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Short Communication

Trends on Isolation of *Staphylococcus aureus* and Distribution of the Virulence Genes in Hospital Universiti Sains Malaysia, Kelantan

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ABSTRACT

Staphylococcus aureus is one of the most common pathogens isolated from clinical specimens in many hospitals worldwide. The objectives of the present study was to determine the trends of the *S. aureus* isolated from clinical specimens and to detect the virulence genes of *S. aureus* namely *mec*A, *LukS*, *can*, *ica*A, *SdrE* and *hlg*. Data of *S. aureus* isolation in Hospital Universiti Sains Malaysia (HUSM) from 2002-2014 which is available in our WHONET system was analyzed. *Staphylococcus aureus* isolates were randomly collected from the archived culture and the virulence genes were detected by PCR. A total of 15176 *S. aureus* were isolated and reduction in the MRSA was observed during the study. This study showed that *S. aureus* infection is still an unresolved issue, where clinical specimen from swab contributes the highest number. The percentage of MRSA also is fluctuating and serves as a good indicator for a more vigilant infection control activity in the hospital setting.

Keywords: Malaysia, MRSA, trends, virulence genes

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INTRODUCTION

Staphylococcus aureus infection can be acquired through both hospital and community settings. The ability of *S. aureus* to cause disease depends on a wide range of virulence factors that contributes to colonization and disease in the host. In Malaysia, an increase in *S. aureus* isolation was observed from a total of 32,611 isolations in 2012, to 34,492 isolations in 2013, whereas the prevalence of methicillin-resistant *S. aureus* (MRSA) in Malaysian hospital had decreased from 26% in 2008 to 17.7% in 2013 (Akpaka et al., 2006). In the United States, an increase of 62% of *S. aureus*-related hospitalizations and an increase in the number of MRSA-related hospitalizations more than doubled (Klein et al., 2007).

There are limited studies on the prevalence of *S. aureus* over a longer period of time in Malaysia specifically in Kelantan. The study on trends of isolation of specific types of organisms helps the policy makers to make certain regulation especially related to infection prevention and control. Therefore, in this present study, the retrospective analysis of *S. aureus* isolation from 2002 till 2014 was done to determine the trends of *S. aureus* and MRSA isolation in Hospital Universiti Sains Malaysia (HUSM), Kelantan, Malaysia. Moreover, the prevalence of its virulence genes and its association with the specific clinical presentation of *S. aureus* infection were also determined. A comparison with *other* community in different geographical areas *may provide valuable information which may be important in the area of study*.

MATERIALS AND METHODS

Data of *S. aureus* infection of patients (location of wards or clinics and type of specimen) in HUSM, Kelantan from 2002 to 2014 was obtained retrospectively from WHONET database, a software used for the management and analysis of microbiology laboratory data. The types of wards include medical wards, intensive care units (ICUs), orthopedic wards, surgical wards, pediatric wards, and others. While the types of specimen collected in HUSM were swab, blood, pus, tracheal aspirates, wound, tissue, and others. The data was analyzed statistically by independent t-test and one-way ANOVA test using IBM SPSS Statistic software (version 21.0). Independent t-test was used to compare the prevalence of MRSA between years while one-way ANOVA test was used to compare the difference in frequency between each virulence genes. In this study, *S. aureus* ATCC 25923 and 80 clinical *S. aureus* isolates were randomly selected from the Medical Microbiology and Parasitology Laboratory, HUSM to study the virulence genes distribution (Table 1). For confirmation of *S. aureus*, all isolates were also evaluated for the presence of the species-specific gene *fem*A by PCR using forward primers CGCAAACTGTTGGCCACTAT and reverse primer CTCGCCATCATGATTCAAGT (Nik Zuraina, 2018).

DNA extraction was carried out by boiling method according to Queipo-Ortuño et al. (2008) with slight modification. Specific primers were used to amplify the virulence genes (Table 2). The PCR amplification was performed in 20 µl

of reaction mixture containing 20 ng of template DNA, 1 pmol/µl of primers (Integrated DNA Technologies, Singapore), 0.16 mM of dNTPs, 0.75 U Taq DNA polymerase, 2.5 mM $MgCl_2$ and 1X Taq polymerase buffer (Thermo Scientific, USA). The PCR was performed using a DNA thermal cycler MJ Research PTC-200 DNA Engine (MJ Research, Canada). All 80 isolates were amplified individually for all six genes using the specific primers, with 1 cycles of denaturation at 95°C for 1 min, 30 cycles of annealing at 56 °C for *LukS/F-PV* and *mec*A, 54°C for *ica*A, 48°C for *sdrE*, and 55.8°C for *hlg* and 46°C for *cna* for 1 min and elongation at 72 °C for 1.5 min; and followed by final extension at 72°C for 10 min. The same PCR cycling condition was used for amplification of *fem*A gene with an annealing temperature of 60°C for 1 min. Then, the PCR products were stained using DNA fluorosafe stain (1st Base Laboratories, Malaysia), and electrophoresed in 1.5% agarose gel at 90 V for 60 minutes. The PCR products were visualized using Alpha Innotech ChemiImager 5500 UV illuminator and image capturing unit (California, USA). The DNA band was analyzed and the size was determined by referring to the size of 100 bp DNA marker (Fermentas, USA).

Table 1
List of bacterial strains used in this study

Bacteria	Sources	No. of Strain
Staphylococcus aureus ATCC™ 25923	ATCC	1
S. aureus B1 – B20	Blood	20
S. aureus T1 – T20	Tissue	20
S. aureus SP1 – SP20	Sputum	20
S. aureus S1 – S20	Swab	20
Total		81

Table 2
Description of primers used for amplification of virulence genes

Gene	Putative function	Primer sequence (5'- 3')	Product size (bp)	References
LukS/ F-*PV*	Bicomponent leukocidin.	F: CAG GAGGTAATGGTTCATTT R: ATGTCCAGACATTTTACCTAA	151	(Al-Talib et al., 2009)
*mec*A	Methiclin resistance	F: ACGAGTAGATGCTCAATATAA R: CTTAGTTCTTTAGCGATTGC	293	(Al-Talib et al., 2009)
cna	Adhesin for collagen	F:AGTGGTTACTAATACTG R: CAGGATAGATTGGTTTA	744	(Peacock et al., 2002)
hlg	Bicomponent leukotoxins	F: GCCAATCCGTTATTAGAAAATGC R: CCATAGACGTAGCAACGGAT	937	(Peacock et al., 2002)
*ica*A	Polysaccharide intercellular adhesin	F: GATTATGTAATGTGCTTGGA R: ACTACTGCTGCGTTAATAAT	770	(Peacock et al., 2002)
sdrE	Putative adhesins	F: AGTAAAATGTGTCAAAAGA R: TTGACTACCAGGCTATATC	767	(Peacock et al., 2002)

RESULTS AND DISCUSSION

Over the period of 13 years (2002-2014), a total of 15,176 *S. aureus* was isolated in HUSM from 9584 patients. Whilst, a total of 4469 (29.4%) of *S. aureus* isolates were MRSA isolated from 2092 patients. This contributes to the MRSA prevalence of 21.8%. The average annual rate of MRSA infections was 344/year and the highest number of *S. aureus* infection was in 2013 (n=1790). The prevalence of *S. aureus* isolation in HUSM has increased by 1.8% from 2002 to 2014 and the prevalence of MRSA has declined from 41.6% in 2002 to 28% in 2014 (Figure 1). The differences between the years were significant (P<0.05). Similarly, a National Surveillance of Antibiotic Resistance Report by the Ministry of Health (MOH) (2013) reported that *S. aureus* isolation had increased from 32,611 *S. aureus* isolated in 2012 to 34,492 *S. aureus* isolated in 2013. Meanwhile, the MRSA prevalence has decreased from 26% in 2008 to 17.7% in 2013 (MOH, 2013).

In comparison, the MRSA prevalence in Hospital Kuala Lumpur (HKL) and Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in 2007 to 2008 and 2009 were 44.1% and 26.6% respectively, were higher compared to the present study (Ghaznavi-Rad et al., 2010; Sapri et al., 2013). The MRSA rate among *S. aureus* isolates in this study was 29.4% which is within the range reported in Western Europe which was between 5% and 54% during 2000-2010 (Dulon et al., 2011). However, the rate cannot be compared directly with the previous study because of the different socio-demographic characteristics of the patients and hospitals. While in the USA, the MRSA infection rates declined 54% between 2005 and 2011, with 30,800 less severe MRSA infections (Dantes et al., 2013).

The MRSA isolation showed varying trends where a progressive decrease in MRSA infection rates was observed through 2002 to 2006, subsequently, the occurrence started to increase moderately from 2007 to 2010 and rapidly increased MRSA isolation through 2011 to 2013. Then, the MRSA isolation was slightly declined from 2013-2014 (Figure 1). The progressive decrease in MRSA infection rates during 2002 to 2006 in HUSM might be due to new prophylactic measures used, including strict isolation of patient whether suspected or proven to have MRSA infection; education improvements, hand hygiene, and use of hydro-alcoholic solutions; impact of surveillance; and better quality of care as a whole (Al-Talib et al., 2010).

The highest *S. aureus* isolated in HUSM were from medical wards (21.1%), followed by ICUs (18.0%), orthopedic wards (15.9%), surgical wards (14.2%), pediatric wards (3.9%) and others (26.9%). Other locations including Accidents and Emergency Department, Obstetrics and Gynecology Department, and Oncology and Ophthalmology Department. While in HKL, the highest *S. aureus* isolated from 2007 to 2008 were medical wards (20.1%), pediatrics (9.2%), surgical wards (11.2%), orthopedics (12.8%) and ICUs (5.1%). Findings in our current study

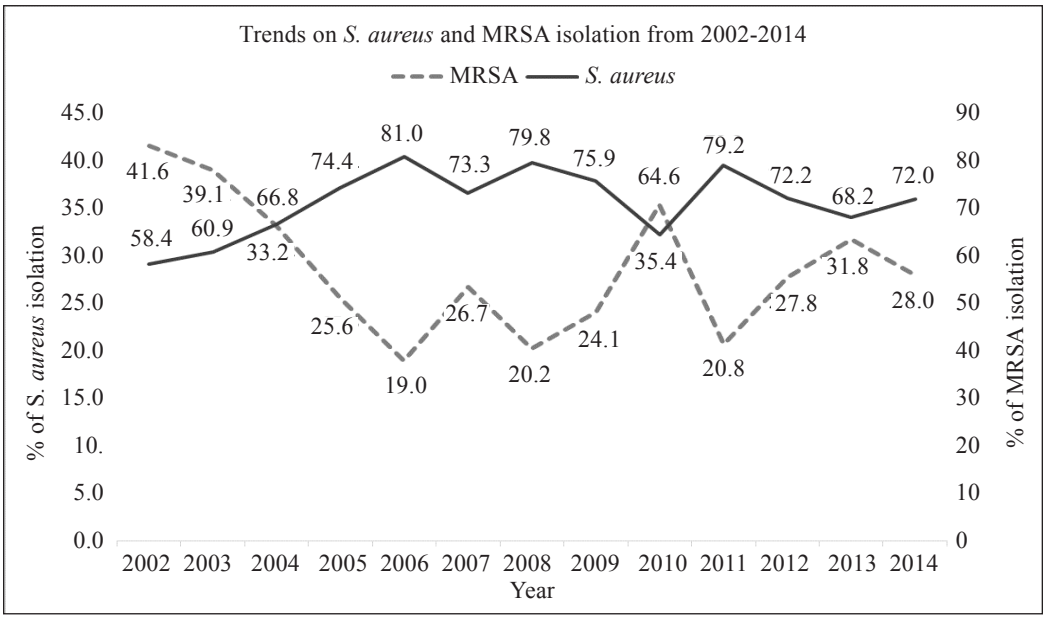

Figure 1. Trends of *Staphylococcus aureus* isolation (left) and MRSA isolation (right) from 2002 – 2014

are in agreement with the previous study conducted in HKL, Malaysia which showed that *S. aureus* was most frequently isolated from medical wards (Ghaznavi-Rad et al., 2010).

High-frequency *S. aureus* isolation from medical wards, orthopedic wards, and surgical wards reflects that these wards usually accommodate chronic patients that require prolonged hospitalization. Infected or colonized patients act as reservoirs, with transient hand carriage by healthcare workers and caretakers of these patients being the predominant mode of transmission from one person to another (McDonald, 1997). It could also be due to the traumatic and postoperative immunological suppression of the patients and environmental factors that were probably related to the higher rate of MRSA infection in these locations (Al-Talib et al., 2013).

In this study, swab specimen had the highest number of *S. aureus* isolation (22.61% of the total in HUSM) followed by blood (17.24%), pus (14.42%), tracheal aspirates (10.62%), wound (7.74%), tissue (7.53%) and others (19.84%). Other specimen includes urine, abdominal fluid, and cerebrospinal fluid. Additional PCR result showed the *fem*A gene (293 bp) was detected in all 80 isolates indicated the isolates were *S. aureus*. Then, PCR amplification of six virulence genes showed that only five genes (*mec*A, *LukS, cna, ica*A, *SdrE*) were present in some isolates except for *hlg* gene. The *mec*A gene was amplified in 13 isolates (16.25%), while 11 isolates (13.75%) were positive for carrying *LukS* gene, 4 isolates (5.0%) for *cna* gene, 20 isolates (25%) for *ica*A gene and 23 isolates (28.75%) for *SdrE* gene. However,no isolate was positive for carrying *hlg* gene.

The genes amplification did not occur consistently in all isolates where different genes were available in different isolates. However, some of the isolates were carrying two to three virulence genes. The highest coexistence of different genes was *mec*A + *SdrE* genes while the lowest frequency of gene coexistence were *mec*A + *cna*, *mec*A + *LukS* +*SdrE*, *mec*A + *cna* + *SdrE*, *LukS* + *ica*A and *LukS* + *SdrE* where each combination was detected in a single isolate. The frequency of virulence genes in this study was also lower compared with the previous study in the United Kingdom and India (Bhatty et al., 2013; Peacock et al., 2002).

The swab specimens were also recorded to have the highest number of virulence genes in 27 (33.8%) *S. aureus* isolates, followed by 19 (23.8%) tissue, 17 (21.4%) sputum, and 8 (10%) blood. The results showed that the separate frequency of each gene in *S. aureus* isolates indicated a significant difference, with $P<0.05$. There were also recorded 35 isolates were devoid of any gene. It is also suggested that *S. aureus* commonly causes non-complicated superficial skin infection in human. The largest number of virulence gene detected was putative adhesion (*SdrE*) suggested the gene play a role in virulence of *S. aureus* infection.

CONCLUSIONS

This study shows that *Staphylococcus aureus* infection is still an unresolved issue, where clinical specimen from swab contributes the highest number. The percentage of MRSA is fluctuating and serves as a good indicator for a more vigilant infection control activity in the hospital setting to curb the increasing trends of *S. aureus* and MRSA isolation. The present study has also demonstrated a variation in the occurrence of virulence genes populations of *S. aureus* as compared to other region. Studies related to the molecular diversity of *S. aureus* will be very helpful in understanding the molecular epidemiology of *S. aureus* in this hospital.

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