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Short report

High prevalence of *qacA/B* carriage among clinical isolates of meticillin-resistant *Staphylococcus aureus* in Malaysia

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SUMMARY

The minimum inhibitory concentrations (MICs) of 60 meticillin-resistant *Staphylococcus aureus* (MRSA) isolates from Malaysia to three antiseptic agents — benzalkonium chloride (BZT), benzethonium chloride (BAC) and chlorhexidine digluconate (CHG) — were determined. All isolates had MICs ranging from 0.5 to 2 mg/L. Antiseptic resistance genes *qacA/B* and *smr* were detected in 83.3% and 1.6% of the isolates, respectively. Carriage of *qacA/B* correlated with reduced susceptibility to CHG and BAC. This is the first report of the prevalence of *qacA/B* and *smr* gene carriage in Malaysian MRSA isolates, with a high frequency of *qacA/B* carriage. The presence of these antiseptic resistance genes and associated reduced susceptibility to antiseptic agents may have clinical implications.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing a wide range of diseases from localized skin infection to life-threatening conditions such as pneumonia and endocarditis.

Control of nosocomial MRSA is a challenge to infection control teams. The use of biocides including antiseptics plays an increasingly important role in maintaining adequate hygiene practices and infection control in hospitals. Recent efforts to improve the quality of infection control procedures in healthcare settings has led to the increased use of disinfectants and antiseptics.

Antiseptics are antimicrobial substances applied externally to destroy or inhibit the growth of micro-organisms in or on living tissue (skin, mucous membrane and wounds). A wide variety of chemical agents including quaternary ammonium compounds (QACs) such as benzalkonium chloride (BAC) and benzethonium chloride (BZT) and cationic biocides such as chlorhexidine digluconate (CHG) are commonly used in antiseptic preparations and have significantly contributed to preventing nosocomial infection in healthcare settings.[1]

Excessive usage of antiseptic agents may result in the emergence of MRSA with reduced susceptibility to antiseptics

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or even antiseptic resistance.[2] *qacA*, *qacB* and *smr* genes can be responsible for reduced susceptibility to certain antiseptic agents.[2–4] *qacA* and *qacB* genes are very closely related and polymerase chain reaction (PCR) results are usually designated as '*qacA/B* positive or negative'.[3] *qacA/B* can be encoded on multi-resistance plasmids from clinical isolates of *S. aureus* and confers reduced susceptibility to a wide range of antimicrobial organic cations, including QACs and biguanidines.[3] The *smr* (staphylococcal multidrug resistance, also known as *qacC/D*) encodes a small protein that belongs to a small multidrug resistance family and confers reduced susceptibility to QACs.[4]

At present, biocides are an integral and essential component of healthcare practice and the emergence of MRSA strains with reduced antiseptic susceptibility is likely to pose a major challenge to hospital infection control teams.

Based on the limited data available on MRSA susceptibility to antiseptics in Malaysia, the current study was performed to investigate the prevalence of the antiseptic resistance genes *qacA/B* and *smr* among the isolates and to assess the efficacy of three different antiseptic agents, CHG, BZT and BAC, against Malaysian MRSA.

Methods

Isolates

Sixty non-consecutive clinical isolates of MRSA were collected from the clinical laboratory of the largest public hospital in Kuala Lumpur, Malaysia, from February to May 2009. The isolates were obtained from pus ($N = 19$), tracheal aspirate ($N = 9$), blood ($N = 9$), nasal swab ($N = 12$), abscess ($N = 6$), and sputum ($N = 5$). Only one isolate was included per patient.

Minimum inhibitory concentration (MIC) determination

All antiseptics CHG, BAC and BZT were purchased from Sigma Aldrich (St Louis, MO, USA). A stock of antiseptics containing 100 mg/L of CHG, BAC and BZT in deionized water was prepared and stored at 4°C. Additional dilutions were prepared in Müller–Hinton broth before each experiment as required.

MICs of antiseptics were determined by the broth microdilution method according to the Clinical and Laboratory Standards Institute.[5] Isolates were considered susceptible to BAC and BZT with an MIC $\leq$3 mg/L[6] and considered susceptible to CHG with an MIC $\leq$1.0 mg/L, and to have reduced susceptibility with an MIC between 1.5 and 3.0 mg/L.[7] Susceptibility tests for the isolates against antiseptics were repeated on different days.

PCR amplification

Total genomic DNA from MRSA isolates were extracted using GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Malaysia), as described by the manufacturer. All isolates were confirmed as MRSA through the detection of the *mecA* gene by PCR. The isolates were screened for the presence of *mecA*, *qacA/B* and *smr* as described previously.[4]

Three amplicons for *qacA/B* (isolates NCM 1, NCM 4 and NCM 10) and one for *smr* (NCM 10) genes from the MRSA clinical isolates were sequenced (First BASE Laboratories, Malaysia) and used as positive control. The sequenced PCR product homology to the published sequences in the GenBank was checked using the BLASTN software, available at http://www.ncbi.nlm.nih.gov.

Statistical analysis

Chi-squared analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) to determine the association between the carriage of genes and the degree of susceptibility towards antiseptics and clinical origin of isolates. $P < 0.05$ was considered statistically significant.

Results

Minimum inhibitory concentrations

All isolates were inhibited by BZT at a concentration of 1 mg/L. Ten (16.6%) MRSA isolates showed susceptibility towards BAC and CHG (MIC: 0.5 mg/L), while 50 (83.3%) were less susceptible to the BAC and CHG (MIC: 2 mg/L). None of the isolates exhibited resistance to any of the antiseptic tested (Table I).

Detection of resistance genes

The *mecA* gene was detected in all 60 isolates (100%), confirming them as MRSA. The *qacA/B* gene was detected in 50 isolates (83.3%), whereas the *smr* gene was amplified in only one isolate (1.6%). The *qacA/B* and *smr* genes were detected concomitantly in 1.6% of the isolates. All isolates that carried *qacA/B* had reduced susceptibility to CHG and BAC ($P < 0.0001$) (Table I). There was no association between the carriage of *qacA/B* and the clinical origin of the MRSA ($P > 0.05$).

The comparison of the sequenced *qacA/B* and *smr* genes to the published sequences showed 100% identity to the corresponding GenBank sequences. One isolate (NCM 1) which

Table I
Frequency of *qacA/B* and *smr* gene carriage, source and susceptibility of the isolates

Antiseptic genes and isolates ($N = 60$)	BAC		BZT	CHG	
	0.5 μg/mL	2 μg/mL	1 μg/mL	0.5 μg/mL	2 μg/mL
qacA/B	0	50	50	0	50
smr	1	0	1	1	0
Pus ($N = 19$)	3	16	19	3	16
Tracheal aspirates ($N = 9$)	2	7	9	2	7
Blood ($N = 9$)	1	8	9	1	8
Nasal swab ($N = 12$)	2	10	12	2	10
Abscess ($N = 6$)	1	5	6	1	5
Sputum ($N = 5$)	1	4	5	1	4

BAC, benzalkonium chloride; BZT, benzethonium chloride; CHG, chlorhexidine digluconate.

showed 98% similarity was submitted to GenBank and is available under accession number JN043515.

Discussion

The treatment and prevention of infections caused by *Staphylococcus aureus*, especially MRSA strains, has become more difficult due to their ability to acquire resistance to major groups of antimicrobial agents. This has contributed in part to the ability of MRSA to persist in healthcare environments and transmit among patients. Therefore, the use of contact precautions including standard hygiene and disinfection is crucial to reduce cross-transmission of this pathogen. Considerable efforts have been made in recent years to improve the quality of infection control procedures including the increased use of disinfectants and antiseptics. Many studies have investigated antimicrobial resistance in MRSA, but relatively few have evaluated antiseptic susceptibility. Thus, the surveillance of antiseptic susceptibility in clinical isolates of MRSA could provide useful information to support infection control and prevention of nosocomial infection.

We examined the antiseptic susceptibility and distribution of antiseptic resistance genes in MRSA isolated in Malaysia. The MICs of the three antiseptics tested for 60 MRSA isolates were <3 mg/L and all the isolates tested were inhibited at MIC values ranging between 0.5 and 2.5 mg/L, which is lower than the actual in-use concentration of each antiseptic (5000, 2000 and 1000 mg/L for CHG, BAC and BZT respectively).[1] Our findings are in agreement with a previous study showing that CHG and QACs have comparable efficacy against MRSA.[8] Hence, the antiseptics commonly used in the hospital environment should be effective against clinical isolates of MRSA if used at recommended in-use concentrations. However, a significant association was identified between the presence of *qacA/B* genes and degree of susceptibility to CHG and BAC ($P < 0.001$) (Table I). This means that isolates carrying *qacA/B* may be able to persist on the skin where concentrations of disinfectants may be lower than in-use concentrations.

The high rate of carriage of *qacA/B* (83.3%) and relatively low rate of carriage of *smr* (1.6%) is in accordance with some studies but in contrast to others. For example, *qacA/B* was carried by 45.9% of isolates in a study from Japan, 62.6% in a European study, and 61.1% in a Chinese study.[2,4,9] The rate of 1.6% of *smr* gene carriage in our study is lower than the 3% reported in Japan and 6.4% in Europe.[4,9]

In conclusion, this is the first time that the carriage rate of *qacA/B* and *smr* gene has been reported for Malaysian MRSA isolates. The presence of these antiseptic resistance genes is potentially a serious concern. The findings of the present study emphasize that the carriage of *qacA/B* is associated with reduced susceptibility, albeit in the susceptible range. Continuous monitoring to ensure proper usage of antiseptics in the hospital is recommended together with continued surveillance of resistance gene carriage.

Conflict of interest statement
None declared.

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