

Toxin gene profiling of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from a Malaysian teaching hospital

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Abstract

Enterotoxin and exfoliative toxin gene profiling of 237 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) were carried out via PCR amplification. Among the tested toxin genes, *sei* was found to be the most prevalent (54.9%).

Text

Methicillin-resistant *Staphylococcus aureus* (MRSA) garners more research attention rather than methicillin-susceptible *S. aureus* (MSSA), due to the fact that MRSA are usually found to be multidrug resistant and hence complicate treatment options [1, 2]. Nevertheless, in a study on *S. aureus* isolated from our medical centre, a four-toxin gene (collagen adhesion-encoding gene (*cna*), staphylococcal enterotoxin h (*seh*), Panton-Valentine Leukocidin (PVL) and Toxic Shock Syndrome Toxin (TSST-1)) profiling study revealed a higher prevalence of these genes in MSSA isolates compared to MRSAs isolated during the same period [3]. Staphylococcal enterotoxin and exfoliative toxin genes are important virulent factors of the bacteria associated with food poisoning and staphylococcal scalded skin syndrome (SSSS), respectively [4]. In addition, staphylococcal toxin gene profiling is also important as a tool for molecular epidemiological studies [4]. In this study, we determined the prevalence of seven staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *sei*) and two exfoliative toxin genes (*eta*, *etb*) in MSSAs isolated from UKM Medical Centre (UKMMC) in 2009.

A total of 237 MSSA infections were recorded in UKMMC in 2009. The first MSSA isolate of each infection was included into the study. Chromosomal DNA of the MSSA strains were extracted using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). Toxin gene profiling was carried out using primers and cycling conditions as described previously [5, 6]. Amplification of toxin genes for all strains was carried out using a Bio-rad MyCycler™, PCR products were electrophoresed on a 1% agarose gel and stained with

Lonza GelStar™ Nucleic Acid Gel Stain (Thermo Fisher Scientific Inc.) for band visualization.

Results of the toxin gene profiling are listed in Table 1. More than half (181 strains, 76.4%) of the MSSA tested in our study carried at least one toxin gene, where *sei* was found to be the most prevalent toxin gene detected (54.9%). Most strains carried the *seg* + *sei* combination (96 strains, 40.5%). *seg-sei* toxin genes are clustered as the enterotoxin gene cluster (*egc*), which comprises the *seg-sei-sem-sen-seo* and sometimes *seu* genes [7]. Interestingly, our study strains did not harbour the *sed* or *see* genes. On the other hand, the prevalence of *eta* and *etb* genes was found to be low in our study strains. Only 4 strains (1.7%) harboured *eta*, while prevalence of *etb* was slightly higher (23 strains, 9.7%). A total of 56 (23.6%) strains did not harbour any toxin genes.

Reports on toxin gene profiling of Malaysian MSSA strains remains limited; as far as we know, only 3 reports on this subject have been published [8, 9, 10]. Two of these reports involved MSSA strains isolated from Hospital Kuala Lumpur (HKL) and University Malaya Medical Centre (UMMC), respectively [8, 9]. These two hospitals are located at the central region of the west coast of Peninsular Malaysia. The third study was conducted on MSSAs isolated from Terengganu, a state located at the East Coast of Peninsular Malaysia [10].

Prevalence of the *seg* and *sei* genes in our strains (41.8% and 54.9%, respectively) were comparable to that of MSSAs isolated from HKL (36.5% and 36.9%,

Table 1: Toxin gene profiles of MSSA strains used in this study.

Toxin gene profile	n (%)	Toxin gene	n (prevalence, %)
<i>sea</i>	31 (13.1)	<i>sea</i>	117 (49.4)
<i>sea + seb</i>	7 (3.0)	<i>seb</i>	71 (30.0)
<i>seb</i>	2 (0.8)	<i>sec</i>	55 (23.2)
<i>sea + sec</i>	4 (1.7)	<i>sed</i>	0 (0)
<i>sec</i>	3 (1.3)	<i>see</i>	0 (0)
<i>seg</i>	2 (0.8)	<i>seg</i>	99 (41.8)
<i>sea + seb + seg</i>	1 (0.4)	<i>sei</i>	130 (54.9)
<i>sea + sei</i>	8 (3.4)	<i>eta</i>	4 (1.7)
<i>sei</i>	8 (3.4)	<i>etb</i>	23 (9.7)
<i>sea + seb + sei</i>	7 (3.0)		
<i>seb + sei</i>	5 (2.1)		
<i>sea + sec + sei</i>	2 (0.8)		
<i>sea + seb + sec + sei</i>	1 (0.4)		
<i>seb + sec + sei</i>	1 (0.4)		
<i>sea + seg + sei</i>	6 (2.5)		
<i>seg + sei</i>	13 (5.5)		
<i>sea + seb + seg + sei</i>	14 (5.9)		
<i>seb + seg + sei</i>	7 (3.0)		
<i>sea + sec + seg + sei</i>	14 (5.9)		
<i>sec + seg + sei</i>	7 (3.0)		
<i>sea + seb + sec + seg + sei</i>	6 (2.5)		
<i>seb + sec + seg + sei</i>	6 (2.5)		
<i>seg + sei + eta</i>	3 (1.3)		
<i>sec + seg + sei + eta</i>	1 (0.4)		
<i>sea + etb</i>	1 (0.4)		
<i>etb</i>	1 (0.4)		
<i>sea + sei + etb</i>	1 (0.4)		
<i>sea + seb + sei + etb</i>	1 (0.4)		
<i>sea + seg + sei + etb</i>	3 (1.3)		
<i>seg + sei + etb</i>	1 (0.4)		
<i>sea + seb + seg + sei + etb</i>	5 (2.1)		
<i>sea + sec + seg + sei + etb</i>	1 (0.4)		
<i>sec + seg + sei + etb</i>	1 (0.4)		
<i>sea + seb + sec + seg + sei + etb</i>	4 (1.7)		
<i>seb + sec + seg + sei + etb</i>	4 (1.7)		
no toxin gene	56 (23.6)		

respectively), which is one of the largest public hospitals in Malaysia [8]. Nevertheless, our MSSA strains had a higher prevalence of *sea* (49.4%), *seb* (30.0%) and *sec* (23.2%) compared to the UMMC (*sea* – 13.4%, *seb* – 4.5%, *sec* – 16.4%) and HKL (*sea* – 24.2%, *seb* – 23.8%, *sec* – 11.9%) [8, 9] strains. Intriguingly, the *seg-sei* genes were not detected in MSSAs isolated from Terengganu [10], even though these genes were commonly found in the central West Peninsular Malaysia MSSAs [8, 9]. Interestingly, even though its prevalence was only 28.6%, the *sea* gene was the most dominant toxin gene detected in the Terengganu's MSSAs. The *sed* and *see* genes seemed to be rare in Malaysian MSSA strains as they were not detected in previous studies as well as in our study [8, 9, 10].

The prevalence of the *eta* (1.7%) and *etb* (9.7%) exfoliative genes in our study strains was slightly lower and higher, respectively, compared to the HKL study (*eta* = 2.3%, *etb* = 0.8%) [8]. On the other hand, no *eta* or *etb* gene was detected in the UMMC study [9]. Similarly, these genes were also not found in the Terengganu study isolates [10].

As a summary, MSSA strains of this study and those isolated from the central region of West Peninsular Malaysia appeared to have a more similar toxin gene profile compared to MSSAs from East Peninsular Malaysia. More toxin gene profiling studies, especially for MSSA isolates from the northern or southern states of Peninsular Malaysia, together with East Malaysia (Borneo), will provide a more complete

picture of the molecular epidemiology of MSSA in Malaysia.

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