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Prevalence and Antibiotic Resistance of *Staphylococcus* spp. Isolated from the Respiratory Tract of Meat Goats in Can Tho City, the Mekong Delta, Vietnam

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ABSTRACT

The research aimed to determine the presence and antibiotic resistance of *Staphylococcus* spp. in goat herds in Can Tho City, center of the Mekong Delta, Vietnam. A total of 456 nasal samples were collected from meat goats. These goats were of all ages, breeds, genders, and health conditions raised in small-scale farms and households in this city. *Staphylococcus* spp. isolates were isolated according to the instructions of Vietnamese standard TCVN 4830-2:2005 and the guideline of Barrow and Feltham; the results indicated that *Staphylococcus* spp. was detected in 24.78% of nasal samples. The prevalence of *Staphylococcus* spp. depended on age, breed, and farm styles, independent of health conditions. The Kirby-Bauer method was used to define the antimicrobial susceptibility of *Staphylococcus* spp. to eleven examined antibiotics. Those strains were sensitive to most of the examined antibiotics but were relatively resistant to ampicillin (41.59%), and four strains (3.54%) exhibited resistance to two antibiotics. The PCR detected antibiotic-resistance genes, including *blaampC, aadA1*, *tetA*, *qnrA*, *sulII*, and *cat1*. The PCR results exhibited that those *Staphylococcus* spp. strains harbored the gene *blaampC* at the highest rate (59.29%), followed by *aadA1* (10.62%), but none of *sullII* and *cat1* was detected. Moreover, 15.04% of *Staphylococcus* spp. strains insisted on two antibiotic-resistance genes. The high prevalence of *Staphylococcus* spp. exhibiting antibiotic resistance and diverse antibiotic-resistance genes has been a potential risk for causing severe respiratory diseases and difficulty treating goats in this region.

Key words: Antibiotic resistance, Can Tho, Resistant genes, Goats, *Staphylococcus*

INTRODUCTION

Goat farming has been increasingly developing, increasing the total herd count yearly in the Mekong Delta and bringing stable income to farmers (Nguyen et al. 2022). According to statistics from the Vietnamese General Statistics Office (Vietnam Husbandry 2022), the goat herd in the Mekong Delta has reached over 400,000. At the same time, goats are livestock with high salinity tolerance, adapting to salinity intrusion and climate change. Currently, most goat farming models in the Mekong Delta are still relatively primitive and small-scale, with few large farms and inadequate veterinary hygiene conditions; these are conditions for pathogens to invade and cause disease. Among common diseases, respiratory diseases are widespread in small ruminants worldwide (Leite-Browning et al. 2021). Respiratory diseases increase goat production costs due to expensive treatments.

Staphylococcus spp. are Gram-positive cocci and common pathogens of humans and other mammals. They are present on the skin, mucous membranes of the upper respiratory tract, lower urinary tract, and genital tract and may also be in the gastrointestinal tract (Kosecka-Strojek et al. 2017; Azam et al. 2024). In animals, *Staphylococcus* spp. causes several diseases, such as pyoderma, mastitis, exudative epidermolysis bullosa, and respiratory disease (Quin et al. 2011; Cheung and Otto 2022; Faccin et al. 2023; Nadi et al. 2024). Moreover, *Staphylococcus* is one of the most common pathogens that cause respiratory infections and mortality in goats (Rashid et al. 2013; Asare et al. 2016; Ugochukwu et al. 2017; Puvarajan et al. 2020).

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In addition, bacteria's antibiotic resistance in treating respiratory diseases in goats is also increasing (Berge et al. 2006; Zhou et al. 2017). Although vaccines have been used to prevent respiratory diseases in goats during the breeding process, the disease still occurs frequently, and the specific cause of the disease has not been determined. Therefore, the prevention and treatment of respiratory diseases in goats have been ineffective. Several reports have been published on *Staphylococcus*'s presence and antibiotic resistance in small ruminants, including goats. In these reports, *Staphylococcus* was present at a relatively high rate and resistant to antibiotics, such as amoxicillin, lincomycin, furazolidone, and trimethoprim (Sedeek and Thabet 2001; Aher et al. 2013; Zhou et al. 2017; Puvarajan et al. 2020).

Can Tho City is located in the center of the Mekong Delta, Vietnam, and has a high number of goats in this region. Goats supply meat and milk for consumers and benefit farmers. However, disease management in goats, including respiratory diseases caused by *Staphylococcus*, has been a potential concern. Therefore, this study aimed to determine the presence and antimicrobial susceptibility of *Staphylococcus* spp. isolated from the respiratory tract of goats in Can Tho City. From there, epidemiological recommendations and methods for preventing and treating respiratory diseases caused by *Staphylococcus* in goats will be provided.

MATERIALS AND METHODS

Ethical approval

This study was conducted following the guidelines outlined in the Helsinki Declaration. The Animal Ethic Committee and the Safety Principles of Can Tho University, Can Tho City, Vietnam, accepted animal experiment procedures in this research.

Sample collection from goats

This study determined the presence of *Staphylococcus* spp. in meat goats (hybrid Boer goats) raised in two small-scale farms (<50 herd/farm) and two households (<10 herd/house) in Can Tho City. The survey was conducted cross-sectionally, entirely randomly, with two sampling times, from March to June 2024.

Nasal swab specimens of meat goats collected using sterile tampons were performed according to the goat 2019 blood and swab sample collection records (USDA's NAHMS 2022). The expected number of samples was calculated using the formula of Fathman (2003) with p=43.24% (Zhou et al. 2017). In this study, a total of 456 nasal samples were collected in these farms and households. The nasal samples were collected directly from goats' nostrils in the morning. After that, samples were taken and kept in separate sterilized Cary-Blair transport medium (Merck, Germany) in cool conditions (4-8^{o}C) for transport to the laboratory to detect *Staphylococcus* spp. within 24 hours.

Isolation and identification of Staphylococcus spp. in goats' nasal samples

Staphylococcus spp. was isolated from blood agar supplemented with 5% sheep blood (BA, Merck, Germany). The nasal samples were streaked on the blood agar and after incubation at 37^{o}C for 24h, colonies suspected to be *Staphylococcus* spp. (opaque white, small, non-slimy, with/without hemolysis on blood agar) were cultured on Nutrient Agar (NA, Merck, Germany) to test biochemical properties (KIA, VP, MR, oxidase, catalase) according to the instructions of Vietnamese Standard TCVN 4830-2:2005 and Barrow and Feltham (2003). These *Staphylococcus* isolates were identified and subcultured on trypticase soy agar (TSA, Merck, Germany) for further experiments.

The antimicrobial susceptibility test of *Staphylococcus* spp. isolated from nasal samples

In this study, one *Staphylococcus* isolate representative of one positive sample was selected to examine antimicrobial susceptibility. The antimicrobial susceptibility test was performed according to Kirby-Bauer's disk diffusion method (Bauer et al. 1966). The selected antibiotics and results determine the sensitivity or resistance of bacterial strains to antibioics based on the CLSI (2021) zone diameter standards.

The antibiotic disc was provided by Nam Khoa Biotek Company (Ho Chi Minh City, Vietnam). It included ampicillin (Am, 10µg), (amoxicillin/clavulanic acid (Ac, 20/10µg), ceftazidime (Cz, 30µg), amikacin (Ak, 30µg), gentamycin (Ge, 10µg), doxycycline (Dx, 30µg), tetracycline (Te, 30µg), levofloxacin (Lv, 5µg), ofloxacin (Of, 5µg), chloramphenicol (Cl, 30µg), trimethoprim/sulfamethoxazole (Bt, 1.25/23.75µg). *Staphylococcus aureus* ATCC 25923 were used as controls in the study.

Detection of antibiotic-resistance genes in *Staphylococcus* spp. isolated from goats' nasal samples

The *Staphylococcus* isolates used in the antimicrobial susceptibility test were examined using the PCR method to detect six antibiotic-resistance genes. These genes are present in commonly used antibiotic categories.

The DNA of *Staphylococcus* isolates was extracted using the ISOLATE II Genomic DNA kit (Bioline, Canada) following the manufacturer's instructions. The sequence of primers and PCR conditions for detecting the antibiotic-resistance genes followed the guidelines, including *blaampC* (Forward et al. 2001), *aadA1* and *tetA* (Randall et al. 2004), *qnrA* (Cattoir et al. 2007), *sulII* (Sáenz et al. 2004) and *cat1* (Van et al. 2008).

The mixture for a PCR reaction (25.0µL) includes Master Mix 2X (Bioline, Canada) (12.5µL); forward and reverse primers at a concentration of 10µM (0.5µL/primer); purified distilled water (9.5µL); and template DNA (2.0µL). PCR products were electrophoresed at 50V for 60 min on a 1.5% agarose gel and stained with an Ethidium bromide solution. The results were detected by photographing the gel under UV light. The standard ladder was 100 bp (Bioline, Canada), the positive control was bacterial strains carrying antibiotic resistance genes, and the negative control was purified water without DNA and RNA.

Statistical analysis

Data were analyzed to compare the presence rate of *Staphylococcus* spp. in the goat respiratory tract and the presence rate of genes encoding antibiotic resistance using the Chi-square method with 95% confidence on Minitab 16.0 software (USA).

RESULTS

The presence of *Staphylococcus* spp. isolated from goats' nasal samples

A total of 456 goats' nasal swabs were collected from small-scale farms and households in Can Tho City to determine the presence of *Staphylococcus* spp. in goats. The analysis results are presented in Table 1.

Table 1: The presence of *Staphylococcus* spp. in goats in Can Tho City, the Mekong Delta, Vietnam

Factor	Goats Examined			Chi-Square Value	P Value
	No.	Positive No.	%		
Farm style					
Small-scale farms	379	60	15.83	96.44	P<0.05
Households	77	53	68.83		
Gender					
Male	218	89	40.83	57.69	P<0.05
Female	238	24	10.08		
Age					
<6 months	158	60	37.97	28.11	P<0.05
≥ 6 months	167	21	12.57		
≥ 1 year	131	32	24.43		
Health condition					
Respiratory diseases	214	60	28.04	2.29	P>0.05
Healthy	242	53	21.90		
Total	456	113	24.78		

The results showed that goats raised in households, male goats, goats <6 months infected with *Staphylococcus* spp. higher than others (P<0.05); however, there was no significant difference between healthy goats and respiratory disease goats (P>0.05). It indicated *Staphylococcus* spp. was present frequently in the goats' respiratory tract, and the presence could depend on age and rearing conditions.

The antimicrobial susceptibility of *Staphylococcus* spp. isolated from goats

One *Staphylococcus* isolate, represented by one positive sample, was selected to examine the antimicrobial susceptibility to eleven antibiotics. The results are shown in Table 2.

Table 2: The antibiotic resistance of *Staphylococcus* spp. isolated from goats (n=113)

Antibiotics	Sensitivity		Resistance	
	No. of isolates	%	No. of isolates	%
Ampicillin	66	58.41	47	41.59
Amox/clav.*	112	99.12	1	0.88
Ceftazidime	111	98.23	2	1.77
Amikacin	113	100.00	0	0.00
Gentamycin	107	94.69	6	5.31
Doxycycline	112	99.12	1	0.88
Tetracycline	113	100.00	0	0.00
Levofloxacin	113	100.00	0	0.00
Ofloxacin	111	98.23	2	1.77
Chloramphenicol	113	100.00	0	0.00
Bactrim	110	97.35	3	2.65

*Amox/clav.: amoxicillin/clavulanic acid

Table 2 showed that *Staphylococcus* isolates were susceptible to most examined antibiotics, especially to amikacin (100%), tetracycline (100%), levofloxacin (100%), and chloramphenicol (100%). However, these isolates were highly resistant to ampicillin (41.49%).

Although *Staphylococcus* isolates in this study were sensitive to most examined antibiotics, a few isolates (3.54%) showed resistance to two antibiotics in two antibiotic categories (Table 3).

Table 3: The pattern of multiple antibiotic resistance in *Staphylococcus* spp. (n=113)

No. antibiotics	Patterns	No. of isolates	%
2	Am + Ac	1	0.88
	Am + Ge	2	1.77
	Am + Dx	1	0.88
Total		4	3.54

Am: ampicillin; Ac: amoxicillin/clavulanic acid; Ge: gentamycin; Dx: doxycycline

The presence of antibiotic-resistance genes in *Staphylococcus* spp. isolated from goats

The PCR assay was used to detect antibiotic-resistance genes, including *blaampC, aadA1, tetA, qnrA, sulII,* and *cat1*, in *Staphylococcus* spp. isolated from goats' nasal samples. The results are shown in Table 4. The results showed that those *Staphylococcus* spp. strains harbored the gene *blaampC* at the highest rate (59.29%), followed by *aaadA1* (10.62%), but none of *sulII* and *cat1* was detected. Moreover, 15.04% of *Staphylococcus* isolates harbored two antibiotic-resistance genes. The pattern of *blaampC* + *aadA1* was the most frequent (8.85%) (Table 5).

Table 4: The presence of antibiotic-resistance genes in *Staphylococcus* spp. isolated from goats (n=113)

Genes	No. of positive isolates	%
blaampC	67	59.29
aadA1	12	10.62
tetA	10	8.85
qnrA	4	3.54
sulII	0	0.00
cat1	0	0.00

Table 5: The combination of antibiotic-resistance genes in *Staphylococcus* spp. isolated from goats (n=113)

No. of genes	Patterns	No. of isolates	%
2	*blaampC + aadA1*	10	8.85
	blaampC + tetA	5	4.42
	blaampC + qnrA	2	1.77
Total		17	15.04

DISCUSSION

In this study, *Staphylococcus* spp. was present at a high rate (24.78%) in the goats' respiratory tract, and the presence could depend on age and rearing conditions. The survey at goat small-scale farms and households in the survey area was still rudimentary, not ensuring veterinary hygiene conditions (wooden barns, open barns, lack of solidity, etc.); these factors contribute to the retention and spread of pathogens in the livestock environment. Tombarkiewicz et al. (2009) reported that the number of microorganisms exceeding the threshold and the presence of pathogenic organisms in the air of goat barns in Poland is evidence of poor hygiene conditions of goat barns and is the cause of pollution of the external environment as well as causing diseases for livestock. Aher et al. (2013) detected *Staphylococcus* spp. at a high rate (29.9%) from nasal swabs and tissue samples in healthy and sick goats in India. Puvarajan et al. (2020) also determined the respiratory microbiota of goats with respiratory disease in Tamilnadu, India and *Staphylococcus* spp., *Pasteurella multocida*, *Streptococcus pyogenes*, and *E. coli* were present frequently. These bacterial strains resided in the lungs and trachea of infected goats. Furthermore, Sireesha et al. (2023) indicated that *Staphylococcus* spp. (37.73%) was the most frequent pathogen isolated from respiratory lesions in the lungs of goats in India. It revealed *Staphylococcus* spp. could be one of the severe pathogens causing respiratory diseases in goats.

Furthermore, studies on the impact of age, sex, and breed on the prevalence of respiratory diseases in livestock have had different findings. In Nigeria, Eneojo et al. (2019) reported that the incidence of pneumonia in goats was sex- and age-dependent, with males predominating, and breed also played an essential role in the susceptibility of goats to pneumonia. Gay et al. (2021) conducted a study on genders in infectious diseases; it pointed out that genetic differences may influence the outcome of infection, which is related to both the susceptibility and resistance of microorganisms. Zhou et al. (2023) clarified the correlation of respiratory pathogens in cattle in Northeast China and noted that cattle (meat or dairy) were equally susceptible to pathogens. Therefore, further in-depth epidemiological studies are needed to accurately assess the causes of *Staphylococcus* spp.'s presence in the goats’ respiratory tract in the survey region. However, goat herds' management and health care must also be strengthened to prevent diseases from occurring at all stages and influencing factors.

Staphylococcus isolates were susceptible to most examined antibiotics in this study, except resistance to ampicillin (41.49%). In fact, at the sampling sites, antibiotics were rarely used due to goats' natural resistance and farmers' limiting the impact on the milk quality of female goats. Therefore, the resistance to ampicillin in these isolates may be due to bacteria-carrying factors that create natural resistance to ampicillin. Jubeh et al. (2020) found that Gram-positive pathogens exhibit a great genetic capacity to acquire and develop resistance to most clinically used antibiotics. In addition, according to Tenover (2006), antibiotic resistance often occurs not with just one but a combination of several resistance mechanisms. Resistance can occur in bacteria through continuous exposure to an antibiotic or the acquisition of resistant genetic elements via plasmids and transposons. For example, *S. aureus* could become drug-resistant by genetic mutations to alter the target DNA gyrase or reduce outer membrane proteins, thus reducing drug accumulation (Kime et al. 2019; Yang et al. 2020). In China, nasal swabs of healthy goats were found to contain *S. aureus*. Most of them were sensitive to multiple antibiotics, except for trimethoprim, furazolidone, amoxicillin, lincomycin, and roxithromycin, which were highly resistant at rates of 50.00, 40.63, 37.5, 28.13, and 21.88%, respectively (Zhou et al. 2017). Doley and Ahmed (2017) reported that *Staphylococcus* spp. recovered from healthy goats in India were found sensitive to ampicillin (100%), amoxicillin (100%), tetracycline (91.67%), chloramphenicol (83.34%), streptomycin (75%) and penicillin-G (75%), while resistant to erythromycin and amikacin. In Egypt, Fouad et al. (2022) reported that *Staphylococcus* spp. isolated from the respiratory tract of small ruminants, including sheep and goats, revealed multi-drug resistance patterns. Those strains were highly resistant to ampicillin, amoxicillin + clavulanic acid, ampicillin + sulbactam, oxacillin, and doxycycline but sensitive to ciprofloxacin, amikacin, aztreonam and imipenem. Thus, it exhibited a significant prevalence of multidrug resistance, which could pose a concern for public health there.

Staphylococcus isolates in this study showed resistance to two antibiotics in two antibiotic categories. Abdalhamed et al. (2021) pointed out that multidrug-resistant bacteria are a global problem in ruminants today due to inappropriate use of antibiotics. Resistance genes in bacteria can be transmitted between ruminants, humans, and the environment through food consumption (meat, milk, and dairy by-products) and direct or indirect contact. In Bangladesh, Dutta et al. (2023) pointed out that about 91% of *S. aureus* isolated from goats, even in healthy goats, were multidrug-resistant (resistant to ≥3 antimicrobial classes). In India, Verma et al. (2024) examined multiple antibiotic resistance (MAR) among MRSA (methicillin-resistant *Staphylococcus aureus*) isolated from nasal swabs and milk samples of sheep and goats. They exhibited that the MAR indices of those strains were relatively high, from 0.3 to 0.8, and the prevalence of MRSA in these animals might pose a possible public health concern. Therefore, controlling multidrug-resistant *Staphylococcus* in the respiratory tract of goats raised in Can Tho City in the early stage is a concern to protect the health of animals and humans.

The presence of antibiotic-resistance genes of *Staphylococcus* spp. (Table 4) was compatible with the results of the antimicrobial susceptibility test in Table 2. The high resistance to ampicillin in *Staphylococcus* spp. and the high presence of the *blaampC* gene were expressed in these strains. However, these *Staphylococcus* isolates harbored genes *aadA1* and *tetA* at a relatively high rate (10.62% and 8.85%, respectively); these strains did not resist these antibiotics in the antimicrobial susceptibility test. This highlights the difference between carrying genes encoding antibiotic resistance and expressing resistance outwardly. Bacteria express resistance to an antibiotic when the pressure to use that antibiotic is high or due to the interaction of many different factors. Furthermore, some antibiotic-resistance genes appear silent ones that are often not expressed or expressed at low levels, even when exposed to antibiotics (Stasiak et al. 2021). Mobile genetic elements, especially plasmids and transposons, significantly contribute to disseminating antimicrobial resistance genes in animal staphylococci. They enable the transfer of resistance genes between staphylococci of animal and human origin and among other Gram-positive bacterial species (Wendlandt et al. 2013). Our study could be the first report on *Staphylococcus* isolated from goats in the Mekong Delta and Vietnam. A few reports were published on the presence of antibiotic-resistance genes of *Staphylococcus* spp. isolated from goats’ respiratory tracts in the world. In Tunisia, *Staphylococcus* isolates from nasal samples of healthy goats were sensitive to all examined antibiotics, and only gene *blaZ* encoding for penicillin resistance was detected (Gharsa et al. 2015). *Staphylococcus* isolates harbor antibiotic-resistant genes, a crucial issue affecting the effective treatment of respiratory diseases in goats, causing a risk of antibiotic-resistance transmission to other animals and humans in the research area.

In addition, although the number of isolates carrying multiple genes was limited in this study (Table 5), *Staphylococcus* spp. isolates also created many different gene patterns, like the antibiotic resistance expression of these isolates in the antimicrobial susceptibility test method. Depenbrock et al. (2024) surveyed respiratory diseases in newly weaned heifers in the US; it found that the co-occurrence of antibiotic resistance genes in a bacterial isolate was expected, with clusters of genes encoding antibiotic resistance to phenicol, macrolide, elfamycin, β-lactams (cephalosporins, penam cephamycin), aminoglycosides, tetracyclines, and sulfonamides. It indicated that *Staphylococcus* spp. isolated from the goat's respiratory tract could resist antibiotics in several categories, causing a potential risk in the failure of antibiotic treatment for respiratory diseases in goats in Can Tho City.

Conclusion

Staphylococcus spp. was present at a high rate in the respiratory tract in both healthy and sick goats. In this study, factors such as age, gender, and rearing conditions affected the presence of *Staphylococcus* in goats in the survey area. Although these *Staphylococcus* isolates were susceptible to most examined antibiotics, they naturally resisted ampicillin. Moreover, these isolates harbored several antibiotic-resistance genes corresponding to the antimicrobial susceptibility test. The results indicated that *Staphylococcus* spp. in goats' respiratory tract could threaten goat's health, even for humans. It is essential for all stakeholders to collaborate and for additional research to be conducted to mitigate the adverse effects of antibiotic resistance from domestic animals.

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Data availability statement: The data supporting this study's findings are available on request from the corresponding author.

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