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Research Article

Occurrence, antibiotic resistance profile and molecular characterization of *Staphylococcus aureus* isolated from pork and chicken meat sold in Gia Lam District, Hanoi City

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Abstract

Staphylococcus aureus (*S. aureus*) is one of the most important zoonotic pathogens causing diseases for both humans and animals. Food, particularly meat, is considered the main vector for the transmission of this bacterium to humans. The findings of this study indicate that *S. aureus* contamination rates of pork and chicken meat samples were 24% and 16%, respectively. *S. aureus* isolates showed the highest resistance rates to penicillin (90%) and ampicillin (75%), and the lowest resistance to meropenem (5%) and linezolid (10%). Notably, 100% of the isolates were resistant to at least one antibiotic, and 65% were classified as multidrug-resistant strains. The results of molecular characterization revealed that all *S. aureus* isolates were positive for *spa* and 15% carried *mecA*. The detection rates of *sea*, *seb*, *sed*, and *see* genes were 20%, 5%, 15%, and 5%, respectively.

Keywords: *Staphylococcus aureus; toxin; MRSA; antibiotic resistance*.

1. INTRODUCTION

Food poisoning caused by *S. aureus* is one of the most common foodborne diseases in the world [1]. In Japan, consumption of milk powder contaminated with *S. aureus* enterotoxin resulted in more than 10,000 hospitalizations in 2000 [2]. In the United States, the annual cost of treating food poisoning caused by *S. aureus* is estimated at one billion dollars [3]. In Europe, *S. aureus* was responsible for 9.9% of all cases of food poisoning caused by microorganisms in 2015 [3]. In addition to causing food poisoning, *S. aureus* can

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produce panton-valentine leukocidin (PVL) toxin, which can destroy white blood cells, weakening the immune system, and subsequently exacerbating the disease [4-6]. *S. aureus* is commonly found on the skin, fur, and mucous membranes of healthy animals [7]. It is therefore a major challenge to prevent this bacterium from contaminating animal carcasses during the slaughter process and being transmitted to humans via the food chain.

2. MATERIALS AND METHODS

2.1. Materials

Meat samples (pork and chicken) were obtained from retail markets in Gia Lam district, Hanoi city. Media used in this study for the isolation, identification, antimicrobial susceptibility test, and PCR of *S. aureus* were purchased from commercial companies (Oxoid, UK and Thermo Fisher Scientific, US).

2.2. Methods

2.2.1. Sample collection

A total of 100 meat samples (50 pork and 50 chicken meat) were purchased from 10 retail markets in Gia Lam district, Hanoi, kept in ice boxes, and promptly transported to the laboratory of the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Vietnam National University of Agriculture for isolating *S. aureus.*

2.2.2. Isolation of S. aureus from pork and chicken meat

To isolate *S. aureus*, 25 g of meat sample was homogenized with 225 mL of Buffered Peptone Water (BPW, Oxoid, Thermo Fisher, Hants, UK) and then incubated overnight at 37°C. Subsequently, the homogenate was streaked onto Baird-Parker agar (BP, Oxoid, Thermo Fisher, Hants, UK) supplemented with egg yolk tellurite emulsion and 6.5% NaCl and incubated at 37°C for 24-48 h. Colonies with typical morphology of *S. aureus* (black center surrounded by opaque halo) were picked up to inoculate into the Brain Heart Infusion (BHI, Oxoid, Thermo Fisher, Hants, UK) broth for Gram-staining and coagulase test. Biochemically confirmed *S. aureus* isolates were then preserved at -86°C.

2.2.3. Antimicrobial susceptibility profile of S. aureus isolates

The antibiotic susceptibility test of *S. aureus* isolates was carried out by the agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [9]. The antibiotics used in this study included: penicillin, ampicillin, cefazoline, cefoxitin, cefotaxime, meropenem, gentamicin, tetracycline, linezolid, quinupristin-dalfopristin, chloramphenicol, erythromycin, clindamycin, ciprofloxacin, sulfamethoxazole/trimethoprim, rifampin. *S. aureus* ATCC 25923 was used as a quality control strain.

2.2.4. Detection of antibiotic resistance and virulence-associated genes of S. aureus isolates

Gene group 1 (*spa*, *mecA*, *mecC*, and *pvl*) and gene group 2 (*sea*, *seb*, *sec*, *sed*, and *see*) were detected by 2 multiplex PCR protocols as previously described [10, 11]. Primers used in this study are shown in Table 1.

Table 1. *Primers for the detection of antibiotic resistance and virulence-associated genes of S. aureus isolates*

Target gene	Primer name	Nucleotide sequence (5'-3')	Product (bp)	Reference
spa	1113F	TAAAGACGATCCTTCGGTGAGC	200-600	[10]
	1514R	CAGCAGTAGTGCCGTTTGCT		
mecA	mecA-F	TCCAGATTACAACTTCACCAGG	162	[10]
	mecA-R	CCACTTCATATCTTGTAACG		
mecC	mecC-F	GAAAAAAAGGCTTAGAACGCCTC	138	[10]
	mecC-R	GAAGATCTTTTCCGTTTTCAGC		
pvl	pvl-F	GCTGGACAAAACTTCTTGGAATAT	85	[10]
	pvl-R	GATAGGACACCAATAAATTCTGGATTG		
sea	SEA-F	GCAGGGAACAGCTTTAGGC	520	[11]
	SEA-R	GTTCTGTAGAAGTATGAAACACG		
seb	SEB-F	ACATGTAATTTTGATATTCGCACTG	667	[11]
	SEB-R	TGCAGGCATCATGTCATACCA		
sec	SEC-F	CTTGTATGTATGGAGGAATAACAA	248	[11]
	SEC-R	TGCAGGCATCATATCATACCA		
sed	SED-F	GTGGTGAAATAGATAGGACTGC	171	[11]
	SED-R	ATATGAAGGTGCTCTGTGG		
see	SEE-F	TACCAATTAACTTGTGGATAGAC	385	[11]
	SEE-R	CTCTTTGCACCTTACCGC		

2.2.5. Data analysis

The data in this study was analyzed using Microsoft Office Excel 2021.

3. RESULTS AND DISCUSSION

3.1. Occurrence of *S. aureus* in pork and chicken meat

The contamination of *S. aureus* in food is considered a potential risk to consumers and causes serious economic losses [12]. Among 100 meat samples tested in this study, 20/100 (20%) samples were positive for *S. aureus*, with 12/50 (24%) of pork samples and 8/50 (16%) of chicken samples (Table 2, Figure 1, Figure 2 and Figure 3).

Table 2. *Occurrence of S. aureus in pork and chicken meat*

Source	No. of samples	No. of positive samples	Detection rate (%)
Chicken	50	8	16
Pork	50	12	24
Total	100	20	20

Similar results were noted in a study conducted by Manh et al. in Ben Tre, showing that the contamination rate of *S. aureus* in chicken meat was 13.3% [13, 14]. The high

incidence (76.82%) of *S. aureus* in pork was reported in a study carried out by Lan and Binh at retail markets in some provinces in the North of Vietnam [15].

Figure 1. *Colony morphology of S. aureus on Baird-Parker agar*

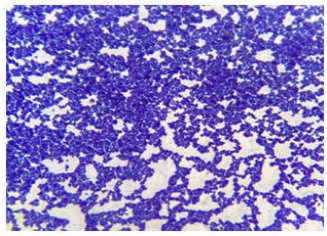

Figure 2. *Morphology of S. aureus under microscopy*

On the contrary, low and moderate occurrence rates of *S. aureus* in chicken and pork were reported in China (12.8% and 9.8%) [16] and South Korea (33.2% and 15.1%) [17]. In the United States, the incidence of *S. aureus* ranges from 11% to 41% in chicken and 12% to 42% in pork [18-21]. Another study in Denmark found that the contamination rates of *S. aureus* in chicken and pork were as high as 75% and 60%, respectively [22]. The differences in the prevalence of *S. aureus* in the previous studies and this study may be due to the differences in location, food processes, and isolation method.

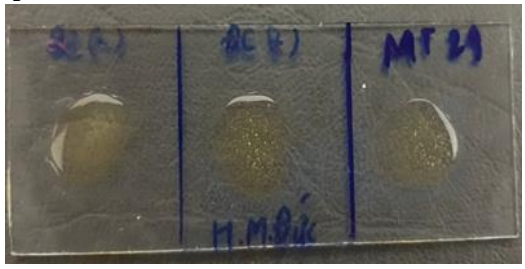

Figure 3. *The coagulase test of S. aureus isolate (Negative control (A), Positive control (B), Treatment (C))*

3.2. Antibiotic resistance profile of *S. aureus* isolates

The emergence of antibiotic-resistant bacteria in pork and chicken meat has been widely reported in many countries worldwide. The overuse and misuse of antibiotics in animal husbandry may contribute to the development of antibiotic resistance. In this study, *S. aureus* isolates showed the highest resistance rates to penicillin (90%), followed by ampicillin (75%), cefazolin (55%) and tetracycline (50%) (Figure 4). On the other hand, the lowest resistance rates were observed with meropenem (5%) and linezolid (10%). All *S. aureus* isolates were found to be sensitive to rifampin (Table 3). Our results are consistent

with a study conducted by Chujun Ou et al. in China reported that *S. aureus* strains were highly resistant to penicillin (82.4%), followed by erythromycin (57.6%), tetracycline (27.9%) [23]. Similar findings were recorded in another study in China, in which *S. aureus* isolates of meat origin exhibited high resistance rates to penicillin (86%) and ampicillin (87%) [24]. The high resistance rates of *S. aureus* to penicillin (100%) and ampicillin (100%) were also documented in the United States [5]. The resistance rate of tetracycline in this study was lower than reported in the US (54.1%) but higher than in China (42.8%) and South Korea (27.9%) [16, 17, 25].

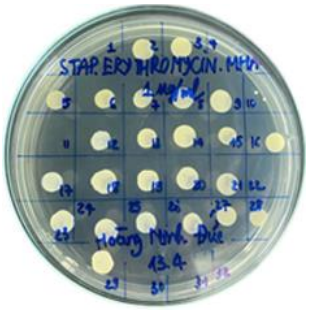

Figure 4. *Determination of antibiotic resistance of S. aureus to erythromycin (1 µg/mL) by agar dilution method*

Table 3. *Antibiotic resistance profile of S. aureus isolates*

Antibiotics	Chicken (n=8)		Pork (n=12)		Total (n=20)	
	No. of resistant isolates	Resistance rate (%)	No. of resistant isolates	Resistance rate (%)	No. of resistant isolates	Resistance rate (%)
Penicillin	8	100	10	83.33	18	90
Ampicillin	7	87.5	8	66.67	15	75
Cefazoline	6	75	5	41.67	11	55
Cefoxitin	1	12,5	2	16.67	3	15
Cefotaxime	2	25	2	16.67	4	20
Meropenem	0	0	1	8.33	1	5
Gentamicin	2	25	3	25	5	25
Tetracycline	3	37.5	7	58.33	10	50
Linezolid	1	12.5	1	8.33	2	10
Quinupristin-dalfopristin	1	12.5	2	16.67	3	15
Chloramphenicol	2	25	4	33.33	6	30
Erythromycin	3	37.5	5	41.67	8	40
Clindamycin	1	12.5	2	16.67	3	15
Ciprofloxacine	2	25	3	25	5	25
Sulfamethoxazole/ Trimethoprim	3	37.5	5	41.67	8	40
Rifampin	0	0	0	0	0	0

In this study, all *S. aureus* isolates were resistant to at least one antibiotic, with 50% being resistant to 1-4 antibiotics, 35% to 5-8 antibiotics, and 15% to 9-16 antibiotics (Table 4). Notably, 65% of *S. aureus* isolates were identified as multidrug-resistant strains.

The prevalence of multidrug-resistant *S. aureus* isolates in chicken and pork was 75% (6/8) and 58.33% (7/12), respectively. Similarly, in a study performed by Water et al. in the USA, the rates of multidrug-resistant *S. aureus* in pork and chicken were reported at 64% and 26%, respectively [21]. Another study in China showed that the prevalence of multidrug-resistant *S. aureus* in pork and chicken was 43.7% and 44.4%, respectively [23].

Table 4. Antibiotic resistance patterns of S. aureus isolates

No. of antibiotics	Antibiotic resistance patterns	No. of resistant isolates
1	TET	1
	GEN	1
2	PEN-CFZ	1
	PEN-TET	1
	PEN-AMP	1
3	PEN-AMP-CIP	1
	PEN-AMP-TET	1
4	PEN-AMP-CFZ-SXT	2
	PEN-AMP-CFZ-TET	1
5	PEN-AMP-ERY-CLI-CIP	1
	PEN-AMP-CFZ-TET-CHL	1
	PEN-AMP-CFZ-ERY-SXT	1
	PEN-ERY-CLI-CIP-SXT	1
6	PEN-AMP-CFZ-CTX-GEN-TET	1
7	PEN-AMP-TET-CHL-ERY-CIP-SXT	1
8	PEN-AMP-CFZ-GEN-TET-SYN-CHL-ERY	1
9	PEN-AMP-CFZ-FOX-CTX-LNZ-CHL-ERY-SXT	1
12	PEN-AMP-CFZ-FOX-CTX-GEN-TET-LNZ-SYN-CHL-ERY-SXT	1
14	PEN-AMP-CFZ-FOX-CTX-MEM-GEN-TET-SYN-CHL-ERY-CLI-CIP-SXT	1

PEN (penicillin), AMP (ampicillin), CIP (ciprofloxacin), CFZ (cefazoline), TET (tetracycline), SXT (sulfamethoxazole/trimethoprim), ERY (erythromycin), CLI (clindamycin), GEN (gentamicin), FOX (cefoxitin), CTX (cefotaxime), LNZ (linezolid), MEM (meropenem), SYN (quinupristin-dalfopristin), CHL (chloramphenicol)

3.3. Detection of antibiotic resistance and virulence-associated genes of *S. aureus* isolates

Identification and typing *of S. aureus* through the detection of *spa* genes by PCR have advantages over Multi-Locus Sequence Typing (MLTS) due to the ability to accurately

determine the diversity of *S. aureus* in a short time and at a low cost [26]. In this study, the detection rate of the *spa* gene of *S. aureus* isolates was 100%.

The mecA and *mecC* are commonly used indicator genes for the identification of MRSA, which encode for PBP-2A that confer resistance to methicillin [27]. The results of multiplex PCR (Figure 5) showed that 3 (15%) out of 20 *S. aureus* isolates carried *mecA* gene, of which, 2 (16.67%) were isolated from pork and 1 (12.5%) from chicken meat. The *mecC* gene was not detected in all *S. aureus* isolates. These results are lower than those in a study conducted in the US, in which the detection rates of *mecA* gene in *S. aureus* isolates from pork and chicken meat were 18% and 20.4%, respectively [28]. In contrast, the incidence of *mecA* gene observed in the present study was higher than reported in Denmark (4% and 15%) and South Korea (0.9% and 1%) [17, 29].

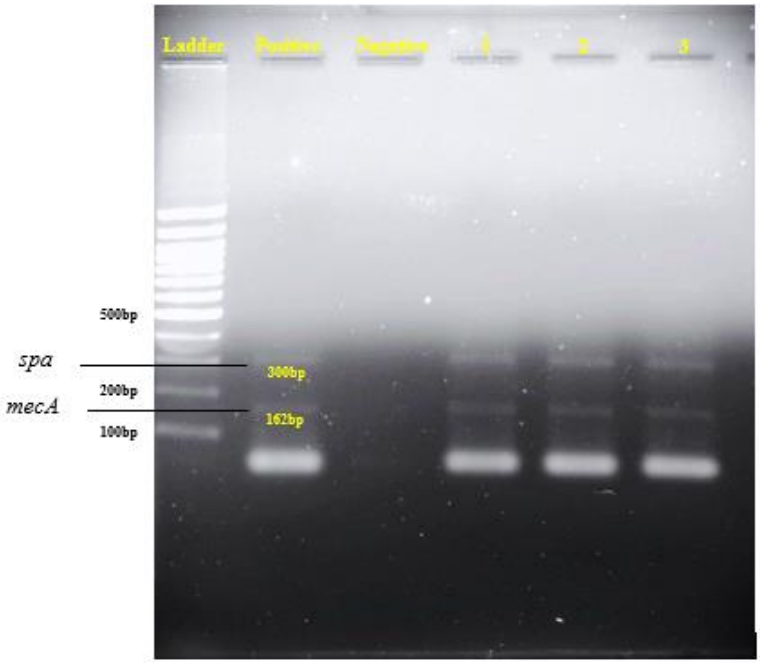

Figure 5. *Multiplex PCR detecting spa, mecA, mecC, and pvl of S. aureus isolates (Lane 1: Ladder; Lane 2: Positive control; Lane 3: Negative control; Lanes 4-6: S. aureus isolates)*

S. aureus can produce various toxins and extracellular enzymes. Among them, the heat-stable enterotoxin (SE) of *S. aureus* is known to be one of the most dangerous toxins and a major cause of food poisoning [30]. Currently, the emergence of new types of SEs has been reported but their role is still unclear [16]. Therefore, in this study, we focused only on detecting the most important enterotoxin genes including *sea, seb, sec, sed,* and *see*. The PCR results revealed that *sea*, *seb*, *sed,* and *see* genes were detected at 20%, 5%, 15%, and 5%, respectively (Table 5). These findings align with a study in China, reporting that *sea* and *seb* were the dominant enterotoxin genes in *S. aureus* strains of chicken meat origin with prevalence ranging from 4% to 14.5% and 3.2% to 14.5%, respectively, while the prevalence of these genes in *S. aureus* strains of pork origin was 23.80% to 33.3% and 12.82% to 60% [31-33]. Similarly, in Japan, *seb* was also the most prevalent enterotoxin gene in *S. aureus* strains isolated from chicken and pork with rates ranging from 1.42% to 64.1% and 4% to

13.8%, respectively [34-36]. Panton-valentine leukocidin is another notable toxin of *S. aureus*, which consists of two components, LukS-PV and LukF-PV. These two components are secreted before they gather and form a hole in the neutrophil membrane, resulting in neutrophil lysis [37]. All *S. aureus* strains isolated from meat in this study were negative for *pvl gene.*

***Table 5**. Detection rate of enterotoxin of S. aureus isolates*

Gene	Chicken (n=8)		Pork (n=12)		Total (n=20)	
	No. of positive isolates	Positive rate (%)	No. of positive isolates	Positive rate (%)	No. of positive isolates	Positive rate (%)
sea	2	25	2	16.67	4	20
seb	1	12.5	0	0	1	5
sec	0	0	0	0	0	0
sed	1	12.5	2	16.67	3	15
see	0	0	1	8.33	1	5

4. CONCLUSION

The results of our study show that the contamination rate of *S. aureus* in pork and chicken meat sold at traditional markets in Gia Lam district, Hanoi city was 24% and 16%, respectively. *S. aureus* isolates exhibited the highest resistance rates to penicillin and ampicillin as well as the lowest resistance rates to meropenem and linezolid. A high rate of *S. aureus* isolates was determined as multidrug-resistant strains. The *spa* and *mecA* gene was detected in 100% and 15% of *S. aureus* isolates. On the contrary, *mecC* and *pvl* were not detected in this study. The *sea* and *seb* were the predominant enterotoxins found in this study.

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