RESEARCH LETTER

Integron-bearing methicillin-resistant coagulase-negative staphylococci in South China, 2001–2004

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Abstract

A total of 53 methicillin-resistant coagulase-negative staphylococci strains isolated in a hospital in Guangzhou, China, were analyzed to detect class 1 integrons and SCC*mec* typing. Thirty strains had the class 1 integrase (*intI1*) gene and 26 strains possessed the 3′ conserved region of *qacEΔ1-sul1*. Four different types of gene cassette arrays were found and a highly prevalent array of *dfrA12-orfF-aadA2* gene cassettes was observed. Thirty class 1 integron-positive coagulase-negative staphylococci strains were subjected to Southern hybridization analysis; the result showed that class 1 integrons were located on chromosome, not plasmid. According to the results of SCC*mec* typing for 30 integron-bearing MRCNS strains, five, 15 and five strains belonged to type I, II and III SCC*mec*, respectively, and five strains were untypeable. For 23 non-integron-bearing methicillin-resistant coagulase-negative staphylococci strains, four, nine and seven strains belonged to type I, II and III SCC*mec*, respectively, and three strains were untypeable. None of the strains belonged to type IV or V. Twenty-three coagulase-negative staphylococci isolates of three *Staphylococcal* species that contained the *dfrA12-orfF-aadA2* gene cassette array were phylogenetically unrelated to each other by randomly amplified polymorphic DNA, indicating that the gene cassettes might be disseminated in the clinical strains by a horizontal gene transfer.

Introduction

Coagulase-negative staphylococci (CoNS) are a frequent cause of nosocomial infection and bacteremia, especially in patients with indwelling medical devices (Ben-Ami *et al.*, 2003). 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 (Nouwen *et al.*, 1998). 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 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* (Katayama *et al.*, 2000). Methicillin-resistant coagulase-negative staphylococci (MRCNS), which are more frequent carriers of SCC*mec* than *Staphylococcus aureus*, are postulated to be the reservoir for the transfer of methicillin resistance to *S. aureus*. One assumes that the *ccr* and *mec* genes were brought together in CoNS from an unknown source, where deletion in the *mec* regulatory genes occurred before the genes were transferred into *S. aureus* (Hanssen *et al.*, 2004).

In recent years, the role of integrons and gene cassettes in the spread of antibiotic resistance has been well documented (Stokes & Hall, 1989; Bennett, 1999; Rowe-Magnus & Mazel, 2002). Since the class 1 integron was first described by Stokes & Hall in 1989, integron-mediated resistance to antibiotics has been reported in clinical isolates of various gram-negative bacteria (Stokes & Hall, 1989; Sallen *et al.*, 1995; Yu *et al.*, 2003; Zhang *et al.*, 2004). An integron comprises three elements: the integrase gene (*intI*) encoding an integrase, a recombination site, *attI* and a promoter gene (Navia *et al.*, 2004). At this time, six distinct classes of integrons have been identified based on the homology of their respective integrase genes (Collis *et al.*, 2002; Dean & Mazel,

2002; Correia *et al.*, 2003), of which the class 1 integron is the most ubiquitous among resistant clinical isolates of gram-negative bacteria (Nesvera *et al.*, 1998). Class 1 integron can capture the gene cassettes, which also contain the *attC* site for recombination, via a site-specific recombination event between *attI* and *attC*. The *attC* sites (also known as 59 base elements, or 59-be sites) comprise a family of diverse sequences which vary in size from 57 to 141 bp; only the boundaries are conserved sequences (Mazel *et al.*, 2000). The 3′ conserved segment (3′CS) of class 1 integrons possesses the genes *qacEΔ1* and *sul1*, encoding resistance to quaternary ammonium salts and sulfonamide, respectively (Recchia & Hall, 1997). Although the role of class 1 integrons is well known in the spread of antibiotic resistance genes in gram-negative bacteria, much less is known about gram-positive bacteria and very few studies have reported the presence of class 1 integrons in gram-positive bacteria (Clark *et al.*, 1999; Nandi *et al.*, 2004), so increasing antibiotic resistance mediated by integrons in gram-positive bacteria has become a great concern in the medical field. In a previous study, we performed a general detection of class 1 integrons in some species of gram-positive bacteria and found that some CoNS strains were positive for *intI1* and gene cassettes (Shi *et al.*, 2006).

During 2001–2004, a total of 222 CoNS isolates were collected at a local hospital in South China (37 isolates in 2001, 61 isolates in 2002, 89 isolates in 2003 and 35 isolates in 2004), 53 of which were MRCNS strains. Class 1 integrons and SCC*mec* typing were investigated on all 53 MRCNS strains, and 30 CoNS strains were found to be integron positive (16 were included in the previous study). These 30 CoNS strains were further characterized by Southern hybridization. Moreover, fingerprinting by randomly amplified polymorphic DNA (RAPD) was performed on 23 CoNS isolates of three *Staphylococcal* species carrying a highly prevalent array of *dfrA12-orfF-aadA2* gene cassettes. The results indicated that the widespread association of *dfrA12-orfF-aadA2* was most probably due to the horizontal transfer of integrons.

Materials and methods

Bacterial strains and medical setting

During 2001–2004, a total of 53 methicillin-resistant coagulase-negative staphylococci isolates of four *Staphylococcal* species from human sources (24 *Staphylococcus epidermidis*, 15 *Staphylococcus hominis*, 10 *Staphylococcus haemolyticus* and four *Staphylococcus warneri* strains) were isolated from various clinical specimens at the First Affiliated Hospital of Jinan University (FAHJU), an 850-bed tertiary-level teaching hospital. The FAHJU staff performed active surveillance and data collection for coagulase-negative staphylococci-colonized or -infected patients during this period. A tracking log was used to identify epidemiologic relatedness between patients to select isolates for testing and thus document cluster. Each isolate was from an individual subject, and no repeat isolates were included. Only the first patient isolates obtained from cultures performed more than 48 h after admission were included in the analysis. The isolates were identified using standard procedures: colony morphology, Gram staining, catalase test, the Vitek 2 automated system and the API-Staph commercial kit (API systems SA, La Balme Les Grottes, France) as described previously (Qi *et al.*, 2005). Methicillin resistance was determined by susceptibility testing, confirmed by latex agglutination for PBP2a (Nakatomi & Sugiyama, 1998) and *mecA* detection by PCR.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by standard disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2005). Antimicrobial drugs tested included amikacin (30 μg), amoxicillin-clavulanic acid (20/10 μg), cefuroxime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), levofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), tetracycline (30 μg), vancomycin (30 μg) and teicoplanin (30 μg). Spectinomycin and streptomycin were determined as described previously (De Sousa *et al.*, 2000). The minimum inhibitory concentration (MIC) of oxacillin was determined and interpreted as described previously (Hanssen *et al.*, 2004): CoNS strains for which the MICs were $\leq 0.25\,\mu g\,mL^{-1}$ were considered oxacillin susceptible, and CoNS strains for which the MICs were $\geq 0.5\,\mu g\,mL^{-1}$ were considered oxacillin resistant. *Staphylococcus aureus* ATCC29213 was used as a reference control strain. The results were analyzed according to the breakpoints of the CLSI for all CoNS strains (Clinical and Laboratory Standards Institute, 2005).

Template DNA preparation

Genomic DNA used as templates for PCR amplification was prepared as described previously (Hanssen *et al.*, 2004). Plasmid DNA used for Southern blot hybridization was prepared using standard protocols.

Detection of class 1 integrons

PCR analysis was carried out on 53 MRCNS strains for class 1 integrons as described previously (Zhang *et al.*, 2004). Fifty-three strains were screened by PCR for the class 1 integrase gene (*intI1*), variable region and 3′-conserved

region (*qacEΔ1-sul1*) using primer sets intI1-U with intI1-D, in-F with In-B and qacEΔ1-F with sul1-B, respectively.

SCC*mec* typing

SCC*mec* types were assigned by PCR analysis of the cassette chromosome recombinase (*ccr*) and *mec* gene complexes (*ccr* – *mec* genes) (Okuma *et al*., 2002) and confirmed by a multiplex PCR (Oliveira & Lencastre, 2002). The primers of Ito *et al*. (2001) and Hisata *et al*. (2005) were used to amplify the *ccr* and *mec* genes, respectively. To ensure reproducibility, PCR was repeated with all of the strains.

DNA sequencing and nucleotide sequence accession number

The PCR products of variable regions 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). The ligation mixture was transformed into *Escherichia coli* DH5α strain and the recombinants were selected on Luria Bertani agar containing ampicillin ($100\,\mu g\,mL^{-1}$). Recombinant plasmid DNA was purified by standard methods and subjected to 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).

Nucleotide sequences of gene cassettes *aadA2, dfrA17-aadA5, dfrA12-orfF-aadA2 and aacA4-cmlA1* from *S. epidermidis, dfrA17-aadA5, dfrA12-orfF-aadA2 and aacA4-cmlA1 from S. hominis, dfrA12-orfF-aadA2 from S. haemolyticus* and *S. warneri* have been deposited in GenBank under accession nos. AB291065, AB291061, AB297447, AB291063, AB291062, AB297448, AB291064, AB297449 and AB297450, respectively.

Southern blot hybridization

To determine the location of *intI1*, Southern blot hybridization using PCR-amplified *intI1* as gene probes on 30 integron-bearing CoNS strains was performed as described previously (Zhang *et al*., 2004). The PCR products of *intI1* were purified and nonradioactively labeled by the DIG High Prime DNA labeling and detection kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. Genomic DNA was digested by *Eco*RI for 2 h. Uncut plasmid DNA and digested genomic DNA were resolved on 0.7% (w/v) agarose gels electrophoresis in Tris-borate-EDTA (0.5 M Tris-HCl, 0.5 M borate, 5 mM EDTA) at $1\,V\,cm^{-1}$, and then transferred from agarose gels to positively charged nylon membranes (Roth, Karlsruhe, Germany) using the capillary blot procedure. Hybridization and signal detection were performed according to the recommendations given by the manufacturer (Roche Diagnostics). Hybridization and washing were carried out under stringent conditions. The nylon membrane was placed in prehybridization solution ($5 \times$ SSC, 0.1% N-laurolysarcosine, 0.02% SDS and 1% blocking reagent) and incubated at 42 °C for 1 h with gentle agitation. DIG-labeled *intI* probes ($10\,ng\,mL^{-1}$) were added to fresh prehybridization solution, and incubated at 42 °C with gentle agitation overnight. The protocol of washing and detection was carried out according to the kit manual.

DNA fingerprinting analysis by RAPD

Twenty-three CoNS isolates of three staphylococcal species carrying a highly prevalent array of *dfrA12-orfF-aadA2* gene cassettes were subjected to DNA fingerprinting analysis by RAPD using primers KZ and M13 as described previously (Obayashi *et al*., 1997). Three individual RAPD assays were performed on every species. The resulting PCR products were electrophoresed on 1.5% agarose gel, followed by ethidium bromide staining for 10 min, and documented with the Gel Doc EQ system (BIO-RAD, Hercules, CA). Computer analysis was carried out using QUANTITY ONE (version 4.5; Applied BIO-RAD) software. The similarity to fingerprinting was calculated with the Dice coefficient. Cluster analysis was performed using the unweighted pair-group method with average linkages (UPGMA). This experiment was performed twice to ensure reproducibility.

Results

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of 53 MRCNS strains showed that all of them were multi-drug resistant, showing resistance to at least six of the antimicrobials used (Table 1). Among 30 integron-bearing MRCNS strains, the number that were amoxicillin/clavulanic acid resistant was the highest (93%, $n = 28$), followed by trimethoprim-sulfamethoxazole (90%, $n = 27$), streptomycin (80%, $n = 24$) and spectinomycin (77%, $n = 23$). The percentage of resistance to erythromycin, cefuroxime, ciprofloxacin, levofloxacin, clindamycin, gentamicin, tetracycline, chloramphenicol and amikacin ranged from 40 to 70% (Table 2). None of the tested isolates showed resistance to vancomycin or teicoplanin. The MIC of oxacillin ranged from 16 to $512\,\mu g\,mL^{-1}$.

Table 1. Prevalence of antibiotic-resistant strains among 53 MRCNS strains

	No. of isolates resistant to														
Strain (no. of isolates)	Ac	Am	Ch	Ci	Cl	Cx	E	G	L	Sm	Sp	Sx	Tc	Te	Va
S. epi (*n* = 24)	22	10	10	15	15	16	15	10	17	15	14	15	15	0	0
S. hom (*n* = 15)	14	6	5	8	7	10	9	6	7	8	7	9	8	0	0
S. haes (*n* = 10)	9	1	2	6	4	5	5	4	5	5	6	6	4	0	0
S. war (*n* = 4)	4	0	0	3	2	2	2	1	3	1	1	2	1	0	0
Total (*n* = 53)	49	17	17	32	28	33	31	21	32	29	28	32	28	0	0

Ac, amoxicillin/clavulanic acid; Am, amikacin; Ch, chloramphenicol; Ci, ciprofloxacin; Cl, clindamycin; Cx, cefuroxime; E, erythromycin; G, gentamicin; L, levofloxacin; Sm, streptomycin; Sp, spectinomycin; Sx, sulfamethoxazole; Tc, tetracycline; Te, teicoplanin; Va, vancomycin.

Table 2. Phenotypic and genotypic characteristics of 30 integron-bearing MRCNS strains

				Integron		SCCmec		
Strain	Species	Year	Source	Gene cassette	3′CS	*ccr*	*mec*	Resistance pattern
012216*	*S. epi*	2001	Bronchu	*dfrA12-orfF-aadA2*	+	2	A	AcChCiCxELSmSpSxTc
012219*	*S. epi*	2001	Sputum	*dfrA12-orfF-aadA2*	+	3	A	AcAmCiClCxGLSmSpSx
012228*	*S. epi*	2001	Stool	*dfrA12-orfF-aadA2*	+	2	A	AcCiClCxLSmSpSx
022212*	*S. epi*	2002	Pus	*dfrA12-orfF-aadA2*	+	3	A	AcAmChEGLSmSpSxTc
022218	S. epi	2002	Sputum	*dfrA12-orfF-aadA2*	+	2	A	AcCiClCxELSxTc
022225	*S. epi*	2002	Pus	*dfrA12-orfF-aadA2*	+	1	B	AcChCiCxSmSpSx
022237*	*S. epi*	2002	Exudate	*dfrA12-orfF-aadA2*	+	2	A	AcClEGLSmSpSxTc
022244*	*S. epi*	2002	Sputum	*dfrA12-orfF-aadA2*	+	3	A	AcAmChCiClCxELSxTc
022256*	*S. epi*	2002	Sputum	*dfrA12-orfF-aadA2*	+	NT	NT	AcClEGLSmSpSx
022258*	*S. epi*	2002	Urine	*dfrA12-orfF-aadA2*	+	NT	NT	AcAmChEGLSmSpSxTc
032211	*S. epi*	2003	Urine	*dfrA12-orfF-aadA2*	+	NT	NT	AcAmChEGLSmSpSxTc
032224	*S. epi*	2003	Stool	*dfrA12-orfF-aadA2*	+	1	B	ChCiCxSmSpSx
012303	*S. hom*	2001	Sputum	*dfrA12-orfF-aadA2*	−	1	B	AcCxEGSmSpTcSx
012305*	*S. hom*	2001	Sputum	*dfrA12-orfF-aadA2*	+	1	B	AcChELSmSpSx
012306*	*S. hom*	2001	Urine	*dfrA12-orfF-aadA2*	+	1	B	EGSmSpSxTc
022303*	*S. hom*	2002	Exudate	*dfrA12-orfF-aadA2*	+	2	A	AcAmChClCxEGLSxTc
032309	*S. hom*	2003	Stool	*dfrA12-orfF-aadA2*	+	2	A	AcClESmSpSxTc
042315	*S. hom*	2004	Urine	*dfrA12-orfF-aadA2*	+	2	A	AcAmCiCxESmSpSx
022405*	*S. hae*	2002	Sputum	*dfrA12-orfF-aadA2*	+	2	A	AcCiClCxESx
022407*	*S. hae*	2002	Prostate	*dfrA12-orfF-aadA2*	+	2	A	AcChEGLSmSpSx
022411*	*S. hae*	2002	Sputum	*dfrA12-orfF-aadA2*	+	2	A	AcEGLSmSpSx
022413*	*S. hae*	2002	Pus	*dfrA12-orfF-aadA2*	+	NT	NT	AcCiCxSmSpSxTc
042403	*S. hae*	2004	Pus	*dfrA12-orfF-aadA2*	+	NT	A	AcCiCxSmSpSxTc
012501*	*S. war*	2001	Choler	*dfrA12-orfF-aadA2*	+	2	A	AcCiClCxEGLSmSpSx
022230	*S. epi*	2002	Sputum	*aadA2*	−	2	A	AcClEGLSmSpTc
032237	*S. epi*	2003	Sputum	*aacA4-cmlA1*	−	2	A	AcAmChCiClCxEGSmTc
042219	*S. epi*	2004	Sputum	*aacA4-cmlA1*	−	2	A	AcAmChCiClCxEGL
042306	*S. hom*	2004	Sputum	*aacA4-cmlA1*	+	2	A	AcAmChCiClCxEGLSxTc
032315	*S. hom*	2003	Pus	*dfrA17-aadA5*	+	3	A	AcAmChCiCxLSmSpSx
042237	*S. epi*	2004	Sputum	*dfrA17-aadA5*	+	3	A	AcAmCiCxSmSpSxTc

*Strain included in a previous study (Su *et al.*, 2006).

S. epi, *S. epidermidis*; *S. hom*, *S. hominis*; *S. hae*, *S. haemolyticus*; *S. war*, *S. warneri*.

+, carrying a 3′CS of *qacEΔ1-sul1*; −, not carrying 3′CS; NT, not typeable.

Ac, amoxicillin/clavulanic acid; Am, amikacin; Ch, chloramphenicol; Ci, ciprofloxacin; Cl, clindamycin; Cx, cefuroxime; E, erythromycin; G, gentamicin; L, levofloxacin; Sm, streptomycin; Sp, spectinomycin; Sx, sulfamethoxazole; Tc, tetracycline.

Detection of class 1 integrons and characterization of the variable region

Thirty MRCNS strains yielded a 923-bp amplicon and 26 strains yielded an 800-bp amplicon in the PCR amplification of the *intI1* and 3′-conserved regions of *qacEΔ1-sul1*. Variable region was determined by PCR with primers in-F and in-B. A 975-bp product was obtained from one strain, and the sequence demonstrated an *aadA2* gene cassette encoding

resistance to aminoglycoside. A 1664-bp product was obtained from two strains and the sequence confirmed the presence of gene cassettes *dfrA17* and *aadA5*, which were resistant to trimethoprim and aminoglycoside, respectively. Twenty-four strains gave a 1913-bp PCR product, the sequence of which demonstrated a *dfrA12-orfF-aadA*2 array of gene cassettes. The *dfrA12* and *aadA2* conferred resistance to trimethoprim and aminoglycoside, respectively; the second ORF encodes an unknown function protein. An amplicon of 2360-bp was found in three strains, harboring *aacA4* and *cmlA1* genes which conferred resistance to aminoglycoside and chloramphenicol (Table 2).

SCC*mec* typing

The distribution of SCC*mec* type in 53 MRCNS strains showed that the classic nosocomial SCC*mec* type (I, II and III) dominated among the tested strains (Table 3): for 30 integron-bearing MRCNS strains, five strains belonged to type I SCC*mec* with type 1 *ccr* and class B *mec*, 15 strains belonged to type II SCC*mec* with type 2 *ccr* and class A *mec*, five strains belonged to type III SCC*mec* with type 3 *ccr* and class A *mec*, and five strains were untypeable, which produced new patterns by multiplex SCC*mec* and *ccr* typing. For 23 non integron-bearing MRCNS strains, four, nine and seven strains belonged to type I, II and III SCC*mec*, respectively, and three strains were untypeable. None of the tested strains belonged to type IV or V.

Southern blot hybridization

To analyze the location of class 1 integrons, uncut plasmid DNA and *Eco*RI-digested genomic DNA from 30 integron-bearing strains were prepared and analyzed by Southern blot hybridization using *intI1* gene probes. According to the results (data not shown), there was no signal on plasmid DNA, whereas the PCR-generated *intI* probes were hybridized with genomic DNA from all 30 strains, showing that the class 1 integrons were located on chromosomes, not plasmid.

Table 3. Distribution of SCC*mec* types in 53 MRCNS strains

	SCC*mec* type					
Strain (no. of isolates)	I	II	III	IV	V	NT
Integron-bearing MRCNS ($n = 30$)	5	15	5	0	0	5
Non integron-bearing MRCNS ($n = 23$)	4	9	7	0	0	3
Total ($n = 53$)	9	24	12	0	0	8

NT, not typeable.

DNA fingerprinting analysis by RAPD

To determine whether the high prevalence of the gene cassettes *dfrA12-orfF-aadA2* was caused by the spread of a specific clone in the hospital environment or the horizontal transfer of integrons, three individual RAPD analyses were performed on 23 CoNS strains carrying an array of *dfrA12-orfF-aadA2*. RAPD yielded a complex banding pattern which was consisted of 5–12 bands ranging from 0.3 to 4.0 kb. To evaluate similarity, the fingerprintings were recorded, followed by computerized analysis of the acquired data. DNA relatedness, based on the unweighted pair group method (differences in band staining intensity were neglected) using arithmetic averages (UPGMA) of Dice coefficients, was shown in the dendrogram (Fig. 1). Apparently, 23 CoNS strains exhibited different RAPD patterns with low Dice coefficients, indicating that they were phylogenetically unrelated. The results suggested that the widespread association of *dfrA12-orfF-aadA2* in the different clones of CoNS strains may be mainly due to the horizontal transfer of integrons.

Discussion

In this study, we identified four species of CoNS strains (*S. epidermidis*, *S. hominis*, *S. haemolyticus* and *S. warneri*) simultaneously carrying two mobile genetic elements: gene cassettes within a class 1 integron and SCC*mec*, including four different types of gene cassette arrays and three different types of SCC*mec*. This was the first time, to our knowledge, that four species of integron-bearing methicillin-resistant CoNS have been identified. The integron system, as a natural cloning and expression system, allows bacteria to incorporate gene cassettes and convert them to functional genes. Integrons are now well known key players in the dissemination 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 (Rowe-Magnus & Mazel, 2001). It is conceivable that any ORF can be structured as a gene cassette and it is vital to decipher the mechanism governing cassette genesis. So, through the recombination platform (the integrase and *attI* site), integrons have the apparently 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. Also, hundreds of gene cassettes, all types of other mobile DNA elements such as conjugative plasmids, transposons, insertion sequences, and even entire chromosome, would probably be vast reservoirs of integron, lending support to the long-standing concept of a single massive genetic pool that is available and shared among bacteria (Rowe-Magnus & Mazel, 2001). SCC*mec* is a genomic island (Gisland) inserted at the 3′end of *orfX* and located near the

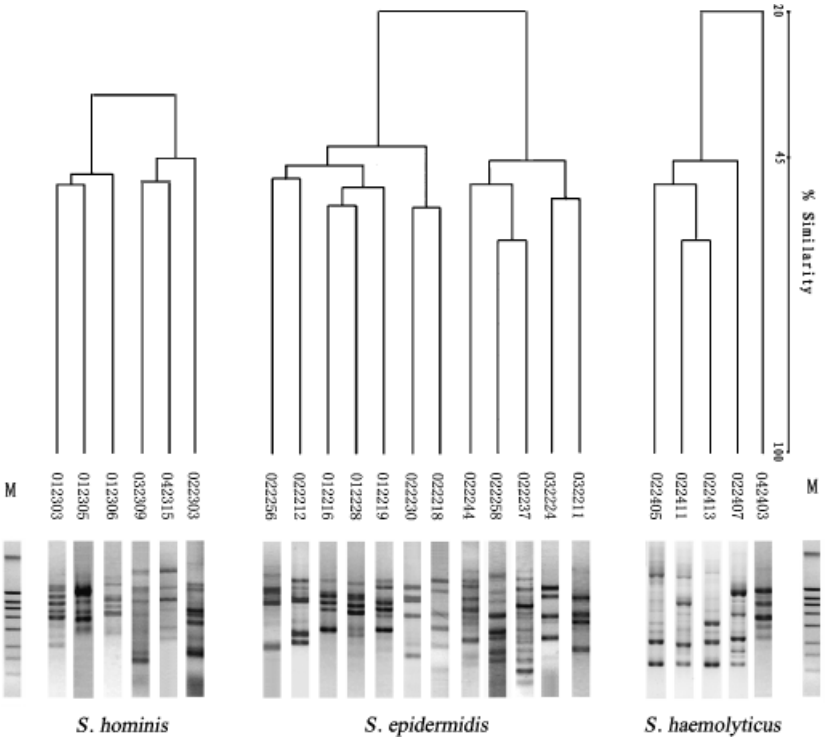

Fig. 1. Dendrogram analysis by RAPD of DNA fingerprinting obtained from 24 CoNS strains carrying *dfrA12-orfF-aadA2* gene cassettes.

replication origin of *Staphylococci* (Kuroda *et al.*, 2001), which is defined as a basic mobile genetic element demarcated by a pair of direct repeats and inverted repeats, with a set of site-specific recombinase genes (*ccrA* and *ccrB*) required for its movement and carrying the *mecA* gene complex. SCC*mec* 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 the *mecA* gene) alone; it might be serving as a vehicle for the exchange of useful genes for the better survival for staphylococci in various environments, which means that SCC*mec* is a general genetic information exchange system of staphylococci with *ccrA* and *ccrB* involved in the recombination events (integration and excision) (Ito *et al.*, 2001). Similar to Qi *et al.* (2005), in our study the MRCNS tended to amplify more than one representative of different *ccr* complexes, suggesting that the MRCNS was harboring multiple new types of SCC*mec* and might be the breeding ground for new SCC*mec* elements. Integron and SCC*mec* serve as the reservoir of many kinds of genes and possess the function to exchange genes between species, so whether the simultaneous existence of integron system and SCC*mec* would speed up the gene exchange and genome evolution in staphylococci requires further investigation.

As demonstrated by the RAPD fingerprinting analysis, the high prevalence of *dfrA12-orfF-aadA2* array of gene cassettes in the different clones of CoNS strains may be mainly due to the horizontal transfer of integrons. Interestingly, these *dfrA12-orfF-aadA*2 arrays of gene cassettes (GenBank accession no. AB154407) of the class 1 integron were also present in the gram-negative enteroinvasive *E. coli* O164 strain RIMD05091045 isolated from a patient in Japan (Ahmed *et al.*, 2005). What is more, the same combinations of gene cassettes, such as *aadA2*, *aacA4-cmlA1* and *dfrA17-aadA5*, have also been detected in clinical isolates of various negative bacteria (Nesvera *et al.*, 1998; Tauch *et al.*, 2002; Yu *et al.*, 2003; Yang *et al.*, 2004). Su *et al.* performed integron detection on clinical *E. coli* strains isolated in South China during 1998–2004, and found the same gene cassettes arrays as above in *E. coli* strains; a similar high prevalence of *dfrA12-orfF-aadA*2 array of gene cassettes was also observed (Su *et al.*, 2006). Although class 1 integrons have been reported to be able to spread among gram-negative bacteria, and the class 1 integrons carried by a plasmid can be transferred from an *Escherichia faecalis* strain W4770 to a recipient strain of *E. faecalis* (Clark *et al.*, 1999), no report is available on the dissemination of class 1 integrons between gram-positive and gram-negative bacteria. In the present study, class 1 integrons found in CoNS strains were identical to those in gram-negative bacteria reported previously (Nesvera *et al.*, 1998; Tauch *et al.*, 2002; Yu *et al.*, 2003;

Yang *et al*., 2004; Su *et al*., 2006), though the relationship between them remains unclear at present. Further studies are needed to determine what the origins of class 1 integrons in gram-positive bacteria are and whether class 1 integrons can spread through gram-positive and gram-negative bacteria.

In recent years, regulations on the appropriate use of antibiotics have been enforced effectively in developed countries, community-associated methicillin-resistant staphylococci (CA-MRS) have emerged and infections caused by CA-MRS have been obtained with increasing frequency in different countries and regions (Chambers, 2001; Gillet *et al*., 2002; Centers for Disease Control and Prevention, 2003; Vandenesch *et al*., 2003; Zetola *et al*., 2005). CA-MRS, which has several distinctive properties such as a more susceptible antimicrobial phenotype, the presence of different exotoxin gene profiles and a much smaller SCC*mec* (type IV or V), is becoming more and more prevalent and is tending to replace traditional hospital-associated methicillin-resistant staphylococci (HA-MRS) in many regions (Salmenlinna *et al*., 2002; Naimi *et al*., 2003). However, in South China, according to our study, classic nosocomial SCC*mec* still dominates, with no type IV or V SCC*mec* strains. The 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 prevalence of integrons and gene cassettes, and was reflected by the domination of nosocomial SCC*mec* and the prevalence of integrons in the present study. In South China, as trained practitioners were unavailable in many areas, regulations on the clinical use of antibiotics were poorly enforced or absent and the surveillance system was not as effective, and there may be more opportunity for the inappropriate use of antibiotics, resulting in heavier antibiotic selective pressure. So it is reasonable to presume that in this region CoNS strains developed into a different direction, and tended to carry genetic elements that would endow more fitness and advantage, resulting in their rapid adaptation to selective pressure and survival.

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