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Original Article

Detection, molecular characterization, and antibiogram of multi-drug resistant and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from pets and pet owners in Malaysia

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Abstract

Background: The emergence of multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Staphylococcus aureus* (MDRSA) in animals and humans with continuous contact are a great zoonotic concern. **Aims:** This cross-sectional study was performed to investigate the carriage rate, genotypic characteristics, and to determine the antibiogram of *S. aureus* isolated from pets and pet owners in Malaysia. **Methods:** Nasal and oral swab samples from 40 cats, 30 dogs, and 70 pet owners were collected through convenient sampling. Presumptive colonies on mannitol salt agar were subjected to biochemical identification. *S. aureus* and MRSA were confirmed by PCR detection of *nuc* and *mecA* genes, respectively. Molecular profiles for antimicrobial resistance and virulence genes in *S. aureus* were also determined. The antibiogram was carried out via Kirby-Bauer test using 18 antibiotics. **Results:** 17.5% of cats, 20% of dogs, and 27% of pet owners were *S. aureus* positive. MRSA was also detected in dogs, and pet owners. *S. aureus* isolates displayed high resistance against penicillin (72.7%), and amoxicillin/clavulanate (66.7%). 39.4% of *S. aureus* isolates showed multidrug-resistance traits, phenotypically. Molecular characterization of *S. aureus* revealed the presence of *mecA*, *tetk*, *tetL*, *ermA*, *ermB*, *ermC*, *msrA*, *scn*, *chp*, *sak*, *sep*, and *sea* genes. **Conclusion:** This study showed the emergence of MRSA and MDRSA in pets and pet owners in Malaysia. The antibiogram findings showed resistance of *S. aureus* to multiple antibiotics. Furthermore, molecular analysis of immune evasion cluster (IEC) strongly suggests the spread of animal-adapted *S. aureus* lineages among pets and pet owners.

Key words: Antibiogram, Methicillin-resistant *Staphylococcus aureus*, Multidrug-resistant *Staphylococcus aureus*, Pet animals, Pet owners

Introduction

Staphylococcus aureus is a Gram-positive bacterium that causes a wide array of infections in both humans and animals. In humans, *S aureus* is considered to be a major human pathogen in both community and medical settings, causing a variety of diseases ranging from mild skin infections to potentially fatal diseases such as toxic shock syndrome, pneumonia, and endocarditis (Suhaili *et al*., 2018; Che Hamzah *et al*., 2019). Meanwhile, *S. aureus* in dogs and cats was previously associated with several clinical conditions, including skin, and wound infections, otitis, conjunctivitis, upper respiratory disease as well as post-surgical infections (Qekwana *et al*., 2017; Bierowiec *et al*., 2019). Although *S. aureus* infections are considered to be relatively rare in veterinary settings, domestic animals such as cats and dogs can act as vectors for the direct transmission and colonization of *S. aureus* in humans and animals (Peacock and Paterson, 2015).

Recently, the emergence of antimicrobial resistance (AMR) bacteria that are resistant to multiple classes of antibiotics, such as methicillin-resistant *S. aureus* (MRSA), is a public health threat in both humans and animals (Kanagarajah *et al*., 2017). Several previous studies reported the emergence of multidrug-resistance bacterial pathogens from different origins such as pet, livestock, fishes as well as animal products that could be

transmitted to humans and cause serious illness (Enany *et al*., 2018; Algammal *et al*., 2020; Chai *et al*., 2020; Miranda *et al*., 2021). The widespread of multidrug-resistance pathogenic bacteria in humans and animals often is related to persistent infections, a higher rate of complications incidences, and increase in morbidity and mortality (Kanagarajah *et al*., 2017). Previous studies showed that *S. aureus* often harbors various antimicrobial resistance (AMR) genes, making treatment of staphylococcal infections a great challenge (Lim *et al*., 2012; Suhaili *et al*., 2018; Che Hamzah *et al*., 2019). Furthermore, *S. aureus* is capable of producing virulence factors that allow the bacterium to evade the immune responses of the host and prevent bacterial elimination (Van Wamel *et al*., 2006). Five of these virulence factors commonly found in *S. aureus* from humans include staphylococcal complement inhibitory protein (SCIN), staphylokinase (SAK), chemotaxis inhibitory protein (CHIPS), staphylococcal enterotoxin type A (SEA), and staphylococcal enterotoxin type P (SEP). These immune modulators are encoded by immune evasion cluster (IEC) comprising of *scn*, *chp*, *sak*, *sea*, and *sep* genes (Van Wamel *et al*., 2006). In addition, *S. aureus* is capable of causing severe infections in human through the secretion of toxic shock syndrome toxin 1 (TSST-1) and Panton-Valentine leukocidin (PVL) (Lim *et al*., 2012). PVL toxin induces the expression of proinflammatory cytokines and lysing of inflammatory cells, which in turn exaggerate the host inflammatory response while TSST can causes neonatal toxic shock syndrome-like exanthematous disease and staphylococcal Purpura fulminans (Bien *et al*., 2011; Lim *et al*., 2012).

In Malaysia, the carriage rate, as well as the genotypic characteristics of *S. aureus* and MRSA isolated from humans in medical and community background, were well studied (Sit *et al*., 2017; Suhaili *et al*., 2018). However, information on the latest prevalence and antibiogram of *S. aureus* from pet owners and pets in Malaysia is limited. In addition, the occurrence rates of tetracycline and erythromycin resistance as well as IEC genes in Malaysian pet animals were not previously reported. Therefore, this study aims to investigate the carriage rate, antibiotic susceptibility profile, and genotypic characteristics of *S. aureus* isolated from both pets and pet owners.

Materials and Methods

Sampling

In the cross-sectional study, a total of 70 pet owners constantly exposed to pet animals were invited to join this research voluntarily. 140 swab samples (70 nasal and 70 oral) were collected from the pet owners using sterile cotton swabs. Besides, swab samples from 70 pet animals (70 nasal and 70 oral swabs), including 30 dogs and 40 cats were also collected. The collected swab samples were kept in transport media under 4°C and transported to Microbiology Laboratory in UniSZA, Besut Campus for further analysis.

Isolation and identification of *S. aureus*

The swab samples were streaked onto mannitol salt agar (MSA, Oxoid, UK) and incubated at 37°C up to 48 h. Yellow colonies with yellow zones that grew on MSA were suspected to be *S. aureus*. The suspected *S. aureus* colonies were then sub-cultured onto nutrient agar (NA, Oxoid, UK) supplemented with 6.5% sodium chloride (NaCl) and incubated at 37°C for 24 overnight. The bacterial colonies that grew on NA were then subjected to DNA extraction using heat lysis method as described by Suhaili *et al*. (2018). The extracted DNAs were kept in -20°C prior to polymerase chain reaction (PCR). PCR was carried out to screen the presence of the *nuc* gene of *S. aureus* and *mecA* gene of MRSA using the primers and protocol described by Saiful *et al*. (2006). Bacteria isolates with the presence of DNA bands of 278 bp were *S. aureus*, while isolates with both *nuc* and *mecA* (533 bp) genes were identified as MRSA.

Antimicrobial susceptibility test (AST)

The antimicrobial susceptibility profile of *S. aureus* isolated from swab samples of pet owners, cats, and dogs in Peninsular Malaysia were determined using the Kirby-Bauer test on Mueller-Hinton agar (Oxoid, UK), according to the Clinical and Laboratory Standards Institute (CLSI). *S. aureus* isolated from pets and pet owners were first suspended in sterile Mueller-Hinton broth (Oxoid, UK) adjusted to a 0.5 McFarland standard. The bacterial isolate in the broth was then streaked on Mueller-Hinton agar (Oxoid, UK) plates. Selected antibiotic disks listed in Table 1 were placed on the streaked Mueller-Hinton agar plates and incubated at 37°C up to 24 h. The diameter of inhibition zones of each isolate was measured and compared to the antibiotic susceptibility breakpoints according to CLSI (2018). Antibiotics commonly used to treat staphylococcal infections in humans and animals were selected in this test. *S. aureus* ATCC 700699 was used as the control strain for the AST test. For the screening of cefoxitin-resistant *S. aureus*, 30 µg of cefoxitin discs (Oxoid, UK) were tested against isolated *S. aureus*. Cefoxitin-resistance *S. aureus* were considered to be MRSA, phenotypically. *S. aureus* resistant to at least one antibiotic, from three or more antimicrobial categories, are classified as multidrug-resistant *S. aureus* (MDRSA) as suggested by Magiorakos *et al*. (2012).

Detection of antimicrobial resistance and virulence genes

S. aureus and MRSA isolates were further screened using PCR to detect the presence of various antimicrobial resistance genes, including methicillin-resistant genes (*mecA*, *mecB*, and *mecC*), tetracycline-resistant (*tet*K, *tet*L, *tet*M, and *tet*O), erythromycin-resistant (*erm*A, *erm*B, *erm*C, and *msr*A), and vancomycin-resistant (*van*A). *S. aureus* isolates harboring *mecA*, *mecB*, and *mecC* genes were considered to be MRSA. In addition, the presence of virulence determinants, including *tst*, *lukPV*, and IEC gene cluster (*scn*, *chp*, *sak*, *sea*, and *sep*), among the *S. aureus*

isolates have also been investigated using PCR. *S. aureus* ATCC 700699 was used as the positive control for *nuc* and *mecA* genes detection. The primer sequences and their band sizes are shown in Table 3. In regard to IEC genes, *S. aureus* isolates were later classified into 8 different IEC types as described by (Ariyarad *et al*., 2019). The presence of *scn* gene is mandatory for the consideration of the IEC types (Van Wamel *et al*., 2006).

Statistical analysis

The occurrence rates of the different genes were counted and presented in percentages (%.) Categorical data were analysed and compared using Chi-square and Fisher's exact test (Minitab 19, 2019) with a 95% confidence interval ($P<0.05$) was set to indicate the significant difference. The antibiotic resistance rate was calculated as the proportion of the isolates having an inhibition zone below the respective antibiotic breakpoint. The relationships between antibiotic exposure and overall antibiotic resistance in *S. aureus* isolates were assessed using a multiple antimicrobial resistance index (MARI). MARI was calculated as the proportion of antibiotics tested to which the isolate was phenotypically resistant. A dendrogram was generated via Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method using BioNumerics version 8.0 software (Applied Maths, Texas) to visualize the relatedness between *S. aureus* isolates based on their phenotypic antibiotic resistance profile.

Ethics approval

Written informed consent was obtained from each pet owner before obtaining swabs samples. The study and method for sampling of pet owners were approved by the University Sultan Zainal Abidin Human Research Ethnic Committee (UHREC) with protocol code: UniSZA/UHREC/2019/85. The study and method of sampling involving pet animals were approved by the University Sultan Zainal Abidin Animal and Plant Research Ethnic Committee (UAPREC) with Protocol code: UAPREC/04/18/006.

Results

The presence of *S. aureus* and MRSA

After *nuc* gene (Fig. 1) detection, 20 (28.5%; 20/70)

Table 1: The antibiogram of MRSA from dogs and pet owners (n=3)

Isolates	Antimicrobials	Disk potency	Number of isolates (%)		
			Resistant	Intermediate	Sensitive
MRSA (n=3)	Penicillin	10 units	3 (100)	0 (0)	0 (0)
	Amoxicillin/clavulanate	10 µg	3 (100)	0 (0)	0 (0)
	Norfloxacin	10 µg	3 (100)	0 (0)	0 (0)
	Oxacillin	1 µg	3 (100)	0 (0)	0 (0)
	Cefoxitin	30 µg	3 (100)	0 (0)	0 (100)
	Erythromycin	15 µg	2 (66.7)	0 (0)	1 (33.3)
	Tetracycline	30 µg	2 (66.7)	0 (0)	1 (33.3)
	Cephalothin	30 µg	1 (33.3)	0 (0)	2 (66.7)
	Chloramphenicol	30 µg	1 (33.3)	0 (0)	2 (66.7)
	Clindamycin	2 µg	1 (33.3)	0 (0)	2 (66.7)
	Doxycycline	30 µg	1 (33.3)	0 (0)	2 (66.7)
	Cefotaxime	30 µg	0 (0)	2 (66.7)	1 (33.3)
	Amikacin	30 µg	0 (0)	0 (0)	3 (100)
	Ciprofloxacin	5 µg	0 (0)	0 (0)	3 (100)
	Gentamicin	30 µg	0 (0)	0 (0)	3 (100)
	Linezolid	30 µg	0 (0)	0 (0)	3 (100)
	Quinupristin/Dalfopristin	15 µg	0 (0)	0 (0)	3 (100)
	Trimethoprim/sulfamethoxazole	25 µg	0 (0)	0 (0)	3 (100)

Table 2: The distribution of the multi-drug resistance patterns, antimicrobial resistance genes, and virulence genes among the MDRSA strains (n=13)

No.	Phenotypic resistance pattern	Antimicrobial resistance genes	Virulence genes
1	AMC-P-OX-FOX-E-NOR	*mecA*	*scn*
2	AMC-P-FOX-KF-TE-E-DA-LZD	*mecA*, *tetL*, *ermB*	*scn*, *sep*
3	AMC-P-OX-TE-E-C	*tetK*, *ermB*	*scn*
4	AMC-QD-CTX-FOX-KF-DA-E-LZD-P	*ermA*, *ermC*	*scn*, *chp*
5	AMC-CTX-FOX-KF-DA-E-LZD-P	*ermA*, *ermC*	*scn*
6	AMC-DO-NOR-OX-P-TE	*mecA*, *tetK*	*scn*, *sak*
7	AMC-QD-FOX-DA-E-OX-P	*ermB*	*scn*
8	AMC-QD-FOX-KF-DA-E-OX-P-TE	*tetL*, *ermA*, *msrA*	-
9	P-E-TE	*tetK*, *ermC*	-
10	AMC-CTX-FOX-DA-E-OX-P	*ermC*	*scn*, *chp*, *sak*
11	AMC-QD-FOX-KF-DA-E-OX-P	*ermC*	-
12	AMC-QD-FOX-KF-DA-E-OX-P	*msrA*	-
13	AMC-QD-FOX-DA-E-OX-P	*ermB*	*scn*, *sak*

Table 3: Primer sequences used for the detection of antimicrobial and virulence genes in *S. aureus*

No.	Primers	Primer sequence (5´-3´)	Amplicon band size (bp)	Accession number	Reference
1	*nuc*	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	278	MN704287	Saiful *et al*. (2006)
2	*mecA*	F: AAAATCGATGGTAAAGGTTGG R: AGTTCTGCAGTACCGGATTTG	533	NG_047945	
3	*mecB*	F: TTAACATATACACCCGCTTG R: TAAAGTTCATTAGGCACCTCC	2264	MK422159	Becker *et al*. (2018)
4	*mecC*	F: GAAAAAAAGGCTTAGAACGCCTC R: GAAGATCTTTTCCGTTTTCAGC	138	NG_047955	Stegger *et al*. (2013)
5	*ermA*	F: GTTCAAGAACAATCAATACAGAG R: GGATCAGGAAAAGGACATTTTAC	421	MG778116	Ma *et al*. (2018)
6	*ermB*	F: CCGTTTACGAAATTGGAACAGGTAAAGGGC R: GAATCGAGACTTGAGTGTGC	359	LC146993	
7	*ermC*	F: GCTAATATTGTTTAAATCGTCAATTCC R: GGATCAGGAAAAGGACATTTTAC	572	MG787087	
8	*msrA*	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	X52085	
9	*tetK*	F: TCGATAGGAACAGCAGTA R: CAGCAGATCCTACTCCTT	169	KR362247	Ng *et al.* (2001)
10	*tetL*	F: TCGTTAGCGTGCTGTCATTC R: GTATCCCACCAATGTAGCCG	267	JN970906	
11	*tetM*	F: GTGGACAAAGGTACAACGAG R: CGGTAAAGTTCGTCACACAC	406	AY057894	
12	*tetO*	F: AACTTAGGCATTCTGGCTCAC R: TCCCACTGTTCCATATCGTCA	515	KC915030	
13	*vanA*	F: ATGAATAGAATAAAAGTTGC R: TCACCCCTTTAACGCTAATA	1032	MG592387	Ma *et al.* (2018)
14	*scn*	F: AGCACAAGCTTGCCAACATCG R: TTAATATTTACTTTTTAGTGC	258	MF185201	Van Wamel *et al*. (2006)
15	*sak*	F: AAGGCGATGACGCGAGTTAT R: GCGCTTGGATCTAATTCAAC	223	EF122253	
16	*sea*	F: AGATCATTCGTGGTATAACG R: TTAACCGAAGGTTCTGTAGA	408	MT906848	
17	*sep*	F: AATCATAACCAACCGAATCA R: TCATAATGGAAGTGCTATAA	500	EF534985	
18	*chp*	F: GAAAAAGAAATTAGCAACAACAG R: CATAAGATGATTTAGACTCTCC	410	MF185201	De Haas *et al.* (2004)
19	*luk*PV	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAASTGTATTGGATAGCAAAAGC	433	MK975993	Lina *et al*. (1999)
20	*tst*	F: TTATCGTAAGCCCTTTGTTG R: TAAAGGTAGTTCTATTGGAGTAGG	398	MT906812	Zarizal *et al*. (2018)

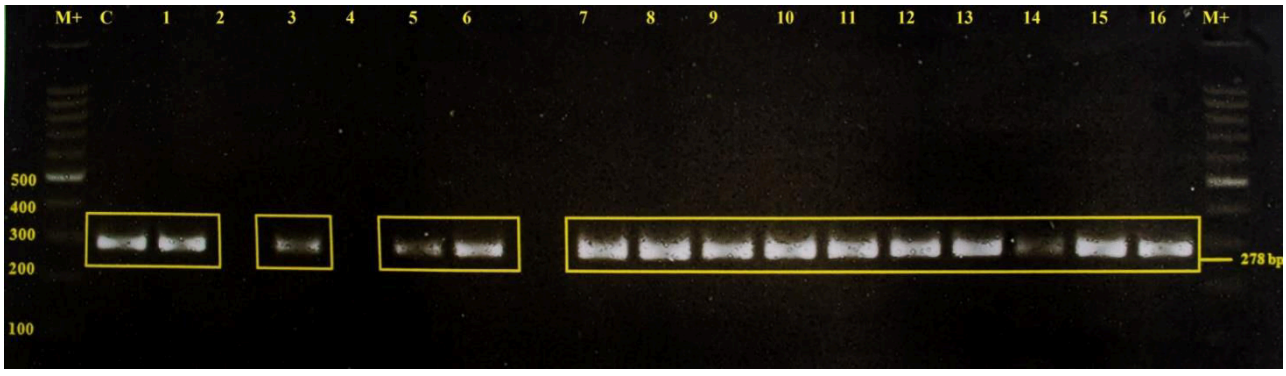

Fig. 1: Representative agarose gel electrophoresis image of *nuc* gene (278 bp). Lane M+: 100 bp DNA markers, and Lane C: Positive control (ATCC700699)

Table 4: The antibiogram of *S. aureus* from pets and pet owners (n=33)

Isolates	Antimicrobials	Disk potency	Number of isolates (%)		
			Resistant	Intermediate	Sensitive
S. aureus (n=33)	Penicillin	10 units	24 (72.7)	0 (0)	9 (27.3)
	Amoxicillin/clavulanate	10 µg	22 (66.7)	0 (0)	11 (33.3)
	Oxacillin	1 µg	20 (60.6)	0 (0)	13 (39.4)
	Erythromycin	15 µg	12 (36.4)	2 (6.1)	19 (57.5)
	Clindamycin	2 µg	10 (30.3)	3 (9.1)	20 (60.6)
	Cefoxitin	30 µg	9 (27.3)	0 (0)	24 (72.7)
	Quinupristin/Dalfopristin	15 µg	7 (21.2)	0 (0)	26 (78.8)
	Cephalothin	30 µg	6 (18.2)	0 (0)	27 (81.8)
	Tetracycline	30 µg	5 (15.2)	1 (3.0)	27 (81.8)
	Cefotaxime	30 µg	3 (9.1)	6 (18.2)	24 (72.7)
	Linezolid	30 µg	3 (9.1)	0 (0)	30 (90.9)
	Norfloxacin	10 µg	2 (6.1)	0 (0)	31 (93.9)
	Chloramphenicol	30 µg	1 (3.0)	2 (6.1)	30 (90.9)
	Doxycycline	30 µg	1 (3.0)	1 (3.0)	31 (94.0)
	Trimethoprim/sulfamethoxazole	25 µg	0 (0)	1 (3.0)	32 (97.0)
	Amikacin	30 µg	0 (0)	2 (6.1)	31 (94.0)
	Gentamicin	30 µg	0 (0)	0 (0)	33 (100)
	Ciprofloxacin	5 µg	0 (0)	0 (0)	33 (100)

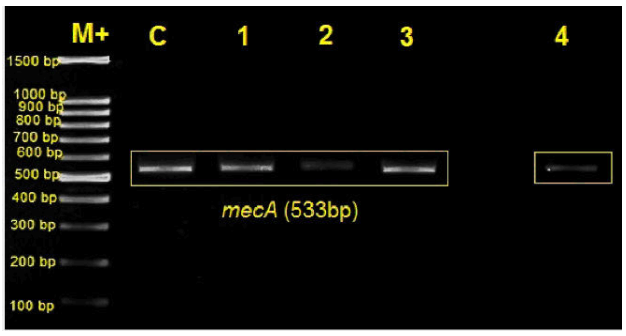

Fig. 2: Representative agarose gel electrophoresis image of *mecA* gene (533 bp). Lane M+: 100 bp DNA markers, and Lane C: Positive control (ATCC700699)

Table 5: Multiple antibiotic resistance index (MARI) of *S. aureus*

Number of antibiotic	Number of isolates	Total (%)	MARI
0	4	12.1	0
1	4	12.1	0.05
2	6	18.2	0.11
3	6	18.2	0.16
4	2	6.1	0.21
5	1	3.0	0.26
6 and above	10	30.3	0.32 and above

pet owners were found carrying *S. aureus*, while the carriage rates of *S. aureus* in cats and dogs were 17.5% (7/40), and 20% (6/30), respectively. Screening of *mecA* gene (Fig. 2) revealed that three (9.1%; 3/33) of the *S. aureus* isolates were MRSA of which one belonged to the pet owner (1.4%; 1/70) and another two were from dogs (6.7%; 2/30). MRSA was not detected in cat samples.

Antibiotic susceptibility test (AST)

AST results for both MRSA and *S. aureus* are summarized in Table 1 and Table 4, respectively. Kirby-Bauer test revealed that *S. aureus* isolates of pets and pet owners were highly resistant against penicillin (72.7; 24/33), followed by amoxicillin/clavulanate (66.7; 22/33) and oxacillin (60.6; 20/33). 27% of the isolates showed cefoxitin-resistance and were considered to be MRSA, phenotypically. Nonetheless, the *S. aureus* isolates were fully susceptible to gentamicin and ciprofloxacin. On the other hand, *mecA* gene-positive MRSA was fully resistant (100% resistant) against penicillin, amoxicillin/clavulanate, norfloxacin, cefoxitin, and oxacillin. Meanwhile, three of the *mecA*-positive MRSA isolates were susceptible to amikacin, ciprofloxacin, gentamicin, linezolid, quinupristin/dalfopristin, and trimethoprim/sulfamethoxazole. *S. aureus* from humans tend to be more resistant against penicillin and amoxicillin/clavulanate compared to isolates from cats and dogs ($P<0.05$). 87.8% (29/33) of *S. aureus* isolates from humans and pets were resistant to at least one antibiotic. Furthermore, 13 *S. aureus* isolates were confirmed to be MDRSA as they were resistant to agents from 3 and above the different classes of antibiotics. All MDRSA were also found to have a multiple antimicrobial resistance index (MARI) of 2.0 and above, suggesting the isolates originated from an environment with high antibiotic usage (Table 5). Figure 3 shows the dendrogram illustrating relatedness between *S. aureus* isolates from cats, dogs, and pet owners based on their phenotypic antibiotic resistance profile.

Detection of antimicrobial resistance and virulence genes

The number of antimicrobial resistance and virulence genes detected from both pets and pet owners is summarized in Table 6. The *mecA* genes (9.1%; 3/33) were detected from the *S. aureus* isolates, with the absence of *mecB* and *mecC* genes. For tetracycline resistance genes, only *tetK* and *tetL* genes (9.1%; 3/33) were detected from both humans and animals *S. aureus* isolates. Meanwhile, all four erythromycin resistance

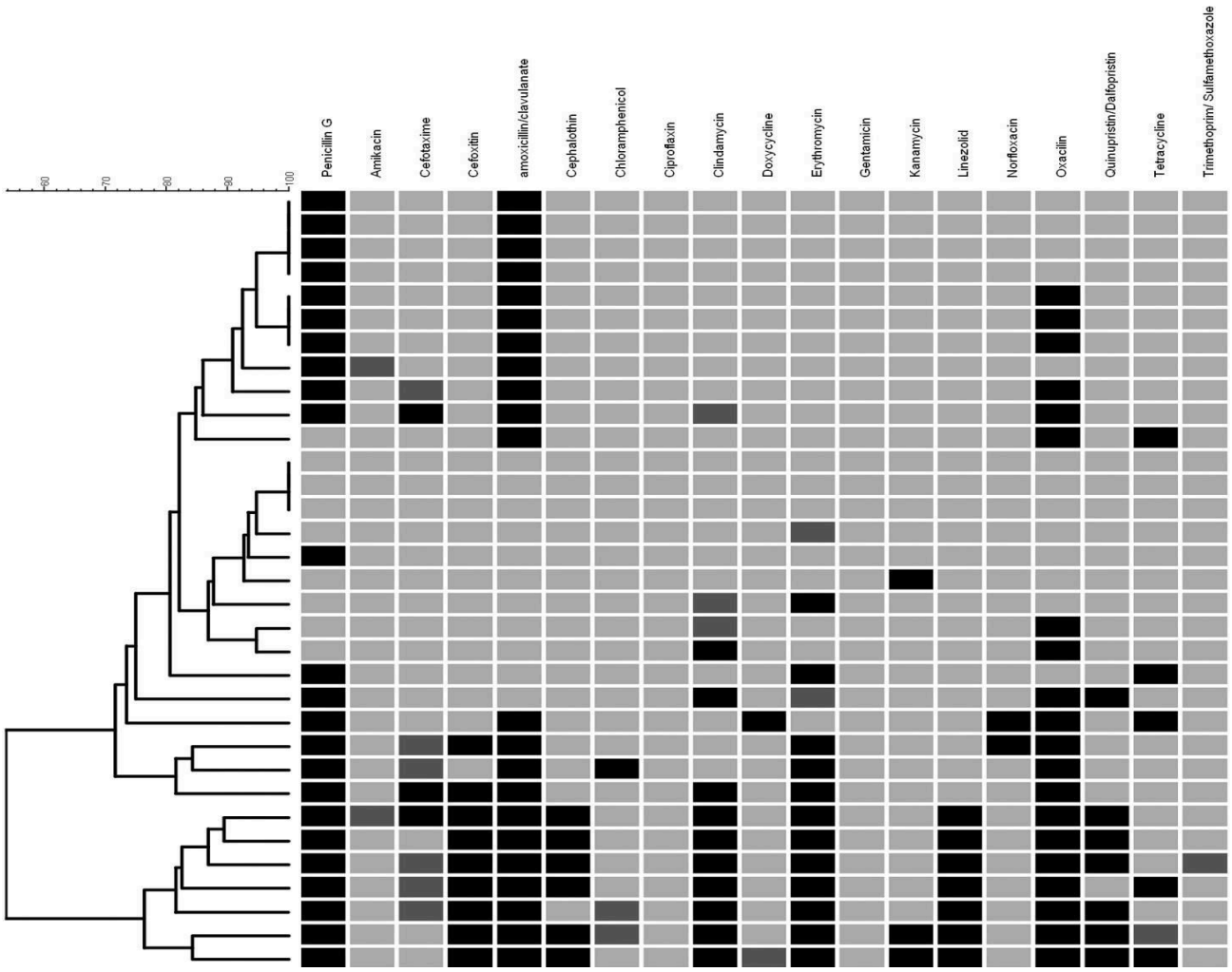

Fig. 3: Dendrogram of relatedness based on antibiogram of *S. aureus*. Black columns represent resistance, and dark grey columns represent intermediate resistance, while light grey columns represent antibiotic susceptibility

Table 6: Prevalence rate of antimicrobial resistance and virulence genes in *S. aureus* (n=33)

Genes	*S. aureus* (n=33)		
	Number of genes in pet owners (n=20) (%)	Number of genes in dog and cats (n=13) (%)	Total number of genes (%)
Antimicrobial resistance genes			
mecA	1 (5.0)	2 (10.0)	3 (9.1)
mecB	0 (0)	0 (0)	0 (0)
mecC	0 (0)	0 (0)	0 (0)
tetM	0 (0)	0 (0)	0 (0)
tetK	1 (5.0)	2 (10.0)	3 (9.1)
tetL	2 (10.0)	1 (7.8)	3 (9.1)
tetO	0 (0)	0 (0)	0 (0)
ermA	1 (10.0)	1 (7.7)	2 (6.0)
ermB	3 (15.0)	2 (15.4)	5 (15.2)
ermC	3 (15.0)	3 (23.10)	6 (18.2)
msrA	2 (10.0)	0 (0)	1 (3.0)
vanA	0 (0)	0 (0)	0 (0)
Virulence genes			
tst	0 (0)	0 (0)	0 (0)
luk-PV	0 (0)	0 (0)	0 (0)
scn	9 (45.0)	8 (61.5)	17 (51.1)
sak	12 (60.0)	5 (25.0)	17 (51.1)
chp	4 (20.0)	3 (15)	7 (21.2)
sea	0 (0)	1 (7.7)	1 (3.0)
sep	0 (0)	1 (7.7)	1 (3.0)

Table 7: Summary of IEC types of *S. aureus* from pets and pet owners (n=33)

IEC type	IEC genes composition	*S. aureus* isolates (n=33)		Total number of isolates (%)
		Pet owners (n=20)	Pets (n=13)	
A	*scn*, *chp*, *sak*, *sea*	0	0	0 (0)
B	*scn*, *chp*, *sak*	3	0	3 (9.1)
C	*scn, chp*	0	1	1 (3.0)
D	*scn*, *sak*, *sea*	0	0	0 (0)
E	*scn*, *sak*	5	2	7 (21.2)
F	*scn*, *chp*, *sak*, *sep*	1	0	1 (3.0)
G	*scn*, *sak*, *sep*	0	0	0 (0)
H	*Scn*	1	3	4 (12.1)
Non-typeable	Absent of *scn* gene	3	3	6 (18.2)
No type	Absent of all IEC genes	7	4	11 (33.3)
Total		20	13	33 (100)

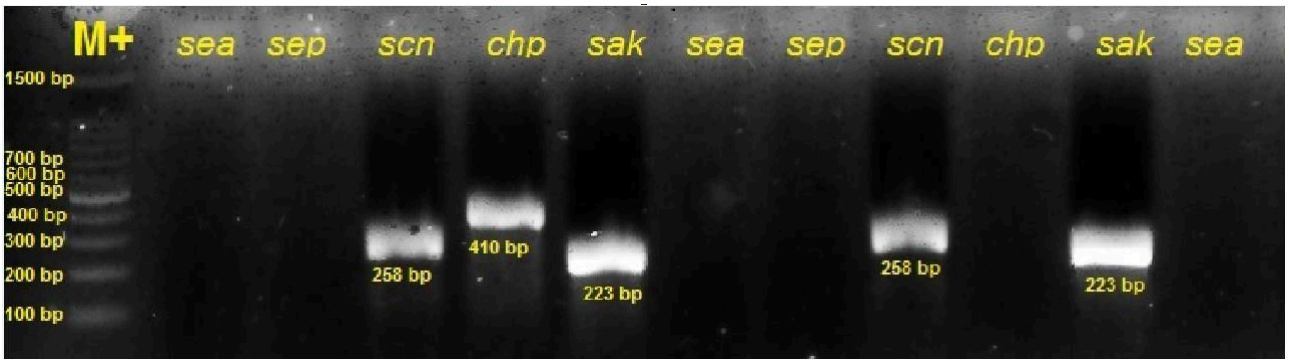

Fig. 4: Representative agarose gel electrophoresis image of IEC genes (*scn* gene at 258 bp, *chp* gene at 410 bp and *sak* gene at 223 bp). M+: 100 bp DNA markers

genes were detected from the 33 *S. aureus* isolates, with *ermB*, the prominent gene (15.2%; 5/33). None of *tetM*, *tetO*, and *vanA* genes were detected. In regards to virulence genes, high prevalence rates of *scn* (48.5%), and *sak* (45.5%) were detected from the *S. aureus* as demonstrated by Fig. 4. Six *S. aureus* carried *chp* (18.2%), while one isolate from dog harbored *sep* gene (3.3%). Eleven *S. aureus* isolates (35.0% of humans, and 30.1% of animal isolates) did not carry any IEC genes. A total of sixteen *S. aureus* harbored *scn* gene and thus, can be categorised into IEC types (Table 7). The predominant IEC type in this study was type E (7 isolates). Six *S. aureus* isolates (3 pet owners, and 3 pets) could not be categorised into any IEC types due to the absence of *scn* gene. No *vanA*, *tst*, *luk-PV*, and *sea* genes were detected from any of the *S. aureus* isolates. The antibiotic resistance pattern and genotypic profile of MDRSA isolates were summarized in Table 2.

Discussion

The existence of zoonotic bacteria is a reason for concern owing to their capability of resistance against multiple antibiotics among animals and their human handlers. This study demonstrated the presence of AMR strains of *S. aureus*, particularly MRSA and MDRSA in both pets and pet owners in Malaysia, highlighting the possibilities of further outspread and transmission of such bacteria in the coming future. In the present study, the carriage rates of *S. aureus* among pet owners and pets are slightly higher compared to previous studies (Boost *et al*., 2008; Walther *et al*., 2012; Van Balen *et al*., 2017). Meanwhile, both cefoxitin resistant test and PCR detection confirmed the MRSA isolates in dog and pet owners. In Malaysia, multiple previous studies have reported the presence of MRSA in pet animals and pet handlers from different settings with the carriage rate ranging from 1.9% to 30% (Saleha *et al*., 2006; Ahmad *et al*., 2009; Saleha and Zunita, 2010; Aklilu *et al*., 2012; Aklilu *et al*., 2013; Kanagarajah *et al*., 2017). The main differences in carriage rates may be due to different sampling sizes, years, and geographical factors. In addition, different detection methods, phenotypic or genotypic methods, may also affect the prevalence rate of MRSA. In this study, the cefoxitin disc diffusion (phenotypic) method and PCR (genotypic) detection of methicillin-resistant genes were used to identify the presence of MRSA. Indeed, the cefoxitin disc diffusion test showed a higher prevalence rate of MRSA compared to PCR assay. However, the absence of *mecA* gene within cefoxitin-resistant staphylococcal isolates was previously reported (Kandel *et al*., 2020). Although the expression of *mecA* gene is considered to be an important mechanism for the expression of methicillin resistance in *S. aureus*, other mechanisms such as hyperproduction of beta-lactamase and alterations of amino acids of protein binding protein cascade (PBPs 1, 2, and 3) by *S. aureus* could be the basis of beta-lactam resistance (Kandel *et al*., 2020). Furthermore, the expression of cefoxitin resistance may also be affected by variants such as differences in types of medium, inoculums size, temperature, and sodium chloride concentration in the

medium (Kandel *et al*., 2020).

The development of antimicrobial resistance in microorganisms is nearly always the result of repeated therapeutic or indiscriminate use of antibiotics (Ariffin *et al*., 2019). More than often, antibiotic resistance in bacteria is acquired through the transfer of antimicrobial resistance genes (Nicolaou and Rigol, 2018). Therefore, it is possible to foresee that *S. aureus* isolates resistant against commonly used antibiotics such as penicillin, tetracycline, and erythromycin carry resistance genes encoding the production of defensive enzymes, efflux pumps, or ribosomal protection mechanisms (Vyletělova *et al*., 2011; Foster, 2017). Indeed, further genotypic detection of antimicrobial resistance genes revealed the presence of several antibiotic resistance genes, where the majority of the genes were detected from MDRSA. The presence of such a wide variety of AMR genes in *S. aureus* isolated from pets and pet owners alarms that these genes can be transmitted to new clones or other bacteria, causing the further emergence of multidrug-resistant strains (Zehra *et al*., 2017). Nonetheless, all MDR isolates were fully susceptible to gentamicin and ciprofloxacin, suggesting that these two antibiotics can be used to treat persistent *S. aureus* infections in pets and pet owners in Malaysia. However, the usage of gentamicin and ciprofloxacin should be strictly regulated as they are critically important antibiotics by the World Health Organization (Collignon *et al*., 2016). In addition, further investigations involving more isolates of *S. aureus* from pets and their owners with different environmental exposures should be carried out to gain more insightful data on the antibiogram of the bacteria.

Immune evasion virulence factor is one of the important virulence factors produced by *S. aureus* that aid the survival of the pathogens in the human host. Past studies have mentioned the importance of IEC genes in disrupting or inhibiting the normal function of the human immune system as well as causing food poisoning (Rooijakkers *et al*., 2005; Van Wamel *et al*., 2006). In the current study, the prevalence rate of IEC genes among pet handlers was lower than in previous studies (Van Wamel *et al*., 2006; Verkaik *et al*., 2011; Ariyarad *et al*., 2019). The low prevalence of IEC positive *S. aureus* isolates in this research may be due to different samples origin. Cuny *et al*. (2015) suggested that IEC genes are highly specific to humans and the absence of *scn* gene is a strong indicator for animal-derived strains. Thus, *S. aureus* isolate colonizing the pet owners may originate from pets or other animals. Similar findings are also reported where the majority of *S. aureus* isolates from individuals with constant exposure to animals lacked IEC genes (Hau *et al*., 2015; You *et al*., 2018). Interestingly, some of MDRSA from pets carried IEC genes, highlighting the possibilities of transmission of *S. aureus* from humans to animals. The predominant IEC type in this study is type E (seven isolates) similar to the study of Ariyarad *et al*. (2019). Nonetheless, multiple studies have reported that type B was the predominant IEC type (Van Wamel *et al*., 2006; Verkaik *et al*., 2011; Hau *et al*., 2015; Ahmadrajabi *et al*., 2017). These findings suggest that there are variations in IEC types among *S. aureus* isolated from different geographical regions (Ariyarad *et al*., 2019). In addition, all *S. aureus* isolates, including MRSA found in this study were negative for the *luk-PV* and *tst* genes coding PVL, and TSST. Our finding is similar to the results by Köck *et al*. (2009), and Neela *et al*. (2009) that they reported the absence of PVL and TSST encoding genes among MRSA isolates. However, Rankin *et al*. in 2005 reported the presence of PVL encoding genes among MRSA isolated from dogs in the USA. Huang and Chou (2019) also reported the *tst* gene-positive MRSA isolated from dogs in Taiwan.

In conclusion, this study demonstrates the presence of *S. aureus* and MRSA in both pets and pet owners in Peninsular Malaysia. 39.3% of *S. aureus* were considered to be MDRSA, suggesting the emergence of multidrug-resistance *S. aureus* in both human and pet owners, in this study. The isolated *S. aureus* showed the highest resistance rate against penicillin, but was fully susceptible to gentamicin and ciprofloxacin. Furthermore, *S. aureus* isolates carry several antibiotic resistance genes which may cause the further establishment of antimicrobial resistance in bacteria from pets and pet owners. However, the isolates were found to be less pathogenic as they carry a relatively low number of tested virulence genes. Nonetheless, the screening of IEC genes in this research suggests the spread of animal-adapted *S. aureus* lineages among pets and pet owners. Due to the rapid emergence of multidrug-resistant strains observed in both animals and humans, coordinated antibiotic stewardship programs, and continuous surveillance on the development of AMR patterns in *S. aureus* are highly recommended in order to determine the suitable antibiotics for successful treatment.

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Conflict of interest

None of the authors have any potential conflict of interest to declare.

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