African Journal of Biotechnology Vol. 11(47), pp. 10785-10791, 12 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB12.867
ISSN 1684-5315 ©2012 Academic Journals

Full Length Research Paper

Prevalence and molecular typing of the antiseptic resistance genes *qacA/B* among *Staphylococcus aureus* strains isolated in a teaching hospital

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Accepted 30 April, 2012

The *qacA/B* genes are found in *Staphylococcus aureus* and confer resistance to various antiseptics and disinfectants. Herein, the prevalence of the *qacA/B* genes in methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) was investigated. Molecular typing systems were used to analyse the relatedness of these *qacA/B*-positive strains. 176 six strains of clinically isolated *S. aureus* were collected between July, 2008 and June, 2010. The strains carrying the *qacA/B* genes were characterised by pulse-field gel electrophoresis (PFGE) typing, *Staphylococcus* protein A (*spa*) typing, Panton-Valentine leucocidin (*pvl*) polymerase chain reaction (PCR) detection, staphylococcal chromosomal cassette (SCC) *mec* typing, and antimicrobial resistance profiles. Strains carrying the *qacA/B* genes composed 9.1% of the strains isolated, but the incidence of *qacA/B* genes in MRSA strains was significantly higher than that in MSSA strains (14.6 versus 4.3%, $p < 0.05$). Additionally, two predominant PFGE (B and A) and *spa* types (t037 and t042) were identified along with two major antimicrobial resistance profiles. All of these *qacA/B*-positive strains strains were *pvl*-negative by PCR. The *qacA/B*-positive MRSA strains all contained the group III SCC*mec* element. These strains were obtained mainly from patients in surgical wards; therefore, the neurosurgical ward and ICU may be considered as a source of MRSA strains carrying the *qacA/B* genes. Finally, the strain identified as s*pa* type t037 is likely to be an epidemiological clone. The presence of the antiseptic resistance genes *qacA/B* by MRSA could potentially lead to MRSA strain prevalence. Thus, the optimal usage of antiseptics and disinfectants is warranted. A policy of molecular typing needs to be implemented to track the possible dissemination of these resistance genes.

Key words: *Staphylococcus aureus, mecA*, *qacA/B* and *pvl* genes, methicillin-resistant, *Staphylococcus aureus (*MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA).

INTRODUCTION

Staphylococcus aureus is one of the leading pathogens causing both severe nosocomial and outpatient infections. A variety of antiseptics and disinfectants are used in hygienic practices, such as hand sanitation and environmental decontamination, and are crucial interventions to control infection. However, the emergence of antiseptic-resistant strains of *S. aureus* poses a new challenge. Genome sequencing has discovered numerous genes that can potentially impart multidrug resistance and that are responsible for the extrusion of antiseptics, dyes and certain antimicrobials from this pathogen (McDonnell and Russell, 1999; Mayer et al., 2001; Noguchi et al., 2005). Among the antiseptic and disinfectant resistance genes, *qacA* is the most important

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and confers resistance to a variety of monovalent and divalent cations, including dyes, quaternary ammonium salts, diamidines, and biguanidines. This gene is located on multidrug-resistant plasmids, such as pSK1, which has resulted in its rapid spread among different strains. Another relevant gene is *qacB,* which is also located on multidrug-resistant plasmids, such as pSK23, and confers resistance to monovalent antiseptics and dyes. Sequencing has shown that these two genes differ only by seven or nine base pairs, rendering them virtually indistinguishable using simple polymerase chain reaction (PCR) assays (Brown and Skurray, 2001). An epidemiological study in China showed that the prevalence of *qacA/B* genes in methicillin-resistant *S. aureus* (MRSA) was as high as 61.1% (Wang et al., 2008). According to the 2009 Chinese bacterial resistance surveillance study, *S. aureus* isolates ranked the most prevalent (32.5%) among Gram-positive strains. MRSA strains accounted for 52.7% of all drug-resistant *S. Aureus* strains, which is alarming because the multidrug resistance properties of MRSA imply a longer hospital stay and higher mortality. Few data have elaborated on the detailed molecular epidemiology of the *qacA/B* genes in *S. aureus* isolates in China. To track and limit the possible dissemination of these genes, molecular typing systems are often used to confirm the relatedness of groups of organisms. The goal of our study was to investigate the distribution of the *qacA/B* gene among *S. aureus* isolates from a university hospital in southwestern China and to determine the relatedness of the specimens by pulse-field gel electrophoresis (PFGE) typing, staphylococcus protein A (*spa*) typing, Panton-Valentine leucocidin (*pvl*) PCR detection and staphylococcal chromosomal cassette (SCC) *mec* typing.

MATERIALS AND METHODS

Bacterial strains

A total of 176 non-duplicate *S. aureus* isolates were collected over a two-year period (July, 2008 to June, 2010) at a university hospital in Chongqing, located in southwestern China. The isolates were sampled from the sputum, purulent discharge, blood and urine of patients. All strains were identified by a Gram stain, an API STAPH strip (bioMérieux, France) and a Latex Agglutination Kit (Bio-rad, USA). *S. Aureus* strain ATCC25923 was used as a negative control in the *qacA/B* detection assay, while strain TS77 was used as a positive control. *S. aureus* strains ATCC29213 and ATCC43300 were used as the standard strains for MSSA and MRSA, respectively. Clinical data regarding the ward and condition of the patients sampled were also collected. Before participating in the study, patients were required to sign an informed consent statement approved by the local ethics review board. This protocol was approved by the appropriate ethics committees in the hospital and carried out in compliance with the declaration of Helsinki.

Antimicrobials

The antimicrobials used for testing included ampicillin-sulbactam, chloramphenicol, ciprofloxacin, erythromycin, fusidic acid, fosfomycin, gentamicin, imipenem, moxifloxacin, oxicillin, penicillin G, rifampin, tetracycline, teicoplanin, vancomycin and dalfopristin; all were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Linezolid was kindly provided by its manufacturer (Pfizer, China).

Minimum inhibitory concentration (MIC) assay

A MIC assay was performed in triplicate using the agar double-dilution method using an anti-bacterial determiner (SAKUMA, Japan). Susceptibility of the isolates to the previously listed antimicrobials was determined using break points set by the Clinical and Laboratory Standards Institute Clinical and Laboratory Standards Institute (CLSI) (2010). The MIC of each antimicrobial was determined 24 h after plating by scoring the lowest concentration at which no growth was observed.

PCR amplification of the *mecA*, *qacA/B* and *pvl* genes

Briefly, the isolates were suspended in 100 µl of ddH_2O (double distilled water). The suspension was heated at 100°C for 10 min followed by centrifugation at 10956 x *g* for 10 min. 1 µl of the supernatant was used as a template for the PCR assay. Primers were designed to amplify 533 bp of *mecA* (5'-AAAATCGATGGTAAAGGTTGGG-3' and 5'-AGTTCTGCAGT-ACCGGATTTGC-3'), 629 bp of *qacA/B* (5′-GCTGCATTTATGA-CAATGTTTG-3′ and 5′-AATCCCACCTACTAAAGCAG-3'), and 433 bp of *pvl* (5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3' and 5'-GCATCAASTGTATTGGATAGCAAAAGC-3').

Typing by spa, PFGE and SCC*mec*

In *qacA/B*-positive strains, the short sequence repeat regions of *Spa* were amplified, sequenced, and analysed using the spa-*server* at Ridom Bioinformatics (http://www.ridom.de/spaserver) (Oliveira et al., 2001). The primers used were 5′-GACGATCCTTCAGT-GAGCAAAG-3′ and 5′-GCAGCAATTTTGTCGGCAGTAG -3′. These same strains were also typed by PFGE using SmaI (New England Biolabs, Beijing Ltd) as the restriction enzyme according to a previously described method (Goering and Winters, 1992). Finally, a multiplex PCR strategy was used to type the MRSA strains by SCC*mec* (Oliveira and de Lencastre, 2002).

Statistical analysis

A chi-square (χ^2) test was used to test the hypotheses, and values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Prevalence and antimicrobial resistance profile of *qacA/B*-carrying strains

MIC tests showed that 82 out of 176 *S. aureus* strains could be characterised as MRSA (46.7%); this was further confirmed by PCR verification of the presence of the *mecA* gene. PCR analysis found 16 *qacA/B*-carrying

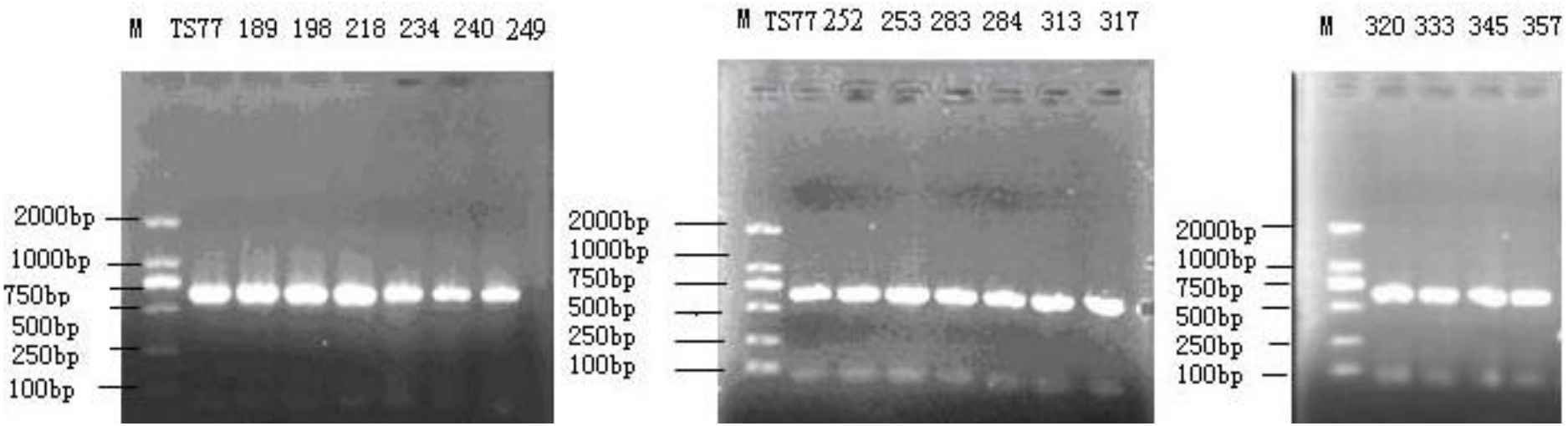

Figure 1. PCR amplification of the *qacA/B* genes. A, PCR assay revealed 16 *qacA/B*-carrying strains among the 176 *S. Aureus* isolates. The size of amplification product was 629 bp. M, DL2000 Marker; TS77, positive control for *qacA/B* detection.

strains among the 176 isolates (9.1%). Of these, 12 strains were identified as MRSA (12/82, 14.6%) and four as MSSA (4/94, 4.3%). The prevalence of the *qacA/B* gene in MRSA isolates was significantly higher than in MSSA isolates (p = 0.005). The PCR results from *qacA/B* amplification are shown in Figure 1. The *pvl* gene was not detected in any of the *qacA/B*-carrying strains.

Typing by *spa*, PFGE and SCC*mec*

On the basis of the sequence and number of *spa* repeats, seven distinct *spa* types were found among the 16 strains harbouring the *qacA/B* gene, including t548, t421, t008, t091, t895, t932 and t037 (Table 1). Spa type t037 was the most frequent (found in 50% of the isolates), followed by *spa* type t421 (18.8%).

Using PFGE, the 16 *qacA/B*-positive strains were also separated into seven pulse-field types based on analysis using BioNumerics software and using an > 80% relatedness threshold to define the types as recommended by the CDC (Tenover et al., 1995). The most predominant PFGE clone was type B (6 isolates), and there were two unique subtypes for type A. The different PFGE subtypes were delineated by changes in the PFGE banding patterns (up to six band differences) that were still > 80% related to their parent type strains. All of the prevalent types were MRSA strains. The relatedness between type A and type B was nearly 80%.

SCC*mec* typing showed that all of the MRSA strains harbouring the *qacA/B* genes were SCC*mec* type III (Figure 2).

Association of bacterial genotype with patient ward distribution

We analysed the departmental distribution of the *qacA/B*-carrying *S. aureus* isolates and found that 81% were isolated from patients in surgical wards. All PFGE type B (spa type 037) isolates were obtained from patients with lung infections in the neurosurgical intensive care unit (ICU), whereas all PFGE type A isolates originated from the sputum samples of patients housed in adjoining surgical wards. Three of the four *mecA*-negative isolates (Strains 189, 283 and 345) were sampled from the purulent discharge of different inpatients from various surgical wards. However, the

Table 1. Characteristics and frequency of *spa* types among the *qacA/B*-positive strains.

Strain number	*Spa* sequence	*spa* type	Sequence length (bp)
189	r26r23r17r34r17r20r17r12r16	t548	350
198	r15r12r16r02r25r17	t421	309
218	r15r12r16r02r25r17	t421	309
234	r15r12r16r02r25r17	t421	309
240	r15r12r16r02r25r17r24	t037	310
249	r15r12r16r02r25r17r24	t037	310
252	r15r12r16r02r25r17r24	t037	310
253	r15r12r16r02r25r17r24	t037	310
283	r26r23r17r02r17r12r17r16	t895	325
284	r15r12r16r02r25r17r24	t037	310
313	r12r16r16r24r24	t932	350
317	r15r12r16r02r25r17r24	t037	310
320	r15r12r16r02r25r17r24	t037	310
333	r15r12r16r02r25r17r24	t037	310
345	r07r23r21r17r34r12r23r02r12r23	t091	382
357	r11r19r12r21r17r34r24r34r22r25	t008	386

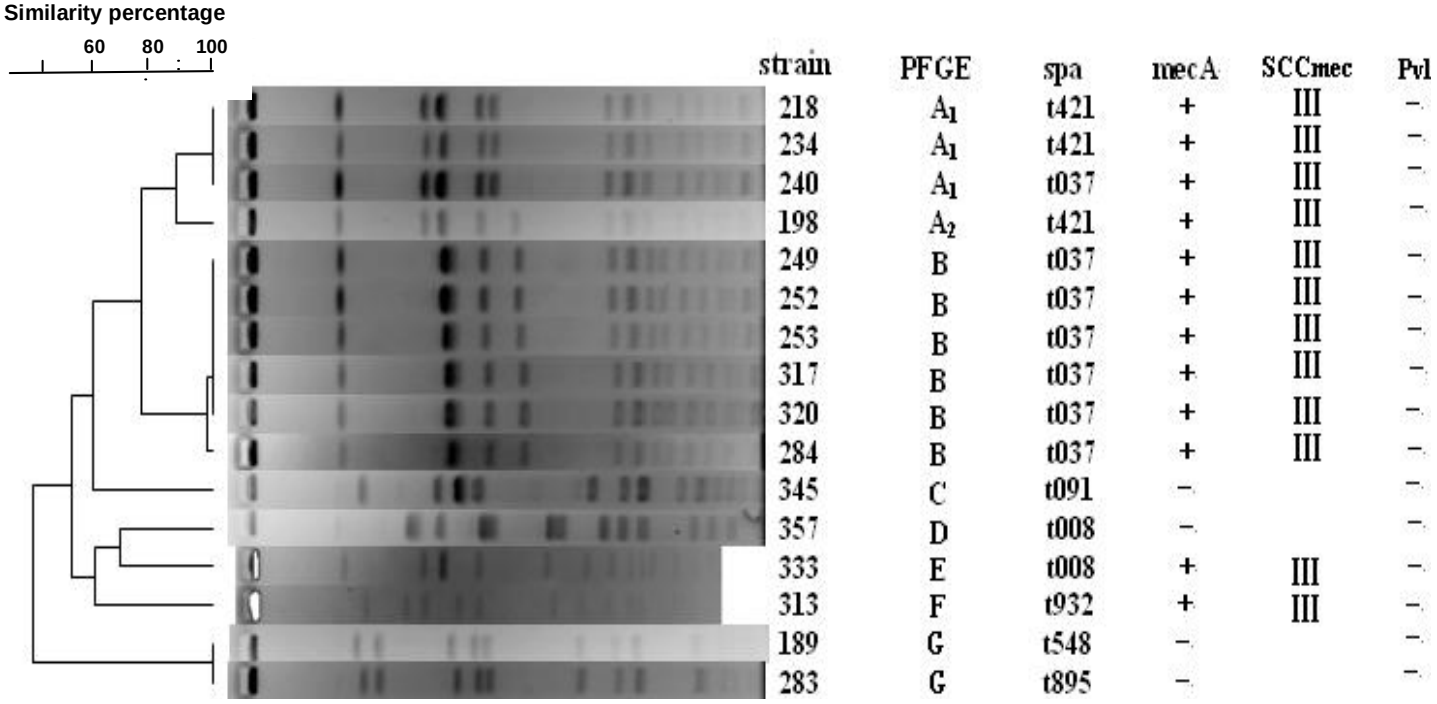

Figure 2. Molecular characteristics of the 16 *qacA/B*-positive *S. aureus* strains. The molecular characteristics of the 16 *qacA/B*-positive *S. aureus* strains are presented in a PFGE dendrogram, along with analysis of *mecA* gene detection, *spa* type, *SCCmec* typing, and *pvl* gene detection. Isolate clusters were delineated with an 80% similarity cut-off value.

remaining strains were recovered from patients in the gerontology department or the neurological and neurosurgical wards.

Antimicrobial resistance profile

A total of six antimicrobial resistance profiles were

Table 2. Antimicrobial phenotypes of resistant *qacA/B*-positive strains.

qacA/B (+) Strain	Resistant	Sensitive	Pattern
189/283*	CHI+ERY+ PEN	SAM+CIP+FUS+FOS+FOX+GER+MFX+PEN+TCY+ IPM+LNE+RIF+TEC+VAN+QDA	A
198/218/249/252/313/317/ 320#	SAM+CHI+CIP+ERY+FUS+FOS+FOX+GER+MFX +PEN+TCY	IPM+LNE+RIF+TEC+VAN+QDA	B
234/240/253/284#	SAM+CHI+CIP+ERY+FOS+FOX+GER+MFX+PEN +TCY	IPM+LNE+RIF+TEC+VAN+QDA+ FUS	C
333#	SAM+CHI+ERY+FOX+GER+PEN	CIP+FUS+FOS+MFX+TCY+ IPM+LNE+RIF+TEC+VAN+QDA	D
345*	SAM+CHI+CIP +ERY+PEN	FUS+FOS+FOX+GER+MFX+PEN+TCY+ IPM+LNE+RIF+TEC+VAN+QDA	E
357*	FUS+PEN	SAM+CHI+CIP+ERY+FOS+FOX+GER+MFX+PEN+TCY+ IPM+LNE+RIF+TEC+VAN+QDA	F

SAM, Ampicillin-sulbactam; CHI, Chloramphenicol; CIP, Ciprofloxacin; ERY, Erythromycin; FUS, Fusidic Acid; FOS, Fosfomycin; GER, Gentamicin; IPM, Imipenem; LNE, Linezolid; MFX, Moxifloxacin; OXA, Oxacillin; PEN, Penicillin G; RIF, Rifampin; TCY, Tetracycline; TEC, Teicoplanin; VAN, Vancomycin; QDA, Dalfopristin, * indicating mecA (-) strain; # indicating mecA (+) strain.

identified among these *qacA/B*-carrying *S. Aureus* isolates. Two predominant, multidrug-resistance profiles spanned PFGE types A and B (spa types t421 and t037) (Table 2). Pattern A differed from pattern B with regard to resistance to fosfomycin. All isolates were susceptible to imipenem, linezolid, rifampin, teicoplanin, vancomycin and dalfopristin.

DISCUSSION

The prevalence of the multidrug-resistance genes *qacA/B* among *S. aureus* strains in general, and among MRSA strains in particular, has raised great concern. Previous studies have shown that this prevalence is a widespread problem in European hospitals with an occurrence rate of 42% (210/497) in *S. aureus* and 63% in MRSA (Mayer et al., 2001). The incidence of these genes in one Brazilian hospital reached 80% among 74 MRSA isolates (Miyazaki et al., 2007). Noguchi et al. (2005) found that MRSA expressing *qacA/B* comprised 41.6% (372/894) of the strains isolated across 11 Asian countries (Noguchi et al., 2005). The frequency of the *qacA/B* gene in China was first reported to be 61.1% among 131 MRSA strains (Wang et al., 2008). In our study, the *qacA/B* gene was found in 9.1% of all *S. aureus* and in 14.6% of MRSA isolates. These findings are significantly lower than the statistics reported in other studies both home and abroad. This difference implies a distinctly regional discrepancy. Further phenotypic and genotypic analyses provided the three findings described below.

First, it is notable that significantly more MRSA strains possessed the *qacA/B* genes than did the MSSA strains (14.6% versus 4.3%), which is consistent with previous observations (Alam et al., 2003; Opacic et al., 2010). The resistance of MRSA strains is attributed to mutated chromosomes and plasmids carrying clusters of resistance determinants. Previous studies have found large multidrug-resistant plasmids harbouring the β-lactamase and *qacA* genes in *Staphylococcus* (Fraise, 2002). Because the *qacA/B* genes are located on multidrug-resistant

plasmids, the higher rate of occurrence of the *qacA/B* gene in MRSA strains is understandable. The *qacA/B* genes usually confer low-level resistance to antiseptics and dyes, that is, the difference in the MICs of antiseptic agents for a resistant strain and a susceptible strain is small; thus, using a phenotypic assay to determine the carriage of *qacA/B* genes is difficult.

Second, these antiseptic-resistant strains were further characterised by phenotypic and genotypic methods including clinical data collection, antimicrobial resistance profiles, *pvl* gene detection, SCC*mec* typing (for MRSA strains), and a combination of PFGE typing and *spa* typing. None of the strains isolated in the study contained the *pvl* gene, including isolates from skin and soft tissue infections, which suggests they might be nosocomial in origin. Using SCC*mec* typing, all MRSA isolates in this study were identified as SCC*mec* type III, which is usually associated with Hospital-acquired (HA)-MRSA (Vandenesch et al., 2003). PFGE is considered the gold standard of band-pattern-based typing to establish clonal relationships among strains, although the results are difficult to compare among laboratories. *Spa* typing, which is based on the sequence of an internal fragment, is the sequence-based method of choice to determine the genetic relatedness of *S. aureus* strains. Combining these two typing methods provides both the powerful discriminatory and classification abilities for a local epidemiological study and the possibility of detecting relationships among distant isolates (Faria et al., 2008; Vindel et al., 2009; Aires-de-Sousa et al., 2006). Our study found that PFGE type A-spa t037 and t042 were *qacA/B*-carrying strains from surgical wards located on different floors of the same building; however, all of the patients with this strain had been transferred from the same surgical ICU, indicating the existence of a single MRSA clone with antiseptic resistance genes. Similarly, an identical PFGE type (type B-spa t037) was observed among 6 MRSA strains isolated from different patients in the neurosurgical ICU during a one-year period, indicating the prevalence of a second clone in the hospital. Because all of the strains were isolated from sputum or tracheal aspirates, the colonisation and failure of measures to control the spread of this pathogen in surgical areas was highly likely. It is reasonable that these antiseptic-resistant strains would be isolated from surgical wards because there is more frequent use of antiseptics in those areas. Increased antiseptic use provides more selective pressure for the incorporation of antiseptic tolerance encoded by the *qacA/B* genes. The acquisition of antiseptic resistance may be one of the important reasons for the survival and transmission of MRSA in these areas. Because these two clusters show > 75% similarity by PFGE, they could be mutants arising from the same clone. MRSA is not as stable as MSSA; therefore recombination is a more frequent event in MRSA. Their similar resistance profiles to various antimicrobials also suggest an identical origin. Further investigation is needed to identify the reservoir of antiseptic-resistant MRSA by examining samples from apparatuses, surfaces in the environment, the hands of medical personnel, disinfectant bottles, etc. The PFGE and spa typing revealed the presence of two different *qacA/B*-positive clones in this hospital. Previous studies have identified spa t037 as an epidemic MRSA clone in other Chinese hospitals (Liu et al., 2009; Pierre-Yves et al., 2007). Therefore, an endemic MRSA clone that expresses *qacA/B* genes is likely a common problem in China, and thus, a national survey is warranted.

The clinical data show that four MSSA strains with *qacA/B* genes may have been community-acquired (CA), although no *pvl* genes were detected by PCR. It is difficult to judge the source of these genes carried by the CA-MSSA. Their presence may be due to the increased selective pressure from biocides that these isolates encounter in the community setting. The increased use of biocides increases the likelihood of the selection of biocide-resistant strains. Another possibility is that purulent samples were collected several days after the patients entered the hospital and that the patients were housed in surgical wards, allowing the horizontal spread of HA-MRSA. All of the MSSA strains were multidrug-resistant (resistant to ≥ two antimicrobial classes), likely due to the selective pressure caused by antiseptics to carry a multidrug-resistant plasmid containing the *qacA/B* genes (Fraise, 2002). Genetic typing showed four *spa* types and three PFGE types. Here, *spa* typing seemed more discriminating than PFGE, although the reverse is usually true at the subtype level. Because *spa* typing is based on the alignment of sequence repeats found in the polymorphic region of the *spa* gene and these repeats are mutated more quickly during evolution, PFGE typing cannot discriminate these tiny mutations (Oliveira et al., 2001).

The results of our study suggest a potential persistence of antiseptic-resistant MRSA epidemic clones in the hospital. The presence of the *qacA/B* genes in clinical *S. aureus* isolates highlights the necessity of rationally employing antiseptics and the importance of implementing specific measures to control MRSA colonisation, including changing or increasing the concentration of disinfectants and antiseptics, more rigorous screening, educating personnel in the ICU and isolating colonised or infected patients.

This study was limited by its relatively small sample size. In addition, these isolates were all collected from a teaching hospital and may not necessarily be representative of the general epidemiology of hospital-acquired infections in China. However, our investigation provides data that will allow different regions or countries to be compared. We are currently collecting more *S. aureus* isolates from other hospitals with the goal of fully characterising the *qacA/B* genes in *S. aureus*. Also, the

presence of the *smr* gene in *S. aureus* strains is reported to be the reason for resistance to quaternary ammonium compounds, although such strains are usually present at a much lower abundance than strains with the *qacA/B* genes. Thus, more strains are needed for further molecular typing.

ACKNOWLEDGEMENTS

We thank Dr. N. Noguchi from the Tokyo University of Pharmacy and Life Science for providing us with the TS77 strain and the National Natural Scientific Fund Committee (NSFC), No. NSFC30973592 for funding the work.

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