SCC*mec* typing of clinical isolates *Staphylococcus haemolyticus*

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ABSTRACT

A total number of 38 clinical strains of *Staphylococcus haemolyticus* originally obtained from Hospital Tuanku Ampuan Rahimah, Klang Selangor, were used in this study. The antimicrobial susceptibility of these nosocomial pathogens was tested against penicillin and cefoxitin. Except for one strain (B200) which was sensitive, the rest of the isolates were resistant to penicillin. Similarly, only one strain (F4) was sensitive against oxacillin while the rest were resistant. For cefoxitin, 34 or 89.5% of the isolates were resistant to this antibiotic and classified as Methicillin resistance *Staphylococcus haemolyticus* (MRSH). Amplification of the *mec*A gene showed among the MRSH strains 19 or 55.9% harbour the *mec*A gene but 15 or 44.1% were found negative for this gene. This could be due to the *mec* gene complex which does not only consist of the *mec*A gene but could also harbour other classes of *mec* genes such as *mec*B, *mec*C, *mec*D or *mec*E. However, one of the Methicillin-sensitive *Staphylococcus haemolyticus* (MSSH) strains susceptible against cefoxitin was also found to harbour the *mec*A gene. All the 20 isolates positive for *mec*A gene were further subjected to Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type I, II, III, IV and V. The results showed that two or 10.0% of the strains (B14 and R27) expressed SCC*mec* type II while only one isolate (P29) expressed SCC*mec* type III. For the remaining 17 isolates, SCC*mec* typing was not detected suggesting that these isolates do not harbour SCC*mec* I, IV or V. Hence, there is a possibility that these isolates carry other types of SCC*mec*.

Keywords: *MRSH, MSSH, mecA, SCCmec, typing.*

INTRODUCTION

Coagulase Negative Staphylococci (CoNS) is a group of *Staphylococcus* without the ability to coagulate blood plasma due to the absence of coagulase. These bacteria are commensals commonly found in the normal flora of humans such as inguinal areas, perineum, axillae and mucous membrane [1]. Hence, their role as pathogens was underrated among *Staphylococcus* species until the late 1970s [2]. Among CoNS, *S. haemolyticus* is one of the most well-known pathogens emerging as major hospital-acquired opportunistic infections in immune-compromised patients such as surgical and diabetic patients and also individuals undergoing dialysis. [3–5].

There were a few case reports regarding multi-drug resistance *S. haemolyticus* which can emerge as a threat. Antibiotic resistance is defined as the ability of the microorganisms to resist any effects of an antibiotic when being exposed to the antibiotic [1]. *S. haemolyticus* was reported to show a high rate of resistance against antiseptic agents and a wide spectrum of antibiotics which include penicillins, cephalosporins, macrolides, tetracyclines, quinolones, aminoglycosides, glycopeptides and fosfomycin [3,6]. This multi-resistant ability was found with an increasing frequency even though this species seems lack of the important virulence attributes described in other CoNS species [2]. Multi-resistant strains of *S. haemolyticus* also pose a serious problem in animal pathology due to the possibility of transmission between human and animals whereby animals can act as reservoirs for multidrug-resistant strains of *S. haemolyticus* [7].

The use of β-lactam antibiotics in medicine is restricted due to methicillin resistance in *Staphylococcus*. Methicillin was first introduced in 1959 for clinical use and in 1961, the first strain of methicillin-resistant CoNS was isolated in the UK [8]. The mechanism of methicillin-resistant is associated with *mec*A gene which encodes the modified penicillin-binding protein (PBP2a) which is responsible for cell wall synthesis. During the 1970s, methicillin resistance was found to be more frequent in CoNS as compared to Methicillin-resistance *Staphylococcus aureus* (MRSA). However, the species of the CoNS are often not specified hence the contribution of *S. haemolyticus* towards methicillin resistance is not well documented.

The *mec*A gene is part of SCC*mec* cassette, a mobile genetic element which could be transferred horizontally. SCC*mec* cassette is comprised of *mec* gene complex (*mec*A, *mec*R1, *mec*I, ccr gene complex and junkyard (J) region). At present, there are 13 types of SCC*mec* cassette identified in *S. aureus* strains [4,9]. For *S. haemolyticus*, the most frequently identified cassette is type V although type IV and VI had also been reported in a small number [2]. *S. haemolyticus* have been described as a reservoir of SCC*mec* elements for other staphylococci due to its ability to transfer the gene to other species.

In Malaysia, knowledge regarding *S. haemolyticus* and its SCCmec typing is still limited. The archetypal of SCC*mec* elements conferring resistance to antibiotics is widespread among staphylococcal species which carrying necessary genes for survival under stressful conditions [10–12]. The correlation between the ability of this species to resist antibiotics and the type of SCC*mec* they harbour is poorly understood. This study would provide data on the distribution of the type of SCC*mec* in clinical isolates of *S. haemolyticus*. It is hoped that the data collected would contribute to a better understanding of SCC*mec* typing of local isolates of *S. haemolyticus* and may lead to better management therapy against the infections caused by this bacterium.

EXPERIMENTAL

Bacterial culture and maintenance

A total number of 38 clinical isolates strains of *S. haemolyticus* used in this study which originally obtained from Hospital Tuanku Ampuan Rahimah, Klang. These strains were first grown on Brain Heart Infusion (BHI) Agar followed by Mannitol Salt Agar (MSA) to check for the purities of isolates. A series of biochemical tests were later performed to verify the isolates. The pure cultures were then maintained in 25% glycerol stock and stored at -80ºC. Prior to usage, the bacterial cultures were sub-cultured on BHI broth.

Antibiotic susceptibility test

Antibiotic susceptibility test was conducted by using the Kirby Bauer disk diffusion method. The samples of *S. haemolyticus* were cultured overnight in Mueller-Hinton Broth (MHB) at 37ºC, 150rpm. The following day, 1ml of the bacterial culture was transferred to fresh MHB at 1:100 dilutions. The cultures were further incubated for 2-3 hours prior to adjustment as 0.5 McFarlands standard which is equivalent to 1 X 10^8 cfu ml^{-1}. The culture was swab on fresh MHA and the antibiotic disk was placed on the agar. Penicillin (10µg), Oxacillin (1µg) and Cefoxitin (30µg) antibiotic disc were purchased from Oxoid (UK) and stored at -20ºC [13].

Genomic DNA extraction

Genomic DNA extraction by using DNeasy Blood and Tissue kit (Qiagen) according to manufacturer's instructions and DNA were stored at -20ºC.

*mec*A gene determination

The 38 samples of DNA extracted from clinical isolates *S. haemolyticus* were subjected to *mec*A gene amplification. Amplification of *mec*A gene sequence of the 38 isolates was using MecA1-F (5'- CTT TGC TAG AGT AGC ACT CG-3') and MecA1-R (3'- GCT AGC CAT TCC TTT ATC TTG-5') which amplify mecA gene at 531bp [14]. The PCR reaction mix was prepared using Gotaq Flexi kit (Promega) in a total volume of 50 µL; 10 µL of 5X Go-Taq Buffer, 1 µL of a 200 µM concentration of each dNTP, 4 µL of 25 mM $MgCl_2$ solution, 4 µL of 10 µM of each primer, 0.25 µL of 1 U of Go-Taq DNA polymerase (Promega) and 5 µL of 150 ng of DNA as the template.

The PCR was performed in Mastercycler (Eppendorf) with the following conditions: 1 min at 94°C for initial denaturation followed by 30 cycles of amplification of the followings; 1 min at 94°C for denaturation, 1 min of annealing at 62°C and 45 sec of extension at 72°C. The last cycle was performed at 72°C for 5 min [15]. The PCR products were verified under gel electrophoresis by using 1% agarose gel at 90 V for 120 minutes using amplified *mec*A of *S. aureus* ATCC 33591 as a positive control.

SCC*mec* typing

All samples positive for *mec*A were amplified to determine their SCC*mec* typing of Type I, II, III, IV and V according to Zhang *et, al.* (2005) [14]. The PCR reaction mix was prepared using Gotaq Flexi kit (Promega) in a total volume of 50 µL; 10 µL of 5X Go-Taq Buffer, 1

µL of a 200 µM concentration of each dNTP, 4 µL of 25 mM $MgCl_2$ solution, 4 µL of 10 µM of each primer, 0.25 µL of 1 U of Go-Taq DNA polymerase (Promega) and 5 µL of 150 ng of DNA as the template.

The PCR was performed in Mastercycler (Eppendorf) with the following conditions: 5 min at 94°C for initial denaturation followed by 30 cycles of amplification of the followings; 1 min at 94°C for denaturation, 1 min of annealing at 62°C and 2 min of extension at 72°C. The last cycle was performed at 72°C for 10 min [15]. After the PCR, the products were verified under gel electrophoresis by using 1% agarose gel, 90 V for 120 minutes.

RESULTS AND DISCUSSION

Except for B200 and F4, which was sensitive against penicillin and oxacillin respectively, the rest of the *S. haemolyticus* strains were resistant to both antibiotics at 97.4%. Both B200 and F4 were also found to be susceptible against cefoxitin together with two other strains which were U4 and P28. The remaining 34 or 89.5% of the *S. haemolyticus* clinical strains were found to be resistant against cefoxitin as shown in Figure 1.

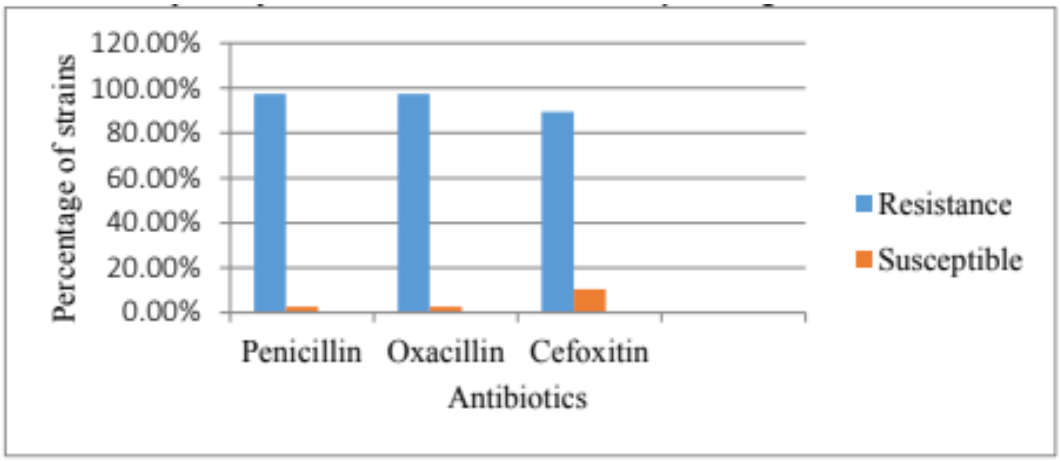

Figure 1: Antibiotic susceptibility of clinical strains of *S. haemolyticus* against selected antibiotics

Majority of the *S. haemolyticus* strains tested were found to be resistant against penicillin, probably due to the production of penicillinase which degrades penicillin [16]. The use of oxacillin and cefoxitin to determine MR *Staphylococcus* have also been reported in many studies [17–19]. This is important as MR *Staphylococcus* are responsible for a large proportion of infections both in hospitals and community settings with an increasing trend in antimicrobial resistance patterns. The accurate and rapid determination of methicillin resistance is therefore crucial, especially in hospital environment.

However, the results in this study showed that only one strain of *S. haemolyticus* was sensitive against oxacillin as compared to an additional of four strains which were found sensitive against cefoxitin. This suggests that *S. haemolyticus* is more resistance towards oxacillin in comparison to the disc diffusion cefoxitin test which has also been reported in other studies [17,20]. The use of cefoxitin disc to detect MR *Staphylococcus* is accepted by many reference group including Clinical and Laboratory Standards Institute (CLSI)

guidelines [18,21]. Cefoxitin is an intense inducer of the *mec*A regulatory system and appeared to be better than oxacillin particularly in low-level MR strains. Cefoxitin test results are simple to be interpreted and sensitive for the detection of the *mec*A gene than oxacillin.

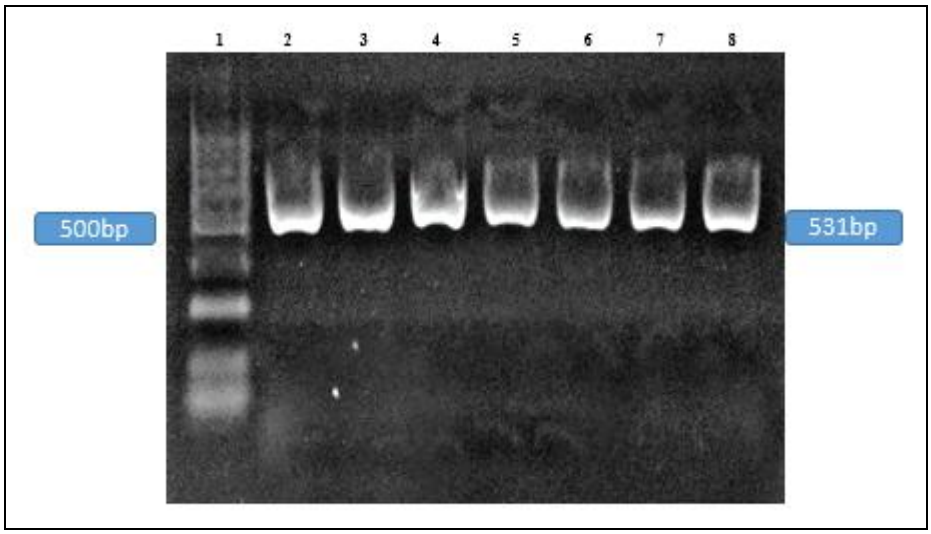

Figure 2: Amplification of the mecA gene in S. haemolyticus. Lane 1: 100bp ladder; lane 2: positive control ATCC 33591; lane 3: B75; lane 4: R8; lane 5: B22; lane 6: R23; lane 7: R29 and lane 8: R27

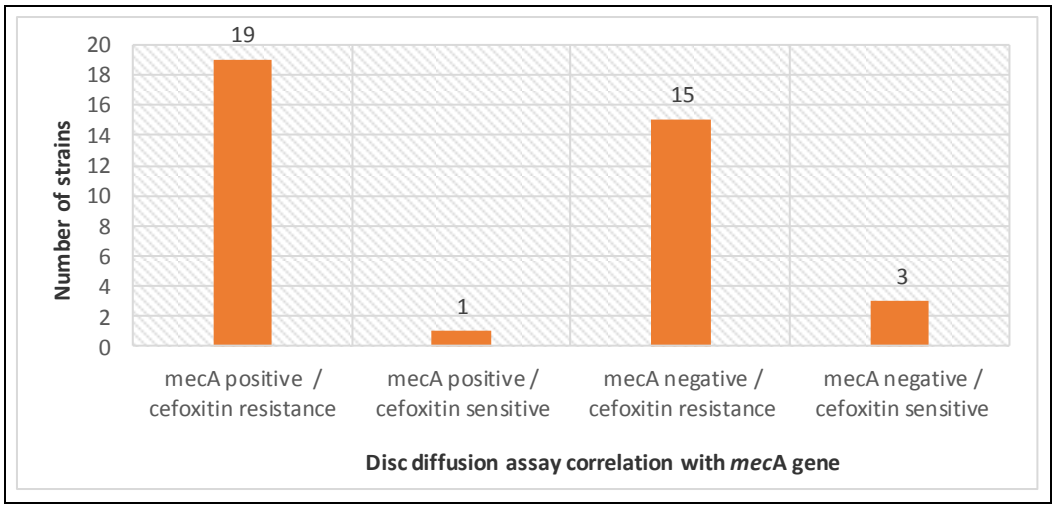

Figure 3: Results of the cefoxitin disc diffusion assay in *S. haemolyticus* and correlation with *mec*A. A correlation between *mec*A and antibiotics activity

Amplification of the *mec*A gene showed that only 19 from the 34 MRSH strains harbour the *mecA* gene. However, 15 of the MRSH strains did not harbour the *mec*A gene. Such findings have also been reported in other studies. It was suggested that these strains are lacking chromosomal *mec*A which had been eliminated by complete excision of the SCC*mec* cassette element but the mechanism of cefoxitin resistant is still active due to the presence of other kind penicillin-binding protein such as PBP4a [22]. This due to resistance to α β-lactam antibiotic, without *mecA* gene, maybe the overproduction or overexpression of penicillinase or by alteration of other penicillin-binding proteins [23].

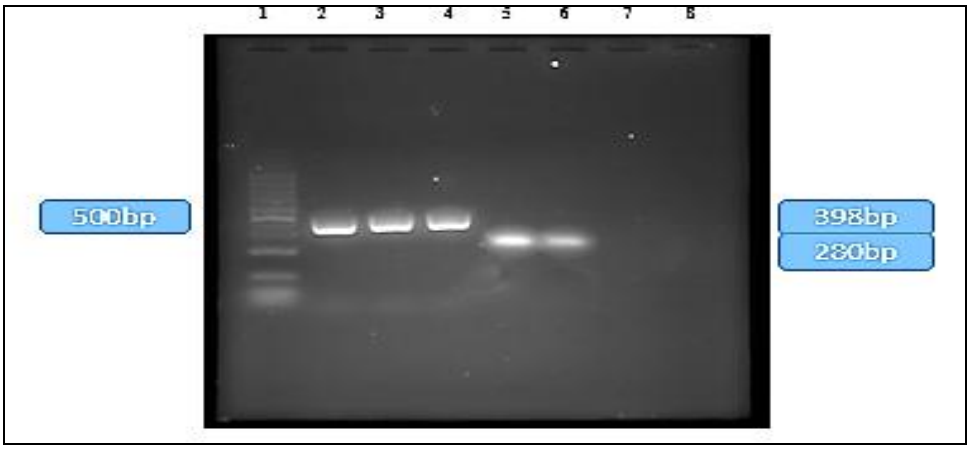

Figure 4: SCC*mec* typing of *S. haemolyticus* clinical isolates. Lane 1: 100bp ladder; lane 2: Positive control Type II SCC*mec* ATCC 33591; lane 3: Type II SCC*mec* R27; lane 4: Type II SCC*mec* B14; lane 5: Positive control Type III SCC*mec* ATCC 29970; lane 6: Type III SCC*mec* P29

The 20 *mec*A positive *S. haemolyticus* strains were further subjected to SCC*mec* typing. Results displayed in Figure 4 showed that two of the strains was of SCC*mec* type II while the other one was of SCC*mec* type III. None of the *mec*A in the remaining isolates was of SCC*mec* type I, II, III, IV or V. In a study conducted by Ruppé and friends in four countries which were Algeria, Cambodia, Moldova and Mali, SCC*mec* type V was reported as the most prevalent among MRSH strains followed by SCC*mec* type IV. It was also suggested that a higher percentage of multidrug resistance carried a higher percentage of SCC*mec* type V and a lower percentage of SCC*mec* type IV [24].

In contrast, *SCCmec* types II, III and V have been detected in *S. haemolyticus* collected in China whereby SCC*mec* type III was the most prevalent [25]. It is believed that the occurrence of different SCC*mec* types in China might reflect the genetic background of *S. haemolyticus* strains, connected with geographical locations [26]. Another study in Brazil showed that SCC*mec* type I was most prevalent among *S. haemolyticus* followed by type II [24]. One of the possibilities that might cause the difference in these studies is the geographical factor whereby in the first study the samples were collected from four different countries while the second study was conducted in China.

It is difficult to ascertain the prevalence of SCC*mec* typing in this study as the samples used were small and the majority of the strains were non-typeable. As the study was conducted only on SCC*mec* type I, II, III, IV, and V, there is a possibility that these strains might harbour the SCC*mec* gene that did not belong to any of these types. It was also suggested that non-typeable *Staphylococcus* could be due to "unclassified" combination of *ccr* and *mec* complexes or it could also be due to the absence of amplification for one of the two complexes [27].

CONCLUSION

In this study, 34 from 38 or 89.5% of the *S. haemolyticus* strains were resistant against cefoxitin and classified as MRSH. Among the MRSH strains, 19 or 55.9% of the MRSH harbour *mec*A gene but 15 or 44.1% were negative for the gene even though they are resistant against cefoxitin. One MSSH strain was also found to harbour *mec*A gene. Two of these strains were of SCC*mec* type II while only one isolate was of SCC*mec* type III. None of the remaining *S. haemolyticus* strains were of type I, II, III, IV or V. It is hoped that study would provide data on distribution on the type of SCC*mec* of clinical isolates of *S. haemolitycus*. However, a bigger sample is necessary for a more comprehensive conclusion.

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