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# Multidrug resistance profile and SCCmec typing of *Staphylococcus haemolyticus* in commensal and clinical isolates

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**ABSTRACT**

Long regarded as a contaminant in clinical cultures, *Staphylococcus haemolyticus* has emerged as a major bacterial species responsible for a variety of serious human infections. This species is increasingly prevalent as an opportunistic agent, and the evolution of *S. haemolyticus* as a multiple drug-resistant strain is emerging as a major threat in worldwide healthcare facilities. In this study, 50 commensal and 98 clinical strains of *S. haemolyticus* were isolated and confirmed via superoxide dismutase gene sequence analysis. All isolates were tested against a series of antibiotics from ten different classes and screened for *Staphylococcus* Chromosomal Cassette (SCC) Types I, II, III, IV, and V. *Staphylococcus haemolyticus* resistance to antibiotics was observed in both the clinical and commensal strains isolated from healthy adults. In clinical isolates, the highest resistance was observed against erythromycin at 79.6% followed by cefoxitin (71.4%), while a high percentage of the commensals were resistant against cefoxitin (56.0%) and tigecycline (40.0%). More than half of the *S. haemolyticus* clinical isolates are multidrug resistance (MDR) strains at 54.1%, while 20.0% of the *S. haemolyticus* commensals are MDR strains. The majority of these MDR strains are methicillin-resistant *S. haemolyticus* (MRSH) suggesting a close relationship between the methicillin-resistant strains and resistance to multiple classes of antibiotics. SCC*mec* Type II is the most abundant type observed in both commensal (92.0%) and clinical (99.0%) isolates of *S. haemolyticus* followed by Type V at 38.0% and 46.9%, respectively. The similar pattern of typing observed indicates the possibility that the clinical isolates of *S. haemolyticus* could have originated from the commensal strains that had successfully entered the host and caused infections. The antibiotic profile indicates the natural resistance of *S. haemolyticus* to antibiotics, even among the commensal strains. It also appears that transmission of genetic determinants for antibiotic resistance is common and widespread among all the *S. haemolyticus* isolates. Commensal strains of *S. haemolyticus* may thus serve as reservoirs for the transmission of antibiotic resistance and the development of opportunistic strains.

## INTRODUCTION

Among the coagulase-negative *Staphylococcus* (CoNS), *Staphylococcus haemolyticus* is one of the most abundant species of the human skin microbiome besides *Staphylococcus epidermidis* and *Staphylococcus capitis* (Ahmadunissah *et al*., 2022; Kitti *et al*., 2018; Socohou *et al*., 2020). As part of the human microflora, *S. haemolyticus* is found abundantly on the axillae, perineum, and inguinal areas due to the existence of apocrine glands in this area (Adeghate *et al*., 2020; Becker *et al*., 2014; Natsis and Cohen, 2018).

*S. haemolyticus* is also a prominent species frequently isolated from clinical cases of human infections. Long dismissed as contaminants, *S. haemolyticus* has recently emerged as a notorious

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species responsible for many types of opportunistic infections in their host (Barros *et al*., 2012; Chaudhry and Patil, 2020). In blood infections including sepsis, *S. haemolyticus* was reported as the second most frequently isolated CoNS from clinical cases after *S. epidermidis*. This versatile species is also responsible for a variety of other infections including peritonitis, otitis, urinary tract infection, male infertility, and meningitis (AL-Ghizzawi and Jomaa, 2018; Czekaj *et al*., 2015; Shi *et al*., 2019). In Malaysia, *S. haemolyticus* is listed as among the top three CoNS isolated from nosocomial infections in hospitals (Abdul-Aziz, 2020; Sukri *et al*., 2022). In addition, *S. haemolyticus* is among the most frequently isolated agents from infections related to indwelling medical devices such as prosthetic implantations, intravascular catheters, urinary catheters, surgery devices, and orthopedic implants (Czekaj *et al*., 2015; Lacalzada *et al*., 2010; Socohou *et al*., 2020). This evidence indicates the emergence of *S. haemolyticus* as a potential major threat in healthcare facilities (Cave *et al*., 2021).

Epidemiological data showed a rising number of commensals or nonpathogenic bacteria becoming implicated as causative agents of serious infections (Leal-Lopes *et al*., 2015; Marshall *et al*., 2009). It is believed that commensal bacteria could adapt and transform into pathogenic strains under selective pressure through the acquisition of virulence or resistance genes from pathogen counterparts (Adegoke and Okoh, 2012). In 2015, Czekaj *et al*. (2015) suggested that *S. haemolyticus* should no longer be dismissed as contaminants because their role in pathogenicity has become increasingly vital, a statement echoed by both Barros *et al*. (2012) and Chaudhry and Pathil (2020). Recommended treatment for infections by *S. haemolyticus* includes glycopeptide antibiotics such as vancomycin and teicoplanin while linezolid is used as a last-resort drug (Czekaj *et al*., 2015; Daniel *et al*., 2014).

Unlike *S. aureus* and *S. epidermidis*, published data regarding the mechanisms of pathogenesis and virulence factors of *S. haemolyticus* are scarce and incomprehensive (Pain *et al*., 2019; Wolden *et al*., 2020). However, the majority of the studies on *S. haemolyticus* showed a common feature in which this species shows high resistance against many antibiotics (AL-Ghizzawi and Jomaa, 2018; Cavanagh *et al*., 2016; Czekaj *et al*., 2015; Shi *et al*., 2019). The level of resistance observed in *S. haemolyticus* against several major antibiotic classes has emerged as a cause of public health threats, especially with the rise in the reports of multidrug resistance (MDR) strains. An MDR strain is defined as a microorganism that has the capability to be resistant to three or more classes of antibiotics (Peters *et al*., 2019; Schmidt *et al*., 2020). *Staphylococcus haemolyticus* strains have shown resistance against antibiotic classes including the β-lactams, aminoglycosides, macrolides, lincomycin, quinolones, and tetracyclines (Bathavatchalam *et al*., 2021; Manoharan *et al*., 2021; Sarker, 2021). However, these findings were focused only on clinical isolates while data on the antibiotic resistance profile of the commensal strains of *S. haemolyticus* is lacking. It would be interesting to see if the antibiotic resistance ability of *S. haemolyticus* is also extended to the "nonpathogenic" commensal counterparts. Hence, in this study, the resistance patterns of both clinical and commensal *S. haemolyticus* isolates against ten different classes of antibiotics were evaluated.

The second point of concern is the rising incidence of methicillin-resistant *S. haemolyticus* (MRSH) isolates from clinical settings (Gómez-Sanz *et al*., 2019; Suhartono *et al*., 2019; Teeraputon *et al*., 2017). The methicillin-resistant phenotype is associated with the ability of *S. haemolyticus* to accumulate penicillin-binding protein PBP2a encodes by the *mec*A gene, which has reduced affinity to β-lactam antibiotics and subsequently decreased susceptibility to methicillin and other penicillin derivatives (Mbah and Isokpehi, 2013; Santiago *et al*., 2014). The *Staphylococcus* Chromosomal Cassette (SCC)*mec* is a unique class of mobile genetic elements found in *Staphylococcus* spp. which carries the *mec*A gene conferring resistance to methicillin. SCC*mec* can also carry genes conferring resistance to non-β-lactam antibiotics (Baig *et al*., 2018; Liu *et al*., 2016). The *mec*A gene is the part of *mec* gene complex which comprises specific insertion sequences and two regulatory genes, *mec*R1 and *mec*I (Chovanová *et al*., 2016; Shore and Coleman, 2013). The components of the *mec* gene complex include the *ccr* gene and junkyard (J) regions and are used to define the SCC*mec* types (Kaya *et al*., 2018; Liu *et al*., 2016). Unfortunately, in the majority of the documented studies on *Staphylococcus*, the SCC*mec* typing among clinical *S. haemolyticus* isolates was commonly reported not as an individual species but as a subset of CoNS instead. In this study, the distribution of SCC*mec* typing of Types I, II, III, IV, and V among the isolates would be further investigated. It is hoped that this study would help to define SCC*mec* elements associated with the resistance against different classes of antibiotics which are widespread among *S. haemolyticus* strains.

## MATERIALS AND METHODS

### Bacterial sampling and isolation

The collection of both clinical and commensal isolates was conducted with the approval of the Research Ethics Committee, UiTM [REC 600-IRMI (5/1/6)].

Presumptive commensal isolates of *Staphylococcus* were collected from 150 healthy adults via the skin swabbing method (Batista *et al*., 2019). The healthy adult group comprises volunteers aged between 21 and 45 years old, and they were recruited from employees and students from the university where the study took place. A healthy adult is defined as an adult without antibiotic exposure and no hospitalization or any healthcare affiliation for the previous three months (Cavanagh *et al*., 2016; Pain *et al*., 2019). The sterile cotton swab was dipped into a test tube containing sterile 0.80% (w/v) saline and was pressed to the sides of the tubes to remove excess water. Each of the swabs was then rubbed on the skin of the axillae or the groin several times. These anatomical parts were selected as they represent the most common areas where *S. haemolyticus* resides (Strasheim *et al*., 2013). The swab was then inoculated in fresh brain heart infusion (BHI) broth and incubated overnight at 37°C, 150 rpm.

*Staphylococcus* clinical isolates were obtained from the microbiology laboratories of several hospitals in the Klang Valley area in Selangor. The samples were previously isolated from various clinical specimens such as blood, respiratory fluid, urine, pus, and tissue swabs. Upon collection, a loopful of each of the clinical specimens was cultured in BHI broth and incubated overnight at 37°C, 150 rpm.

A loopful of each of the overnight cultures was then streaked on mannitol salt agar and incubated at 37°C for 18–24 hours. Pink or white colonies which indicate the growth of CoNS were selected and subcultured to obtain pure colonies. Presumptive

identification of *S. haemolyticus* isolates was performed via a series of standard biochemical tests including Gram stain, catalase, coagulase, and urease test (Kloos and Bannerman, 1994).

**Species identification**

All the presumptive *Staphylococcus* isolates were further subjected to PCR amplification of the superoxide dismutase (*sod*A) gene (Abdul-Aziz *et al*., 2015) using the primers d1 (5′ CCI TAY ICI TAY GAY GCI YTI GAR CC ′3) and d2 (5′ARR TAR TAI GCR TGY TCC CAI ACR TC ′3). The amplicon has an expected size of 426 bp and represents 83.0% of the *sod*A gene. *Staphylococcus haemolyticus* ATCC29970 was used as a positive control.

Genomic DNA was extracted using a commercial kit (Qiagen DNeasy, USA) according to the manufacturer's protocol. PCR was performed using MyTaq Master Mix (Bioline, UK) on a Mastercycler nexus gradient (Eppendorf, Germany) with the following conditions: 3 minutes at 95.0°C for the initial cycle, followed by 30 cycles of amplification of the followings: 30 seconds of denaturation at 95.0°C, 60 seconds of annealing at 37.0°C, and 45 seconds of elongation at 72.0°C. The last cycle was 72.0°C for 10 minutes. The PCR purification was performed via ExoSap (New England Biolabs, USA) and the clean PCR products were sequenced by Bio Basic, Singapore. The resulting sequence data were analyzed through the GenBank database using the BLAST interface. The match with the highest percentage of similarity with a minimum of 99.0% similarity and 99.0% coverage was confirmed as *S. haemolyticus*.

**Antimicrobial susceptibility profile**

The antimicrobial susceptibility profile of the *S. haemolyticus* isolates was performed using the Kirby-Bauer disc diffusion method against ten antibiotics from different classes: β-lactam (cefoxitin, 30.0 µg), fluoroquinolone (ciprofloxacin, 5.0 µg), lincomycin (clindamycin, 2.0 µg), macrolide (erythromycin, 15.0 µg), aminoglycoside (gentamicin, 10.0 µg), oxazolidinone (linezolid, 30.0 µg), ansamycin (rifampicin, 5.0 µg), tetracycline (tetracycline, 30.0 µg), glycylcycline (tigecycline, 15.0 µg), and glycopeptide (vancomycin, 30.0 µg). A loopful of *S. haemolyticus* culture was inoculated into Mueller Hinton Broth and incubated overnight at 37.0°C, 150 rpm. The culture was then diluted to 1:100 and further incubated for approximately 2 hours until it reached the exponential phase. The turbidity of the culture was adjusted to 0.5 McFarland standard (Wiegand *et al*., 2008) and swabbed onto Mueller Hinton Agar plate with antibiotic discs placed on the agar. The plates were incubated overnight at 37.0°C and the inhibition zones were measured after 18 to 24 hours of incubation. The antibiograms of the isolates were interpreted according to the 2018 guidelines by the Clinical and Laboratory Standard Institute (CLSI, 2018). For vancomycin, the efficacy of this antibiotic was tested using the *E*-test instead as recommended by CSLI. The bacteria culture was prepared in the same manner as the disc diffusion method, and a vancomycin *E*-test strip (M.I.C.E, UK) was applied in place of a disc.

***mec*A gene**

The presence of *mec*A gene was determined in the *S. haemolyticus* isolates by PCR using primers *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 gives an expected amplicon of 531bp (Al-Khulaifi Manal *et al*., 2009; Zhang *et al*., 2005a). *Staphylococcus aureus* ATCC 33591 was used as the positive control. The amplification conditions were 1 minute at 94.0°C for initial denaturation followed by 30 cycles of amplification of the followings: 1 minute at 94.0°C for denaturation, 1 minute of annealing at 62.0°C and 45 seconds of extension at 72.0°C. The last cycle was performed at 72.0°C for 5 minutes. The PCR products were observed using gel electrophoresis in a 1.2% agarose gel.

**SCC*mec* Typing I, II, III, IV, and V**

The *S. haemolyticus* isolates were subjected to SCC*mec* typing for Types I, II, III, IV, and V using the multiplex PCR method of (Zhang *et al.*, 2005b, 2012) with the following conditions: 5 minutes at 94.0°C for initial denaturation followed by 30 cycles of amplification of the followings 1 minute at 94.0°C for denaturation, 1 minute of annealing at 62.0°C, and 2 minutes of extension at 72.0°C. The last cycle was performed at 72.0°C for 10 minutes. Positive amplifications were also confirmed using a single PCR method. The amplicons were observed using gel electrophoresis in a 1.5% agarose gel at 80V for 120 minutes. *Staphylococcus capitis* B102 was used as a positive control (Abdul-Aziz *et al.*, 2018).

## RESULTS

**Isolation of *S. haemolyticus* from commensal and clinical samples**

A total of 50 commensal strains of *S. haemolyticus* were successfully isolated from healthy adults while 98 strains of clinical isolates were obtained from three hospitals. All isolates used in this study were confirmed as *S. haemolyticus* by *sod*A gene sequence analysis. Figure 1 shows the amplification of the *sod*A gene in some of the isolates.

**Antibiotics resistance patterns of *S. haemolyticus***

The resistance patterns for both commensal and clinical isolates of *S. haemolyticus* against selected antibiotics are summarized in Figure 2.

Among the clinical isolates, the highest resistance was observed against erythromycin at 79.6%, followed by cefoxitin (71.4%), ciprofloxacin (40.8%), tigecycline (37.6%), and gentamicin (33.7%). On the other hand, the majority of the commensal isolates were resistant against cefoxitin at 56.0% followed by tigecycline (40.0%), clindamycin (34.0%), and clindamycin (34.0%).

In general, clinical isolates of *S. haemolyticus* displayed higher resistance against most of the antibiotics as compared to the commensals. An exception was observed in clindamycin and linezolid whereby a slightly higher degree of resistance to these antibiotics at 34.0% and 6.0% were seen in the commensal isolates as compared to the clinical isolates at 11.2% and 3.1%, respectively. Both clinical and commensal isolates showed an almost similar level of resistance against tigecycline at 40.0% and 37.6%, respectively.

**Methicillin-resistant *S. haemolyticus* (MRSH)**

Cefoxitin resistance (30 µg) is regarded as the gold standard for validation and identification of methicillin-resistant isolates among *Staphylococcus* sp. as suggested by the CLSI and

Centres for Disease Control and Prevention (Fernandes *et al*., 2005; Ibrahim *et al*., 2017; Jain *et al*., 2008). Hence, any *Staphylococcus* strain resistant to cefoxitin is determined as methicillin-resistant *Staphylococcus*.

Among the clinical isolates of *S. haemolyticus*, 70 (71.4%) were MRSH while the remaining 28% or 28.6% were MSSH. This is in agreement with reports from other studies, demonstrating the clinical significance of MRSH. Data on commensal *S. haemolyticus* MRSH, however, are lacking. Figure 2 shows results from this study indicating that the incidence of MRSH among the commensal isolates is surprisingly high at 56.0%.

### MDR profiles

Table 1 shows that 53% or 54.1% of the *S. haemolyticus* clinical isolates were MDR strains. From this, 17 (17.5%) were resistant to three antibiotic classes, five (5.1%) were resistant to four antibiotic classes, 13 (13.3%) were resistant to five antibiotic classes, and 14 (14.3%) were resistant to six different classes of antibiotics. Two of the clinical isolates were resistant to seven classes of antibiotics, and one isolate was resistant to all the antibiotics except for linezolid and vancomycin, while another isolate was only susceptible to vancomycin. The most prevalent resistance profile was observed in Pattern 22 (erythromycin-cefoxitin-ciprofloxacin-tigecycline-gentamicin-tetracycline) with a frequency of nine or 9.2% of the isolates.

The presence of MDR strains among the *S. haemolyticus* commensal isolates was also observed albeit at a smaller number of ten or 20.0%. Of these, three isolates (6.0%) were resistant against three and four antibiotics, respectively, while two isolates (4.0%) were resistant against five and six antibiotic classes, respectively. The most prevalent resistant patterns were Pattern 5 (erythromycin-cefoxitin-clindamycin) and Pattern 13 (erythromycin-cefoxitin-clindamycin-tigecycline) at 6.0%, respectively. Interestingly, none of the clinical and commensal isolates displayed similar resistance patterns against the antibiotics tested.

All the MDR strains were resistant to erythromycin (90.5%) or cefoxitin (96.8%). Coresistance to erythromycin and cefoxitin appears to be highly frequent and was observed in 87.3% (55/63) of the MDR strains. Among the commensals, nine out of ten strains displayed coresistance to erythromycin and cefoxitin. However, among the clinical isolates, 46 out of 53 strains (86.8%) have the erythromycin-cefoxitin profile. It is thus tempting to speculate that the "progenitor" *S. haemolyticus* MDR strain first acquired resistance to erythromycin and cefoxitin, and the subsequent accumulation of other resistance genes gave rise to the plethora of MDR strains observed. In erythromycin-cefoxitin-resistant strains, 22.2% show coresistance to ciprofloxacin, while

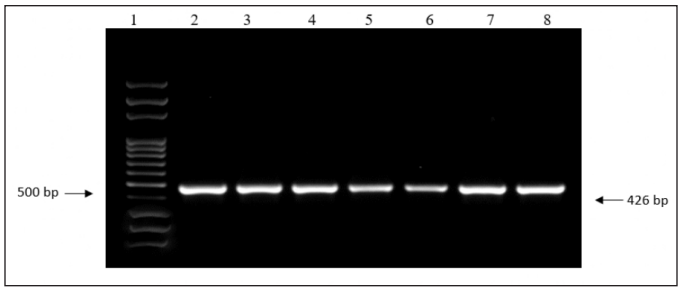


**Figure 1.** Amplification of *sod*A gene in *S. haemolyticus*. Lane 1: 100 bp of DNA ladder (Solis BioDyne) Lane 2: T11, Lane 3: S6, Lane 4: E1, Lane 5: A114, Lane 6: C49, Lane 7: D50, and Lane 8: *S. haemolyticus* ATCC 29970 (control).

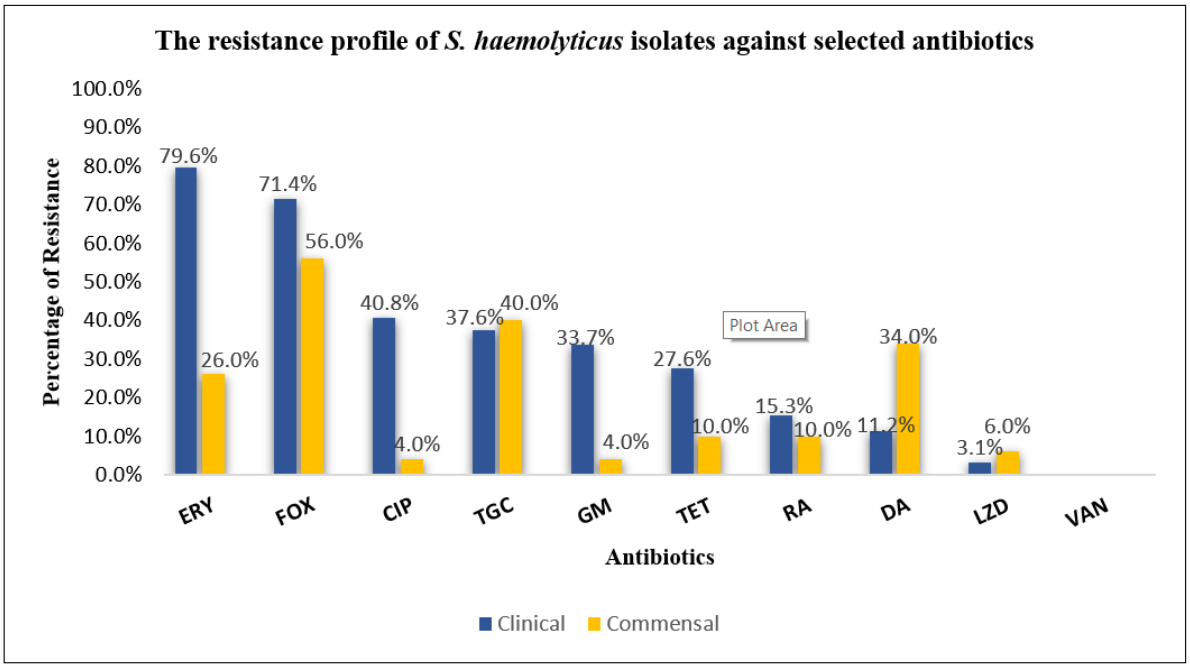


**Figure 2.** The resistance patterns of *S. haemolyticus* isolates against selected antibiotics. Erythromycin (ERY), Cefoxitin (FOX), Ciprofloxacin (CIP), Tigecycline (TIG), Gentamicin (GM), Tetracycline (TET), Rifampicin (RIF), Clindamycin (DA), Linezolid (LZD), and Vancomycin (VAN).

**Table 1**. Pattern of MDR among the *S. haemolyticus* isolates.

| Resistance pattern | Phenotypic pattern | Commensals number (%) | Clinicals number (%) |
|---|---|---|---|
| 1 | ERY-FOX-CIP | | 4 (4.1) |
| 2 | ERY-FOX-TGC | | 7 (7.1) |
| 3 | ERY-FOX-GM | | 1 (1.0) |
| 4 | ERY-FOX-TET | | 1 (1.0) |
| 5 | ERY-FOX-DA | 3 (6.0) | |
| 6 | ERY-TGC-TET | | 1 (1.0) |
| 7 | FOX-CIP-RA | | 1 (1.0) |
| 8 | FOX-TGC-TET | | 1 (1.0) |
| 9 | FOX-TGC-RA | | 1 (1.0) |
| 10 | ERY-FOX-CIP-TET | | 1 (1.0) |
| 11 | ERY-FOX-CIP-DA | | 1 (1.0) |
| 12 | ERY-FOX-TGC-TET | | 1 (1.0) |
| 13 | ERY-FOX-TGC-DA | 3 (6.0) | |
| 14 | FOX-CIP-GM-TET | | 1 (1.0) |
| 15 | FOX-CIP-GM-RA | | 1 (1.0) |
| 16 | ERY-FOX-CIP-TGC-GM | | 3 (3.1) |
| 17 | ERY-FOX-CIP-GM-TET | | 5 (5.1) |
| 18 | ERY-FOX-CIP-GM-RA | | 1 (1.0) |
| 19 | ERY-FOX-CIP-GM-DA | | 3 (3.1) |
| 20 | ERY-FOX-RA-DA-LZD | 2 (4.0) | |
| 21 | ERY-CIP-TGC-TET-DA | | 1 (1.0) |
| 22 | ERY-FOX-CIP-TGC-GM-TET | | 9 (9.2) |
| 23 | ERY-FOX-CIP-TGC-GM-RA | | 3 (3.1) |
| 24 | ERY-FOX-CIP-TGC-GM-DA | | 1 (1.0) |
| 25 | ERY-FOX-TGC-GM-TET-DA | | 1 (1.0) |
| 26 | ERY-FOX-TGC-RA-DA-LZD | 1 (2.0) | |
| 27 | FOX-CIP-GM-TET-RA-DA | 1 (2.0) | |
| 28 | ERY-FOX-CIP-TGC-GM-RA-DA | | 1 (1.0) |
| 29 | ERY-FOX-CIP-TGC-GM-RA-LZD | | 1 (1.0) |
| 30 | ERY-FOX-CIP-TGC-GM-TET-RA-DA | | 1 (1.0) |
| 31 | ERY-FOX-CIP-TGC-GM-TET-RA-DA-LZD | | 1 (1.0) |
| | TOTAL | 10 | 53 |

Erythromycin (ERY), Cefoxitin (FOX), Ciprofloxacin (CIP), Tigecycline (TGC), Gentamicin (GM), Tetracycline (TET), Rifampicin (RA), Clindamycin (DA), and Linezolid (LZD).

20.6% show coresistance to tigecycline. There is however no clear correlation observed between the antibiotic resistance profiles and the tendency to cause infections, although the erythromycin-cefoxitin-ciprofloxacin pattern appears to be enriched among the MDR clinical isolates.

### SCC*mec* Types I, II, III, IV, and V

There are currently 13 types of SCC*mec* classified based on the nature of their *mec* and *ccr* gene complexes (Kaya *et al*., 2018; McClure *et al*., 2020; Singh-Moodley *et al*., 2019). The majority of these SCC*mec* types are present among *S. aureus* isolates, while SCC*mec* Types I, II, III, IV, and V are reported to be common among CoNS. All the isolates in this study were found to harbor the *mec*A gene. Figures 3 and 4 display the results of the amplification of SCC*mec* Types I, II, III, IV, and V among some of the isolates while the details of the typing are tabulated in Table 2.

Our study showed that SCC*mec* Type II is the most abundant SCC*mec* type in both commensal (92.0%) and clinical (99.0%) isolates of *S. haemolyticus*, present either as a single type or in combination with other SCC*mec* types (Table 2). This is followed by Type V, which is present in 38.0% and 46.9% of the commensals and clinical isolates, respectively. Other SCC*mec* types include Type I (20.0% in commensals, 17.3% in clinicals), Type IV (8.0% in commensals, 7.1% in clinicals), and Type III (2.0% in commensals and 0% in clinicals).

## DISCUSSION

The ability of *S. haemolyticus* to resist killing by antibiotics has been reported in several studies worldwide. In

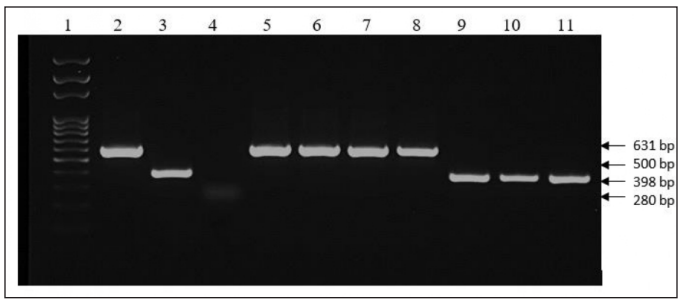


**Figure 3.** SCC*mec* typing for Types I, II, and III of some *S. haemolyticus* isolates. Lane 1: 100 bp of DNA ladder (Solis BioDyne). Lane 2–4: SCC*mec* Types I, II, III (control). SCC*mec* Type I (631 bp), Type II (398 bp), and Type III (280 bp).

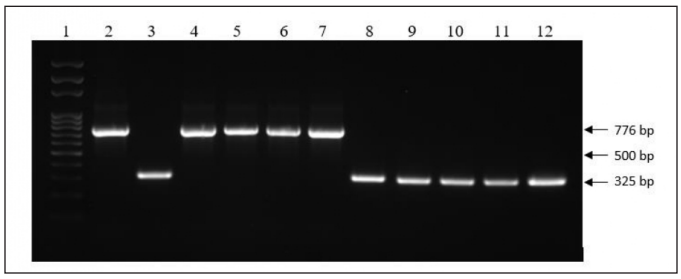


**Figure 4.** The SCC*mec* typing for Types IV and V of some *S. haemolyticus* isolates. Lane 1: 100 bp of DNA ladder (Solis BioDyne), Lanes 2–3: SCC*mec* Types IV and V (control), Type IV (776 bp), and Type V (325 bp).

**Table 2**. Distribution of SCC*mec* types among the *S. haemolyticus* isolates.

| SCC*mec* type | Commensals | | Clinicals | |
|---|---|---|---|---|
| | Number | (%) | Number | (%) |
| I | - | - | - | - |
| II | 23 | 46.0 | 36 | 36.7 |
| III | - | - | - | - |
| IV | 1 | 2.0 | - | |
| V | 1 | 2.0 | 1 | 1.0 |
| I and II | 2 | 4.0 | 13 | 13.3 |
| I and V | 1 | 2.0 | - | |
| II and IV | 4 | 8.0 | 3 | 3.1 |
| II and V | 9 | 18.0 | 37 | 37.8 |
| I, II, and V | 5 | 10.0 | 4 | 4.1 |
| II, IV, and V | - | - | 4 | 4.1 |
| I, II, IV, and V | 2 | 4.0 | - | - |
| II, III, IV, and V | 1 | 2.0 | - | - |
| None | 1 | 2.0 | - | - |
| TOTAL | 50 | | 98 | |

2012 in Brazil, Barros *et al*. (2012) isolated 64 clinical isolates of *S. haemolyticus* from human blood cultures. A high degree of resistance to cefoxitin was observed at 88.0%, while 72.0% and 64.0% of the isolates were resistant against ciprofloxacin and erythromycin, respectively (Barros *et al*., 2012). In another study in Hamadan, Iran, cefoxitin resistance was observed in all 23 strains of *S. haemolyticus* isolated from blood and urine, while 39.1% and 26.1 of the isolates were also resistant against erythromycin and gentamicin, respectively (Hajiahmadi *et al*., 2017).

On the other hand, 48 *S. haemolyticus* isolated from blood and catheter from a local hospital in Ankara, Turkey, showed 91.7% are resistant to cefoxitin followed by erythromycin (87.5%), ciprofloxacin (81.3%), and gentamicin (77.1%) (Tekeli *et al*., 2020). In general, reports from other studies show that the clinical isolates *S. haemolyticus* displayed high resistance against cefoxitin, ciprofloxacin, erythromycin, gentamicin, and tigecycline, causing problems in public health systems (Eltwisy *et al*., 2022).

Nosocomial isolates of *S. haemolyticus* are known to display the highest incidences of antibiotic resistance among the CoNS (Cavanagh *et al*., 2014). Various factors have been suggested to contribute to this phenomenon especially the presence of resistant genes and inappropriate use of antibiotics (Becker *et al*., 2014). Although these findings are in agreement with this study, the current study also shows a high level of antibiotic resistance among commensal strains of *S. haemolyticus*, albeit at a lower percentage. Coresistance to different classes of antibiotics was also observed, although the incidence of MDR is lower as compared to clinical isolates. This suggests that the incidence of antibiotic resistance in commensal *S. haemolyticus* may have been underestimated. Opportunistic infections caused by these antibiotic-resistant commensal strains will be difficult to treat (Brown and Horswill, 2020). Similarly, a comparative study by Cavanagh *et al*. (2019) on exoproteome profiling of the clinical and commensal strains of *S. haemolyticus* showed that proteins conferring antimicrobial resistance are more abundantly found in the clinical isolates, reflecting their more virulent traits (Cavanagh *et al*., 2019). Results from this study also show that none of the isolates were resistant to vancomycin, suggesting the potential of this antibiotic as a treatment for *S. haemolyticus* infections. Resistance against glycopeptides in *Staphylococcus* is rare although *S. haemolyticus* is predicted to play an important role if such resistance occurs among the members of this genus (Czekaj *et al*., 2015). On the other hand, the increased resistance to -lactams among *S. haemolyticus* is a growing phenomenon and a public health challenge.

The correlation between the MDR strains and MRSH among the *S. haemolyticus* isolates is summarized in Table 3.

Reichmann and Pinho (2017) proposed that one main concern of antibiotics susceptibility profile among staphylococci is the incidence of methicillin resistance which has been widely reported. In this study, all the MDR strains of the commensal isolates were found to be MRSH while the majority of the MDR strains in the clinical isolates were also MRSH, suggesting a close relationship between the methicillin-resistant strains and resistance to other tested antibiotics. In agreement, numerous case studies concerning the high number of MRSH in clinical settings have been documented globally in which MRSH was reported as the aetiological agent in a variety of infections. In 2018, a study in South-West Iran reported that at 45.4%, both MRSH and MRSE (methicillin-resistant *S. epidermidis*) were the most dominant agents of CoNS isolated from blood, urine, and wounds of nosocomial patients (Abbasi Montazeri *et al*., 2020). The rising numbers of MRSH in India were also reported in a study by

**Table 3.** The correlation between MDR and MRSH among *S. haemolyticus* isolates.

| *Staphylococcus haemolyticus* | Commensals | | Clinical | |
|---|---|---|---|---|
| | MRSH (%) | MSSH (%) | MRSH (%) | MSSH (%) |
| MDR | 10 (20.0) | 0 | 51 (52.0) | 2 (2.1) |
| Non-MDR | 18 (36.0) | 22 (44.0) | 19 (19.4) | 26 (26.5) |
| Total | 28 (56.0) | 22 (44.0) | 70 (71.4) | 28 (28.6) |

MRSH = Methicillin-resistant *S. haemolyticus*; MSSH = Methicillin-sensitive *S. haemolyticus*; MDR = Multidrug resistance.

Singh *et al*. (2016) whereby 67.9% of MRSH were found to be dominant among the 34 CoNS strains isolated from patients suffering from bacteremia. Similarly, in Chiang Rai of Northern Thailand, among the 31 MR-CoNS isolated from patients suffering from bacteremia, 58.1% were found to be MRSH (Kitti *et al*., 2018). Similarly, MRSH was found to be the responsible agent causing infections among NICU patients at a hospital in Brazil (Salgueiro *et al*., 2019).

Thus, the increased level of antibiotic resistance is observed not only among clinical strains of *S. haemolyticus,* but also in commensal strains isolated from healthy individuals. One important factor might be the ability to acquire multiresistance against available antimicrobial agents by both groups. Taking into consideration the high adaptability and the ability to survive in the hospital environment, especially on medical devices, *S. haemolyticus* has the potential to become a major source of nosocomial infections. Virulent strains of *S. haemolyticus* are often characterized by a large number of acquired insertion sequences and single nucleotide polymorphisms (Cavanagh *et al*., 2014; Pain *et al*., 2019). This unusual genome plasticity may lead to the enhanced ability of this species to acquire antibiotic-resistance genes and form a potential reservoir for the dissemination of such genes. This may represent a major clinical concern as it will provide *S. haemolyticus* with the same genetic resources to develop into a successful pathogen like *S. aureus.* The presence of a pool of MDR commensal *S. haemolyticus* on hospital personnel and visitors will likely fuel future outbreaks of hospital-acquired infections, which may then eventually develop into community-acquired infections.

This study showed that the majority of the *S. haemolyticus* isolates harbor SCC*mec* Type II. However, several other studies have reported that SCC*mec* Type V is more common among clinical strains of *S. haemolyticus*. These include studies in Poland (Hosseinkhani *et al*., 2016; Hajiahmadi *et al*., 2017; Szczuka *et al*., 2016; Szemraj *et al*., 2020), neonates in Rio de' Janeiro (Salgueiro *et al*., 2019), and Ankara (Turkey) (Tekeli *et al*., 2020). In addition, there were several reports on SCC*mec* Type I being the predominant type among *S. haemolyticus* as in India (Ghosh *et al*., 2016; Singh *et al*., 2016), Brazil (Ternes *et al*., 2013), and Iran (Abbasi Montazeri *et al*., 2020), while SCC*mec* Type III predominates among clinical strains of *S. haemolyticus* in Chengdu, China (Zong *et al*., 2011) and Brazil (Pedroso *et al*., 2018). This suggests that the dominant SCC*mec* type in *S. haemolyticus* can vary, depending on factors such as the host and geographical locations (Azharollah *et al*., 2021; Ebrahim-Saraie *et al*., 2015; Yilmaz *et al*., 2015).

SCC*mec* Type II was also found to be a dominant type among the commensal *S. haemolyticus*. In contrast, a study on commensal strains of *S. haemolyticus* conducted by Ruppé *et al*. (2009) in Algeria, Cambodia, Mali, and Moldova reported that SCC*mec* Type V was most prevalent followed by SCC*mec* Type IV. Similar findings were also observed in Iran (Iravani Mohammad Abadi *et al*., 2015) and in Chennai, India (Murugesan *et al*., 2015). However, in these studies, the *S. haemolyticus* strains were isolated from the nasal of healthy adults while, in this study, *S. haemolyticus* were isolated from other areas of the body which include the groins, axillae, and perineum instead. This suggests that the variety of SCC*mec* typing could also depend on different anatomical areas of the human body (Azharollah *et al*., 2021).

It is also interesting to note that the patterns of dominance in the SCC*mec* typing for both the commensal and clinical isolates are very similar to each other. Such striking similarity strongly suggests that the clinical strains isolated from the hospital settings could have originated from the commensal strains that had successfully converted into opportunistic pathogens. Hence, a similar pattern of SCC*mec* typing is observed in *S. haemolyticus* among both the community- and hospital-acquired infection isolates.

While the results from this study showed the ability of both commensal and clinical strains of *S. haemolyticus* to become MDR pathogens, the scope of the study is limited to one geographical region in Malaysia. Also, the markers used for tracking are limited to antimicrobial resistance genes and SCC*mec* cassettes. While similar observations have been reported elsewhere, a global analysis using whole genome sequencing data will provide more information on the evolutionary trajectory of this important opportunistic pathogen.

## CONCLUSION

This study highlighted the natural ability of *S. haemolyticus* to develop resistance to commonly used antibiotics. Such a phenomenon was observed not only in clinical strains isolated from infected patients in hospital settings, but also in commensal strains isolated from healthy individuals. MDR and MRSH strains of *S. haemolyticus* are not only a concern in clinical settings, but the same phenotypes are also present among the commensal isolates, albeit at a lower percentage. SCC*mec* Type II is the most abundant SCC*mec* type in both clinical and commensal isolates, followed by Type V, suggesting the ability of this species to transform from a commensal strain to an opportunistic pathogen. It also suggests that genetic determinants are widespread among the *S. haemolyticus* isolates which could serve as vehicles for the transmission of antibiotic-resistant genes and which may also be transmitted to other CoNS species horizontally (Ahmadunissah *et al*., 2022). The findings could be useful in antibiotic prescription policies and infection control strategies for *S. haemolyticus*.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study was conducted under the approval of the Research Ethics Committee, UiTM [REC 600-IRMI (5/1/6)].

## DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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