Malaysian Journal of Microbiology, Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

The growing threat of multidrug-resistant coagulase-negative *Staphylococcus* spp. on hospital lift buttons in Malaysia

Izzati Muhammad[1], Niza Shamsuddin[1], Raja Noor Zaliha Raja Abd Rahman[2], Norhidayah Kamarudin[3] and Norsyuhada Alias[1]*

[1]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia.
[2]Department of Microbiology, Enzyme and Microbial Technology Research Centre, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.
[3]Kulliyyah of Medicine, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia.
Email: norsyuhada_alias@iium.edu.my

Received 9 February 2024; Received in revised form 10 March 2025; Accepted 17 March 2025

ABSTRACT

Aims: This study investigated the presence of coagulase-negative *Staphylococcus* spp. (CoNS) and its antimicrobial resistance properties, isolated from lift buttons in public and teaching hospitals in Pahang, Malaysia.
Methodology and results: Purposive swab sampling was conducted thrice at two-week intervals. The samples were processed using standard microbiological methods to identify the species. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. CoNS resistant to cefoxitin (4/16) were further analyzed for the presence of the methicillin-resistance gene (*mec*A) through polymerase chain reaction (PCR). Seven *Staphylococcus* species were identified from 16 isolates and classified as CoNS, which are commonly found as part of the human skin microbiome. Approximately 82% (13/16) of the CoNS exhibited antibiotic resistance, including resistance to penicillin, fusidic acid, and erythromycin. *Staphylococcus haemolyticus* had a higher multiple antibiotic resistance (MAR) index than the others, suggesting it originated from a high-risk contamination source, likely associated with heavy antibiotic usage. Among the four cefoxitin-resistant CoNS, *S. haemolyticus* was resistant to nine antibiotic classes and carried the *mec*A gene. The other three CoNS were resistant to four antibiotic classes.
Conclusion, significance and impact of study: This study highlights the presence of multidrug-resistant (MDR) CoNS on hospital lift buttons, posing an increased risk of severe infection for immunocompromised patients. Future research should focus on elucidating the mechanisms of antibiotic resistance in MDR CoNS, assessing the efficacy of current antimicrobial treatments, and implementing continuous surveillance to monitor the spread of resistance.

Keywords: Coagulase-negative staphylococci, fomites, hospital-acquired infection, lift button, multidrug resistance

INTRODUCTION

A lift serves as a pivotal mode of transportation, particularly in multi-level buildings, including hospitals. Contaminant transmission occurs when every other person's bodily fluid, including respiratory droplets, sweat and saliva, share direct contact with the same lift buttons (Gooch, 2020). The risk of contaminant transmission is further compounded by unhygienic lift users, confined spaces, and an ineffective cleaning routine of the lifts (Ministry of Health Malaysia, 2021). The adverse effects of contaminants were more pronounced in patients than in visitors, primarily attributable to the compromised immune systems of patients, exacerbating the severity of their conditions. Despite these challenges, the current methods for detecting contaminants on lift buttons are limited to laboratory testing, reflecting the constraints imposed by existing technology.

Hospital-acquired infections (HAIs) are significant concerns in hospitals, with 15 in 100 patients in low- and middle-income countries contracting them [World Health Organization (WHO), 2022]. On average, one in ten patients with HAI succumbs to the disease, and this fatality rate increases two to threefold when the disease is caused by antimicrobial resistance (AMR) pathogens (WHO, 2022). The treatment of HAIs, especially those with AMR pathogens, necessitates prolonged hospital stays, changes in antibiotic regimens, frequent physician visits, and increased use of healthcare equipment. Shamsuddin *et al*. (2016) noted that antibiotic procurement costs increased 2.6-fold from 2011-2013 to meet growing demands for antibiotics. The expenditure of

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

an upper-middle-income country on hospital antibiotics alone is projected to surpass RM 165 million, with common amoxicillin costing RM 0.46 per capsule. The gradual rise in antibiotic prices per year, coupled with the surge in AMR-associated infections, has led to a substantial increase in the country and patient expenditures.

Coagulase-negative *Staphylococcus* (CoNS) is a contributor to the prevalence of HAIs in the hospitals of Southeast Asian Countries, accounting for 22% of the cases from the year 1990 to 2022 (Goh *et al*., 2023). The highest reported prevalence of HAIs was observed in Indonesia, followed by Cambodia, Vietnam, Malaysia, Thailand and Singapore. There was no notable difference between the prevalence of HAIs in public and private hospitals. This could be attributed to CoNS exhibiting notable resistance to methicillin antibiotics, as observed in *Staphylococcus haemolyticus* isolated from the skin of a healthy person (Azharollah *et al*., 2021). The presence of mobile genetic elements, housing antimicrobial resistance and virulence genes as part of integrated transposons or plasmids in CoNS, is believed to facilitate the dissemination of these genes through horizontal transmission (Smith and Andam, 2021). Nonetheless, the intricate mechanism underlying its resistance is still the subject of ongoing investigation. Consequently, the use of antibiotics, even with a prescription, may induce mutation in CoNS and lead to the emergence of multidrug-resistant (MDR) CoNS.

To the authors' knowledge, no recent information is available regarding the potential fomites inhabiting the lift buttons, particularly in a healthcare facility in a Southeast Asian country. This study seeks to address this gap by investigating the presence of AMR CoNS isolated from two hospitals in Pahang, Malaysia, a Southeast Asian country with a standard ideal population suitable for preliminary observation (Department of Statistics Malaysia, 2022). This study specifically aims to understand whether the lift buttons served as reservoirs for CoNS and accommodated AMR CoNS. Addressing these issues is critical in light of the current state of AMR in hospitals and would greatly benefit the healthcare and pharmaceutical workers. This preliminary data offers valuable insights into the prevalence of AMR CoNS, laying the foundation for further studies on AMR CoNS in Malaysia, which could benefit both the economy and society in the long term.

MATERIALS AND METHODS

Study setting

The hospitals involved in this study adhered to a stringent cleaning protocol throughout the sampling process conducted during the coronavirus disease 2019 (COVID-19) pandemic. Their cleaning regimen consisted of three key routines: daily cleaning, weekly decontamination, and incident-specific decontamination. The daily cleaning routine was performed twice daily, once before visiting hours and again after, using a multipurpose detergent to clean all surfaces throughout the hospital. Weekly decontamination occurred over the weekend when there were fewer visitors. Incident-specific decontamination was carried out only in response to specific events, such as bodily fluid spills. According to personal communication with Mr. Amir, the hospital's Radicare officer, on 13th March 2021, the decontamination routines employed a chlorine-based disinfectant known for its broad-spectrum bactericidal properties. The choice of chemicals, concentration, and application followed the general disinfection guidelines established by the Ministry of Health Malaysia (2021). However, it is essential to note that no specific cleaning routine was dedicated to the lift buttons in the hospital.

Identification of CoNS

The study focused on assessing the microbiological contamination of hospital lifts based on specific criteria. Lifts selected for investigation were those accessible to all patient floors, with dimensions suitable for a standard hospital bed. Lifts involved in transporting COVID-19-related matters were excluded from the study. The button selection was strategic, targeting areas previously reported as harbouring contaminants, including main entrances, operation theatres, intensive care units, paediatric wards, and general wards (Olise and Simon-Oke, 2018; De Paula Menezes *et al*., 2022).

A sterile cotton swab immersed in 0.1% peptone water (Merck) was used to swab the lift buttons transversely and longitudinally along a 14 cm^2 stencil for 10 sec (Carrascosa *et al*., 2018). The selected buttons included those from key areas, and controls were implemented using lift buttons disinfected with 70% ethanol. The samples were kept on ice until further processing at the lab. A total of 100 lift button samples were collected in a single sampling process, comprising both interior (horizontal and vertical panels) and exterior lift buttons. The sampling was repeated thrice at two-week intervals, with one sampling each time during the COVID-19 Movement Control Order.

Subsequent laboratory processes involved spreading 50 µL of the swab samples onto nutrient agar plates, followed by aerobic incubation at 37 °C for 24-48 h. The morphology of the isolates was recorded, and distinct colonies, identified through Gram staining, were selected for further investigation. The species-level identification of selected isolates (16/103) was performed by amplifying the 16S ribosomal deoxyribonucleic acid (rDNA) region using the primers (Forward primer: 5'-GAGTTTGATCCTGGCTCAG-3', reverse primer: 5'-CGGCTACCTTGTTACGACTT-3') suggested by Mohd Fauzi (2018). The polymerase chain reaction (PCR) was performed using GoTaq® Green Master Mix (Promega, United States) under the following conditions: a pre-denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 1 min.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

The amplified DNA products that underwent gel electrophoresis using 1% agarose gel showed approximately 1400 bp in size, as depicted in Figure 1. These DNA products were purified using the innuPREP DOUBLEpure Kit (Analytika Jena, Germany) and subsequently subjected to sequencing analysis. The obtained reads were subjected to basic trimming and noise removal. Microbial identification was performed by comparing the obtained sequencing reads against the National Center for Biotechnology Information (NCBI, USA) nucleotide database using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameters. This process identified bacterial taxa based on the sequences with the highest percentage of identity.

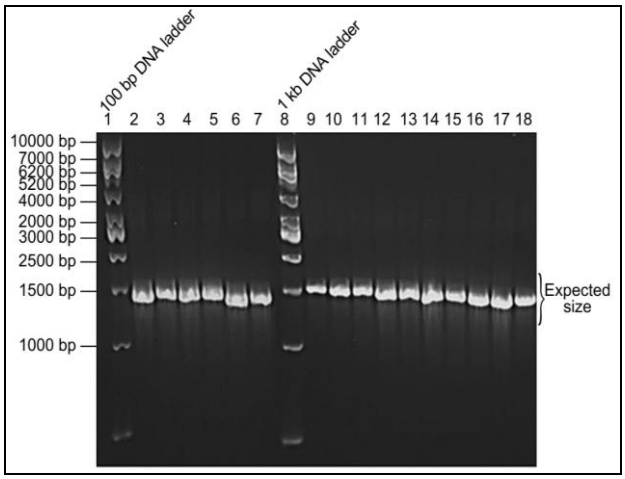

Figure 1: Visualization result of PCR for the identification of CoNS. Annotation: (Lane 1 and 8): 1kb DNA ladder (Promega, United States); (Lane 2-7 and 9-18): PCR amplicons of samples isolated from the hospitals. A total of 16 PCR amplicons were analyzed via gel electrophoresis to assess the DNA quantification and qualification, with an estimated base pair size of approximately 1400 bp.

Antimicrobial susceptibility test

The antimicrobial susceptibility test employed the Kirby-Bauer disc diffusion method. A standardized overnight subculture (0.5 McFarland standard) was spread onto Mueller-Hinton agar using a sterile cotton swab. In a zig-zag pattern, the inoculum covered one side and was rotated to ensure even coverage before placing the impregnated commercial discs. Antibiotics representing 11 classes were utilized, including 10 µg fusidic acid (Fusidanes), 30 µg cefoxitin (Cephalosporin), 5 µg ciprofloxacin (Fluoroquinolone), 2 µg clindamycin (Lincosamide), 15 µg erythromycin (Macrolide), 5 µg rifampin (Macrolide), 10 µg gentamicin (Aminoglycoside), 200 µg mupirocin (Carboxylic acid), 10 U penicillin (Beta-lactam), 1.25/23.75 µg trimethoprim-sulfamethoxazole (Sulphonamides), and 30 µg doxycycline (Tetracycline). *Staphylococcus aureus* and *Escherichia coli* were used as positive and negative controls, respectively.

The plates were incubated at 35 °C for 16-20 h, except for the cefoxitin plates, which were incubated for 24 h as suggested by the Clinical and Laboratory Standards Institute (CLSI) M100 (2020) guidelines. The test was conducted in triplicates. The zones of inhibition were measured, and the average of these measurements was used for interpretation. The interpretation of all antibiotics, except for fusidic acid, followed the CLSI (2020) guidelines, while fusidic acid was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 12.0 (2022) guidelines. The antibiotic interpretation was categorized into three parts: susceptible, intermediate resistance, and resistance (CLSI, 2020).

The multiple antibiotic resistance (MAR) index was used to track the source of bacteria. MAR index was determined by dividing the number of antibiotics that each isolate was resistant to by the total number of antibiotics tested. An index ≥ 0.2 indicates an isolate originated from a source that used a large amount of antibiotics (Magiorakos *et al.*, 2012; Mir *et al.*, 2022). The classification of MDR bacteria in this study was based on CoNS exhibiting resistance to one or more antibiotics across at least three antibiotic classes, following the criteria outlined by Magiorakos *et al.* (2012). Results indicating intermediate resistance were not classified as complete resistance and were therefore excluded from the resistance category in this analysis.

Identification of methicillin resistance gene

The CoNS identified as cefoxitin-resistant, indicating potential methicillin resistance, were selected for PCR to determine the resistance gene. The PCR reaction was performed in a 50 µL reaction using Promega GoTaq® Green Master Mix (Promega, United States) containing PCR reagent as recommended by the manufacturer. Forward primer MECA P4 (5'-TCCAGATTACAACTTCACCAGG-3') and reverse primer MECA P7 (5'-CCACTTCATATCTTGTAACG-3') were employed in the reaction (Oliveira and de Lencastre, 2002; Pardo *et al*., 2024).

The amplification process involved pre-denaturation for 4 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 53 °C for 30 sec, and extension at 72 °C for 1 min. Post-extension occurred for 4 min at 72 °C, and the reaction was then cooled to 4 °C for further analysis. The PCR amplicon was visualized on a 2% agarose gel, as observed in Figure 2. Next, the 162 bp amplicon underwent purification, followed by sequencing and sequence analysis and comparison of reads using BLAST.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

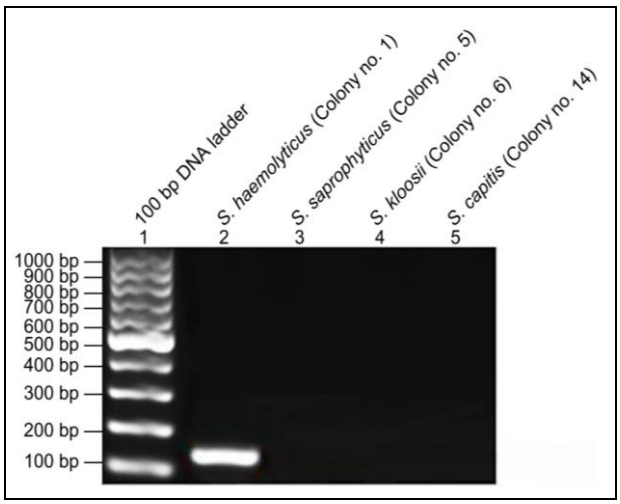

Figure 2: Result of PCR for the identification of *mec*A gene. Annotation: (Lane 1): 100 bp DNA ladder (Promega, United States); (Lane 2-5): PCR amplicons of isolates suspected to carry *mec*A gene based on their resistance to cefoxitin. Four PCR amplicons were subjected to gel electrophoresis for qualitative evaluation. The appearance of a distinct band in the *S. haemolyticus* lane indicated the successful amplification of the target gene.

RESULTS AND DISCUSSION

Presence of CoNS on lift buttons

Sixteen colonies of CoNS, identified through previous confirmation methods, consisted of seven species of *Staphylococcus*, as illustrated in Table 1, namely *S. haemolyticus*, *S. saprophyticus*, *S. kloosii*, *S. capitis*, *S. caprae*, *S. cohnii*, and *S. warneri*. It has been established previously that the presence of CoNS on fomite is unavoidable because they are a part of the human skin microflora. There is no apparent pattern in the diversity of CoNS isolated in hospitals since the isolated CoNS could have come from humans, animals, or the environment. The absence of *S. epidermidis* in this study, which is recognized as the most prevalent HAIs, proved this conjecture (Sukri *et al*., 2022). In contrast, *S. haemolyticus* is perceived as the second highest prevalence in the hospital despite having the lowest prevalence in this study. Other CoNS like *S. warneri*, *S. capitis*, *S. cohnii* and *S. saprophyticus* came in third, sixth, eighth and ninth place in species distribution, respectively. The presence of *S. kloosii* and *S. caprae* was not reported in the previous study (Masri *et al*., 2020). The variability in CoNS prevalence can be attributed to the dynamic and ever-changing hospital environment, fostering conditions that promote the survival of CoNS.

Table 1: Summary of the BLAST searches using 16S rDNA gene PCR amplicons.

Colony No.	BLAST search results		
	Percentage of identity (%)	Species identified	Accession no.
1	99.80	*S. haemolyticus*	LC647817
2	99.76	*S. capitis*	MK629839
3	99.17	*S. capitis*	JX094954
4	99.75	*S. caprae*	MT071654
5	99.45	*S. saprophyticus*	KU550151
6	99.38	*S. kloosii*	MN733166
7	99.75	*S. saprophyticus*	KU550151
8	99.92	*S. kloosii*	OL454812
9	99.80	*S. saprophyticus*	CP054438
10	99.29	*S. caprae*	MT071654
11	99.37	*S. kloosii*	KJ888121
12	99.59	*S. cohnii*	MT122816
13	99.66	*S. warneri*	OL636003
14	97.99	*S. capitis*	OM992223
15	99.70	*S. warneri*	MT914211
16	99.42	*S. warneri*	MW181167

Annotation: The BLAST results revealed the species with the highest percentage of identity, representing one out of one hundred results obtained. The identification of similar species originating from different colonies highlights the bacterial diversity present on the lift buttons.

Resistance of CoNS to common antibiotics

Out of 16 CoNS strains, 13 exhibited resistances to antibiotics with varying patterns, as detailed in Table 2. Most of the CoNS identified are resistant to common antibiotics, including penicillin and fusidic acid. *S. haemolyticus* showed the highest degree of resistance compared to other CoNS, where it resisted eight antibiotic classes except for sulphonamides and tetracycline, as seen in Figure 3. *S. capitis* was resistant to penicillin, erythromycin, and cefoxitin. *S. saprophyticus* isolates exhibit a similar resistance to fusidic acid, penicillin, and erythromycin. *S. kloosii* commonly resisted fusidic acid, while *S. cohnii* was resistant to fusidic acid and penicillin. *S. warneri* shared a similar resistance profile with *S. kloosii* and *S. cohnii*, with an additional gentamicin resistance.

A detailed observation and analysis of the antibiotic susceptibility tests revealed that each CoNS species exhibited similar resistance patterns. For instance, *S. haemolyticus* showed consistent resistance to oxacillin, erythromycin, aminoglycosides, clindamycin, and rifampicin, with no significant differences between strains taken from neonates and adults (Magnan *et al.*, 2024). It is well established that methicillin-resistant *S. haemolyticus* produces an altered penicillin-binding protein, similar to methicillin-resistant *S. aureus*, resulting in reduced affinity and rendering beta-lactam antibiotics ineffective (Panchal *et al.*, 2020; Azharollah *et al.*, 2021; Wacnik *et al.*, 2022). A clinical study in India, identified

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

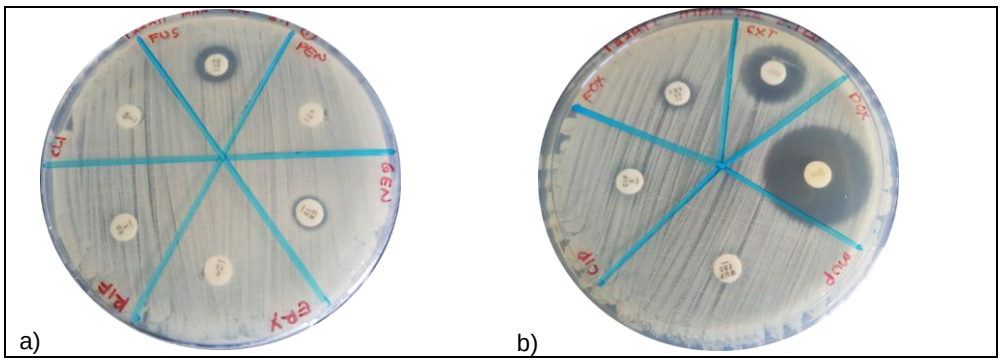

Figure 3: Zone of antibiotic inhibition in *S. haemolyticus*. Annotation: (FUS): Fusidic acid; (PEN): Penicillin; (GEN): Gentamicin; (ERY): Erythromycin; (RIF): Rifampin; (CLI): Clindamycin; (FOX): Cefoxitin; (SXT): Trimethoprim-sulfamethoxazole; (DOX): Doxycycline; (MUP): Mupirocin; (CIP): Ciprofloxacin. *S. haemolyticus* exhibited resistance to the majority of administered antibiotics, with notable exceptions being susceptibility to DOX and intermediate resistance to SXT. *S. haemolyticus* demonstrated the highest level of antibiotic resistance in comparison to other CoNS, establishing its prominence as the most significant strain in this study.

Table 2: Antibiotic resistance and MAR index in isolated CoNS.

Bacteria species (Colony no.)	MAR index	Antibiotics										
		FUS	PEN	GEN	ERY	RIF	CLI	FOX	SXT	DOX	MUP	CIP
S. capitis (2)	0.1		R									
S. capitis (3)	0.1		R		I							
S. capitis (14)	0.3		R		R			R				
S. caprae (4)	0.0											
S. caprae (10)	0.0											
S. cohnii (12)	0.2	R	R		I		I					
S. haemolyticus (1)	0.9	R	R	R	R	R	R	R	I		R	R
S. kloosii (6)	0.2	R						R				
S. kloosii (8)	0.1	R										
S. kloosii (11)	0.1	R										
S. saprophyticus (5)	0.4	R	R		R			R				
S. saprophyticus (7)	0.4	R	R		R				R			
S. saprophyticus (9)	0.4	R	R		R				R			
S. warneri (13)	0.0											
S. warneri (15)	0.3	R	R	R	I							
S. warneri (16)	0.2		R	R								

Annotation : (MAR index): Multiple antibiotic resistance index; (FUS): Fusidic acid (Fusidanes); (PEN): Penicillin (Beta-lactam); (GEN): Gentamicin (Aminoglycoside); (ERY): Erythromycin (Macrolide); (RIF): Rifampin (Macrolide); (CLI): Clindamycin (Lincosamide); (FOX): Cefoxitin (Cephalosporin); (SXT): Trimethoprim-sulfamethoxazole (Sulfonamide); (DOX): Doxycycline (Tetracycline); (MUP): Mupirocin (Carboxylic acid); (CIP): Ciprofloxacin (Fluoroquinolone); Colour coding: R showed resistance, I showed intermediate resistance, S showed susceptible. Three colonies (4, 10, and 13) were found to be vulnerable to all antibiotics tested. CoNS demonstrated susceptibility to DOX, while intermediate resistance was observed in CoNS tested against ERY, CLI, and SXT. Additionally, four CoNS exhibiting resistance to cefoxitin were classified as methicillin-resistant CoNS. The MAR index across the colonies ranged from 0.0 to 0.9, with a mean of 0.2, and over half of the colonies had an index of 0.2 or higher. The highest MAR index was recorded in *S. haemolyticus*, while the lowest was observed in *S. warneri* and *S. caprae*.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

S. haemolyticus to be resistant to cefoxitin, tetracycline, trimethoprim-sulfamethoxazole, and macrolides (Manoharan *et al.*, 2021). Consistent with other findings, *S. haemolyticus* in this study also showed similar resistance to beta-lactams, macrolides, and fluoroquinolones, suggesting it might be resistant to these antibiotics in general.

Comparable results were also observed in *S. saprophyticus*, a bacterium frequently linked to urinary tract infection (UTI), which was mainly resistant to fusidic acid, penicillin, and erythromycin. The bacteria displayed resistance patterns similar to those of UTI-causing *Escherichia coli*, displaying resistance to fusidic acid, erythromycin, clindamycin, gentamicin, ciprofloxacin, tetracycline at similar concentrations (Hashemzadeh *et al*., 2020; Hossain *et al*., 2024). A change in the 50S subunit of ribosomal ribonucleic acid (rRNA) and penicillin-binding protein was believed to lead to *S. saprophyticus* being naturally resistant to cephalosporins, macrolides, and beta-lactam antibiotics.

Previous studies revealed that most isolates of *S. capitis* from clinical samples of neonates were resistant to fusidic acid, methicillin, aminoglycoside and beta-lactam antibiotics (Felgate *et al*., 2023; Sala *et al*., 2024). Given the phenotypic similarity of *S. capitis*, it is hypothesized that this species primarily exhibits resistance to beta-lactams and aminoglycosides. Interestingly, *S. caprae*, susceptible to all antibiotics used in this study, has been reported to show resistance to gentamicin with isolates recovered from the hands of dental patients and staff (Hirose *et al*., 2021). Another study found that *S. caprae*, isolated from a spinal infection, was susceptible to gentamicin but resistant to clindamycin, erythromycin and penicillin (Sulaiman *et al*., 2024). These studies suggested no consistent pattern of antibiotic resistance in *S caprae*. *S. cohnii*, a rare *Staphylococcus* to be found in humans, has been shown to exhibit resistance to clindamycin, erythromycin, fusidic acid, tetracycline and beta-lactam antibiotics in livestock isolates (Lienen *et al*., 2021). A similar resistance profile was found in clinical isolates and this study (Garza-González *et al*., 2011). This led to the hypothesis that *S. cohnii* may mainly resist beta-lactam antibiotics, commonly used in treating mastitis in livestock (Wieland, 2024).

A study on *S. warneri* isolated from animals displayed resistance to vancomycin, cephalexin, sulfisoxazole penicillin, amoxicillin, and ampicillin (Liu *et al*., 2020). However, this isolate also exhibited resistance to gentamicin, possibly due to alterations in the 30S ribosomal subunit that prevent its interaction with 16S rRNA (Chaves and Tadi, 2023). Humans and animals might be exposed to various antibiotics with different concentrations, which might account for the slight difference in antibiotic resistance between the two isolates. Previously, *S. kloosii* was reported to be cefoxitin-sensitive, which slightly differs from the current study (Blondeau *et al*., 2021). However, these studies managed to isolate *S. kloosii* from humans who had no interaction with animals, a common source of *S. kloosii* infection. The current study proved that *S. kloosii* infection might happen in hospitals due to users' unhygienic practices.

The analysis of the MAR index revealed that *S. haemolyticus* had a higher MAR index compared to other CoNS, as observed in Table 2. Mir *et al.* (2022) reported that all isolates in their study had a MAR index of higher than 0.2, indicating a high-risk source of contamination. In this study, the mean MAR index of all isolates was 0.2, suggesting that most isolates had a high potential of originating from a source with high antibiotics usage (Mir *et al.*, 2022). *S. haemolyticus,* with the highest MAR index of 0.9, strongly indicated that it originated from a high-risk source of contamination. Since the isolates were obtained from lift buttons, there was a high possibility that human touch contributed to this contamination. However, further analysis of bacterial transmission pathways is necessary to confirm this hypothesis.

CoNS showed resistance to multiple classes of antibiotics

Several colonies exhibited resistance to more than three antibiotic classes, as displayed in Figure 4. *S. haemolyticus*, *S. saprophyticus* (colonies 5, 7, and 9), *S. capitis* (colony 14), and *S. warneri* (colony 15) were resistant to more than three antibiotic classes and were classified as MDR bacteria. *S. haemolyticus* demonstrated resistance to nine antibiotic classes, surpassing previous reports of resistance to seven classes (Qin *et al*., 2022). The majority of MDR bacteria in this study were resistant to four antibiotic classes, while others were resistant to three.

It has been hypothesized that *S. haemolyticus* may possess an efflux mechanism similar to that found in *Staphylococcus urealyticus*, which expels a broad range of antibiotics at high intracellular concentrations. This mechanism could significantly enhance its MDR potential compared to other CoNS species (Lienen *et al*., 2021; Muhammad *et al*., 2024). Additionally, this study found that *S. saprophyticus* exhibited resistance to four classes of antibiotics, while *S. warneri* and *S. capitis* were resistant to three classes each. Research on MDR in other CoNS species has been less extensive compared to *S. haemolyticus*. MDR *S. saprophyticus* has been reported in women with UTIs, where 58% of the isolates were MDR, with erythromycin being the most commonly resisted antibiotic (Hashemzadeh *et al*., 2020). A study in Japan focusing on the oral cavity found that more than 50% of *S. capitis* isolates were MDR, with most showing resistance to flomoxef, a second- or fourth-generation cephalosporin (Hirose *et al*., 2021). However, there was no clear indication of the number of antibiotic classes resisted by these CoNS isolates.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 21(2) 2025, pp. 250-258
DOI: http://dx.doi.org/10.21161/mjm.240592

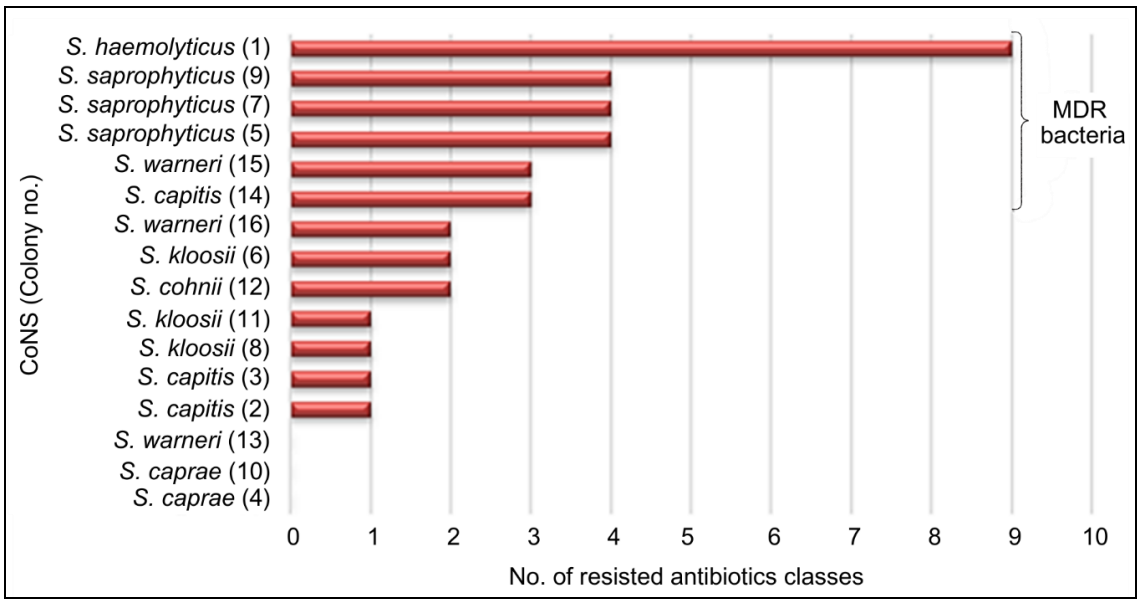

Figure 4: Number of antibiotic classes resisted by CoNS. Annotation: (MDR): multidrug resistance. Three colonies were susceptible to all antibiotic classes tested, while the remaining colonies exhibited resistance to at least one class. *S. saprophyticus* displayed a consistent pattern of resistance, with all colonies resisting up to four antibiotic classes. Furthermore, five CoNS colonies were resistant to three or more antibiotic classes, categorizing them as MDR bacteria.

Methicillin-resistance CoNS harboured *mec*A gene

Four CoNS species, specifically *S. haemolyticu*s (Colony 1), *S. saprophyticus* (Colony 5), *S. kloosii* (Colony 6), and *S. capitis* (Colony 14), were resistant to cefoxitin, as indicated in Table 2. Further amplification of the methicillin-resistance gene showed that *S. haemolyticus* carried the *mec*A gene. BLAST analysis of the 162 bp amplicon sequence showed 98.45% significant identity with the *mec*A gene from *S. aureus* (CP162467.1), *S. epidermidis* (CP121529.1), and *S. haemolyticus* (CP102571.2) The *mec*A gene encodes the PBP2a protein, which is part of the family of beta-lactam-resistant penicillin-binding proteins (PBPs). The *mec*A gene is a critical factor in conferring methicillin resistance in *Staphylococcus* sp., where its expression leads to resistance to methicillin-based antibiotics (Smith and Andam, 2021). Additionally, other lesser-known methicillin-resistance gene variants, such as *mec*C, existed as mutations of *mec*A, though these variants are less prevalent (Idrees *et al.,* 2023). However, the *mec*A gene was not expressed in the methicillin-resistant *S. saprophyticus* and *S. capitis*, contrasting with findings by Hirose *et al.* (2021). Notably, no previous studies have confirmed the presence of the *mec*A gene in methicillin-resistant *S. kloosii*. This absence could be due to the expression of *mec*A gene variants. Therefore, further investigation using alternative primers is necessary to validate this hypothesis.

CONCLUSION

In conclusion, hospital lift buttons exhibit varying degrees of contamination with antibiotic resistance CoNS. Predominant resistance was observed against penicillin (62.5%) and fusidic acid (56.3%), with an overall mean MAR index of 0.2. Notably, *S. haemolyticus* exhibited resistance to nine antibiotic classes, had a MAR index of 0.9, and carried the *mec*A gene. Additionally, the study reveals the presence of MDR CoNS, commonly resistant to four antibiotic classes, with some of them resistant to cefoxitin, an indicator of methicillin resistance. The discovery of cefoxitin and fusidic acid resistance in *S. kloosii* is particularly noteworthy, given the rarity of finding this species in human contexts. These findings enhance healthcare workers' understanding of lift button contaminants, advocating improved hygiene practices. This preliminary study identifies potential sources of healthcare-associated infections and underscores the need for further investigations in Malaysian healthcare settings, given the presence of MDR CoNS. Despite limitations in sampling locations, hospitals, sample size, sampling dates, and excluded lifts (e.g., those used for COVID-19 transport, inaccessible to all patient floors and non-standard lift dimensions), the study suggests a potential underestimation of contaminant density. Future research should focus on elucidating the mechanisms of antibiotic resistance in MDR CoNS and evaluating the effectiveness of existing antimicrobial treatments while also establishing continuous surveillance and monitoring to identify and address the emergence and propagation of resistance.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Higher Education, Malaysia, under the Fundamental Research Grant Scheme (FRGS) for Research Acculturation of Early Career Researchers (Project ID: RACER 19-027-0027). The work was done according to the approved protocol by the IIUM Research Ethics Committee (Ref. No.: IREC 2020-115) and the Medical Research and Ethics Committee, Ministry of Health Malaysia [Ref. No.: NMRR-20-2505-55630(IIR)], which does not involve any intervention on human and animal subjects.

REFERENCES

Azharollah, F. H., Abdullah, M. F. F., Mohd Nawi, S. F. A. and Abdul-Aziz, A. (2021). High prevalence of methicillin-resistant *Staphylococcus haemolyticus* isolated from commensals in healthy adults. *Malaysian Journal of Medicine and Health Sciences* **17(1), 145-152.**

Blondeau, L. D., Rubin, J. E., Deneer, H., Kanthan, R., Sanche, S. and Blondeau, J. M. (2021). Isolation of *Staphylococcus kloosii* from an ankle wound of an elderly female patient in rural Saskatchewan, Canada: A case report. *Annals of Clinical Case Reports* **6(1), 2073.**

Carrascosa, C., Velázquez, E. S., Millán, R., Martín, S. M. M., Saavedra, P., Raposo, A. *et al.* (2018). Is the use of supermarket trolleys microbiologically safe? Study of microbiological contamination. *Journal of Applied Animal Research* **47(1), 17-23.**

Chaves, B. J. and Tadi, P. (2023). Gentamicin. StatPearls Publishing, Treasure Island.

CLSI, Clinical and Laboratory Standard Institute (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th Edn. CLSI supplement M100. Clinical and Laboratory Standard Institute, Wayne, Pennsylvania, USA.

De Paula Menezes, R., De Andrade Marques, L., Silva, F. F., Silva, N. B. S., Alves, P. G. V., De Souza Bessa, M. A. *et al.* (2022). Inanimate surfaces and air contamination with multidrug resistant species of *Staphylococcus* in the neonatal intensive care unit environment. *Microorganisms* **10(3), 567.**

Department of Statistics Malaysia (2022). Vital Statistics Malaysia 2022 (ISSN: 0127-466X). Department of Statistic Malaysia, Malaysia.

European Committee on Antimicrobial Susceptibility Testing (2022). Breakpoint tables for interpretation of MICs and zone diameters; version 12.0. EUCAST, Sweden.

Felgate, H., Sethi, D., Faust, K., Kiy, C., Härtel, C., Rupp, J. *et al.* (2023). Characterization of neonatal *Staphylococcus capitis* NRCS-A isolates compared with non NRCS-A *Staphylococcus capitis* from neonates and adults. *Microbial Genomics* **9(10), 001106.**

Garza-González, E., Morfín-Otero, R., Martínez-Vazquez, M., González-Díaz, E., González-Santiago, O. and Rodríguez-Noriega, E. (2011). Microbiological and molecular characterization of human clinical isolates of *Staphylococcus cohnii*, *Staphylococcus hominis*, and *Staphylococcus sciuri*. *Scandinavian Journal of Infectious Diseases* **43(11-12), 930-936.**

Goh, L. P. W., Marbawi, H., Goh, S. M., Asis, A. K. B. A. and Gansau, J. A. (2023). The prevalence of hospital-acquired infections in Southeast Asia (1990-2022). *Journal of Infection in Developing Countries* **17(02), 139-146.**

Gooch, C. M. (2020). Body fluid exposures. StatPearls Publishing, Treasure Island.

Hashemzadeh, M., Dezfuli, A. A. Z., Nashibi, R., Mehr, F. J., and Akbarian, Z. (2020). Study of biofilm formation, structure and antibiotic resistance in *Staphylococcus saprophyticus* strains causing urinary tract infection in women in Ahvaz, Iran. *New Microbes and New Infections* **39, 100831.**

Hirose, M., Aung, M. S., Fujita, Y., Sakakibara, S., Minowa-Suzuki, E., Otomo, M. *et al.* (2021). Prevalence, genetic characteristics, and antimicrobial resistance of staphylococcal isolates from oral cavity and skin surface of healthy individuals in northern Japan. *Journal of Infection and Public Health* **17(8), 102488.**

Hossain, M. J., Azad, A. K., Shahid, M. S. B., Shahjahan, M. and Ferdous, J. (2024). Prevalence, antibiotic resistance pattern for bacteriuria from patients with urinary tract infections. *Health Science Reports* **7(4), e2039.**

Idrees, M. M., Saeed, K., Shahid, M. A., Akhtar, M., Qammar, K., Hassan, J. *et al.* (2023). Prevalence of *mec*A- and *mec*C-associated methicillin-resistant *Staphylococcus aureus* in clinical specimens, Punjab, Pakistan. *Biomedicines* **11(3), 878.**

Lienen, T., Schnitt, A., Hammerl, J. A., Marino, S. F., Maurischat, S. and Tenhagen, B. (2021). Multidrug-resistant *Staphylococcus cohnii* and *Staphylococcus urealyticus* isolates from German dairy farms exhibit resistance to beta-lactam antibiotics and divergent penicillin-binding proteins. *Scientific Reports* **11(1), 6075.**

Liu, C., Zhao, X., Xie, H., Zhang, X., Li, K., Ma, C. *et al.* (2020). Whole genome sequence and comparative genome analyses of multi-resistant *Staphylococcus warneri* GD01 isolated from a diseased pig in China. *PLoS ONE* **15(5), e0233363.**

Magiorakos, A., Srinivasan, A., Carey, R., Carmeli, Y., Falagas, M., Giske, C. *et al.* (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* **18(3), 268-281.**

Magnan, C., Morsli, M., Salipante, F., Thiry, B., Attar, J. E., Di Maio, M. *et al.* (2024). Emergence of multidrug-resistant *Staphylococcus haemolyticus* in neonatal intensive care unit in Southern France, a genomic study. *Emerging Microbes & Infections* **13(1), 2353291.**

Manoharan, M., Sistla, S. and Ray, P. (2021). Prevalence and molecular determinants of antimicrobial resistance in clinical isolates of *Staphylococcus haemolyticus* from India. *Microbial Drug Resistance* **27(4), 501-508.**

Masri, S. N., Wan Abu Bakar, W. N., Ibrahim, R., Noh, M. A. and Hj Nawi, S. (2020). Species distribution and clinical profiles of coagulase-negative staphylococci (CoNS) isolated from blood cultures among paediatric patients in Hospital Kuala Lumpur. *The Medical Journal of Malaysia* **75(3), 266-273.**

Ministry of Health Malaysia (2021). Garis panduan pembersihan dan disinfeksi di tempat awam (Annex 36). Ministry of Health Malaysia.

Mir, R., Salari, S., Najimi, M. and Rashki, A. (2022). Determination of frequency, multiple antibiotic resistance index and resistotype of *Salmonella* spp. in chicken meat collected from southeast of Iran. *Veterinary Medicine and Science* **8(1), 229-236.**

Mohd Fauzi, F. S. (2018). Isolation of bacteria from lift buttons of library and Kulliyyah of Nursing at IIUM Kuantan. B.Sc. Thesis. International Islamic University Malaysia, Malaysia.

Muhammad, I., Samsudin, N., Raja Abd Rahman, R. N. Z., Kamarudin, N. and Alias, N. (2024). Metagenomic analysis of contaminated lift buttons reveals prevalent pathogens with antimicrobial resistance genes: A study in a public hospital in Pahang, Malaysia. *International Journal of Allied Health Sciences* **8(2), 3086-3098.**

Olise, C. C. and Simon-Oke, I. A. (2018). Fomites: Possible vehicle of nosocomial infections. *Journal of Public Health and Nutrition* **01(01), 16-16.**

Oliveira, D. C. and de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy* **46(7), 2155-2161.**

Panchal, V. V., Griffiths, C., Mosaei, H., Bilyk, B., Sutton, J. A. F., Carnell, O. T. *et al.* (2020). Evolving MRSA: High-level β-lactam resistance in *Staphylococcus aureus* is associated with RNA polymerase alterations and fine tuning of gene expression. *PLoS Pathogens* **16(7), e1008672.**

Pardo, L., Mota, M. I., Parnizari, A., Varela, A., Algorta, G. and Varela, G. (2024). Detection of vancomycin resistance among methicillin-resistant *Staphylococcus aureus* strains recovered from children with invasive diseases in a reference pediatric hospital. *Antibiotics* **13(4), 298.**

Qin, M., Chen, P., Deng, B., He, R., Wu, Y., Yang, Y. *et al.* (2022). The emergence of a multidrug-resistant and pathogenic ST42 lineage of *Staphylococcus haemolyticus* from a hospital in China. *Microbiology Spectrum* **10(3), e02342-21.**

Sala, A., Pivetti, V., Vittorini, A., Viggiano, C., Castoldi, F., Fabiano, V. *et al.* (2024). *Staphylococcus capitis* central-line-associated bloodstream infections in the neonatal intensive care unit: A single-center, four-year experience in central- line management during sepsis treatment. *Pathogens* **13(3), 234.**

Shamsuddin, S., Akkawi, M. E., Zaidi, S. T. R., Ming, L. C. and Manan, M. M. (2016). Antimicrobial drug use in primary healthcare clinics: A retrospective evaluation. *International Journal of Infectious Diseases*, **52, 16-22.**

Smith, J. T. and Andam, C. P. (2021). Extensive horizontal gene transfer within and between species of coagulase-negative *Staphylococcus*. *Genome Biology and Evolution*, **13(9), evab206.**

Sukri, A., Zaki, H. H. M. and Zin, N. M. (2022). Antibiotic resistance patterns of coagulase-negative *Staphylococcus* (CoNS) isolates from a major teaching hospital in Kuala Lumpur, Malaysia. *Sains Malaysiana* **51(3), 865-872.**

Sulaiman, Z. I., Tu, P. J., Baer, S. L. and Skidmore, P. J. (2024). A capricious case of *Staphylococcus caprae* thoracic osteomyelitis. *IDCases* **36, e01962.**

Wacnik, K., Rao, V. A., Chen, X., Lafage, L., Pazos, M., Booth, S. *et al.* (2022). Penicillin-binding protein 1 (PBP1) of *Staphylococcus aureus* has multiple essential functions in cell division. *mBio*, **13(4), e0066922.**

WHO, World Health Organization (2022). WHO launches first ever global report on infection prevention and control. https: //www. who.int /news/item/06-05-2022-who-launches-first-ever-global-report-on-infection-prevention-and-control [Accessed on 6 February 2024]

Wieland, M. (2024). Mastitis in cattle. https:// www. msdvetmanual.com/ reproductive-system/mastitis-in-large-animals/mastitis-in-cattle [Accessed on 13 September 2024].

 ISSN (print): 1823-8262, ISSN (online): 2231-7538