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Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse

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ABSTRACT

Despite the association of methicillin-susceptible *S. aureus* (MSSA) with several life-threatening diseases, relatively little is known about their clinical epidemiology in Malaysia. We characterized MSSA isolates ($n = 252$) obtained from clinical and community (carriage) sources based on *spa* sequencing and multilocus sequence typing (MLST). The prevalence of several important virulence genes was determined to further define the molecular characteristics of MSSA clones circulating in Malaysia. Among the 142 clinical and 110 community-acquired MSSA isolates, 98 different *spa* types were identified, corresponding to 8 different *spa* clonal clusters (*spa*-CCs). In addition, MLST analysis revealed 22 sequence types (STs) with 5 singletons corresponding to 12 MLST-CCs. Interestingly, *spa*-CC084/085 (MLST-CC15) ($p = 0.038$), *spa*-non-founder 2 (MLST-ST188) ($p = 0.002$), and *spa*-CC127 (MLST-CC1) ($p = 0.049$) were identified significantly more often among clinical isolates. *spa*-CC3204 (MLST-CC121) ($p = 0.02$) and *spa*-CC015 (MLST-CC45) ($p = 0.0002$) were more common among community isolates. Five dominant MLST-CCs (CC8, CC121, CC1, CC45, and CC5) having clear counterparts among the major MRSA clones were also identified in this study. While the MSSA strains are usually genetically heterogeneous, a relatively high frequency (19/7.5%) of ST188 (t189) strains was found, with 57.8% of these strains carrying the Panton–Valentine leukocidin (PVL). Analysis of additional virulence genes showed a frequency of 36.5% and 36.9% for *seg* and *sei* and 0.8% and 6.3% for *etb* and *tst* genes, respectively. Arginine catabolic mobile element (ACME) was detected in 4 community isolates only. These represent the first isolates harbouring this gene in an Asian region. In conclusion, MSSA from the Malaysian community and their clinical counterparts are genetically diverse, but certain clones occur more often among clinical isolates than among carriage isolates and vice versa.

Introduction

Staphylococcus aureus is a leading human pathogen associated with high rates of hospital-acquired infections worldwide (Faria et al., 2008). It is capable of causing a variety of skin and soft tissue infections and debilitating or even fatal diseases, such as pneumonia and septicaemia. In addition, it can also produce toxins that cause toxin-mediated afflictions such as toxic shock syndrome or food intoxications. *S. aureus* bacteraemia is associated with substantial morbidity and mortality worldwide (Kaech et al., 2006). Apart from being an established nosocomial pathogen, *S. aureus* is also a well-known human colonizer and is frequently isolated from the anterior nares of a large proportion of the healthy human population with a prevalence rate of approximately 30% (van Belkum et al., 2009b). Nasal carriage of *S. aureus* plays a key role in the epidemiology and pathogenesis of staphylococcal infection (Weidenmaier et al., 2004; Wertheim et al., 2005).

Treatment of *S. aureus* infections is often complicated, among others due to the emergence of methicillin-resistant *S. aureus* (MRSA) strains and resistance to other classes of antibiotics. Molecular epidemiological analyses of MRSA isolates have demonstrated that only a relatively limited number of MRSA clones are responsible for epidemic spread (Aires de Sousa et al., 2003; da Silva Coimbra et al., 2003). Because of its pathogenic potential and the complexity of its treatment, MRSA has received more attention

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than its methicillin-susceptible counterpart (MSSA). The limited number of detailed molecular characterization studies involving MSSA shows that there is clear and obvious genetic diversity among MSSA strains (Aires de Sousa et al., 2005; Goering et al., 2008). However, also among MSSA, regionally predominant clones have been described (Melles et al., 2004). A recent study from the United States showed a predominance of sequence type 8 (ST8) among MSSA, whereas other strain collections revealed genetical heterogeneity (Miller et al., 2007). Similar studies from a Swiss university hospital as well revealed genetic diversity among MSSA strains (Fenner et al., 2008). In a previous analysis of over 1000 MSSA strains from The Netherlands, evidence was provided that essentially any MSSA genotype carried by humans can transform into a life-threatening human pathogen, but that certain clones may be more virulent than others (van Belkum et al., 2009a). The knowledge on the epidemiology of MSSA is lagging behind as compared to the expertise on MRSA epidemiology (Chaves et al., 2005). In the present study, our goal was to asses the molecular-epidemiological relationships among MSSA isolated from clinical and community sources in Malaysia.

Materials and methods

Bacterial isolates

A total of 268 MSSA isolates including 158 from clinical and 110 from community sources were used in this study.

One hundred and fifty-eight non-repetitive clinical MSSA isolates isolated from patients admitted at Hospital Kuala Lumpur from January to June 2008 were obtained. Hospital Kuala Lumpur is the largest government tertiary referral hospital with 81 wards and 2502 beds. Four to five thousand clinical *S. aureus* isolates have been obtained annually in this hospital 55–60% of which are MSSA while 40–45% are MRSA. The strains were isolated from different infection sites and from 8 different wards including general medicine, general surgery, paediatrics, orthopaedic surgery, neurosurgery, urology, nephrosurgery, maternity wards, and intensive care units. Demographic and culture data such as age, gender, ethnicity, ward, site of isolation, type of sample were all recorded. All isolates previously species-identified by the hospital laboratory were reconfirmed in our research laboratory stationed at Universiti Putra Malaysia by standard methods including Gram staining, catalase production, tube coagulase testing, mannitol testing, and species-specific PCR (Martineau et al., 1998).

One hundred and ten MSSA isolates isolated upon ethical clearance from nasal swabs obtained from different groups of healthy individuals with no risk factors for hospital-acquired MRSA or MSSA were investigated in this study. The subjects included aborigines ($n = 33$), undergraduate students and staff ($n = 31$), kindergarten children ($n = 18$), and village people ($n = 28$) (Neela et al., 2009).

spa typing and MLST

Chromosomal DNA was extracted using the DNeasy Kit (Qiagen Inc.) according to the manufacturer's instructions. All MSSA isolates, irrespective of their sources, were subjected to *spa* typing according to the method of Shopsin et al. (1999) using the primers SpaF (AGACGATCCTTCGGTGA) and SpaR (CAGCAGTAGTGCCGTTTG). The amplified *spa* gene fragment was purified and sequenced. *spa* types were assigned by using StaphType software (version 1.5; Ridom GmbH, Würzburg, Germany), as described by Harmsen et al. (2003). By application of the BURP algorithm implemented by the software, *spa* types with more than 5 repeats were clustered into different groups, with the calculated cost between members of a group being less than or equal to 4 (Strommenger et al., 2008).

MLST was carried out to determine the sequence types (STs) of local clinical and community MSSA isolates. At least one representative of each *spa* lineage isolate was subjected to MLST. Hundred and six strains were selected for MLST. STs and clonal clusters (CC) were assigned using the *S. aureus* MLST database (www.mlst.net) hosted by Imperial College in London.

PCR-based assays for the detection of virulence factors of S. aureus

Genes for 4 different categories of virulence factors were screened. These included cytolytic toxin genes such as *pvl* (Faria et al., 2008), superantigen genes which included *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *tsst*, *eta*, and *etb* (Ellington et al., 2008), genes encoding adhesins such as *cna* (Aires de Sousa et al., 2003), fnbA (Woods et al., 1992), *ica*, and *icaD* (Genestier et al., 2005), and the newly identified arginine catabolic mobile element (ACME) gene cluster that is involved in bacterial growth and development (Diep et al., 2006; Ellington et al., 2008). Multiplex PCR-based protocols for allotyping of *agr* classes I–IV were performed as described elsewhere (Layer et al., 2006).

Data analysis

The method used for two-dimensional clustering (Fig. 1) of the *S. aureus* isolates (based on the presence or absence of the different virulence factors and *agr* types) was agglomerative (successive) hierarchical. This was performed using the unweighted pair group method with arithmetic mean (UPGMA). The similarity metric used was Tanimoto (Spotfire DecisionSite 7.2; Spotfire), which defines similarity for binary data (0 and 1) based on the number of positive attributes that 2 records have in common. The resulting dendrogram was ordered by average value.

Statistical analyses were performed with SPSS version 14 software (SPSS Inc., Chicago, IL, USA). Categorical variables between 2 groups were compared by means of the Chi-square or Fisher's exact tests if 20% of the expected values were <5. $p < 0.05$ indicated statistical significance.

Results

Demographic characterization of clinical and community MSSA strains

Clinical MSSA

During the study period from January to June 2008, 158 MSSA causing various infections were obtained from Hospital Kuala Lumpur. As the patient history was not available for 16 isolates, only 142 isolates were subjected to further demographic investigations. The frequency of MSSA isolates obtained from various samples/anatomical sites were as follows: wound 44 (31.0%), skin 29 (20.4%), tissue 15 (10.6%), abscess 8 (5.6%), blood 6 (4.2%), urine 6 (4.2%), and others 34 (23.9%). The majority of the strains were isolated from patients admitted to general medicine wards 36 (25.4%) and general surgery 35 (24.6%), while the others where from paediatrics 22 (15.5%), orthopaedic surgery 22 (15.5%), neurosurgery 10 (7.0%), urology/nephrology surgery 6 (4.2%), maternity 5 (3.5%), intensive care units 3 (2.1%), and unknown 3 (2.1%). Among 142 isolates for which age data were available, 11.9%, 70%, 11.1%, and 6.8% were isolated from elderly (>65 years), adults (16–65 years), children (1–16 years), and infants (0–1 year), respectively. Most isolates were obtained from patients in the adult category with a median age of 40 years. The male-to-female patient ratio was equally divided (50%/50%). When stratified according to the 3 major