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Molecular typing and antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* isolates from clinical samples in Malaysia: An update

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Abstract. A total of 120 non-consecutive MRSA isolates were obtained from hospitalized patients at Hospital Kuala Lumpur, Malaysia. All isolates were subjected to antimicrobial susceptibility tests and genotyping based on staphylococcal cassette chromosome *mec*(SCC*mec*), *Staphylococcus aureus* protein A typing (*spa*) and multilocus sequence typing (MLST). Vast majority of MRSA isolates were resistant to more than three classes of antibiotics. Five antibiotic resistance profiles were observed among the MRSA isolates. All isolates tested were still susceptible to vancomycin. Genotyping revealed isolates are highly clonal, where all MRSA belonged to the predominant Asian clone ST239 comprising 4 *spa* types. *Spa* typing revealed four different *spa* types. Continuous monitoring and effective therapeutic options for Asian MRSA clone is recommended.

INTRODUCTION

Staphylococcus aureus is a major Gram-positive pathogen that is capable of causing wide range of infectious diseases such as soft tissue infection, pneumonia, and sepsis. The prevalence of invasive *S. aureus* infections dramatically decreased following introduction of penicillinase-stable penicillins. The introduction of these antibiotics has also contributed to the emergence of methicillin-resistant *S. aureus* (MRSA) strains and increasing numbers of MRSA have been isolated worldwide. Alarmingly these strains are also frequently resistant to other antibiotic classes and they require treatment with second- or third-choice medicines that may be less effective, more toxic and more expensive. Over the last three decades, rates of infection with MRSA have risen rapidly in hospitals worldwide (Russell *et al*., 2009). MRSA is still endemic in most hospitals in the world and accounts for 40-60% of all nosocomial *S. aureus* infections (Campanile *et al*., 2009).

A detailed knowledge on the susceptibility and molecular background of MRSA are necessary for optimal patient management and that facilitates the development of effective strategies to combat the growing problem of resistance.

Elucidation of molecular properties of MRSA has been greatly facilitated by the techniques of molecular epidemiology

typing. Currently, a wide variety of genotype-based typing methods are available for classifying MRSA isolates for epidemiological studies. Staphylococcal cassette chromosome *mec*(SCC*mec*), multi locus sequence typing (MLST) and protein A typing (*spa*) are important typing tool to determine the genetic relatedness of MRSA strains.

The aim of this study was to investigate the genotypes and antibiotic resistance profiles of MRSA isolated from clinical samples in Malaysia.

MATERIALS AND METHODS

Isolates

A total of 120 MRSA isolated from different anatomical sites were obtained from the Medical Microbiology Laboratory, Hospital Kuala Lumpur Figure 1. The isolates were reconfirmed by Gram staining, yellow colonies on mannitol salt agar, coagulase slide and catalase tests. The resistance to methicillin was determined by disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2009) and PCR detection of the *mecA* gene as described earlier (Noguchi *et al*., 2006).

Antibiotic susceptibility

Antimicrobial susceptibility tests were performed by disc diffusion method as recommended by the CLSI (CLSI, 2009). Thirteen antimicrobial agents were tested which include oxacillin (1µg), cefoxitin (30µg), penicillin (1µg), ampicillin (10µg), amoxicillin (1µg), cotrimoxazole (25µg), cephalothin (30µg), erythromycin (15µg), clindamycin (2µg), mupirocin (5µg), rifampicin (30µg) and vancomycin (30µg). *S.aureus* ATCC 25923 was used as the control strain. The resistance or susceptibility of the isolates to the antibiotic discs was determined according to the CLSI standard (CLSI, 2009). Susceptibility to fusidic acid was interpreted following guidelines by the British Society for Antimicrobial Chemotherapy (British Society for Antimicrobial Chemotherapy, 2011).

SCC*mec* Typing

SCC*mec* types were determined using the multiplex PCR strategy described previously (Ghaznavi-Rad *et al*., 2010).

Spa Typing

Amplification of the *spa* repeat region was performed using primers with the following sequence *spa*-f (5-AAAGACGATCCTTC GGTGAGC-3) and *spa*-r (5-CAGCAGTAGT GCCGTTTGCTT-3) as previously described (Harmsen *et al*., 2003). PCR products were purified using the GF-1 PCR clean up kit (Vivantis, Malaysia) according to manufacturers' instruction and sequenced commercially (First base Laboratories Sdn

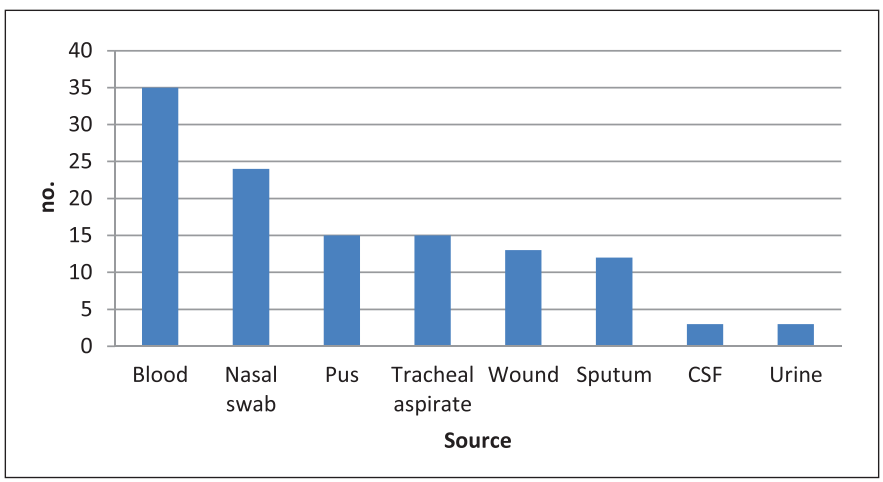

Figure 1. MRSA isolates from different clinical sources.

Bhd). The obtained sequences were subjected to *spa* repeat analysis. *Spa* types (t) were determined by using the Ridom Staph Type software (Ridom GmbH, Germany), which automatically detects *spa* repeats and assigns a *spa* type. By applying the recently developed algorithm BURP (Based Upon Repeat Patterns), *spa* types were clustered into different groups and the clonal complexes (*spa* CC) were automatically assigned by this software.

Multi locus sequence typing (MLST)
MLST was performed as described previously (Enright *et al.*, 2000). The PCR products of seven housekeeping genes were purified and sequenced. The sequence of each house keeping gene was entered into the MLST website (http://saureus.mlst.net), where seven numbers depicting the allelic profiles were assigned which defined the sequence type (ST).

RESULTS

Bacterial identification
All isolates were coagulase and catalase positive with typical *S. aureus* colony morphology of large, round, creamy-golden colonies on MSA plate. The *mecA* gene was detected in all isolates tested.

Antibiotic susceptibility test
Antimicrobial susceptibility testing revealed five resistant profiles among the MRSA isolates (Table 1). All isolates (100%) were resistant to the β-lactam antimicrobial agents; oxacillin, cefoxitin, penicillin, amoxicillin and cephalothin. In addition majority of the isolates also showed resistance to other antibiotic agents such as erythromycin (100%), cotrimoxazole (98.3%), clindamycin (88.3%), rifampicin and mupirocin (6.7%).

Multi-resistance (defined as resistance to three or more antibiotics classes other than β-lactams) was observed in 23 (19.2%) MRSA isolates.

SCC*mec* typing
As all isolates in this study were characterised as HA-MRSA, only three SCC*mec* types I-III were investigated. The SCC*mec* type III was detected in all (100%) isolates.

***Spa* and MLST typing**
A total number of four different *spa* types were detected. Vast majority (n=108) were typed as t037, 10 MRSA isolates as t041, one each as t032 and t138. The BURP algorithm used to group isolates into definite clonal lineages clustered into two cluster groups, Cluster 1: *spa*-CC 030/037 (t037, t138 and t421) and cluster 2: *spa*-CC 790 (t032). For MLST typing, all isolates belonged to ST239.

DISCUSSION

The prevalence and growing rates of MRSA in hospitals are becoming a global problem in many parts of the world including Malaysia. In the present study, the MRSA isolates were evaluated phenotypically for antibiotic resistance to obtain vital information that could enhance better strategy for prevention and treatment of MRSA in patients. All MRSA isolates were

Table 1. Antibiotics resistance profiles of MRSA isolates

No.	Resistance Profiles	*n* (%)
1	OXA FOX AMX AMP CEP PEN TS ERY	97 (80.8)
2	OXA FOX AMX AMP CEP PEN TS ERY	14 (11.7)
3	OXA FOX AMX AMP CEP PEN TS CLI ERY MUP RIF	6 (5)
4	OXA FOX AMX AMP CEP PEN CLI ERY MUP RIF	2 (1.7)
5	OXA FOX AMX AMP CEP PEN TS CLI ERY	1 (0.8)

resistant to all β-lactam antibiotic agents including oxacillin, cefoxitin, penicillin, amoxicillin, ampicillin and cephalothin. This finding is in agreement with well-known feature of MRSA which has been considered to be resistant to all β-lactam antibiotics independent of the susceptibility testing result (Sader *et al*., 2008).

Resistance to β-lactam in MRSA is related to a chromosomal *mecA* gene that codes the production of an abnormal penicillin binding protein called PBP2a. Penicillin-binding proteins are membrane-bound enzymes, which targets all β-lactam antibiotics. PBP2a has a decreased affinity for binding β-lactam antibiotics resulting in resistance not only to methicillin but also to all β-lactams antibiotics (Johnson, 2011).

In contrast to study with very low resistant rates for cotrimoxazole (Dibah *et al*., 2014), we found high resistance (98.3%). It is higher than (83%) previously reported results from Malaysia (V Neela, 2008). Similar results have been s reported elsewhere (Frazee *et al*., 2005).

The macrolide-lincosamide-streptogramin B (MLSB) families of antibiotics are chemically distinct, but have similar inhibitory effects on bacterial protein synthesis by binding to the 50S ribosomal subunits. Erythromycin (a macrolide) and clindamycin (a lincosamide) are commonly used for treatment of *S. aureus* infections (Eksi *et al*., 2011; Weisblum, 1995). Our study revealed high resistance to widely used non β-lactam antibiotic erythromycin in MRSA isolates. Macrolides resistance in MRSA demonstrated that methicillin resistance leads physicians to use different macrolides, mainly erythromycin and lincosamides, such as clindamycin and lincomycin which facilitate development of different MLSB phenotypic patterns, and which mostly end with resistance to macrolides, lincosamide and streptogramin B (MLSB). This reason may explain the increased prevalence of MLSB in geographical area with high prevalence of MRSA, and vice versa (Adaleti *et al*., 2010).

Rifampicin has excellent activity against MRSA infections but cannot be used as a single agent to treat such infections and commonly used in combination with other antibiotics to prevent the emergence of resistance during therapy (Moellering, 2008). The MRSA isolates in this study showed low resistance rate towards this agent. The overall rifampicin resistance rate in Malaysia (6.7%) was relatively close to the 5% reported previously from Malaysia (Norazah, 2002). Similar resistance rate was observed for mupirocin was also close to 5% reported previously from Malaysia (Lim *et al*., 2010).

The use of rifampicin in treatment of MRSA infection should be controlled even in combination with other agents. The recent study reported that rifampicin resistance may occur in clinical practice despite combinations with minocycline, fusidic acid or vancomycin. Therefore, the development of resistance is not prevented completely even when used in combination with other antibiotics (Neogi *et al*., 2009).

Among the antibiotics tested, rifampicin and mupirocin are two still valuable agents, but resistance has already been described. Fusidic acid, erythromycin, clindamycin and cotrimoxazole is not much recommended for treatment of MRSA infections as the percentage of susceptible isolates is very low. The presence of Brazilian clone ST 239 is in accordance with a recent report showing that this clone is currently the most prevalent MRSA clone in Malaysia (Neela *et al*., 2010). As they are clonal, the effective control to prevent spread of this clone will reduce the resistance and infection rates in hospital (Udo and Jacob, 2000).

In conclusion, the discovery and development of new antimicrobials, although necessary, is unlikely to solve the problem of drug resistance in long run. New technologies that lead to improved and more rapid diagnostics, a better understanding of the pathogenesis of staphylococcal disease and non-antimicrobial approaches to the prevention and treatment of infection will also be needed to forestall post-antibiotic era (Chambers and DeLeo, 2009). Hence, the attempts to control the spread of MRSA in hospitals should continue, with reinforcement of hygienic precautions and infection control measures. Hospitals also should develop policies to restrict the use of anti-

biotics and establish monitoring systems with at least *spa* typing technique to rapidly identifyepidemics and determination of circulating and new emerging clones that will lead to improved prevention and treatment strategies.

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