ORIGINAL ARTICLE

High Prevalence of Methicillin-resistant *Staphylococcus haemolyticus* Isolated From Commensals in Healthy Adults

Farhan Haziq Azharollah[1], Mohd Faiz Foong Abdullah[1], Siti Farah Alwani Mohd Nawi[2], Aziyah Abdul-Aziz[1]

[1] School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, Malaysia
[2] Clinical Training Centre, Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor, Malaysia

ABSTRACT

Introduction: *S. haemolyticus* is known to be commensals residing on human skin. However, their ability to develop as pathogens among the healthy community has becoming increasingly vital. **Methods:** In this study, a total of 49 non-duplicated samples of *S. haemolyticus* was isolated from the skin of healthy adults and confirmed via *sod*A gene sequencing method. Cefoxitin (30µg) disc diffusion test was performed to determine methicillin resistance among the *S. haemolyticus* isolates. The isolates were then subjected to *mec*A amplification and *Staphylococcus* Cassette Chromosome (SCC*mec*) typing of I, II, III, IV and V. **Results:** Interestingly, 59.2% of the *S. haemolyticus* commensal isolates were found to be methicillin-resistant (MRSH) while the remaining 40.8% was methicillin-sensitive (MSSH). Amplification of *mec*A gene showed that 43 isolates (87.8%) were positive while only six isolates were negative for the gene. A majority of the positive *mec*A isolates (90.7%) were discovered to harbour SCC*mec* Type II while the remaining 44.2% were Type V followed by 23.3% of Type I and 18.6% of Type IV. Only one of the isolates was found to be SCC*mec* Type III while another isolate, T187 was non-typeable. **Conclusion:** The data indicates the acquisition of SCC*mec* typing circulated among the commensal strains which could be a potential route of pathogenicity among the isolates.

Keywords: Commensals, *mec*A, MRSH, MSSH, SCC*mec*

Corresponding Author:
Aziyah Abdul-Aziz, PhD
Email: aziyah960@uitm.edu.my
Tel: +603-5521 1756

INTRODUCTION

While coagulase positive *S. aureus* has been known as the most virulent species of *Staphylococcus* causing a broad spectrum of infections, coagulase negative *Staphylococcus* or CoNS on the other hand are known as harmless commensals. They are commonly found on the skin and mucous membranes in human, forming a benign or symbiotic association with their host. However, it was later discovered that CoNS have potentials to be pathogens upon gaining access into the host tissue. CoNS was then labelled as a typical opportunistic nosocomial pathogen showing substantially impact towards health issues not only in adults but also among paediatric patients (1,2). The rise of CoNS as aetiological agent for nosocomial infections especially due to the use of intravascular catheters and prosthetic devices among immune-compromised patients has been reported as life-threatening (3).

Among CoNS, *S. haemolyticus* is the second most frequent aetiological agents isolated from staphylococcal infections after *S. epidermidis* (1,4,5). *S. haemolyticus* have been accounted as the responsible agent in severe nosocomial infections which affecting preterm infants and immune-compromised patients such as liver abscess, sepsis and endocarditis (1,6). However, despite the importance of this species in causing infections, there is little information on *S. haemolyticus* as compared to the abundance data on its counterpart, *S. epidermidis*.

The increasing clinical significance of *S. haemolyticus* is unclear as this species was reported to be lacking the important virulence attributes. Despite this, studies have shown that *S. haemolyticus* has the highest level of antimicrobial resistance among CoNS (1,5,7) . Among the main concern is the incidence of Methicillin Resistance *S. haemolyticus* or MRSH (5).

Methicillin resistance determines the resistance towards β-lactam antibiotics which include penicillins, cephalosporins, carbapenems and monobactams (8). It was first discovered in *S. aureus* which reported to be resistant to penicillin due to their ability to produce β-lactamases (9). The mechanism of methicillin resistance is associated with the production of the penicillin binding protein PBP2a encodes by *mec*A gene which decrease

the affinity for β-lactam antibiotics(10). Analysis of *mec*A gene sequences revealed 99.95% similarity in *S. aureus*, *S. haemolyticus* and *S. epidermidis* which suggests the theory of the interspecies transfer of *mec*A gene between the *Staphylococcus* species (1,4).

The *mec*A gene is carried on the staphylococcal cassette chromosome element or SCC*mec* comprise of *mec* gene complex which includes *mec*A, *mec*R1 and *mec*I, the ccr gene complex and junkyard (J) region. At present, there are 13 types of SCC*mec* cassette identified in *S. aureus* strains (11). Majority of the clinical studies showed that SCC*mec* Type I, II, III, IV and V were commonly reported among *S. haemolyticus* strains instead of all the 13 types of SCC*mec* (5,12). Being a mobile genetic element, the cassette is believed could be transferred horizontally among *Staphylococcus* sp.(7). *S. haemolyticus* itself have been proposed as a reservoir of SCC*mec* elements for other staphylococci due to its ability to transfer gene to other species (1,13,14). The acquisition of mobile genetic elements has also been suggested to contribute to the successful hospital adapted clones of *S. haemolyticus* (15).

However, the knowledge regarding *S. haemolyticus* and its SCC*mec* typing within commensal strains is still limited. It is believed that commensal organism could be evolved as pathogen in favourable condition by receiving virulence/resistance genes from pathogen counterpart in order to establish an infection by commensals (16). The dual living mode of *Staphylococcus* between commensalism and parasitism is quite complex in reality to be able to justify the possibility of commensal strains of *S. haemolyticus* waiting to exhibit the intrinsic pathogenic tendencies. This study would provide data on the distribution of the type of SCC*mec* in commensals of *S. haemolyticus* whereby the archetypal of SCC*mec* elements conferring resistance to antibiotics is known to be widespread among staphylococcal species carrying necessary genes for survival under stressful conditions.

MATERIALS AND METHODS

Bacterial sampling and isolation

An approximate number of 150 non-duplicated swab samples were randomly collected from the skin of 150 healthy adults. Healthy adults are defined as adult without antibiotic exposure and no hospitalization or any healthcare affiliation for the previous three months (15,17). The healthy adults recruited were mainly undergraduate students under the approval of the Research Ethics Committee (REC 600-IRMI (5/16)) of Universiti Teknologi MARA, Shah Alam.

Unlike *S. aureus* which is known to colonize the nasal area (18,19), the ecological niches of other human-associated CoNS can be found on other sites of the human body (20). In this study, the isolation of *S. haemolyticus* was focused on anatomical areas such as the axillae, perineum and groins area of the chosen subject. The focus on these areas on the human body is as according to the suggestion by Takeuchi et al., (2005) where *S. haemolyticus* tend to be colonizing the axillae, perineum and inguinal areas of human. This was supported by another study which claimed that the majority of this bacterium can be isolated from areas high in apocrine glands such as the axillae and pubic areas (20).

Each sample was collected using sterile cotton swabs soaked in 0.85% sterile saline solution. Upon swabbing, the samples were placed in Brain-heart infusion (BHI) broth incubated at 37 °C for 18–24 h. The overnight cultures were then streaked on Mannitol Salt agar (MSA) and incubated for 37 °C for 18–24 h. Pink colonies which indicate the growth of CoNS were selected and further identified by standard biochemical test including Gram stain, catalase, coagulase and urease test (21).

Species identification

All presumptive CoNS isolates were further subjected to PCR amplification of the superoxide dismutase (*sod*A) gene to confirm their identity (22). These gene fragment represent approximately 83% of the entire genome. *S. haemolyticus* ATCC29970 was used as a control.

DNA were extracted according to manufacturer's protocol (Qiagen, USA) and PCR master mix was used for amplification (New England Biolab, USA). The PCR was performed by using Mastercycler nexus gradient (Eppendorf) with the following conditions: 3 minutes at 95°C for initial cycle, followed by 30 cycles of amplification of the followings; 60 sec of annealing at 37°C, 45 sec of elongation at 72°C and 30 sec of denaturation at 95°C. The last cycle was 72°C for 10 min. The PCR purification was performed by using ExoSap (New England Biolab, USA and the clean PCR products were sent for sequencing (Bio Basic, Singapore).

The resulting sequence data were analysed via Genbank database using the BLAST interface. The match with highest percentage of similarity with minimum 99% of similarity and 99% coverage were considered as *S. haemolyticus*.

Methicillin-resistant *S. haemolyticus*

Anti-microbial susceptibility of *S. haemolyticus* against cefoxitin, 30 µg (Oxoid, UK) was tested by using a Kirby-bauer disc diffusion test. The samples of *S. haemolyticus* were cultured overnight in Mueller-Hinton Broth (MHB) at 37°C, 150rpm. The following day, the bacterial culture was transferred to fresh MHB at 1:100 dilutions and further incubated for approximately three hours to reach the exponential phase and adjusted to the 0.5 McFarlands standard which is equivalent to 1 X 10^8 cfu ml^{-1} (23). The adjusted cultures were swabbed on fresh MHA plate with the antibiotic disc and incubated at 35 °C for 24 h. *Staphylococcus aureus* ATCC 33591 was

used as positive control. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2017).

*mec*A gene determination

The strains of *S.haemolyticus* were then subjected to *mec*A gene amplification using *mec*A1-F (5'- CTT TGC TAG AGT AGC ACT CG-3') and *mec*A1-R (3'- GCT AGC CAT TCC TTT ATC TTG-5') which amplify the *mec*A gene at 531bp (24).

The PCR was performed 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. The PCR products were verified using 1.5% agarose gel at 70 V for 120 minutes. *S. aureus* ATCC 33591 was used as a positive control.

SCC*mec* typing

All 49 isolates were further amplified to determine their SCC*mec* typing of Type I, II, III, IV and V following protocols as recommended by Zhang and co-workers (24,25). The PCR was performed 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. After the PCR, the products were verified using 2% agarose gel, 65 V for 120. *S.capitis* B102 was used as positive control (26).

RESULTS

Species identification from presumptive CoNS

From a total of 150 non-duplicated swab samples, 49 isolates were identified and verified as *S. haemolyticus*. Figure 1 shows the results of the *sod*A amplification resulting in 429bp amplicons on some of the representative isolates of the *S. haemolyticus*.

Methicillin-resistant test for *S. haemolyticus* isolates

Cefoxitin resistance test is considered as a gold standard for identification of methicillin-resistant isolates in *Staphylococcus* as suggested by CLSI (27–29). From the 49 isolates, 29 or 59.2% were found to be MRSH as they were resistant against cefoxitin while the remaining 20 isolates or 40.8% were MSSH.

Amplification of the *mec*A gene was performed on all of the *S. haemolyticus* isolates. A total of 43 (87.8%) of the isolates were found to harbour the *mec*A gene while six were negative for *mec*A gene. From the 43 isolates, 25 were MRSH while the remaining 18 isolates were MSSH. The results of some of the representative isolates are as shown in Figure 2 displaying 531bp amplicons. The correlations between the *mec*A gene, MRSH and MSSH isolates are summarised in Figure 3.

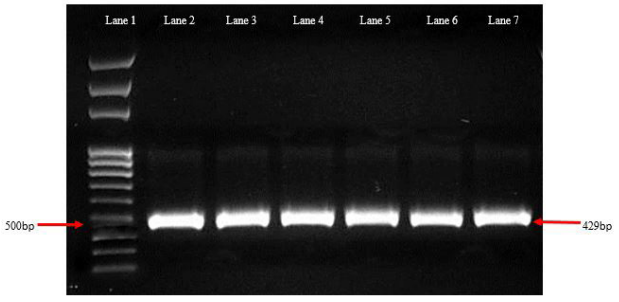

Figure 1: *sod*A gene amplification of *S. haemolyticus* isolates. Lane 1: 100bp DNA ladder, Lane 2: T69, Lane 3: T150, Lane 4: T152, Lane 5: S45, Lane 6: S69, Lane 7: *S. haemolyticus* ATCC 29970 (control).

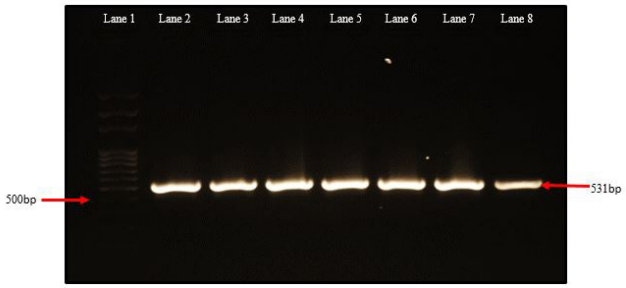

Figure 2: *mec*A gene amplification of *S. haemolyticus* isolates. Lane 1: 100bp DNA ladder, Lane 2: T11, Lane 3: T32, Lane 4: T47, Lane 5: T69, Lane 6: S6, Lane 7: S25, Lane 8: *S. aureus* ATCC 33591 (control).

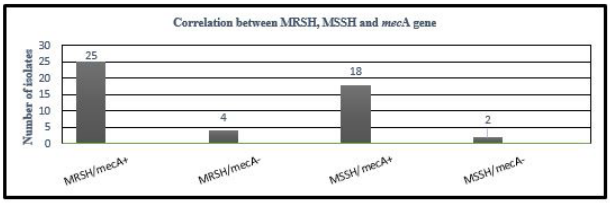

Figure 3: Correlation between MRSH, MSSH and *mec*A gene of the *S.haemolyticus* isolates. Lane 1: 100bp DNA ladder, Lane 2: T11, Lane 3: T32, Lane 4: T47, Lane 5: T69, Lane 6: S6, Lane 7: S25, Lane 8: *S. aureus* ATCC 33591 (control).

SCC*mec* typing of *S. haemolyticus* isolates

The 43 commensal isolates of *S. haemolyticus* which were positive for *mec*A were further subjected for SCC*mec* typing for Type I, II, III, IV and V. The results are as shown in Figure 4 and 5.

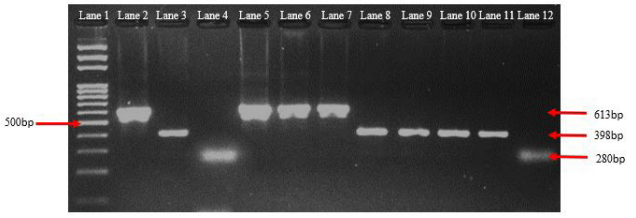

Figure 4: SCC*mec* Type I, II and III of *S. haemolyticus* isolates. Lane 1: 100bp DNA ladder, Lane 2-4: SCCmec Type I, II and III (*S. capitis* B102, control), Lane 5: T149, Lane 6: T150, Lane 7: T152, Lane 8: S6, Lane 9: S25, Lane 10: T11, Lane 11: T32, Lane 12: T69.

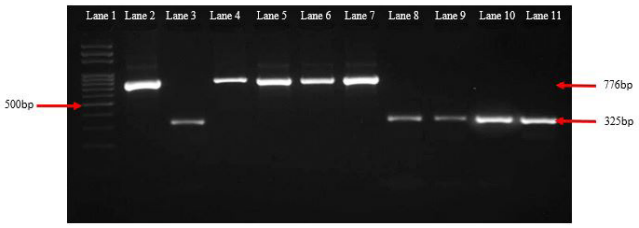

Figure 5: SCC*mec* Type IV and V of *S. haemolyticus* isolates. Lane 1: 100bp DNA ladder, Lane 2-3: SCCmec Type IV and V (*S. capitis* B102, control), Lane 4: T69, Lane 5: T133, Lane 6: T136, Lane 7: S92, Lane 8: S25, Lane 9: S45, Lane 10: S175, Lane 11: T184.

The detail results of the SCC*mec* typing are as shown in Figure 6 and Table 1. A majority of 39 of the *S. haemolyticus* isolates or 90.7% were found to harbour SCC*mec* Type II while 19 isolates harbour the SCC*mec* V (44.2%). This is followed by Type I with ten isolates (23.3%), eight isolates of Type IV (18.6%) and only one of Type III (2.33%). It is interesting to observe that while SCC*mec* Type I, II, III, IV or V were present in both MRSH and MSSH isolates. One MSSH isolate (T187) was found to be non-typeable even though it harbours the *mec*A gene. However, this isolate was MSSH. The results also showed that some of the isolates were found to harbour more than one type of SCC*mec* typing. The number of the SCC*mec* typing found in all the *S. haemolyticus* isolates is summarise in Figure 6.

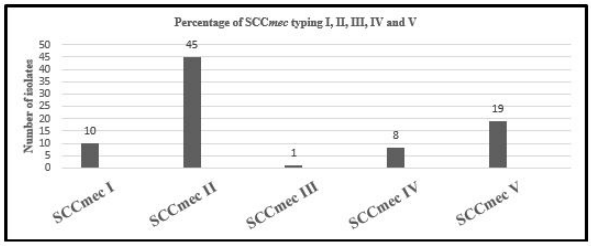

Figure 6: SCC*mec* Type I, II, III, IV and V of *S. haemolyticus* isolates. The figure shows the number of *S. haemolyticus* commensal isolates which harbour SCCmec Type I, II, III, IV and V.

DISCUSSION

The spread of MR-CoNS from the hospital setting into the healthy-community environment has been reported worldwide (30,31). However, in Malaysia the information on the incidence of MR-CoNS in general or MRSH especially among commensal isolates in particular is very limited.

Where MR-*Staphylococcus* is concerned, the definition of methicillin resistance varies in both *S. aureus* and CoNS in general. In some studies, resistant against cefoxitin was used to determine the MR strains for both *S. aureus* (27) and CoNS (28). This test can be executed by using cefoxitin disk (30 µg) whereby minimal inhibitory control (MIC) of ≥8µg/mL was reported as MR. In contrast, the genotypic approach has also been used in determining the MR- *Staphylococcus* strains by

Table I: SCC*mec* typing of the commensal isolates of MRSH and MSSH

Isolates	*mec*A	MR test		SCC*mec* Type				
		MRSH	MSSH	I	II	III	IV	V
T11	/	/		/	/			/
T32	/	/			/			
T48	/	/		/	/			
T57	/	/			/			/
T60	/	/			/			
T69	/	/			/	/	/	/
T71	/	/			/			/
T94	/	/			/			
T133	/	/		/	/		/	/
T134	/	/		/	/			/
T135	/	/			/		/	
T150	/	/		/	/		/	/
T151	/	/			/			
T152	/	/		/	/			
T181	/	/		/	/			/
T220	/	/			/			/
T253	/	/			/			
T280	/	/			/			
S30	/	/			/			
S38	/	/			/			
S45	/	/						/
S47	/	/			/			/
S66	/	/			/			
S90	/	/			/			/
S175	/	/		/	/			/
T47	/		/		/			
T59	/		/		/		/	
T165	/		/	/	/			/
T187	/		/					
T209	/		/		/		/	
T212	/		/		/			
T225	/		/		/			/
T226	/		/		/			
S6	/		/		/		/	
S25	/		/		/			/
S85	/		/		/			
S92	/		/				/	
S112	/		/		/			
S123	/		/		/			
S125	/		/		/			
S126	/		/		/			/
S167	/		/		/			/
S168	/		/	/				/
T29		/						
T56		/						
T254		/						
S37		/						
T27			/					
T247			/					

Distribution of the MRSH & MSSH isolates on *mec*A gene and SCC*mec* Type I, II, III, IV and V.

amplification of *mec*A gene which is responsible for the production of the penicillin binding protein PBP2a, associated with the mechanism of MR (10). Thus, the screening of *mec*A gene is considered vital to determine the MR strains in *S. haemolyticus* (32), CoNS (33) and also in *S. aureus* (29). In addition, there are also studies on CoNS which define MR-*Staphylococcus* strains as both by the presence of *mec*A gene and also resistant against cefoxitin (34,35).

However, the use of cefoxitin disc is known to be a gold standard for identification of MR isolates in *Staphylococcus* (27,28) while Anand et al., (2009) suggested the sensitivity and specificity of the cefoxitin disk method to be 100%,. The guidelines by CLSI (2019) also suggested the use of cefoxitin as a surrogate test for MR detection in *Staphylococcus*. Hence, similar approach was used in this study.

A total of 29 or 59.2% of the *S. haemolyticus* isolates were found to be MRSH while the remaining 20 isolates or 40.8% were MSSH. Subsequent amplification of *mec*A gene showed that the majority of the MRSH strains harbour the gene. However, there were 18 of the *mec*A positive isolates that were negative for cefoxitin. This indicates that the presence of *mec*A did not always result in isolates demonstrating significant levels of resistance to cefoxitin. Similar occurrence was observed in another study which suggested that this could be due to the low expression of PBP2a that probably cause the low levels of minimum inhibitory concentration in cefoxitin (34,36). In another study conducted on oxacillin-susceptible-*mec*A-positive *Staphylococcus* without *mec* regulators, it was found that high expression of BlaI strongly repressed the expression of *mec*A and rendered the susceptible phenotype (37).

In contrast, there were four *S. haemolyticus* isolates that were resistant against cefoxitin but negative for *mec*A gene. Such incidence has also been observed in other studies involving *S. aureus* whereby it was believed that the loss of MR genotype is due to partial excision of the SCC*mec* (38) whereby in *S. haemolyticus*, the isolates were found to be lacking of *mec*A but carry the ccrC complex instead (12). Hence, this suggests that to exclude MR-*Staphylococcus* by the absence of *mec*A gene should strongly be reconsidered.

The present study shows that majority of 90.7% of the *S. haemolyticus* isolates were found to harbour SCC*mec* Type II while 44.2% harbour the SCC*mec* Type V. In contrast, a study by Ruppé and friends in four countries (Algeria, Cambodia, Moldova and Mali), SCC*mec* Type V was the most prevalent among commensals MRSH strains followed by SCC*mec* Type IV. In agreement, a study in French Guinea, Lebeaux et al., (2012) also identified that SCC*mec* type V was predominant followed by SCC*mec* Type IV. Similarly, in two more studies in China and Denmark, SCC*mec* Type V and IV were also found to be the most predominant SCC*mec* isolated (39–41).

Hence, generally, in *S. haemolyticus* from community environment, both SCC*mec* Type IV and Type V were found to be predominant. However, in most of these studies, the *S. haemolyticus* was isolated from the nasal of healthy adults instead. This is because, in these studies, the samples gathered were actually part of CoNS from nasal samples collected during a large epidemiological study devoted to the analysis of *S. aureus* nasal carriage in the community (30,42). In the present study, the *S. haemolyticus* strains were isolated from other area of the body which include the groins, axillae and perineum area instead as the bacterium is believed to be abundance in these area (4,20). In the current literature, evidence of study on *S. haemolyticus* commensal isolates is scarce where any studies on *S. haemolyticus* is actually targeted on CoNS in general while *S. haemolyticus* is just a subset of the study. Hence, there is very little information on studies dedicated solely on *S. haemolyticus*.

In general, the incidence of SCC*mec* element which could be associated with the pathogenicity and generally dispersed among MR-CoNS species was suggested to be rely upon the host species, environments and geographical areas (33,35,36). In addition, the results in this study also suggests the possibilities that the variety of SCC*mec* could also varies depending on the different anatomical areas of human body. However, studies with larger samples is necessary to confirm these findings.

The reasons for the increasing clinical significance among *S. haemolyticus* strains however remain unclear. The data on MRSH among commensal strains are lacking enough to hinder the possibility of evaluating their resistance ability as compared to commensals MRSA. It is predicted that *S. haemolyticus* would contribute to critical health concern for medical practitioners as the commensal MRSH started to manifest a greater issue among community-health environment. This can be seen where SCC*mec* typing profile especially among CoNS was found to have higher capacity of genetic transferability among *Staphylococcus* species (12,44).

In community-acquired MRSA (CA-MRSA) strains, the majority of the SCC*mec* among the strains was also found to be SCC*mec* IV and V (18,45–47). The existence of the same SCC*mec* typing among different species suggested that staphylococci are frequently exchanging genetic material (48). In the presence study, SCC*mec* Type V was also frequently isolated among the MRSH commensal isolates. It was suggested that it might be a possibility of commensals MRSA and MRSH to share the same SCC*mec* typing as their antibiotic resistance genes where genetic transmission between host may occur (49). In 2005, Takeuchi and friends sequenced the *S. haemolyticus* strain JCSC1435 and compared to the genomes of *S. aureus* and *S. epidermidi*s which

served as control strains. The IS of *S. haemolyticus* showed high similarities towards *S. aureus* (86%) and *S. epidermidis* (81%). Later, Barros et al., (2012) reported that horizontal transfer of SCC*mec* Type V from MRSH to methicillin-susceptible *S. aureus* (MSSA), resulted in the transformation of MSSA to MRSA which might be a potential outbreak. *S. haemolyticus* was suspected to have a highly flexible genome that support the frequent exchange and high degree of recombination of DNA either intra or inter-species (7). This indicates that resistance genes could be transferred from *S. haemolyticus* to *S. aureus* or vice-versa via the SCC*mec* cassette (1).

Such rearrangements could have raised the genomic plasticity to this species and contribute to its acquisition of antibiotics resistance. This may pose as a real clinical problem if *S. haemolyticus* or CoNS in general evolved to the level of MRSA, creating yet another 'superbug' by freely exchanging genetic materials. A study by Chen and co-workers revealed that the evolution of hospital acquired *S. haemolyticus* in a recent event was due to the acquisition of SCC*mec* typing that have been circulated among the commensal strains (40). In addition, the possible acquisition of mobile genetic elements in *S. haemolyticus* could also contribute to the success of this species in hospital environment (15).

CONCLUSION

In this study, 59.2% of MRSH among the commensals isolates indicates the significance of this once harmless CoNS in the medical setting. The presence of a variety of SCC*mec* typing in the commensal isolates suggest that the mobile genetic elements can be a point of mutations for resistance genes rearrangement. However, in *S. haemolyticus*, the pathogenicity occurrence could possibly also be associated with arginine catabolic mobile elements (ACME) but the mechanism remains unclear. It was reported that MR-*Staphylococcus* might harbour both SCC*mec* and ACME instead of *mec*A gene by sharing the same nucleotide position in the open reading frame (50). Hence, a continuous surveillance and molecular study is proposed to observe the evolution and spread pattern of resistance genes among commensals *S. haemolyticus*. As such, the multi-drug pattern among these commensal isolates will be further investigated.

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REFERENCES

1. Czekaj T, Ciszewski M, Szewczyk EM. *Staphylococcus haemolyticus* – an emerging threat in the twilight of the antibiotics age. Microbiology. 2015 Nov 1;161(11):2061–8.
2. Kumar S, endra J, Das A, Mane P, Sangwan J, Kumari S. Isolation, Identification and Antibiogram of Coagulase Negative *Staphylococcus* (CoNS) Isolated from Various Clinical Samples at a Tertiary Care Teaching Hospital, Jaipur, India. Int J Curr Microbiol Appl Sci. 2018 Jan 20;7(1):3048–59.
3. Mohamad M, Deabate L, Belaieff W, Bouvet C, Zingg M, Kuczma P, et al. Prosthetic Joint Infections Due to Coagulase-Negative Staphylococci. Int J Infect [Internet]. 2016 Jan 1 [cited 2019 Mar 1];3(1).
4. Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. J Bacteriol. 2005 Nov;187(21):7292–308.
5. Barros EM, Ceotto H, Bastos MCF, dos Santos KRN, Giambiagi-deMarval M. *Staphylococcus haemolyticus* as an Important Hospital Pathogen and Carrier of Methicillin Resistance Genes. J Clin Microbiol. 2012 Jan;50(1):166–8.
6. Krzymińska S, Szczuka E, Kaznowski A. *Staphylococcus haemolyticus* strains target mitochondria and induce caspase-dependent apoptosis of macrophages. Antonie Van Leeuwenhoek. 2012 Nov;102(4):611–20.
7. Cavanagh JP, Hjerde E, Holden MTG, Kahlke T, Klingenberg C, Flaegstad T, et al. Whole-genome sequencing reveals clonal expansion of multiresistant *Staphylococcus haemolyticus* in European hospitals. J Antimicrob Chemother. 2014 Nov 1;69(11):2920–7.
8. Holden MTG, Hsu L-Y, Kurt K, Weinert LA, Mather AE, Harris SR, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res. 2013 Apr;23(4):653–64.
9. Fuller E, Elmer C, Nattress F, Ellis R, Horne G, Cook P, et al. β-Lactam Resistance in *Staphylococcus aureus* Cells That Do Not Require a Cell Wall for Integrity. Antimicrob Agents Chemother. 2005 Dec;49(12):5075–80.
10. Brody T, Yavatkar AS, Lin Y, Ross J, Kuzin A, Kundu M, et al. Horizontal Gene Transfers Link a Human MRSA Pathogen to Contagious Bovine Mastitis Bacteria. Hofreiter M, editor. PLoS ONE. 2008 Aug 27;3(8):e3074.
11. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesшe RL, et al. SCC*mec*Finder, a Web-Based Tool for Typing of *Staphylococcal* Cassette Chromosome *mec* in *Staphylococcus aureus* Using Whole-Genome Sequence Data. mSphere [Internet]. 2018 Feb 14 [cited 2018 Jun 28];3(1).

12. Bouchami O, Achour W, Mekni MA, Rolo J, Ben Hassen A. Antibiotic resistance and molecular characterization of clinical isolates of methicillin-resistant coagulase-negative staphylococci isolated from bacteremic patients in oncohematology. Folia Microbiol (Praha). 2011 Mar;56(2):122–30.
13. Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, et al. Whole-Genome Sequencing of *Staphylococcus haemolyticus* Uncovers the Extreme Plasticity of Its Genome and the Evolution of Human-Colonizing Staphylococcal Species. J Bacteriol. 2005 Nov 1;187(21):7292–308.
14. McManus BA, Coleman DC, Deasy EC, Brennan GI, Connell BO, Monecke S, et al. Comparative Genotypes, *Staphylococcal* Cassette Chromosome mec (SCC*mec*) Genes and Antimicrobial Resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Isolates from Infections in Humans and Companion Animals. PLoS ONE [Internet]. 2015 [cited 2019 Sep 22];10(9).
15. Pain M, Hjerde E, Klingenberg C, Cavanagh JP. Comparative Genomic Analysis of *Staphylococcus haemolyticus* Reveals Key to Hospital Adaptation and Pathogenicity. Front Microbiol [Internet]. 2019 Sep 10 [cited 2019 Oct 19];10.
16. Adegoke A, Okoh A. Commensal *Staphylococcus* spp., *Acinetobacter* spp. and *Stenotrophomonas maltophilia* as reservoirs of antibiotic resistance genes. Afr J Biotechnol. 2012 Jul 31;11:12429–35.
17. Cavanagh JP, Wolden R, Heise P, Esaiassen E, Klingenberg C, Aarag Fredheim EG. Antimicrobial susceptibility and body site distribution of community isolates of coagulase-negative staphylococci. APMIS. 2016 Nov;124(11):973–8.
18. Asiimwe BB, Baldan R, Trovato A, Cirillo DM. Molecular epidemiology of Panton-Valentine Leukocidin-positive community-acquired methicillin resistant *Staphylococcus aureus* isolates in pastoral communities of rural south western Uganda. BMC Infect Dis [Internet]. 2017 Jan 5 [cited 2019 Sep 26];17.
19. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? Clin Microbiol Infect. 2019 Sep;25(9):1071–80.
20. Becker K, Heilmann C, Peters G. Coagulase-Negative Staphylococci. Clin Microbiol Rev. 2014 Oct 1;27(4):870–926.
21. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev. 1994 Jan;7(1):117–40.
22. Abdul-Aziz A, Mohamad SAS, Abdullah MFF. Identification of Coagulase-Negative Staphylococci by SodA Gene Sequence Analysis. Curr Res Bacteriol. 2015 Mar 1;8(3):48–61.
23. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008 Jan;3(2):163–75.
24. Zhang K, McClure J-A, Conly JM. Enhanced multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. Mol Cell Probes. 2012 Oct 1;26(5):218–21.
25. Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome mec Types I to V in Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2005 Oct 1;43(10):5026–33.
26. Abdul-Aziz A, Anuar II, Mohamad SAS, Mohd-Zain Z, Abdullah MFF. Detection of type I-V SCCmec in methicillin-resistant *Staphylococcus capitis* isolates from Malaysia. Malays J Biochem Mol Biol. 2018 Aug 1;21:12–6.
27. Fernandes CJ, Fernandes LA, Collignon P. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother. 2005 Apr 1;55(4):506–10.
28. Jain A, Agarwal A, Verma RK. Cefoxitin disc diffusion test for detection of meticillin-resistant staphylococci. J Med Microbiol. 2008 Aug 1;57(8):957–61.
29. Ibrahim OMA, Bilal NE, Osman OF, Magzoub MA. Assessment of methicillin resistant *Staphylococcus aureus* detection methods: analytical comparative study. Pan Afr Med J [Internet]. 2017 Aug 21 [cited 2019 Oct 20];27.
30. Lebeaux D, Barbier F, Angebault C, Benmahdi L, Ruppй E, Felix B, et al. Evolution of nasal carriage of methicillin-resistant coagulase-negative staphylococci in a remote population. Antimicrob Agents Chemother. 2012 Jan;56(1):315–23.
31. Widerstrцm M, Wistrцm J, Edebro H, Marklund E, Backman M, Lindqvist P, et al. Colonization of patients, healthcare workers, and the environment with healthcare-associated *Staphylococcus epidermidis* genotypes in an intensive care unit: a prospective observational cohort study. BMC Infect Dis. 2016 Dec 9;16(1):743.
32. Hosseinkhani F, Jabalameli F, Nodeh Farahani N, Taherikalani M, van Leeuwen WB, Emaneini M. Variable number of tandem repeat profiles and antimicrobial resistance patterns of *Staphylococcus haemolyticus* strains isolated from blood cultures in children. Infect Genet Evol. 2016 Mar;38:19–21.
33. Salgueiro VC, Seixas MDL, Guimarres LC, Ferreira D de C, Cunha DC da, Nouйr SA, et al. High rate of neonates colonized by methicillin-resistant *Staphylococcus* species in an Intensive Care Unit. J Infect Dev Ctries. 2019 Sep 30;13(09):810–6.
34. Seng R, Leungtongkam U, Thummeepak R, Chatdumrong W, Sitthisak S. High prevalence of methicillin-resistant coagulase-negative staphylococci isolated from a university environment in Thailand. Int Microbiol. 2017;(20):65–73.
35. Talebi M, Shafiee M, Sadeghi J, Moghadam NA, Saifi M, Pourshafie MR. Genotypic Diversity

of Methicillin-Resistant Coagulase-Negative *Staphylococci* Isolated from Inpatients and Outpatients. Microb Drug Resist Larchmt N. 2016 Mar;22(2):147–54.
36. Xu Z, Mkrtchyan HV, Cutler RR. Antibiotic resistance and mecA characterization of coagulase-negative staphylococci isolated from three hotels in London, UK. Front Microbiol. 2015;6:947.
37. Liu P, Xue H, Wu Z, Ma J, Zhao X. Effect of bla regulators on the susceptible phenotype and phenotypic conversion for oxacillin-susceptible mecA-positive staphylococcal isolates. J Antimicrob Chemother. 2016;71(8):2105–12.
38. Wong H, Louie L, Lo RYC, Simor AE. Characterization of *Staphylococcus aureus* Isolates with a Partial or Complete Absence of Staphylococcal Cassette Chromosome Elements. J Clin Microbiol. 2010 Oct 1;48(10):3525–31.
39. Faria NA, Conceiɜro T, Miragaia M, Bartels MD, de Lencastre H, Westh H. Nasal Carriage of Methicillin Resistant Staphylococci. Microb Drug Resist. 2014 Apr;20(2):108–17.
40. Chen X-P, Li W-G, Zheng H, Du H-Y, Zhang L, Zhang L, et al. Extreme diversity and multiple SCCmec elements in coagulase-negative *Staphylococcus* found in the Clinic and Community in Beijing, China. Ann Clin Microbiol Antimicrob [Internet]. 2017 Aug 22 [cited 2019 Sep 26];16.
41. Li L, Chen Z, Guo D, Li S, Huang J, Wang X, et al. Nasal carriage of methicillin-resistant coagulase-negative staphylococci in healthy humans is associated with occupational pig contact in a dose-response manner. Vet Microbiol. 2017 Sep;208:231–8.
42. Ruppé E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, et al. Diversity of Staphylococcal Cassette Chromosome mec Structures in Methicillin-Resistant Staphylococcus epidermidis and Staphylococcus haemolyticus Strains among Outpatients from Four Countries. Antimicrob Agents Chemother. 2009 Feb 1;53(2):442–9.
43. Murugesan S, Perumal N, Mahalingam SP, Dilliappan SK, Krishnan P. Analysis of Antibiotic Resistance Genes and its Associated SCCmec Types among Nasal Carriage of Methicillin Resistant Coagulase Negative Staphylococci from Community Settings, Chennai, Southern India. J Clin Diagn Res JCDR. 2015 Aug;9(8):DC01–5.
44. Vitali LA, Petrelli D, Lamikanra A, Prenna M, Akinkunmi EO. Diversity of antibiotic resistance genes and staphylococcal cassette chromosome mec elements in faecal isolates of coagulase-negative staphylococci from Nigeria. BMC Microbiol. 2014 Apr 26;14:106.
45. Zhang K, McClure J-A, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2009 Feb;53(2):531–40.
46. Reich PJ, Boyle MG, Hogan PG, Johnson AJ, Wallace MA, Elward AM, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect. 2016 Jul 1;22(7):645.e1-645.e8.
47. Funaki T, Yasuhara T, Kugawa S, Yamazaki Y, Sugano E, Nagakura Y, et al. SCCmec typing of PVL-positive community-acquired Staphylococcus aureus (CA-MRSA) at a Japanese hospital. Heliyon [Internet]. 2019 Mar 28 [cited 2019 Sep 26];5(3).
48. Hanssen A-M, Ericson Sollid JU. SCCmec in staphylococci: genes on the move. FEMS Immunol Med Microbiol. 2006 Feb 1;46(1):8–20.
49. Rodrigues AC, Belas A, Marques C, Cruz L, Gama LT, Pomba C. Risk Factors for Nasal Colonization by Methicillin-Resistant Staphylococci in Healthy Humans in Professional Daily Contact with Companion Animals in Portugal. Microb Drug Resist. 2018 May;24(4):434–46.
50. Shore AC, Rossney AS, Brennan OM, Kinnevey PM, Humphreys H, Sullivan DJ, et al. Characterization of a Novel Arginine Catabolic Mobile Element (ACME) and Staphylococcal Chromosomal Cassette mec Composite Island with Significant Homology to *Staphylococcus epidermidis* ACME Type II in Methicillin-Resistant Staphylococcus aureus Genotype ST22-MRSA-IVv. Antimicrob Agents Chemother. 2011 May;55(5):1896–905.