Short Communication

Prevalence of Mupirocin Resistance in Methicillin-Resistant
*Staphylococcus aureus* Strains Isolated from a Malaysian Hospital

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**SUMMARY:** Mupirocin is used topically to treat skin infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA). One hundred eighty-eight strains (isolated in 2003, 2004, 2007, and 2008) were tested for mupirocin susceptibility using disk diffusion method and minimum inhibitory concentration (MIC). Mupirocin resistance was detected in 10 (5%) strains with 2 of them showing MIC of 256 mg/L. PCR detection using gene-specific primers showed that all 10 mupirocin-resistant strains harbored *ileS2* gene whereas *mupA* gene was detected in 2 mupirocin-resistant strains with MIC of 256 mg/L. Amplification of *agr* grouping and SCCmec typing showed that all 10 strains were *agr* group I and SCCmec type III. Sequence analysis of region X of the *spa* gene yielded 4 distinct *spa* types (t037, t363, t421, and t6405) which were clonally related. In conclusion, the rate of mupirocin resistance in Malaysia is still low but is much higher than previous reports in Malaysia.

Mupirocin (pseudomonic acid A) is a topical antimicrobial agent used for treatment of superficial skin infections (1). This topical drug binds competitively to bacterial isoleucyl-tRNA synthetase (IRS) and inhibits protein synthesis (1). High level resistance to mupirocin is often associated with the acquisition of *mupA* gene while low level resistance to mupirocin is due to mutation in endogenous bacterial IRS (2).

The increase usage of this agent has led to the rapid emergence of mupirocin-resistant strains in some parts of the world (3). This mupirocin drug has been used in Malaysian hospitals since 1998 and the only report on mupirocin resistance (1.25%) in Malaysia was reported by Norazah et al. (4). However, in the University Malaya Medical Centre (UMMC), mupirocin is still of limited use as it is only recommended for outpatients and not for inpatients. However, this drug is used for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in medical staff for a period of 5 days and then retested on the 7th day to ensure clearance.

SCCmec typing is used to analyze a mobile genetic element named staphylococcal cassette chromosome * mec* (SCCmec) which contains the *mecA* gene (5). The accessory gene regulator (agr) controls the coordinated production of virulence factor in *Staphylococcus aureus* (6). *spa* typing which involves the sequencing of part of the staphylococcal protein A (*spa*) gene, is known for tracking epidemic strains of *S. aureus* (7).

The purpose of this study was to determine the prevalence of mupirocin resistance for 188 nonrepeat MRSA strains (52 in 2003, 9 in 2004, 16 in 2007, and 111 in 2008) isolated from inpatients who stayed in UMMC during 2003–2004 and 2007–2008 (Table 1). UMMC is a 980-bed referral and premier teaching hospital in Malaysia which has surgical orthopedic, pediatric, medical, obstetrics and gynecology, general intensive care units, psychiatry, pediatric and neurology intensive care units.

The characteristics of the mupirocin-resistant strains were further analyzed in terms of their resistance level, SCCmec typing, *agr* grouping, and *spa* typing.

Antimicrobial susceptibility tests were carried out by disk diffusion (mupirocin 5 μg; Oxoid, Hampshire, UK) on Mueller-Hinton agar plates according to the Clinical and Laboratory Standard Institute (8). A strain is considered susceptible to mupirocin if the inhibition zone is ≥14 mm and resistant if the inhibition zone ≤13 mm (9).

The MICs of mupirocin was determined using the agar dilution method (8). The strain was considered susceptible to mupirocin if MIC was ≤4 mg/L (9). The breakpoints for mupirocin were defined as follows: high level resistance, MIC, ≥512 mg/L and low level resistance, MIC, 8–256 mg/L (4). *S. aureus* ATCC25923 was used as the control for susceptibility testing.

PCR detection of *mupA* and *ileS2* genes was done using DNA templates prepared from direct boiled-cell lysate and extracted plasmid DNA. The PCR primers used were as described earlier (10,11). SCCmec typing, *agr* grouping, and *spa* typing were done as previously described (7,12,13). The amplicons of *mupA*, *ileS2*, and *spa* were purified by a commercial kit (Qiagen, Hilden, Germany) and sequenced to validate their identities.

Of 188 MRSA strains tested, only 10 (5%) were mupirocin resistant. The other 178 strains (95%) were...
sensitive to mupirocin at MIC ≤ 4 mg/l. There was no history of previous mupirocin use in these 10 patients. The mechanism of acquisition of mupirocin resistance in these 10 strains remains unknown and needs further study. Two strains (MRSA0406-8 and MRSA0801-27) showed mupirocin resistance of 256 mg/l while the MIC for the other 8 mupirocin-resistant strains ranged from 8–32 mg/l (Table 2). Overall the mupirocin-resistance rate was relatively higher than the 1.25% rate previously reported by Norazah et al. (4). Similarly, this resistant rate was relatively higher than the 2% mupirocin rate reported in the United States (14). However, the resistant rate reported in Malaysia was lower compared to earlier studies reported in Canada between 1995–2004 (15), Korea in 2006 (16), and Trinidad in 2008 (17).

The mupA gene product (1.65 kb) was detected in 2 mupirocin-resistant strains (MIC, 256 mg/l) while ileS2 gene product (456 bp) was detected in all 10 mupirocin-resistant strains using boiled-cell lysate and plasmid DNA as templates (Figs. 1A–C, Table 2). Sequence analysis of the amplified products indicated 100% homology with the respective gene sequences (mupA and ileS2) in the NCBI database. Both mupA and ileS2 genes have been shown to be exclusively associated with high level mupirocin resistance (1,18). However in this study, both mupA and ileS2 genes were also detected in strains with low level mupirocin resistance and were plasmid borne.

All 10 mupirocin-resistant strains were SCCmec type III and agr group 1. This is not surprising as SCCmec type III strains are often associated with hospital infections (19), while agr group distribution is often correlated with the genetic background of the strains (6).

Sequence analysis of region X of the spa gene gave four distinct spa types (Table 2, Fig. 2). The most frequent spa type was t037 (60%). All 4 mupirocin strains isolated in 2004 shared the same spa type (t037) with strains isolated in 2003 and 2007, indicating the persistence of a particular genotype in the UMMC hospital environment, even though these were isolated from different patient wards and different sources. All strains isolated in 2008 were of three different spa types (t6405, t363, and t421) and were only 98% related to strains isolated from the previous years (Fig. 2).

The emergence of mupirocin-resistant strains signals the potential loss of the use of this drug against MRSA. Vancomycin is still the single most active agent against MRSA infection. The rise in mupirocin resistance should be controlled through appropriate infection control procedure.
Fig. 1. Representative agarose gel of PCR-amplified products using different mupirocin specific primers for MRSA strains. (A) Amplicons with primers for ileS2 gene with crude DNA and plasmid DNA as template. Lanes 1 and 14, 100-bp DNA ladder; lanes 2–10, positive control, crude DNA MRSA0312-35, crude DNA MRSA0402-21, negative control, crude DNA MRSA0402-8, plasmid DNA MRSA0312-35, plasmid DNA MRSA0402-21, plasmid DNA MRSA0402-8, crude DNA MRSA0801-27; lanes 11–13, negative control. (B) Amplicons with primers for mupA gene with crude DNA as template. Lane 1, 1-kb DNA ladder; lanes 2 and 7, 100-bp DNA ladder; lanes 3–6, positive control, MRSA0406-8, MRSA0801-27, negative control. (C) Amplicons with primers for mupA gene with plasmid DNA as template. Lane 1, 100-bp DNA ladder; lanes 2–4, plasmid DNA MRSA0406-8, plasmid DNA MRSA0801-27, negative control.

Fig. 2. Dendrogram of MRSA mupirocin-resistant strains based on spa types. Cluster analysis was based on unweighted pair-grouped method with arithmetic mean algorithm (UPGMA).

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Conflict of interest None to declare.

REFERENCES


