The presence of *qacA/B* gene in Brazilian methicillin-resistant *Staphylococcus aureus*

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A total of 74 methicillin-resistant Staphylococcus aureus *(MRSA) strains isolated from three government hospitals in 2002 and 2003 were examined concerning the distribution of* qacA/B *gene, which is the determinant of resistance to quaternary ammonium compounds largely employed in hospital disinfection. By polymerase chain reaction the* qacA/B *gene was found in 80% of the isolates, which is a significant result considering it is the first time that* qacA/B *gene is being reported for Brazilian MRSA strains and it is presented at a high rate.*

Key words: methicillin-resistant *Staphylococcus aureus* - quaternary ammonium compounds - *qacA/B* gene

The methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which characterize for being resistant to multiple antibiotics, have become one of the most important nosocomial pathogens causing considerable morbidity and mortality in hospitals (Hiramatsu et al. 2001). Clinical isolates of MRSA carry the *mecA* gene that codes for a penicillin-binding protein (PBP) referred to PBP2a or PBP2', an inducible 78-kDa PBP. In susceptible strains there is no *mecA* gene homolog (Chambers 1997). The transmission of some important nosocomial pathogens including MRSA has been linked to environmental contamination and since outbreaks of hospital infections due to MRSA first became a serious problem, counter measures have concentrated on the prevention of infection rather than on medical treatment (Noguchi et al. 1999).

One of the essential measures of hospital infection control is the use of disinfectants for inanimate objects and surfaces, and antiseptics for topical living tissue applications (McDonnell & Russell 1999). Disinfectants and antiseptics containing quaternary ammonium compounds (QAC) are extensively used in health care settings, and some data suggest that MRSA strains may exhibit decreased susceptibility to QAC by *qac* determinants, which confer resistance through efflux pumps that are membrane-bound, proton-motive force-dependent cation export protein (Chapman 2003). In clinical staphylococci, *qac* resistance determinants have been detected on plasmids carrying various antibiotic resistance genes. The large numbers of chemotherapeutic agents employed into clinical practice resulted in the development and spread of antibiotic resistance determinants among bacterial populations. Concerns have arisen regarding the potential emergence of cross-resistance and co-resistance between widely used disinfectants and antibiotics (Noguchi et al. 1999).

The purpose of the present study was to investigate the *qacA/B* determinant distribution among 74 clinical MRSA isolated from Hospitals RJ-A, RJ-B, and RJ-C located in Rio de Janeiro during the years of 2002 and 2003.

To identify the isolates, after Gram staining, coagulase and catalase detection, the staphylococci were characterized to the species level on the basis of a APIStaph kit (bioMérieux, France).

Total genomic DNA of the microorganisms in this study was extracted using Dneasy Tissue Kit (Qiagen Inc., Valencia, CA). The MRSA strains were confirmed through the presence of *mecA* gene by polymerase chain reaction (PCR) technique that is so far regarded as the gold standard method for the detection of methicillin resistance. The nucleotide sequences of the primers used for this detection are listed in the Table and *S. aureus* ATCC 25923 and ATCC 33591 were used respectively as negative and positive control organisms for *mecA* gene in PCR. The *mecA* gene was detected in all 74 isolates (100%), which confirms that they were MRSA.

Concerning to the detection of *qacA/B* gene the nucleotide sequences of the primers are listed in the Table. The *qacA/B* gene was found in 59 (80%) of the 74 MRSA and the distribution rates at the hospitals were: 100% at Hospital RJ-A and Hospital RJ-C, and 42% at Hospital RJ-B. Some of the *qacA/B*-PCR products are showed at the Figure. In order to confirm the *qacA/B*-PCR results, we performed the comparison of the nucleotide sequences of eight bacterial strains with the nucleotide sequence available at GenBank no. X56628. The sequences obtained from the *qacA/B*-PCR were determined by the dideoxy chains-termination method with dye-labeled terminations and T7 DNA polymerase (Applied Biosystems, Fosters City, CA, US) according to manufacturer instructions, followed by analysis with an

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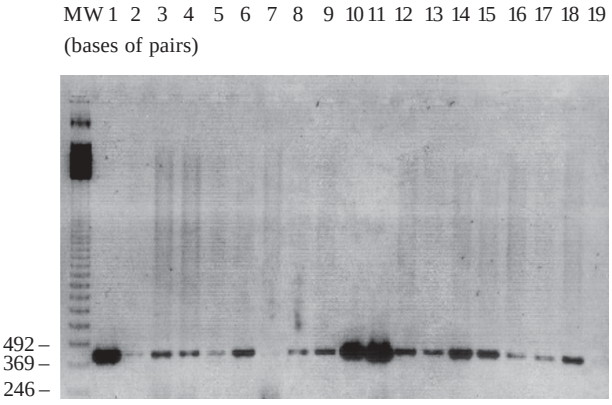
TABLE
Primers used for polymerase chain reaction (PCR)

Gene	Primer	Primers sequences (5'- 3')	Location at the gene	PCR product size (bp)	References
mecA	mecA-R	ACTGCTATCCACCCTCAAAC	1182-1201	163	Mehrotra et al. (2000)
	mecA-F	CTGGTGAAGTTGTAATCTGG	1325-1344		
qacA/B	qacA/B-R	CTATGGCAATAGGAGATATGGTGT	1801-1824	417	Mayer et al. (2001)
	qacA/B-F	CCACTACAGATTCTTCAGCTACATG	2193-2217		

bp: bases of pairs.



The polymerase chain reaction products of *qacA/B* gene of some methicillin-resistant *Staphylococcus aureus* isolates.

ABI Prism 3100 automated sequence apparatus (Applied Biosystems). Nucleotide sequences were aligned with the clustal X program version 1.8/22. The comparison showed the identity of 100% for six of the eight isolates and the other two isolates showed 99% of identity confirming they all harbored that gene, and both nucleotide sequences were submitted to GenBank available under accession numbers EF418547 and EF418548.

The rate of 80% of *qacA/B* in 74 Brazilian MRSA isolates is much higher than 45.9% found in Japan by Noguchi et al. (2006), who performed their studies using 207 MRSA strains collected between 1999 and 2004, and it is even higher than 63% found in Europe by Mayer et al. (2001) in 297 MRSA strains isolated between 1997 and 1999.

We underline the importance of our result since it is the first time that the presence of *qacA/B* is being reported for Brazilian MRSA strains as well as in so high rate. We call attention to the fact that if the *qacA/B* gene is present at 80% of those MRSA, carefulness of controlling their survival in the hospital environment must be considered since the high prevalence of *qacA/B* gene might be either due selective pressure imposed by the large use of QACs disinfectant or due the cross-resistance and co-resistance between QACs and antibiotics (Sidhu et al. 2002). Moreover it is necessary to have trained staff and use the best management practices for disinfecting, employing only the amount of disinfectant necessary to do the job to ensure the killing of microorganisms. Ultimately, best management practices protect patients, employees and the environments.

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