Contamination and antimicrobial resistance testing of *Staphylococcus aureus* isolates from pork in the traditional market at Central Vietnam

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ABSTRACT

Background: *Staphylococcus aureus* is an opportunistic pathogen widely present in humans and food-producing animals. The emergence of antimicrobial resistance (AMR) in *S. aureus* represents a major challenge for both animal and public health.

Aim: This study investigated the presence of *S. aureus* in pork sold in traditional markets in Central Vietnam, analyzing its AMR profile and evaluating potential public health risks.

Methods: We collected 48 retail pork samples from traditional markets in Central Vietnam. The *S. aureus* count (log10 CFU/g) was determined using Baird–Parker (BP) medium and was confirmed by the coagulase reaction. The *S. aureus* isolates were tested for AMR and AMR genes (ARGs) using the disk diffusion method and PCR, respectively.

Results: All 48 retail pork samples (100%) tested positive for *S. aureus*, with *S. aureus* counts ranging from 4.17 to 4.52 log10 CFU/g. AMR was most commonly observed against penicillin (75.0%), followed by amoxicillin (64.6%), tetracycline (54.2%), ampicillin (41.7%), neomycin (39.6%), and vancomycin (39.6%). Multidrug resistance was observed in 85.4% of the *S. aureus* isolates, with penicillin–ampicillin–amoxicillin being the most common resistance pattern. The highest frequency of *S. aureus* isolates carried the tetM (30/48, 62.5%) gene, followed by blaZ (25/48, 52.1%), and femA (20/48, 41.7%). Moreover, the results revealed a positive correlation among β-lactams, specifically between ampicillin and neomycin, and between amoxicillin and erythromycin/neomycin.

Conclusion: The high AMR of *S. aureus* isolates from retail pork samples is alarming and raises public health concerns. Food of animal origin serves as a source of AMR *S. aureus*, which can spread throughout the food supply chain. Therefore, controlling *S. aureus* requires targeting specific entry points based on findings related to risk factors and food contamination drivers.

Keywords: *S. aureus,* Retail, Antimicrobial resistance, Vietnam, Market.

Introduction

Global average annual meat consumption is approximately 15 kg per person for pork, 12.6 kg for poultry, and 9.6 kg for beef. In Europe, these figures are considerably higher, with per capita consumption reaching 34.2 kg for pork, 21.9 kg for poultry, and 16.1 kg for beef (World Health Organization, 2022). Pork is the most popular type of meat in Vietnam, accounting for around 73% of all meat consumption (Nguyen *et al*., 2006). A steady growth of per capita pork consumption was observed in the country, rising from 8.1 kg/capita/year in 1990 to 31.3 kg/capita/year in 2018 (Organisation for Economic Co-operation and Development, 2018). In 2023, pork production amounted to 3.8 million tons, an increase of 2.2% compared with 2017 (Vietnam Livestock Production, 2022). Nevertheless, food safety remains a major concern in Vietnam, which carries a high burden of foodborne illnesses, particularly those linked to pork products (Nguyen *et al*., 2020). Approximately 80% of pork in Vietnam is produced by smallholders, with processing and distribution occurring primarily through informal market channels, such as open-air and traditional markets (Lapar and Tiongco, 2011). Retail pork is frequently contaminated with biological hazards such as *E. coli*, *Salmonella*, *Staphylococcus*

aureus, *Streptococcus suis*, and chemical hazards (Cook and Phuc, 2019). This widespread contamination contributes to the high burden of foodborne illnesses. *Staphylococcus aureus* is a major foodborne pathogen in fresh and processed products and is responsible for a wide range of infections worldwide (Diep *et al*., 2006). *Staphylococcus aureus* is widely distributed, but food is its most important source of infection. After *Salmonella* and *Campylobacter*, the two most common bacteria associated with food poisoning, *S. aureus* is the third most common foodborne pathogen, identified in approximately 241,000 cases globally per year (Wu *et al*., 2018a). In 2013, *S. aureus* caused 12.5% of all foodborne bacterial outbreaks in China (Wei-Wei *et al*., 2018). In Vietnam, 677 foodborne outbreaks involving 21,002 patients were recorded during the four years from 2011 to 2014 (Hung *et al*., 2020). The most frequent source of infection among Staphylococcal species is *S. aureus*, a facultatively aerobic Gram-positive bacteria. It is a component of the skin's and the nasal mucosa's microbiota. Nearly 20% of the general population is consistently positive for *S. aureus*, and this percentage can reach 80% in healthcare workers, frequent needle users, hospital patients, and people with compromised immune systems (Boucher and Corey, 2008; Foster and Geoghegan, 2023). Human and animal infections (zoonotic) caused by the bacteria *S. aureus* typically manifest as impetigo, pus-filled abscesses, swelling, discomfort, and pus-filled redness on the skin (Wertheim *et al*., 2005, 2009). Serious symptoms, such as fever, chills, and low blood pressure, might arise if the bacteria enter the bloodstream and cause sepsis. Myocarditis, pneumonia, osteomyelitis, and mastitis can all be caused by bacteria (Squier *et al*., 2002). Bacteria can cause serious infections when they reach the blood, leading to shock, multiple organ failure, or even death (Igbinosa *et al*., 2016). Food poisoning is a condition that can cause vomiting and fever in individuals who consume contaminated food containing *S. aureus* (Thompson and Brown, 2014).

Over the past few decades, the widespread use of antimicrobials (AMs) to treat infectious diseases has contributed to the emergence of multidrug-resistant (MDR) strains, posing major challenges to public health (Ahmed *et al*., 2024). As a highly adaptable pathogen, *S. aureus* can survive in various environmental conditions and rapidly develop resistance to most AMs (Velasco *et al*., 2022). Currently, an increasing number of MDR *S. aureus* strains have been reported in food poisoning outbreaks and isolated from food products (Papadopoulos *et al*., 2018; Dorjgochoo *et al*., 2023). Furthermore, methicillin-resistant *S. aureus* (MRSA) has gained widespread attention. MRSA has been increasing in prevalence (Savariraj *et al*., 2019; Velasco *et al*., 2022), with recent reports indicating higher resistance rates in foodborne isolates. MRSA often exhibits MDR and was listed by the WHO in 2017 as one of the 12 bacterial families posing the greatest threat to human health (Asokan and Vanitha, 2018). In Vietnam, outbreaks of community-acquired MRSA infections have been documented by Tang *et al*. (2007). MRSA bloodstream infections accounted for 19% of reported cases in Vietnam, according to Song *et al*. (2011). The most frequently identified bacterium from the nasal mucosa was *S. aureus* (110/838; 13.1%), followed by 8.6% (72/838) of MRSA isolates (Thuy *et al*., 2017). A notable height rate of MRSA bacterial isolation was reported by Nguyen *et al*. (2014), with 303 of 1,016 samples (29.8%). These findings and others demonstrate a strong association between MRSA infection and increased morbidity and mortality (Shoaib *et al*., 2023).

However, little information on *S. aureus* contamination in pork meat from Central Vietnam has been available to date. Given the need to better understand the dissemination of *S. aureus* in traditional markets, this study employed microbiological and genetic methodologies to characterize isolates and their resistance profiles. In light of these findings, the present study examined the contamination and AM resistance (AMR) prevalence of *S. aureus* isolates from retail pork meat sold at traditional Vietnamese markets in Hue City, Central Vietnam. Additionally, we compared these profiles with previously reported data from various regions of Vietnam to determine whether foodborne *S. aureus* isolates exhibit geographical variation.

Materials and Methods

Sample size and study area
A total of 48 retail pork samples were collected from 16 distinct meat sellers at four traditional markets in Hue City, Central Vietnam, between June and August 2023. Samples were selected based on [only sell pork (not sold with other meat), the amount of daily sales, about table form, cutting board, and knife], while samples with [the number of customers, location] were discarded. Samples were collected once a month, with 16 samples per collection. Samples were collected from the following traditional markets in Hue City: An Cuu ($n = 12$), Ben Ngu ($n = 12$), Dong Ba ($n = 12$), and Tay Loc ($n = 12$). The samples were preserved in sterile zip-lock plastic bags, placed in cold boxes at temperatures below 4°C, and transported to an accredited laboratory, where microbiological analysis was conducted within 4 hours.

S. aureus counts and identification
Staphylococcus aureus counts and identification were performed according to the Viet Nam Standards and Quality Institute (2015). Briefly, a total of 25 g of each sample was cut into small pieces with sterile scissors, placed in a stomacher bag, mixed with 225 ml of sterile 0.1% Buffered Peptone water (BPW, Oxoid, Thermo Fisher, Hants, UK), and homogenized using a Stomacher® 400 Circulator (Seward, UK). For *S. aureus* counts, 1 ml of each homogenate was used to

prepare 10-fold serial dilutions up to 10^4 in 0.1% (w/v) BPW. Aliquots of 0.1 ml of each dilution were spread in triplicate onto Baird–Parker (BP) agar (Becton, Dickinson, USA, 276840) containing 5% egg yolk tellurite emulsion and 6.5% NaCl. After incubation at 37°C for 24–48 hours, selected plates with 20–200 colonies displaying the typical *S. aureus* morphology (black center surrounded by an opaque halo) were counted. Ten to twenty colonies were randomly selected and identified them as *S. aureus* based on colony morphology, Gram stain appearance, and the coagulase reaction (Lin *et al.*, 2009). Moreover, we confirmed each strain as *S. aureus* by polymerase chain reaction (PCR) testing for the presence of *S. aureus*–specific *nuc* gene (Brakstad *et al.*, 1992). *Staphylococcus aureus* NCTC 6571 (Oxford *Staphylococcus aureus* NCTC 6571, Heatley Oxford, Heatley, UK) strain was used as the quality-control strain. The identified *S. aureus* isolates were stored at −80°C in brain heart infusion (BHI) broth (Difco, USA, 1104930500) and were supplemented with 15% glycerol until further analyses.

AM susceptibility testing

The disk diffusion method was used to assess the AM susceptibility of *S. aureus* isolates using the Mueller–Hinton agar (MH, Oxoid, UK) plates according to the guidelines and recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2020). Penicillin (PEN, 10 UI), amoxicillin (AMO, 10 μg), ampicillin (AMP, 10 μg), streptomycin (STR, 10 μg), neomycin (NEO, 30 μg), amikacin (AMK, 30 μg), gentamicin (GEN, 10 μg), enrofloxacin (ENR, 5 μg), vancomycin (VAN, 30 μg), erythromycin (ERY, 15 μg), doxycycline (DOX, 30 μg), and tetracycline (TET, 30 μg) (Oxoid™, United Kingdom) were used for AM susceptibility testing. The inoculum was prepared from cultures in BHI broth incubated for 4–6 hours before adjusting the turbidity to 0.5 according to the McFarland scale (DensiCHEK™ Plus, ALT, San Diego, US). One hundred microliters of the bacterial suspension was spread on MH agar. The appropriate AM-impregnated disks were placed on the agar surface, and the plates were incubated at 37°C for 24 hours. The zones of inhibition were measured, and the AM susceptibility of the isolates was determined using interpretative standards for *Staphylococcus* species (Eurl, 2018; CLSI, 2020).

AMR gene amplification

For the AM resistance genes (ARGs) of *S. aureus* isolates, the β-lactams (*blaZ*, *rpoB*, and *femA*), macrolides (*ermB*), and tetracycline (*tetM*) were detected using a single PCR method (Akanbi *et al.*, 2017). The predicted sizes of the amplified products of the specific oligonucleotide primers used in this study are presented in Table 1. The PCR assays were performed in a 25 μl reaction volume, which included 2 μl of gDNA template (at >10 ng·μl⁻¹), 1 μl of each primer (25 μmol·l⁻¹), 12.5 μl of 2× Taq PCR master mix (CW Biotech, China), and 8.5 μl of ultrapure

H₂O. PCR products were then electrophoresed in 1.0%–1.5% agarose gels, stained with Gelred (Thermo Scientific, UK), and visualized under an ultraviolet transilluminator (Bio-Rad, USA).

Statistical analysis

All data were managed in Microsoft Excel 2016 (MSO, 16.0.4266.1001) and analyzed using SPSS (IBM SPSS Statistics version 18.0, IBM, Armonk, NY, USA). The bacterial count was expressed in log10 CFU/g (colony-forming units per gram, expressed on a logarithmic scale). The *S. aureus* count was not normally distributed and was $\log_{10}$ transformed before analysis. The isolates resistant to at least two different types of AM agents were classified as MDR isolates. Two indices were used to assess the results obtained: the AMR index (ARI) and the MDR index (MARI), as described by Manjusha *et al.* (2005) and Krumperman (1983). An isolate carrying two or more ARGs was defined as a multiple-ARG isolate. Pairwise analysis of the association between AMR phenotypes was performed using Pearson's correlation analysis. A positive association (>0) indicates a similarity in susceptibility or resistance between two AMs, whereas a negative association (<0) indicates an association between the susceptibility of an individual AM and the resistance to another AM. Differences were considered statistically significant when $p < 0.05$.

Ethical approval

Not needed for this study.

Results

Detection of S. aureus contamination of samples

Overall, 100% of the retail pork samples were positive for *Staphylococcus* with the typical colonies growing on BP-specific agar, and there were confirmed for *S. aureus* by colony morphology, Gram staining, and coagulase. The colonies were determined positive by black color, shiny, rounded, and 1–1.5 mm in diameter, with a wide halo around the colony 2–5 mm (Table 2). As shown in Table 2, the contamination level of all samples with *S. aureus* was 4.40 log10 CFU/g. The *S. aureus* count in pork samples from Dong Ba market (4.52 log10 CFU/g) was highest, followed by the samples from An Cuu and Tay Loc (4.45 log10 CFU/g), while the lowest *S. aureus* count was observed in the samples from Ben Ngu with 4.17 log10 CFU/g. However, the *S. aureus* counts were not significant difference ($p > 0.05$) between the samples from difference markets. According to TCVN 7046:2015 (Viet Nam Standards and Quality Institute, 2015), the permissible limit of *S. aureus* in unprocessed meat products is 10^2 CFU/g sample. The results indicated that the number of *S. aureus* in all samples from traditional markets in Hue city was higher than the minimum allowed limit by TCVN7046:2015 (Viet Nam Standards and Quality Institute, 2015).

Table 1. Primers used for ARG amplification.

Gene	Primer	Oligonucleotide sequence (5'----> 3')	Amplicon size (bp)	Annealing temperature (ºC)	Ref.
blaZ	blaZ-F	ACTTCAACACCTGCTGCTTTC	173	49.0	
	blaZ-R	GACCACTTTTATCAGCAACC			
rpoB	rpoB-F	ACCGTCGTTTACGTTCTGTA	460	45.5	
	rpoB-R	TCAGTGATAGCATGTGTATC			
femA	femA-F	AAAAAAGCACATAACAAGCG	132	45.5	Akanbi *et al.* (2017)
	femA-R	GATAAAGAAGAAACCAGCAG			
ermB	ermB-F	CTATCTGATTGTTGAAGAAGGATT	142	47.0	
	ermB-R	GTTTACTCTTGGTTTAGGATGAAA			
tetM	tetM-F	AGTGGAGCGATTACAGAA	158	45.0	
	tetM-R	CATATGTCCTGGCGTGTCTA			

Table 2. The contamination of *S. aureus* in pork meat samples.

Markets	No. of samples	Positive	%	Log CFU/g
An Cuu	12	12	100	4.45 ± 0.64
Ben Ngu	12	12	100	4.17 ± 0.48
Dong Ba	12	12	100	4.52 ± 0.47
Tay Loc	12	12	100	4.45 ± 0.58
Total	48	48	100	4.40 ± 0.54

AMR profiles of S. aureus isolates

The frequencies of resistance to 12 AM agents in six different groups were assessed. The results are summarized in Tables 3, 4, and 5. As shown in Table 3, the *S. aureus* isolates exhibited the highest rate of resistance to PEN (36/48, 75.00%), followed by AMO (31/48, 64.58%), and TET (26/48, 54.16%). The majority of the isolates were sensitive to AMK (45/48, 93,75%), followed by GEN and ENR (39/48, 81,25%), DOX (32/48, 66.67%), NEO (29/48, 60,42%), and AMP (24/48, 50.00%). Overall, 95.8% (46/48) of the *S. aureus* isolates were resistant to at least one AM (Table 5). The MARI values of the *S. aureus* isolates ranged from 0.17 to 0.75 (Table 5). There were 41 (85.4%) isolates resistant to two or more AMs (considered as multi-AMR), and most of the *S. aureus* isolates (27, 56.3%) resistant to three to five AMs (MARIs were 0.25, 0.33, and 0.42, respectively). The predominant MARI (0.17) was found in four isolates that were resistant to two out of the 12 AMs. Surprisingly, two *S. aureus* isolates were found to have the highest MARI of 0.75. They were resistant to 9 of the 12 AMs. A total of 32 MDR patterns were observed for the *S. aureus* strains for the 12 AMs tested (Table 4). Resistant to PEN-AMP-AMO was most prevalence observed in 4 (9.7%) MDR isolates. The other common patterns were PEN-TET-VAN-resistant and PEN-AMP-AMO-NEO observed in 3 (7.3%) MDR isolates.

Prevalence of ARGs in the S. aureus isolates

The ARG profiles of 48 *S. aureus* isolates from retail pork samples from Hue City are presented in Table 6. The most common in ARGs in the *S. aureus* isolates were *tetM* (30/48, 62.5%), *blaZ* (25/48, 52.1%), and *femA* (20/48, 41.7%). The incidences of *rpoB* (15/48, 31.3%) and *ermB* (12/48, 25.0%) were lower than those of other detected ARGs. The *S. aureus* isolates had multi-ARGs (35/48, 72.9%). Specifically, one isolate carried all five ARGs tested (Table 6).

Correlations between the two AMR statuses of S. aureus

The pairwise correlation analysis of the AMR status of *S. aureus* isolates is presented in Table 7. Table 7 shows that several strong correlations among resistance to different AMs in *S. aureus* isolates were observed. A significant positive correlation was observed among the cell wall synthesis inhibitors, specifically between resistance to PEN and AMP, PEN and AMO, and AMP and AMO, with Pearson's correlation coefficients of 0.39, 0.68, and 0.54, respectively ($p < 0.001$). Moreover, resistance to cell wall synthesis inhibitors was positively correlated with resistance to protein synthesis inhibitors, such as the resistance to AMP and NEO (0.53, $p < 0.001$), AMO and ERY (0.28, $p < 0.05$), and AMO and NEO (0.33, $p < 0.05$). For AMs that inhibit protein synthesis, resistance to STR, GEN, and AMK was positively correlated with resistance to DOX (0.40, $p < 0.01$), TET (0.35, $p < 0.01$), and STR (0.29, $p < 0.05$), respectively.

Table 3. Antimicrobial resistance prevalence among the *S. aureus* isolates.

Groups	Antibiotics	Resistance		Intermediate		Susceptible	
		No.	(%)	No.	(%)	No.	(%)
β-lactam	Penicillin	36	75.00	0	0.00	12	25.00
	Amoxicillin	31	64.58	0	0.00	17	35.42
	Ampicillin	20	41.67	4	8.33	24	50.00
Aminoglycoside	Streptomycin	10	20.83	10	20.83	28	58.33
	Neomycin	19	39.58	0	0.00	29	60.42
	Amikacin	3	6.25	0	0.00	45	93.75
	Gentamicin	6	12.50	3	6.25	39	81.25
Quinolone	Enrofloxacin	2	4.17	7	14.58	39	81.25
Glycopeptide	Vancomycin	19	39.58	20	41.67	9	18.75
Macrolide	Erythromycin	14	29.17	14	29.17	20	41.66
Tetracycline	Doxycycline	4	8.33	12	25.00	32	66.67
	Tetracycline	26	54.16	11	22.92	11	22.92

Discussion

The main objective of this study was to assess the contamination of retail pork sold in traditional markets with *S. aureus*. This is the first study on the prevalence of AMR *S. aureus* from traditional markets in Central Vietnam. This study found a high frequency of *S. aureus* in retail pork samples from all markets (100%). The frequencies of *S. aureus* contamination in retail pork samples ranged from low (5.0%) (Yakubu *et al*., 2020) to high (72.5%) (Owuna *et al*., 2015). A study by Ton *et al*. (2021) in Hue City reported the absence of *S. aureus* in processed meat. However, Fahrion *et al*. (2013) reported that 41% of pork samples in the pork supply chain in Hanoi were positive for *S. aureus*. In other countries such as China, India, and Thailan, the frequencies of *S. aureus* in retail pork samples were 35.0% (Wu *et al*., 2018b), 76,67% (Savariraj *et al*., 2019) and 28.18% (Kanungpean *et al*., 2021), respectively. Numerous factors contribute to the microbial contamination of meat, including slaughtering and transportation processes, as well as the sanitary conditions of the meat counter (Akagha *et al*., 2015; Bodunde *et al*., 2019). Throughout this process, meat is inadequately stored, facilitating the development of microorganisms over time. Moreover, cross-contamination may occur during its sale at the market. The majority of meat found at markets originates from slaughterhouses that employ manual slaughtering processes, which often fail to guarantee safe and hygienic conditions. Consequently, during the slaughtering process, meat can become contaminated with microorganisms present in water, on floors, utensils, slaughter tools, and through contact with workers (Klaharn *et al*., 2022). Furthermore, the sample collection time for this research at the market is from 7 to 9 am, a period of high customer traffic.

During this time, meat undergoes frequent handling by both sellers and buyers, increasing the likelihood of cross-contamination. Additionally, the cutting boards and knives used during the trading process are not adequately maintained, further elevating the risk of microbial contamination in meat (Sekoai *et al*., 2020). The previous study conducted by Ly and Nguyen (2016) revealed that pork samples sold at the market tend to accumulate higher levels of *S. aureus* over time postslaughter. Within 6 hours after slaughter, 70.63% of the samples obtained from local markets contained more than 10^2 CFU/g of *S. aureus*, whereas only 16.67% of samples from supermarkets exhibited similar levels. These findings underscore the significant impact of storage temperature and exposure conditions on product quality. Overall, a comprehensive assessment of food safety levels for fresh meat involves a multidimensional approach that addresses microbial, chemical, and physical hazards throughout the production and distribution process. Regular monitoring, compliance with regulations, and continuous improvement efforts are essential to ensure the quality and safety of fresh pork and processed meat for consumers. There was no significant difference in *S. aureus* counts between the markets ($p > 0.05$), which may reflect the uniformity of trading conditions across sites. These conditions include pork sourced from slaughterhouses with similar hygiene standards and transportation methods, comparable customer volumes, and consistent sampling times. According to Lapar and Tiongco (2011), approximately 80% of pork in the region is produced by smallholders, with processing and retail activities primarily conducted within the informal market value chain, such as open-air and traditional markets.

AM-resistant *S. aureus* isolates from food are common and are a major cause of economic losses in the food

Table 4. Antimicrobial resistance patterns of the *S. aureus* isolate.

No. of antimicrobials	Antimicrobial agents	No. of strains
No resistances		2
1	PEN	1
	TET	3
	NEO	1
2	TET-STR	1
	PEN-AMO	1
	TET-NEO	1
	TET-VAN	1
3	PEN-AMO-VAN	2
	PEN-AMP-AMO	4
	PEN-TET-VAN	3
	PEN-TET-GEN	1
	PEN-AMO-AMK	1
	ERY-NEO-VAN	1
4	PEN-AMP-AMO-NEO	3
	PEN-AMP-AMO-VAN	1
	PEN-AMO-ERY-NEO	1
	PEN-AMO-TET-ERY	1
	PEN-ENR-DOX-STR	1
	AMP-STR-ERY-VAN	1
	AMO-DOX-TET-STR	1
5	PEN-AMO-TET-STR-ERY	1
	PEN-AMO-TET-ERY-VAN	1
	PEN-AMO-ENR-TET-ERY	1
	PEN-AMP-AMO-TET-NEO	1
	PEN-AMP-AMO-ERY-NEO	1
	PEN-AMO-TET-ERY-GEN	1
6	PEN-AMP-AMO-TET-NEO-VAN	1
	PEN-AMO-TET-GEN-NEO-VAN	1
7	PEN-AMP-AMO-DOX-STR-AMK-NEO	1
	PEN-AMP-AMO-TET-ERY-NEO-VAN	1
	PEN-AMP-AMO-TET-GEN-NEO-VAN	1
8	PEN-AMP-AMO-TET-STR-ERY-NEO-VAN	2
	PEN-AMP-AMO-DOX-TET-ERY-NEO-VAN	1
9	PEN-AMP-AMO-TET-STR-GEN-AMK-NEO-VAN	1
	PEN-AMP-AMO-TET-STR-ERY-GEN-NEO-VAN	1
Total		48

Penicillin (PEN, 10 UI), amoxicillin (AMO, 10 µg), ampicillin (AMP, 10 µg), streptomycin (STR, 10 µg), neomycin (NEO, 30 µg), amikacin (AMK, 30 µg), gentamycin (GEN, 10 µg), enrofloxacin (ENR, 5 µg), vancomycin (VAN, 30 µg), erythromycin (ERY, 15 µg), doxycycline (DOX, 30 µg), tetracycline (TET, 30 µg).

supply chain (Richter *et al*., 2012). In the present study, 95.8% *of S. aureus* isolates from retail pork samples were resistant to at least one type of AM. A significantly higher resistance to PEN, AMO, and TET was observed. These results are consistent with previous studies in Vietnam (Hoang *et al*., 2024) and

Table 5. Number of AMRs and ARGs in the *S. aureus* isolates.

No. of AM or ARG	MARI	No. of strains positive for AMR	ARI	No. strain positive for ARG	Rate (%)
0		2	0.04	6	100.0
1	0.08	5	0.10	7	87.5
2	0.17	4	0.08	17	72.9
3	0.25	12	0.25	12	37.5
4	0.33	9	0.19	5	12.5
5	0.42	6	0.13	1	2.1
6	0.50	2	0.04		
7	0.58	3	0.06		
8	0.67	3	0.06		
9	0.75	2	0.04		

ARI = antimicrobial resistance index; MARI = multiple antimicrobial resistance index.

Table 6. Antimicrobial resistance genes prevalence among the *S. aureus* isolates ($n = 48$).

Resistance genes[*]	No. strain	Rate (%)
BlaZ	25	52.1
rpoB	15	31.3
ermb	12	25.0
femA	20	41.7
tetM	30	62.5

[*]*BlaZ* = Encodes the beta-lactamase, *ermB* = Encodes the ribosomal methylase*, femA* = methicillin resistance factor; *rpoB* = encodes the β subunit of bacterial RNA polymerase, *tetM* = Tetracycline resistance genes.

other nations (Ge *et al*., 2017; Kraushaar *et al*., 2017; Wang *et al*., 2017; Wu *et al*., 2018b). A reason for the high level of AMR in *S. aureus* is the widespread use of AMs in animal production (Chu *et al*., 2013; Jibril *et al*., 2021). Vancomycin is a key drug for treating MRSA and other Gram-positive infections (Dasgupta, 2012). In 1997, Hiramatsu *et al*. (1997) first reported vancomycin-resistant *S. aureus* (VRSA). Recently, other studies reported VRSA isolates from pork meat (Kanungpean *et al*., 2021; Sineke *et al*., 2021; Velasco *et al*., 2022). The high frequency of VRSA (39.58%) in food may reduce the effectiveness of VAN for treating important human infectious diseases. In June 2002, the first VRSA strains were identified, carrying the *vanA* operon within the Tn1546 transposon (Centers for Disease Control Prevention, 1997). Tn1546, which confers VAN resistance, was first described in the *Enterococcus* genus in 1988 (Uttley *et al*., 1988). Subsequent research has shown that Tn1546 is expressed in most staphylococcal strains (Wang *et al*., 2022; Vo *et al*., 2024). Glycopeptide resistance encoded by the *vanA* operon is more frequently expressed in *S. aureus* strains harboring mutations in their restriction-modification systems and/or carrying

pSK41-like conjugative plasmids, which increase the likelihood of *vanA* operon transfer (LeBard *et al*., 2008; Rossi *et al*., 2014). The detection of *vanA* in both VRSA and vancomycin-resistant *Enterococcus* (VRE) suggests horizontal gene transfer from *Enterococcus* to *Staphylococcus*. The clinical significance of VRE lies in its ability to transmit vancomycin resistance genes to MRSA.

Moreover, 41 out of 48 (85.4%) *S. aureus* isolates were MDR, and 37 (77.08%) had MARI > 0.25. The results of this study are similar to those of a study in China, in which 94.6% of *S. aureus* isolates were MDR (Wu *et al*., 2018b). Additionally, according to Waters *et al*. (2011), up to 52% of *S. aureus* isolates from pork samples in the US were MDR. In this study, the MARI of *S. aureus* isolates ranged from 0.17 to 0.75. Compared with a previous study in Malaysia, the MARI of *S. aureus* isolates from cutting boards ranged from 0.18 to 0.55 (Mahyudin *et al*., 2019). Moreover, the results in the present study showed that the MARI of *S. aureus* isolates was >0.2 MAR. This indicates that these isolates originated from a high-risk source of contamination with a high frequency of AM use (Davis and Brown, 2016; Onaolapo *et al*., 2016). In 2006, class I integrons previously identified in Gram-negative bacteria were first detected in staphylococci (Shi *et al*., 2006). These were initially found in coagulase-negative staphylococci and later in *S. aureus*. These integrons contained gene cassettes associated with resistance to STR (*aadA2*, *aadA5*), CHL (*cmlA1*), and TXT (*dfrA12*, *dfr17*) (Xu *et al*., 2008). The emergence and spread of such integrons in staphylococci and other Gram-positive bacteria raise significant concerns regarding the potential for rapid horizontal gene transfer across species and genera. This includes the risk of Gram-positive bacteria acquiring resistance genes typically associated with Gram-negative organisms, thereby accelerating the dissemination of AMR. A high frequency of pork samples were contaminated with *S.*

Table 7. Pairwise correlation between two antimicrobial resistance statuses.

	PEN											
PEN	1	AMP										
AMP	0.39**	1	AMO									
AMO	0.68***	0.54***	1	ENR								
ENR	0.12	−0.18	−0.06	1	DOX							
DOX	0.01	0.05	0.07	0.31*	1	TET						
TET	−0.05	−0.16	−0.07	−0.02	−0.03	1	STR					
STR	−0.06	0.19	0.06	0.15	0.40**	0.16	1	ERY				
ERY	0.16	0.11	0.28*	0.10	−0.03	0.22	0.24	1	GEN			
GEN	0.22	0.06	0.15	−0.10	−0.11	0.35**	0.12	0.04	1	AMK		
AMK	0.15	0.13	0.19	−0.05	0.23	−0.11	0.29*	−0.17	0.16	1	NEO	
NEO	0.17	0.53***	0.33*	−0.17	0.06	0.06	0.11	0.23	0.21	0.14	1	VAN
VAN	0.17	0.18	0.07	−0.17	−0.09	0.32*	0.11	0.23	0.21	−0.03	0.22	1

Significant *p*-values corresponding to the correlation coefficient are indicated with asterisk * *p*-value < 0.05; ** *p*-value < 0.01, *** *p*-value < 0.001. Penicillin (PEN, 10 UI), amoxicillin (AMO, 10 µg), ampicillin (AMP, 10 µg), streptomycin (STR, 10 µg), neomycin (NEO, 30 µg), amikacin (AMK, 30 µg), gentamycin (GEN, 10 µg), enrofloxacin (ENR, 5 µg), vancomycin (VAN, 30 µg), erythromycin (ERY, 15 µg), doxycycline (DOX, 30 µg), tetracycline (TET, 30 µg)

aureus (in the present study), *E. coli*, and *Salmonella* (in the same samples of our previous study) (Le *et al*., 2023). The cooccurrence of these bacteria may increase the likelihood of horizontal transfer of antibiotic resistance genes within pork samples. These findings highlight the potential public health risk associated with AMR *S. aureus* isolates from freath foods sold in traditional markets. These findings highlight the potential public health risk associated with AMR *S. aureus* isolates from freath foods sold in traditional markets.

The high frequency of ARGs in S. *aureus* isolates is concerning because it highlights the potential for transmission of resistance traits from the food supply chain to the clinical setting. This study showed that 62.5% of the *S. aureus* isolates carried the *tetM* gene, which encodes for TET resistance. A high prevalence (82.8%) of *S. aureus* isolates from hospitals in Vietnam carrying the tetM/K gene was reported by Son *et al*. (2019). The high frequency of the *tetM* gene may indicate the overuse of TETs in livestock production in recent years (Nguyen *et al*., 2016; Nguyen Van Chao *et al*., 2022). The *tetM* gene binds to the EF-G site on the ribosome, preventing TETs from binding and exerting their effects (Grossman, 2016). Tetracycline resistance may be caused by mutations in chromosomal genes (such as Tet38) that lead to increased expression of TETs efflux pumps on the cell membrane (Truong-Bolduc *et al*., 2022). In agreement with the present study, Akanbi *et al*. (2017) confirmed that *tetM, blaZ, rpoB*, and *ermB* were the most prevalent ARGs in S. *aureus* isolates from food samples. The high resistance to PEN (75.0%) and AMO (64.58%) raises concerns because these AMs are still widely used in veterinary medicine. The presence of the *blaZ* gene in 52.1% of isolates suggests that β-lactamase production is a key driver of resistance.

The more common AMR *S. aureus* isolates from food (as reported in the present study and others) significantly reduced the effectiveness of AMs in treating infectious diseases. Recently, the use of combination therapy instead of monotherapy has been found to be considerably more effective for treating MDR infections and preventing the emergence of new resistant strains (Dai *et al*., 2017). Hu *et al*. (2015) reported that the combination of a beta-lactam and GEN exerted a strong synergistic effect against *S. aureus* infection. However, in this study, the positive correlations among b-lactams (PEN and AMP), b-lactams (AMO), macrolides (ERY), and aminoglycosides (NEO) were investigated. Moreover, high-level resistance to b-lactams (PEN, AMO) and TET in the *S. aureus* isolates were also observed. Yu *et al*. (2020) demonstrated a marked synergistic activity of the combination regimens, including VAN/rifampicin, VAN/oxacillin, levofloxacin/oxacillin, and GEN/oxacillin, in treating *S. aureus* infection. In this study, no significant correlation was observed between VAN and the other AMs (except VAN and TET) was not observed. Therefore, combination therapy with VAN plus NEO or GEN may be an option for the treatment of *S. aureus* infections. Further studies are needed to evaluate the efficacy of AM combinations for treating *S. aureus* infections in humans and animals.

Conclusion

To the best of our knowledge, this is the first study on the prevalence and AMR of *S. aureus* isolates from

retail pork in Hue City, Vietnam. This study found that 100% of the retail pork samples collected from traditional markets in Hue City was contaminated with *S. aureus*. The *S. aureus* counts in all samples were higher than the minimum allowed limit by TCVN7046:2015. The *S. aureus* isolates exhibited high resistance to penicillin, amoxicillin, and tetracycline but remained susceptible to amikacin, gentamicin, enrofloxacin, and doxycycline. The mutiple AMR index ranged from 0.17 to 0.75 (resistance to two to nine different AMs). A high frequency of *S. aureus* isolates was resistant to three different AMs (ARI = 0.25). Pairwise correlation analysis suggested that combining penicillin, ampicillin, and amoxicillin with neomycin may not be an effective option for treating *S. aureus* infections. Furthermore, a high rate of MDR *S. aureus* isolates from retail pork samples from traditional markets underscores the role of foodborne pathogens in the spread of AMR. The findings of this study underscore the need for strengthened surveillance throughout the food supply chain. Additionally, expanding the research scale and conducting studies over extended periods would yield comprehensive insights. Integrating advanced methodologies, such as large-scale genomic epidemiology, metagenomic analysis, and machine learning models for AMR predictions will greatly enhance our understanding of the evolution and dissemination of resistance within the food supply chain.

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