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Detection of virulence genes and antibiotic resistance profiles of *Staphylococcus aureus* isolated from animals

Asinamai Athliamai Bitrus[1], Zakaria Zunita[1*], Siti Khairani Bejo[1], Sarah Othman[2] and Nur Adilah Ahmad Nadzir[1]

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia.
[2]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia.
Email: zunita@upm.edu.my

ABSTRACT

Aims: This study was designed to determine the virulence genes and antibiotic resistance profiles of *Staphylococcus aureus* isolated from dogs, cats, chickens and horses.
Methodology and results: A total of 15 *S. aureus* isolates were used in this study. Antibiogram and screening of virulence genes was carried out using disc diffusion method and polymerase chain reaction. The results obtained showed that a total of 9 *S. aureus* isolates were resistant towards oxacillin (60%), 9 isolates were resistant towards neomycin (60%) and 8 isolates were resistant towards tilmicosin (53%). Resistance to amoxicillin, tetracycline and vancomycin was also observed in 6 (40%) of the isolates. Additionally, 5 (33%) of the isolates showed resistance towards streptomycin and linzolide while 4 (27%) of the isolates were resistant towards rifampin, erythromycin and mupirocin. Lastly, 3 (20%) of the isolates were resistant towards doxycycline. Intermediate resistance to amoxicillin and doxycycline was also observed. Virulence gene profiling showed that 4 (26.7%) of the isolates were positive for *hlβ and Ssp*A, 9 of the isolates (60%) showed positive for *geh* and 12 of the isolates (80%) showed positive for *Set*-1. Similarly, 2 (13.3%) of the isolates showed positive for *et*A and *Seu* while only 1 isolate (6.7%) showed positive for PVL and *hlα*. None of the isolates were positive for *tst*-1 and *et*B.
Conclusion, significance and impact of study: This study revealed reduced susceptibility and multiple drug resistance (MDR) in four isolates, and susceptibility to all antibiotics in two isolates in addition to low carriage rate of virulence gene in all isolates. Thus, indicating resistance development in majority of the isolates and the need to regulate indiscriminate use of antibiotics in animals.

Keywords: antibiotics, polymerase chain reaction, resistance, *Staphylococcus aureus,* susceptibility, virulence

INTRODUCTION

Staphylococcus aureus is a leading cause of bacteremia as well as skin and soft tissue infection in animals and humans. The severity of infection caused by this pathogen is by the ability to rapidly acquire and loss resistance and virulence determinants (Noto *et al.*, 2008). It is a frequent colonizer of the nasal epithelium of animal and humans. In addition to that, studies have shown that nasal colonization predisposes an individual to be at greater risks of infection with *S. aureus.* Furthermore, 20 to 30% of healthy individuals are frequently or persistently colonized by *S. aureus*, while about 70% are intermittently colonized (Kennedy and DeLeo, 2009; Aqel *et al*., 2015; Jenkins *et al.*, 2015).

Staphylococcus aureus causes a spectrum of infection which ranges from mild uncomplicated skin infection to more fatal disease conditions such as Septicemia, endocarditis, botryomycosis in horse, pyaemic dermatitis in dogs and osteomyelitis, necrotic pneumonia and necrotic fasciitis (So and Farrington, 2008). This is as a result of the pathogen's ability to produce a number of putative virulence determinants and to acquire resistance determinants towards almost all classes of clinically important antibiotics. (Kennedy and DeLeo, 2009). A number of cell surface proteins and secretory toxins produced by S*. aureus* have been shown to promote virulence by facilitating evasion of the host immune response. In addition to that, *S. aureus* is regarded as one of the most prevalent cause of food poisoning worldwide and known to produce more than 30 different extracellular metabolites (Aydin *et al.*, 2011).

The clinical importance of *S. aureus* is associated to its high virulence and rapid development of drug resistance. Members of the genus *Staphylococci* are known to harbor a large repertoire of virulence gene coding for pyogenic superantigens, exfoliative, leukocidine, hemolysin, toxic shock syndrome toxin -1, Lipase, biofilm formation proteins, coagulase, fibrinolysin, protease and lipase (Peacock *et al*., 2002). These virulence factors are associated with attachment, persistence, evasion/destruction of host defenses, tissue

***Corresponding author**

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invasion/penetration and toxin-mediated disease symptoms (Moura *et al.*, 2012; Spanu *et al*., 2012). Furthermore, the virulence determinants in *S. aureus* are responsible for a number of life- threatening diseases worldwide and as such determining the virulence and resistance profiles of these isolates will provide the baseline data for evaluating the carriage rate and the level of risk associated with each virulence and resistance determinants. This is because the ability of the pathogen to cause severe and life threatening disease is gaining more attention as a potential zoonosis. In addition, there is a global public health concern with regards to the development of antibiotic resistance in *S. aureus*. Although the situation may vary in different countries, there are indications that some countries are epicentres of antibiotic resistance. This was observed due to the rise in the prevalence of antibiotic resistant strains of *S. aureus* leading to a limited treatment options. There is therefore the need for stakeholders in veterinary medicine to be aware of the current trend of resistance development and have a proper understanding of the use of antibiotics in clinical practice. To this end, the study to determine the antibiotic resistance and virulence gene profile of *S. aureus* isolated from animals was carried out.

MATERIALS AND METHODS

Bacterial isolates

A total of 15 *S. aureus* isolates obtained from horse wounds swabs around Selangor and four other isolates obtained from stock cultures in the Bacteriology Laboratory, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia were used in this study. Swab sample were enriched at 37 °C overnight in tryptic soy broth (Oxoid, UK) before culturing onto blood agar containing 5% horse blood. Pure isolates were confirmed as *S. aureus* by colony morphology, Gram staining for cellular morphology, mannitol fermentation, biochemical tests (catalase and coagulase) and PCR amplification of the thermostable nuclease gene *nuc* as described by Saiful *et al*. (2006) as shown in Table 1.

Table 1: Oligonucleotide Sequence for the amplification of *S. aureus* virulence determinants.

Gene	Primer set	Oligonucleotide sequence	Product size (bp)	References
Nuc	NUC-F NUC-R	5'-GCG ATT GAT GGT GAT ACG GTT-3' 5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'	278	Saiful *et al*. (2006)
mecA	MECA-F MECA-R	5'-ACT GCT ATC CAC CCT CAA AC-3' 5'-CTG GTG AAG TTG TAA TCT GG-3'	533	Merlino *et al*. (2002)
etA	ETA-F ETA-R	5'-GCA GGT GTT GAT TTA GCA TT-3' 5'-AGA TGT CCC TAT TTT TGC TG-3'	93	Mehrotra *et al.* (2000)
etB	ETB-F ETB-R	5'-ACA AGC AAA AGA ATA CAG CG-3' 5'-GTT TTT GGC TGC TTC TCT TG-3'	226	Mehrotra *et al*. (2000)
Geh	GEH-F GEH-R	5'-GCA CAA GCC TCG G-3' 5'-GAC GGG GGT GTA G-3'	319	Said-Salim *et al*. (2003)
Hlα	HLα-F HLα-R	5'-GGT TTA AGC CTG GCC TTC-3' 5'-CAT CAC GAA CTC GTT CG-3'	543	Kumar *et al*. (2011)
Hlβ	HLβ-F HLβ-R	5'-GCC AAA GCC GAA TCT AAG-3' 5'-GCG ATA TAC ATC CCA TGG C -3'	833	Kumar *et al*. (2011)
Pvl	PVL-F PVL-R	5'-ATG TCT GGA CAT GAT CCA A-3' 5'-AAC TAT CTC TGC CAT ATG GT-3'	970	Sapri *et al*. (2011)
Seu	SEU-F SEU-R	5'-ATT TGC TTT TAT CTT CAT-3' 5'-GGA CTT TAA TGT TTG TTT CTG AT-3'	167	Chiang *et al*. (2008)
Set-1	SET-1-F SET-1-R	5'-GGG ACA GAA TAA TAC TAT GAA ATT AA AAA CG-3' 5'-ATC TTT TTG GTT AAA GCG TAC-3'	253	Williams *et al*. (2000)
sspA	SSPA-F SSPA-R	5'-GCG ACA CTT GTG AGT TCT CCA GC-3' 5'-GTT TTA AGA AGT TGC GTA CAT TTT C-3'	772	Zdzalik *et al*. (2012)

Antibiotic susceptibility test

Antibiotic sensitivity test was performed using disc diffusion method as described by Bauer *et al.* (1996). Diameter of inhibition zone were measured and interpreted according to the breakpoints of antibiotics as shown in Table 2. All isolates were tested for susceptibility to eight veterinary critically important antibiotics (VCIA) and three highly important antimicrobial agents (HIA) for human medicine which includes; amoxicillin (AML) 25 µg, doxycycline (DO) 30 µg, rifampin (RD) 30 µg , tetracycline (TE)10 µg, neomycin (N) 10 µg , oxacillin (OX)1 µg, erythromycin (E) 15 µg, tilmicosin (TIL) 15 µg, streptomycin (S)10 µg, vancomycin (VA) 30 µg and linzolide (LZD) 15 µg and two veterinary highly important antimicrobial agents (VHIA) which includes; cefoxitin (FOX) 30 µg and mupirocin (MUP) 5 µg (Oxoid, UK) according to the World Organization for Animal Health (OIE) list of antibiotics and World Health Organisation.

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DNA extraction method

Bacterial genomic DNA extraction was carried out using boiling method as described by Chen *et al*. (2009). A suspension of overnight fresh cultures of *S. aureus* on tryptic soy agar (Oxoid, UK) was prepared in a 1.5 mL microcentrifuge tube containing 1000 µL of sterile distilled water. The suspension was incubated at room temperature for 5 min and then in a dry bath at 96 °C for 10 min. After cooling for about 10 min, the suspension was centrifuged at 13,000 rpm for 5 min, the supernatant was collected in a new 1.5 mL microcentrifuge tube and used as DNA template.

Polymerase chain reaction

PCR amplification of 10 selected target virulence genes was performed using specific primers and PCR cycling conditions as shown in Table 1. The reaction mixture was performed in a 50 µL reaction volume consisting of 25 µL Toptaq master mix (Qiagen) containing DNA polymerase, PCR Buffer (with 3 mM $MgCl_2$), and 400 µM each dNTPs, 10X coral load 5 µL (Qiagen); 1 µL (0.1 µM) of each forward and reverse primer (Integrated DNA technologies, Singapore), 13 µL of RNase free water (Qiagen) and 5 µL of DNA template. The reaction was performed in a Thermal cycler (BIO-RAD) under the following PCR cycling condition as were as those shown in Table 1. Where the initial denaturation step was performed for 5 min at 94 °C, and the final extension was performed for 10 min at 72 °C with exception to SspA where initial denaturation was at 95 °C for 3 min. A total of 30 - 35 PCR cycles was performed.

Agarose gel electrophoresis

Electrophoresis of PCR products was carried out in 2% Agarose (Sigma-Aldrich) prepared in a 0.5X Tris-Borate EDTA buffer. Ten microliter (10 µL) of amplified PCR products each were loaded in a well of submerged gel. The PCR products were then electrophoresed at 80V for 90 min. The gel was stained with ethidium bromide (0.25 mg/mL) for 5 min and then destained with distilled water for 15-20 min. The stained electrophoresed PCR products were then visualized under the transilluminator UV-light using a gel documentation system alpha imager (BIO-RAD) Figure 1 (a) and (b).

RESULTS

Detection of virulence gene of *S. aureus*

The findings of PCR amplification of genes coding for virulence showed that a total of 9 (60%) and 12 (80%) of the isolates were positive for staphylococcal exotoxin-like toxin 1 (*set1*) and lipase encoding gene (*geh*), while 4 (26.7%) of the all the isolates were positive for beta hemolysin (*hlβ*) and V8 protease (*SspA*) respectively (Table 1).

Table 2: Diameter Breakpoints for antibiotics according to CLSI guidelines.

Antibiotic type	Disc content (µg)	S	I	R	Source
Amoxicillin	25	≥21		≤14	CA-SFM (1996)
Streptomycin	10	14-22			CLSI (2014)
Erythromycin	15	≥23	14-22	≤13	CLSI (2014)
Neomycin	10	≥17		≤16	EUCAST (2012)
Tilmicosin	15	17-21			CLSI (2014)
Doxycycline	30	≥16	13-15	≤12	CLSI (2014)
Rifampin	30	≥29		≤14	CA-SFM (1996)
Mupirocin	5	≥14		≤13	CLSI2006
Linezolid	10	21-27			EUCAST (2012)
Oxacillin	1	18-24			CLSI (2014)
Cefoxitin	30	≥22		≤21	CLSI (2014)
Tetracycline	10	≥20		≤19	EUCAST (2012)
Vancomycin	30	17-21			CLSI (2014)

R, Resistant; S, Susceptibility; I, Intermediate.

Additionally, 2 (13.3%) of the isolates were positive for both exfoliative A (*etA*) and staphylococcal enterotoxin u (*Seu*). While only 1 (6.7%) of the isolates were positive for Panton valentine leukocidine (PVL) and alpha hemolysin (*hlα*) respectively (Figure 2). None of the isolates were positive for gene coding for toxic shock syndrome toxin-1 (*tst*) and exfoliative B (*etB*) (Table 3).

Antibiotic sensitivity test

The result of antibiotic susceptibility test revealed that 9 (60%) and 3 (20%) of the isolates were resistant to oxacillin and cefoxitin respectively (Table 4). While resistance to amoxicillin, tetracycline and vancomycin was observed in 6 (40%) of the isolates. Similarly, 5 (33.3%) of the isolates were resistant to streptomycin and linzolide (Table 3). The frequency of resistance to neomycin and tilmicosin was observed in 9 (60%) and 8 (53%) of the isolates respectively while the resistance towards doxycycline was observed in only 3 (20%) isolates. Additionally, resistance to rifampin, mupirocin and erythromycin was observed in 4 (27%) of the isolates. also observed (Figure 3). In this study, reduced susceptibility

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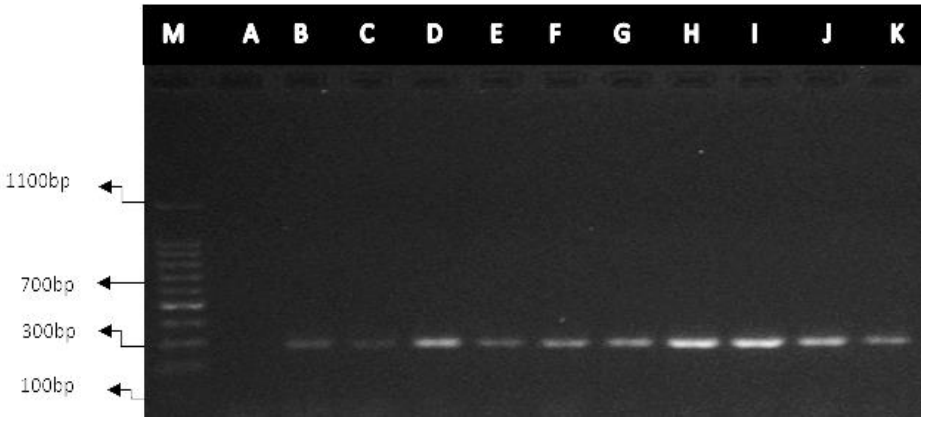
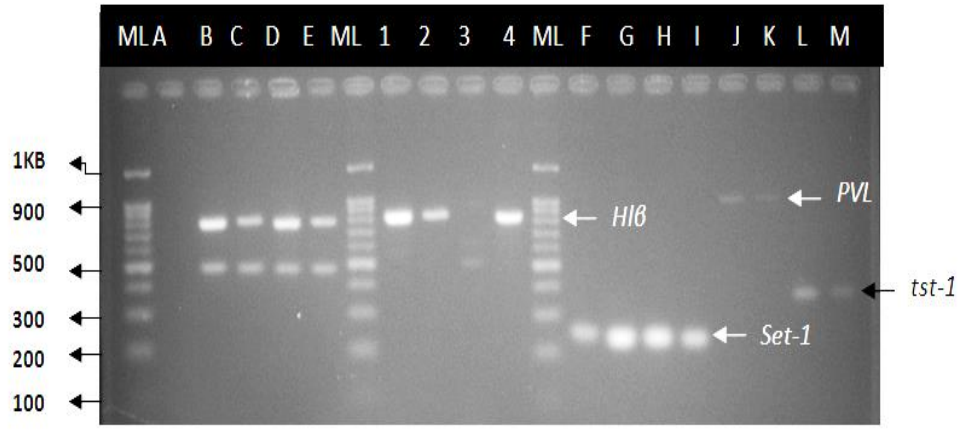


(a)



(b)

Figure 1: (a) PCR amplification of *S. aureus* endonuclease gene *nuc* (276 bp). Lane M, molecular DNA marker 100 bp (Thermo-Fisher Scientific); Lane A, negative control; Lane B, positive control *Staphylococcus aureus* ATCC 25923; Lane C-K, SCH3, SDG4, SH8 and SEQ1-SEQ6 respectively. (b) PCR amplification of virulence gene of *S. aureus* resolved in 2% agarose gel (wt. /Vol). Lane A-E showing SspA (772 bp) and geh (319 bp); Lane 1-4; Hlβ (833 bp) and Hlα (543 bp); Lane F-I, *Set-1* (253 bp), Lane J and K, PVL (970 bp), Lane L and M *tst-1* (385 bp); Lane ML, 100 bp molecular DNA marker (geneExact, Thermofisher Scientific, Malaysia).

and multidrug resistance was observed in four isolates (SEQ5, SEQ7, SEQ10 and SEQ11) and susceptibility to all antibiotics observed was observed in two isolates (SEQ 6 and SCH4) (Table 3).

DISCUSSION

The study on virulence gene and antimicrobial resistance profile of S*. aureus* isolated from animals revealed that the carriage rate of virulence determinants is relatively low in all isolates with the exception of lipase encoding gene and staphylococcal exotoxin –like toxin. The study also revealed an inverse relationship between carriage of virulence gene and phenotypic antibiotic resistance. This is because the higher the carriage of virulence gene, the lower the phenotypic antibiotic resistance and vice versa (Figure 4). Furthermore, reduced susceptibility and multidrug resistance was observed in three isolates, while susceptibility to all antibiotic was observed in one isolate. Additionally, only two isolates were positive for the methicillin resistance determinants *mec*A. The results of antibiotic susceptibility test showed that 9 (60%) and 3 (20%) of the isolates were resistant to oxacillin and cefoxitin. However, only two isolates were positive for the methicillin resistance determinants *mec*A by PCR. Hence, suggesting that in most instances isolates that are phenotypically resistant to oxacillin do not carry methicillin resistance determinant *mec*A. This can be inferred to be due to the development of low-level methicillin resistance or borderline resistance which is observed in certain strains of *S. aureus* (Louie *et al.*, 2000). Phenotypic resistance to oxacillin is not a good indicator of methicillin

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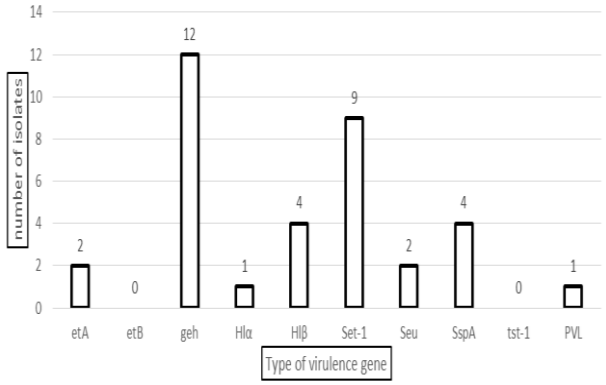

Figure 2: Virulence gene profile of *S. aureus* isolated from animals. *et*A and *et*B, Exfoliative toxin; *geh*, Lipase encoding gene; *hlα* and *hlβ*, Hemolysin; *Set*-1, Staphylococcal exotoxin-like toxin-1; *Seu*, Staphylococcal enterotoxin like U; *SspA*, V8 protease; *tst*-1, Toxic shock syndrome-1; PVL, Panton Valentine Leucocidin.

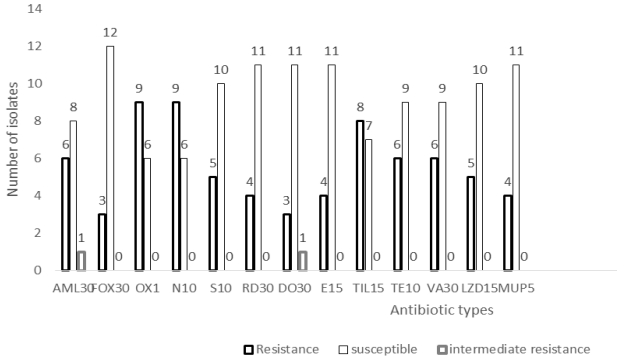

Figure 3: Antibiotic susceptibility profile of *S. aureus* isolates. AML, Amoxicillin; DO, Doxycycline; E, Erythromycin; FOX, Cefoxitin; LZD, Linzolide; MUP, Mupirocin; N, Neomycin; OX, Oxacillin; RD, Rifampin; S, Streptomycin; TE, Tetracycline; TIL, Tilmicosin; VA, Vancomycin.

Table 3: Virulence gene profile of *S. aureus* isolates.

Sample ID	Virulence Gene Profile of *S. aureus* isolates									
	*et*A	*et*B	*Hlα*	*hlβ*	*Set*-1	*Seu*	*Ssp*A	*tst*-1	PVL	*geh*
SH8	-	-	-	+	+	-	+	-	-	+
SDG4	-	-	-	-	+	-	-	-	-	+
SCH3	-	-	-	+	+	-	-	-	-	+
SCH4	+	-	-	-	-	-	-	-	+	-
SEQ1	-	-	-	-	+	-	-	-	-	-
SEQ2	-	-	-	-	-	-	-	-	-	-
SEQ3	-	-	-	-	+	-	+	-	-	+
SEQ4	-	-	-	-	+	-	-	-	-	+
SEQ5	-	-	+	+	-	-	-	-	-	+
SEQ6	-	-	-	-	+	-	-	-	-	+
SEQ7	-	-	-	-	-	-	-	-	-	+
SEQ8	-	-	-	-	-	-	-	-	-	+
SEQ9	-	-	-	-	-	-	-	-	-	+
SEQ10	-	-	-	-	+	+	+	-	-	+
SEQ11	+	-	-	+	+	+	+	-	-	+
% Total	2 (13%)	0	1 (6.7%)	4 (27%)	9 (60%)	2 (13%)	4 (27%)	0	1 (6.7%)	12 (80%)

*et*A and *et*B, Exfoliative toxin; *geh*, Lipase encoding gene; *hlα* and *hlβ*, Hemolysin; *Set*-1, Staphylococcal exotoxin-like toxin-1; *Seu*, Staphylococcal enterotoxin like U; *SspA*, V8 protease; *tst*-1, Toxic shock syndrome-1; PVL, Panton Valentine Leucocidin.

resistance. Even though in some cases, it has been shown to induce the production of *mec*A; however, combination of oxacillin and cefoxitin have been shown to yield a good result in detection of methicillin resistant S. *aureus* (Mimica *et al.*, 2007). This report is consistent with our findings because only two *mec*A positive *S. aureus* isolates were resistant to both cefoxitin and oxacillin. Methicillin resistance arises following acquisition of a genomic island called *SCCmec* which carries methicillin resistance determinant *mec*A that encodes an alternative penicillin binding protein (PBP2') with reduced susceptibility to all beta-lactam antibiotics (Lindsay, 2014). Prior to the early 1960s, methicillin is drug of choice for the treatment of *S. aureus* infection. However, from 1961 onwards *S. aureus* have developed resistance to almost all clinically important antibiotics; thus, reducing their therapeutic value and prolonging the course of clinical disease.

In this study, resistance to tetracycline and neomycin was also observed in 6 (60 %) and 11 (73.3%) of the isolates. Resistance to tetracycline is associated with a reduced intracellular accumulation of the antibiotic. In *S. aureus*, development of resistance to tetracycline was initially associated with reduced uptake. However, resistance to tetracycline is more likely to be the result of a specific efflux mechanism that is similar to what obtains in strains of *Escherichia coli*. In addition, two types of phenotypic resistance was observed in *S. aureus*. The

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Table 4: Antibiotic resistance and virulence gene profile of *S. aureus* isolated from animals.

Sample ID	Antibiotic susceptibility profile of *S. aureus* isolates												
	AML25	FOX30	0X1	N10	S10	RD30	DO30	TIL15	E15	TE10	VA30	LZD10	MUP5
SDG4	12.62=R	24.97=S	16.66=R	0.00=R	14.82=S	30.58=S	25.02=S	15.26=R	21.58=S	26.94=S	16.78=S	20.90=S	23.18=S
SCH3	24.89=S	30.14=S	15.72=R	15.96=S	19.96=S	39.04=S	33.78=S	39.70=S	28.36=S	29.06=S	19.09=S	28.90=S	29.54=S
SCH4	34.92=S	38.34=S	19.19=S	20.46=S	22.98=S	35.34=S	24.26=S	20.58=S	31.46=S	30.20=S	19.30=S	27.10=S	36.08=S
SH8	16.64=I	30.76=S	17.23=R	0.00=R	15.78=S	42.78=S	34.50=S	20.40=S	23.92=S	32.96=S	16.46=S	23.52=S	33.02=S
SEQ1	17.11=I	15.2=R	18.78=S	NZ=R	14.73=S	37.96=S	14.05=I	17.78=S	24.84=S	NZ=R	21.49=S	27.46=S	28.26=S
SEQ2	18.41=I	26.78=S	15.70=R	NZ=R	14.07=S	37.64=S	12.03=R	18.37=S	25.36=S	NZ=R	17.15=S	24.99=S	31.18=S
SEQ3	24.29=S	24.13=S	11.56=R	14.55=R	NZ=R	27.26=S	35.48=S	NZ=R	22.92=S	23.22=S	15.12=R	22.46=S	27.22=S
SEQ4	37.28=S	33.20=S	28.16=S	NZ=R	NZ=R	39.96=S	43.48=S	NZ=R	23.38=S	37.00=S	19.06=S	27.74=S	34.86=S
SEQ5	NZ=R	23.80=S	NZ=R	15.52=S	24.22=S	14.44=R	22.31=S	NZ=R	NZ=R	20.50=S	NZ=R	NZ=R	NZ=R
SEQ6	36.74=S	32.52=S	27.52=S	17.88=S	16.69=S	42.36=S	36.48=S	21.27=S	15.62=S	35.70=S	21.10=S	27.64=S	32.24=S
SEQ7	NZ=R	23.18=S	NZ=R	12.26=R	24.41=S	12.83=R	13.10=I	NZ=R	NZ=R	NZ=R	NZ=R	NZ=R	NZ=R
SEQ8	21.75=S	26.91=S	23.16=S	NZ=R	8.15=R	39.68=S	14.33=S	NZ=R	23.44=S	NZ=R	19.07=S	12.54=R	27.44=S
SEQ9	NZ=R	31.06=S	24.84=S	18.04=S	15.96=S	31.58=S	24.72=S	19.68=S	24.62=S	25.11=S	14.04=R	23.30=S	29.54=S
SEQ10	NZ=R	NZ=R	NZ=R	12.04= R	13.26=R	9.44=R	9.80=R	NZ=R	NZ=R	17.66=R	NZ=R	NZ=R	NZ=R
SEQ11	NZ=R	20.21=R	NZ=R	10.52=R	12.38=R	14.62=R	NZ=R	NZ=R	NZ=R	NZ=R	NZ=R	NZ=R	NZ=R
%R	6 (40%)	3 (20%)	9 (60%)	9 (60%)	5 (33%)	4 (27%)	3 (20%)	8 (53%)	4 (27%)	6 (40%)	6 (40%)	5 (33%)	4 (27%)

SEQ, horse isolate; SCH, chicken isolate; SDG, dog isolate; SH8, human; R, Resistant; S, Susceptibility; I, Intermediate; AML, Amoxicillin; AMC, Amoxicillin-Clauvulanic Acid; FOX, Cefoxitin; DO, Doxycycline; E, Erythromycin; Lev, Levofloxacin; MUP, Mupirocin; N, Neomycin; OX, Oxacillin; RD, Rifampin; S, Streptomycin; TIL, Tilmicosin; TE, Tetracycline; LZD, Linezolid; VA, Vancomycin.

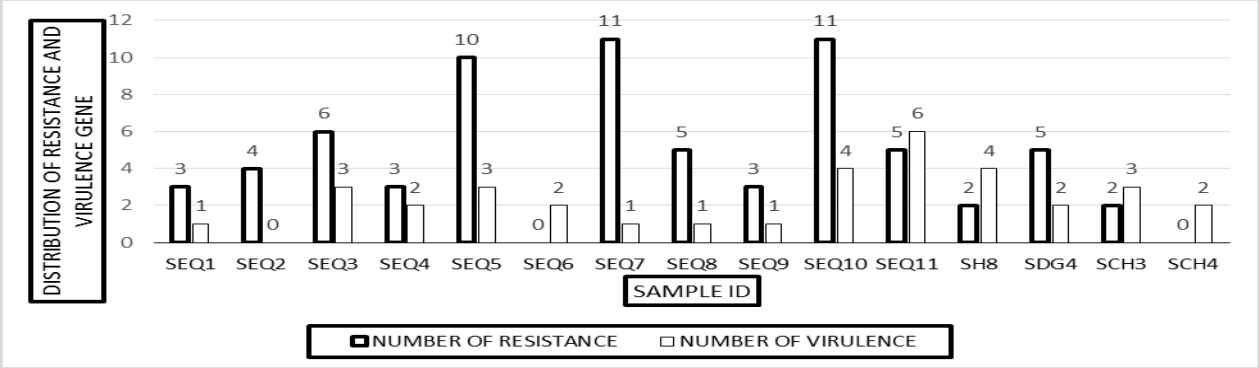

Figure 4: Distribution of antibiotic resistance and virulence gene profile of *S. aureus* isolated from animals. SEQ, horse isolate; SCH, chicken isolate; SDG, dog isolate; SH8, human.

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initial one being resistance to tetracycline that is inductive but not to minocycline a derivative of tetracycline that is plasmid mediated. While the second is a constitutive tetracycline and minocycline resistance encoded by chromosomal determinants. There was reduced susceptibility to neomycin in all of the isolates; this could be due to selective pressure which occurred as a result of prolonged usage of neomycin either as feed additives or for treatment of infection. This finding is supported by the understanding that since isolates from horses were collected from horse wound; the occurrence of phenotypic resistance is as a result of selective pressure due to prolonged usage of antibiotics. Neomycin is reported to be a very good topical antibiotic with a good spectrum of activity against gram negative bacteria. Our findings also showed that resistance to linezolid and vancomycin was observed in 5 (33.3%) and 6 (40%) of the isolates. The number of isolates resistant to linezolid and vancomycin as reported in this study is considered very high. This is because, both drugs are used as drug of last resort in the treatment of *S. aureus* infection and only few cases of resistance development were reported to have occurred. In addition, linezolid is a drug belonging to the oxazolidinone class and one of the newest critically important human antibiotics approved for clinical use in the year 2000. It functions as a synthetic inhibitor of protein synthesis that is therapeutically active against many Gram-positive bacteria (Wilson *et al.*, 2003) including such pathogens as methicillin and vancomycin-resistant staphylococci, vancomycin-resistant enterococci and penicillin-resistant pneumococci (Toh *et al*., 2007; Tsiodras *et al*., 2001). One of the main advantages of linezolid over other clinically important antibiotics is the fact that it is synthetic in nature in comparison with most antibiotics that are derived from natural sources, which in most cases favours the horizontal acquisition of natural resistance. It is rather surprising to observe resistance in 5(33.3%) of the tested isolates since acquisition of resistance to linzolide is not common like other classes of antibiotics. The threat of resistance development to some of this critically important antibiotics lies in the fact that it reduces our option as to which drug combination in our arsenal is to be used in the treatment of infections (Zhang *et al*., 2004). Development of resistance to vancomycin has ushered in a new era in the threat to antibiotic resistance; thus, indicating the loss of therapeutic value of a once considered drug of last resort in the treatment of *S. aureus.* Vancomycin resistance development occurs through mutation and thickening of cell due to accumulation of excess peptidoglycan layer which results in reduction in the pores through which vancomycin moves into the cell (Hiramatsu, 2001). Intermediate resistance to glycopeptides in *S. aureus* is defined as a *S. aureus* isolate with a minimum inhibitory concentration of some are yet to be identified, and studies have shown that not all are involved in cases of staphylococcal food poisoning and toxic shock syndrome (Moulding *et al.*, 1999; Williams *et al.*, 2000).

These enterotoxins have significant clinical implication as they are the major causes of food poisoning. In vancomycin of 8 to 16 µg/ mL. In this study however, disc diffusion method was employed to detect resistance and susceptibility, minimum inhibitory concentration was not determined. In Malaysia, Lim *et al*. (2013) reported that all of the 162 MRSA isolates obtained between 2003 and 2008 and tested for susceptibility against vancomycin were susceptible. Mupirocin resistance was also observed. The rise in resistance to mupirocin is threatening the clinical value of the drug, this is because topical mupirocin is known to play an important role in controlling outbreaks of MRSA and MRS especially in cases of peritoneal dialysis, post-surgical patients and in hemodialysis (Zhang *et al*., 2004). It is also considered as a strategic drug in Malaysia, even though two separate studies carried out on mupirocin resistance revealed that the prevalence was low. However, there is need to regulate the use of the drug (Rohani *et al.*, 2000; Norazah *et al.*, 2001). Resistance to rifampin an important drug for the treatment of tuberculosis and *Rhodococcus equi* infection was also observed. Rifampin is known to act by inhibiting the synthesis of bacterial RNA by attaching to the DNA- dependent RNA polymerase 1 subunit; hence preventing transcription. In *S. aureus* however, the mechanism of resistance to rifampin has not been fully established, but it was believed to be due to altered RNA polymerase 1 subunit with reduced susceptibility to rifampin. Rapid resistance was observed with rifampin when used as a sole agent *in vivo*. Alterations in the chromosomal and not plasmid DNA was observed in however, positive for the gene coding for staphylococcal exotoxin like toxin 1 and Lipase encoding gene. Even though the actual role of lipase encoding gene as a virulence determinant in the pathogenesis of *S. aureus* infection has not been fully established. A number of studies have reported protease and lipase activity in *S. aureus.* For instance, Prakash and Karmegam (2007) reported that protease and lipase play a very significant role in food spoilage which occurred as a result of improper handling of food. In this study, it was observed that 80% and 26.7% of the isolates were positive for gene coding for lipase and protease respectively. Furthermore, studies have shown that *S. aureus* strains with strong lipolytic and proteolytic activities have been isolated from chickens and humans with necrotic dermatitis and acne (Moura *et al.*, 2012). The carriage rate was however lower than the 62.06% and 95.55% as well as 45% and 80% of lipase reported by Gundogan and Torlak (2013); Pinto *et al.* (2015). This could be due to difference in sample size as well as method of detection. High frequency of gene coding for staphylococcal exotoxin- like toxins (*Set* -1) a term used to describe an aggregate group of *S. aureus* toxins such as the members of the superantigens family was observed (Williams *et al.*, 2000). There are about 22 enterotoxins that have so far been identified and addition, staphylococcal enterotoxin like toxin u was also observed in 2 (13.3%) of the isolates. Staphylococcal enterotoxin-like toxin u is a newly identified member of the superantigens family, however; their role in the pathogenesis of staphylococcal food poisoning has not been fully established (Letertre *et al*., 2003).

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In this study, the frequency of hemolysin was relatively low, only 4 (26.7%) and 1 (6.7%) of the isolates were positive for beta and alpha hemolysin, respectively. Similar result was also reported by Bitrus *et al.* (2016); the authors observed a low carriage rate of alpha hemolysin in MRSA isolates. Alpha and beta hemolysin play a very important role in the laboratory diagnosis of *S. aureus* on blood agar by forming a clear zone haemolysis. In addition, studies have shown that *S. aureus* strains are able to evade the antimicrobial activity of mast cell by up-regulating the expression of alpha hemolysin (Goldmann *et al*., 2015). It was demonstrated that recombinant alpha hemolysin induces the alteration of cell shape and formation of paracellular gaps in cell layers (Hermann *et al.*, 2015). However, being one of the most potent cytotoxic virulence factor associated with the pathogenesis of *S. aureus* infection, alpha hemolysin have been shown to serve as a very promising vaccine antigen and target of monoclonal antibody in animal models of *S. aureus* disease (Rouha *et al.*, 2015) . In addition, none of the isolates were positive for toxic shock syndrome toxin and exfoliative toxin B. This could be due to the fact that both toxins are mostly associated with infection in humans and rarely in animals.

CONCLUSION

The finding of this study revealed a relatively low carriage of virulence gene in all isolates. Reduced susceptibility and multi-drug resistance was also observed in four isolates (SEQ5, SEQ7 and SEQ10) while susceptibility to all isolates was observed in two isolate (SCH4 and SEQ6). Furthermore, there was an indirect relationship between carriage of virulence gene and phenotypic antibiotic resistance. The occurrence of multi-drug resistance and resistance to vancomycin calls for concern, since it is considered as the drug of last resort in the treatment of *S. aureus* infection. This finding affirmed the importance of virulence and resistance determinants in the pathogenesis of *S. aureus* infection.

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Author's contribution

The design and execution of this research study is a collective effort of all the authors. All authors were also involved in the critical analysis and review of the manuscript.

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