alam MJ, Li L, Yamasaki S (2008) Integron-bearing methicillin-resistant coagulase-negative staphylococci in South China, 2001–2004. FEMS Microbiol Lett 278:223–230
 96. Xu Z, Li L, Alam MJ, Yamasaki S, Shi L (2008) First confirmation of integron-bearing methicillin-resistant *Staphylococcus aureus*. Curr Microbiol 57:264–268
 97. Xu Z, Li L, Shirliff ME, Alam MJ, Yamasaki S, Shi L (2009) Occurrence and characteristics of class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in Southern China. J Clin Microbiol 47:230–234
 98. Xu Z, Li L, Shirliff ME, Peters BM, Peng Y, Alam MJ, Yamasaki S, Shi L (2010) First report of class 2 integron in clinical *Enterococcus faecalis* and class 1 integron in *Enterococcus faecium* in South China. Diag Microbiol Infect Dis 68:315–317
 99. Xu Z, Li L, Shirliff ME, Peters BM, Li B, Peng Y, Alam MJ, Yamasaki S, Shi L (2010) Resistance class 1 integron in clinical methicillin-resistant *Staphylococcus aureus* strains in Southern China, 2001–2006. Clin Microbiol Infect. doi:10.1111/j.1469-0691.2010.03379.x
 100. Yang H, Chen S, White DG, Zhao S, McDermott P, Walker R, Meng J (2004) Characterization of multiple antimicrobial resistant *Escherichia coli* isolates from diseased chickens and swine in China. J Clin Microbiol 42:3483–3489

101. Yu HS, Lee JC, Kang HY, Ro DW, Chung JY, Jeong YS, Tae SH, Choi CH, Lee EY, Soel SY, Lee YC, Cho DT (2003) Changes in gene cassettes of class 1 integrons among *Escherichia coli* isolates from urine specimens collected in Korea during the last two decades. *J Clin Microbiol* 41:5429–5433
102. Zetola N, Francis JS, Nuermberger EL, Bishai WR (2005) Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis* 5:275–286
103. Zhao X, Li Y, Wang L, You L, Xu Z, Li L, He X, Liu Y, Wang J, Yang L (2010) Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. *Mol Biol Rep* 37:2183–2188