Veterinary Integrative Sciences 2024; 22(3): 871 - 881 DOI; 10.12982/VIS.2024.059

Vet Integr Sci

Veterinary Integrative Sciences

Faculty of Veterinary Medicine
Chiang Mai University

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Detection of Staphylococci from nasal samples from healthy and sick dogs in Ho Chi Minh City, Vietnam

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Abstract

Staphylococci are one of the most commonly found opportunistic bacteria in animals and human. The objective of this study was to determine the prevalence of *Staphylococcus* spp. isolated from healthy and sick dogs visiting veterinary clinics in Ho Chi Minh city, Vietnam from January 2022 to December 2022. A total of 123 nasal swab samples were collected, including 40 samples from healthy dogs and 83 samples from dogs with respiratory symptoms. *Staphylococcus* spp. were isolated from 47.5% of healthy animals and 60.24% of dogs showing respiratory symptoms. The study showed that isolation rates from confined, semi-confined and free-ranging dogs were 75.86%, 54.84% and 40.63%, respectively. Furthermore, the prevalence was higher in dogs over one year old compared to younger dogs (64.10% and 42.22%, respectively). Several subspecies of Staphylococci, including *S. aureus*, *S. pseudintermedius* and *S. epidermidis* were identified, in which *S. pseudintermedius* accounted for the highest proportion (48.28%). The prevalence of *S. pseudintermedius* also varied across different age groups. Coagulase activity of the isolated *Staphylococcus* strains was also determined and indicated that coagulase-positive Staphylococci constituted 80.46% of the isolates. This study represents the first report on the prevalence of Staphylococci in dogs in Vietnam and reemphasizes the zoonotic significance of *Staphylococcus* at the interface between dogs and humans.

Keywords: Dog, Isolation, Respiratory tract, Staphylococci, Vietnam

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Article history; *received manuscript: 15 August 2023*
revised manuscript: 14 November 2023
accepted manuscript: 19 December 2023
published online: 15 January 2024
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

Staphylococcus species are recognized as a prevalent source of disease in domestic animals, especially in domestic dogs and other pets (Worthing et al., 2018; Elshabrawy et al., 2020; Lynch and Helbig, 2021; Suepaul et al., 2021). To date, 71 species of *Staphylococcus* have been identified (Parte et al., 2020) in which *Staphylococcus pseudintermedius* (*S. pseudintermedius*) is reported to be the most important in dogs and cats. *Staphylococcus aureus* (*S. aureus*) is prevalent in humans and various other animals (Suepaul et al., 2021). This species is considered as a major source of disease in dogs and cats raised in Egypt (Elshabrawy et al., 2020). In addition, previous studies have confirmed that the composition of *Staphylococcus* species in domestic dogs is very diverse and closely related to pathogens in humans and other domestic animals (Cox et al., 1988; Misic et al., 2015; Bierowiec et al., 2016). Staphylococci are capable of surviving in a wide range of environmental conditions including adverse environments (Onyango and Alreshidi, 2018). More importantly, the bacteria have been found to cause a variety of opportunistic infection in a wide range of animal species (Weese and van Duijkeren, 2010). *S. pseudintermedius* and *S. aureus* have been reported to be transmissible between humans, domestic dogs, and other animals (Cox et al., 1988; Misic et al., 2015; Bierowiec et al., 2016; Suepaul et al., 2021).

Several studies have reported several bacterial species associated with respiratory tract infections (RTI), particularly *Staphylococcus* spp. (Kalhoro et al., 2017; Moyaert et al., 2017; Moyaert et al., 2019; Vientós Plotts et al., 2019). Ayodhya et al. (2013) detected that 40% of dogs with severe respiratory disease symptoms were infected with Staphylococci. In Europe, Moyaert et al. (2019) conducted a study on 233 dogs suffering from RTI during 2013–2014, and their results also showed that the prevalence of *S. pseudintermedius* and *S. aureus* nasal swabs or bronchoalveolar lavage fluid samples from dogs with respiratory symptoms was 34.3% and 9.9%, respectively. A study in South Africa from 2007–2013 found that 99.4%of isolates recovered from 157 canines with RTI were resistant to at least one antibiotic, 64.7% of isolates listed as multi-drug resistance (MDR), and *Staphylococcus* spp. making up to 7.1% of the MDR isolates (Qekwana et al., 2020).

In Vietnam, investigation on the prevalence of *Staphylococcus* in the upper respiratory tract of dogs remains limited. Most of previous studies have focused on methicillin-resistant *Staphylococcus aureus* (MRSA) for human medicine (Van Nguyen et al., 2014; Quyet et al., 2019; Thai et al., 2019). In a study carried out in Can Tho city, this genera accounted for the highest isolation rate among bacteria isolated from dogs with respiratory tract diseases (Ly, 2017). No study has been conducted to determine the presence of *S. pseudintermedius*, *S. aureus*, or any other *Staphylococcus* strains in pet animals in Vietnam. Therefore, the present study aimed to explore the distribution of staphylococcal species in healthy and sick dogs and identified risk factors associated with the prevalence of *Staphylococcus* in dogs.

MATERIALS AND METHODS

Animals and sampling

All experimental protocols followed the Animal Welfare Assessment (DT2021-04/KNN) and informed consent was obtained from all dog owners. Specimens were collected from 123 dogs, with one sample collected per dog, for the isolation of *Staphylococcus* spp. The samples were collected from five veterinary clinics located in Ho Chi Minh City, specifically Thu Duc City, Districts 1, 3, 11, and Binh Thanh, during the period from January 2022 to December 2022.

The animals were categorized based on their health status (healthy or sick), breed (local, cross, or foreign breed), gender (male or female), level of confinement (confined, semi-confined, or free-ranging), and age (adult, defined as ≥1 year, or juvenile, defined as <1 year). Dogs with respiratory symptoms were identified through the presence of symptoms such as a runny nose with cloudy or purulent mucus, dry or wet cough, and difficulty breathing. The healthy dogs included in this study were selected from those visiting the clinic for routine health check-ups or vaccinations and without any abnormal signs in health. Nasal swab samples were collected by gently inserting the swab tip into the nasal cavity, approximately 2–3 cm from the tip of the nose and 1–2 cm away from the eye. The swab was rotated a few times, held in place for 5 seconds, and then gently pulled out. Each sampled swab was placed in a tube containing sterile Stuart Amies solution, labeled, and stored in containers at 2–8°C. The samples were immediately transported to the Laboratory of Microbiology, Faculty of Veterinary Medicine and Animal Husbandry, HUTECH University, Vietnam for bacterial isolation.

Bacterial strains, isolation and identification

Five pure strains, namely *S. epidermidis*, *S. aureus*, *S. pseudintermedius*, *S. intermedius*, and *S. schleiferi* subsp., were provided by the Laboratory of Microbiology, Faculty of Veterinary Medicine and Animal Husbandry, HUTECH University and served as positive controls.

The isolation procedure followed established methods as described in previous studies (Sahebnasagh et al., 2014; Fungwithaya et al., 2017; Suepaul et al., 2021). Each swab sample was streaked onto blood agar base (Oxoid, UK) and mannitol salt agar (Oxoid, UK) plates. These plates were then incubated aerobically at 37°C for 24 hours. Suspected colonies of *Staphylococcus* spp. were identified based on colony morphology, Gram staining (MOH, 2017), catalase and oxidase tests, and coagulase tests (Chaieb et al., 2005).

One suspicious *Staphylococcus* colony was streaked onto a nutrient agar plate to promote proliferation. Subsequently, the colony was transferred into Brain Heart Infusion Broth (BHI) supplemented with 30% glycerol. All isolates were then stored at -20 °C for further analysis. All presumptive Staphylococci were confirmed and identified at the species level by a multiplex PCR. For DNA extraction, a 500-µL aliquot of each culture suspension was utilized, following the Tris-EDTA-NaCl-Triton X100 (TENT) method as previously described (Hassanzadeh et al., 2016).

Seven primer pairs were used in the multiplex PCR to identify and differentiate *Staphylococcus* species. The 27F and 1492R targeted highly conserved regions of the *16S rRNA* gene among eubacterial species (Weisburg et al., 1991). The Nuc-alF1 and Nuc-alR primers were designed to target conserved regions of the 16S RNA gene specific to *Staphylococcus* species (Sasaki et al., 2010). Additionally, specific primer pairs, namely epi-F/epi-R, au F3/au-nucR, pse-F2/pse-R5, in-F/in-R3, and sch-F/sch-R, were employed to amplify the *nuc* gene for the identification of *S. epidermidis*, *S. aureus*, *S. pseudintermedius*, *S. intermedius*, and *S. schleiferi* subsp., respectively (Hirotaki et al., 2011; Sasaki et al., 2010). Details of the primers used in this study are described in Table 1.

Multiplex PCR reaction was carried out in a total volume of 20 µL reaction mixture which comprised 2 µL of genomic DNA template and 18 µL of the 2x colorless Go-Taq master mix that included $MgCl_2$, 10x PCR buffer, dNTPs, 10 units of Taq DNA polymerase (Cat #M7132, Promega, USA), and 0.5 µL of each 10 µM forward and reverse the primer pairs. PCR amplification was carried out using the thermal profile as follows: 94 °C for 4 min, followed by 35 cycles of 94°C for 60 sec, 35 sec for annealing temperature as described in Table 1, and 72°C for 1.5 min, and a final extension cycle at 72°C for 10 min.

After amplification, the resulting PCR products were electrophoresed on 1.3% tris-acetate-EDTA agarose gels stained with ethidium bromide, alongside a 1,000 bp ladder (Bioline, UK). The gels were then visualized and photographed under UV transillumination.

Table 1 Nucleotide sequences of primers used for detection of *Staphylococcus* species

Target gene	Primer	Sequence (5' – 3')	Amplicon size (bp)	Annealing Tm (°C)	Reference
16S rRNA	27F	CAGAGTTTGATCCTGGCT	1,465	55	Weisburg et al., 1991
	1492R	AGGAGGTGATCCAGCCGCA			
16s rRNA Staphylococcus	Nuc-alF1	CCTATAAGACTGGGATAACTTCGGG	791	52	Sasaki et al., 2010
	Nuc-alR	CTTTGAGTTTCAACCTTGCGGTCG			
nuc (S. epidermidis)	epi-F	TTGTAAACCATTCTGGACCG	251	56	Hirotaki et al., 2011
	epi-R	ATGCGTGAGATACTTCTTCG			
nuc (S. aureus)	au-F3	TCGCTTGCTATGATTGTGG	359	57	Sasaki et al., 2010
	au-nucR	GCCAATGTTCTACCATAGC			
nuc (S. pseudintermedius)	pse-F2	TRGGCAGTAGGATTCGTTAA	926	57	Sasaki et al., 2010
	pse-R5	CTTTTGTGCTYCMTTTTGG			
nuc (S. intermedius)	in-F	CATGTCATATTATTGCGAATGA	430	56	Sasaki et al., 2010
	in-R3	AGGACCATCACCATTGACATATTGAAACC			
S. schleiferi subsp.	sch-F	AATGGCTACAATGATAATCACTAA	526	56	Sasaki et al., 2010
	sch-R	CATATCTGTCTTTCGGCGCG			

Data analysis

The data obtained in this study were processed and analyzed using Microsoft Excel 2016 software. The statistical analysis was performed using the Chi-square test in Minitab version 16.0 software.

RESULTS

Isolation of *Staphylococcus* spp.

The results of nasal carriage of *Staphylococcus* spp. in 123 sampled dogs are presented in Table 2.

Table 2 The prevalence of nasal *Staphylococcus* carriage in the sampled dogs

Survey targets		Disease dogs (N = 83)		Heathy dogs (N = 40)		Total (N=123)	
		n/N	%	n/N	%	n/N	%
Breed	Local	7/15	46.67	3/11	27.27	10/26	38.46
	Cross	31/49	63.27	11/22	50.00	42/71	59.15
	Foreign	12/19	63.16	5/7	71.43	17/26	65.38
Sex	Male	26/40	65.00	9/19	47.37	35/59	59.32
	Female	24/43	55.81	10/21	47.62	34/64	53.13
Confinement	Free-ranging	13/27	48.15	0/5	0.00	13/32	40.63
	Semi-confined	20/35	57.14	14/27	51.85	34/62	54.84
	Confined	17/21	80.95	5/8	62.50	22/29	75.86
Age (years)	< 1	12/26	46.15	7/19	36.84	19/45	42.22
	≥ 1	38/57	66.67	12/21	57.14	50/78	64.10
Total		**50/83**	**60.24**	**19/40**	**47.50**	**69/123**	**56.10**

Note: N – total samples, n – Staphylococci samples

Table 2 shows that the average isolation rate of Staphylococci from the collected dogs was 56.10%. The isolation rate in dogs with respiratory symptoms (60.24%) was higher than in healthy dogs (47.50%). The results of *Staphylococcus* spp. isolation in confined dogs had the highest infection rate (75.86%), followed by semi-confined dogs (54.84%), and the lowest in free-ranging dogs (40.63%). The difference was statistically significant ($P<0.05$). Additionally, the results presented in Table 2 indicate that dogs over one-year-old had a Staphylococci detection rate of 64.10%. A statistically lower isolation rate (42.22%, $P<0.05$) was observed in dogs under one-year-old. Therefore, the results suggest that the factors of confinement and age affect the prevalence of *Staphylococcus* spp. in dogs.

Species identification of *Staphylococcus* isolates

Out of the total 87 staphylococcal isolates obtained from samples of 19 healthy and 50 sick dogs, 48 (55.17%) were successfully identified at the species level by the multiplex PCR (Figure 1). The distribution of species in different groups is shown in Table 3.

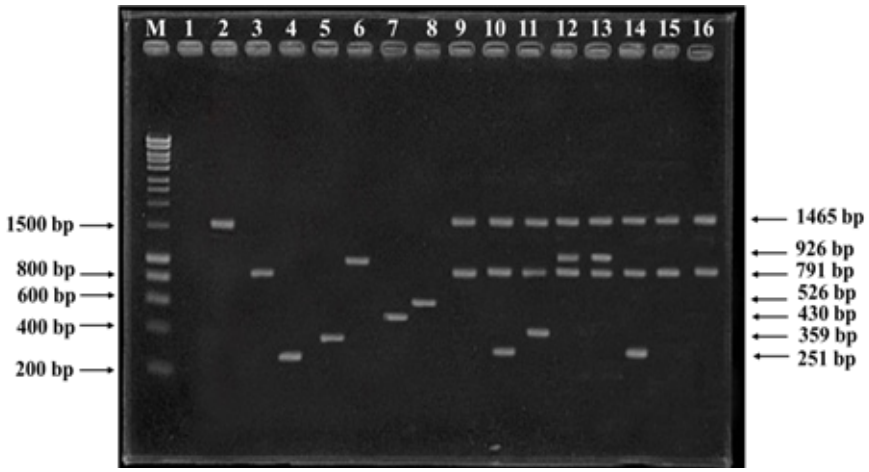

Figure 1 Multiplex PCR for species identification of staphylococcal isolates. Lane M: 1 kbp ladder; lane 1: negative control; lane 2: 16s rRNA, lane 3: 16s RNA staphylococcal; lane 4: positive control, *S. epidermidis*; lane 5: positive control, *S. aureus*; lane 6: positive control, *S. pseudintermedius*; lane 7: positive control, *S. intermedius*; lane 8: positive control, *S. schleiferi*; lanes 9–16: isolates.

Table 3 Distribution of *Staphylococcus* species isolated from dogs

Survey targets		N	*Staphylococcus* spp.							
			S. aureus		*S. pseudintermedius*		*S. epidermidis*		Other	
			n	%	n	%	n	%	n	%
Breed	Local	13	0	-	5	38.46	0	-	8	61.54
	Cross	53	4	7.55	22	41.51	2	3.77	25	47.17
	Foreign	21	0	-	15	75.00	0	-	6	30.00
Sex	Male	45	3	6.67	21	46.67	1	2.22	20	44.44
	Female	42	1	2.38	21	50.00	1	2.38	19	45.24
Confinement	Free-ranging	18	1	5.56	12	66.67	0	-	5	27.78
	Semi-confined	39	3	7.69	14	35.90	0	-	22	56.41
	Confined	30	0	-	16	53.33	2	6.67	12	40.00
Age (years)	< 1	24	1	4.17	7	29.17	0	-	16	66.67
	≥ 1	63	3	4.76	35	55.56	2	3.17	23	36.51
Total		87	4	4.60	42	48.28	2	2.30	39	44.83

Note: N – total Staphylococci isolates, n – Staphylococci species

In this study, a multiplex PCR method was employed to detect five species of Staphylococci. However, the results revealed the presence of only three species: *S. aureus*, *S. pseudintermedius*, and *S. epidermidis*. Table 3 showed that *S. pseudintermedius* was the most frequently isolated species (48.28%). A low proportions of the isolates were identified as *S. aureus* (4.60%) and *S. epidermidis* (2.30%).

The detection rate of *S. pseudintermedius* differed significantly between age groups. Among dogs over one year old, the rate was 55.56%, whereas in dogs under one year old, it was 29.17%. This difference was statistically significant ($P<0.05$), indicating a notable variation in the prevalence of *S. pseudintermedius* among these age groups.

Coagulase test results of the isolates

Out of 87 staphylococcal isolates, 80.46% gave coagulase-positive results (Table 4). Among the coagulase-positive Staphylococci (CoPS) strains, *S. pseudintermedius* was more frequently isolated from sick dogs (36/36). Besides, coagulase-negative Staphylococci (CoNS) species accounted for 27.27% (6/22) of all isolates in healthy dogs was higher than in sick dogs (Table 4).

Table 4 Coagulase test results of staphylococcal isolates

Coagulase test	Diseased dogs (N=65)		Heathy dogs (N=22)		Total (N=87)	
	n/N	%	n/N	%	n/N	%
CoPS	54/65	83.08	16/22	72.73	70/87	80.46
S. aureus	4/4	100.00	0	-	4/4	100.00
S. pseudintermedius	36/36	100.00	6/6	100.00	42/42	100.00
Other Staphylococci	14/23	60.87	10/16	62.50	24/39	61.54
CoNS	11/65	16.92	6/22	27.27	17/87	19.54
S. epidermidis	2/2	100.00	0	-	2/2	100.00
Other Staphylococci	9/23	39.13	6/16	37.50	15/39	38.46

Note: *CoPS: coagulase-positive Staphylococci, CoNS: coagulase-negative Staphylococci.*

DISCUSSION

Staphylococci are opportunistic bacteria found in canine. In this study, 87 isolates were collected from the nasal cavities of dogs exhibiting respiratory symptoms, as well as healthy dogs. Staphylococci naturally inhabit the nasal cavity of healthy dogs as part of their microbiota. However, they can also act as opportunistic agents, causing various infections such as pyoderma, soft tissue infections, urinary tract infections, respiratory infections, and ear infections (Abdullahi et al., 2021; Bertelloni et al., 2021; Lynch and Helbig, 2021; Ruiz-Ripa et al., 2021). Close contact between humans and Staphylococci-infected dogs can result in human infections (Lozano et al., 2017; Carroll et al., 2021). Globally, the prevalence of *S. aureus* and *S. pseudintermedius* nasal carriage among healthy dogs was found to be 10.9% and 18.3%; these figures varied across different regions: Africa (15.3% and 15.4%); Asia (8% and 18.9%); America (6.7% and 17.6%); Europe (7.3% and 18.2%) and Oceania (0% and 44.4%) (Abdullahi et al., 2022). Numerous studies have demonstrated the transmission of Staphylococci between humans and dogs (Loeffler et al., 2005; Sahin-Tóth et al., 2021; Cuny et al., 2022).

The detection rates of Staphylococci in dog nasal fluid samples varied across different studies and regions. In Africa, the study conducted by Elnageh et al. (2020) reported a detection rate of 29%, while Suepaul et al. (2021) in the Americas found a higher rate of 53.4%. Another study by Santana et al. (2023) in the Americas focused on 501 samples from 54 dogs in an intensive care unit therapy, revealing a very high detection rate of Staphylococci at 94%. In Can Tho, Vietnam, Ly (2017) conducted research on dogs with respiratory diseases and found that *Staphylococcus* accounted for the highest percentage at 39.07%. In Ha Noi, Vietnam, Nguyen (2017) detected 3.33% (4/120) positive with MRSA in dogs. The study conducted

by Hoekstra and Paulton (2002) examined the prevalence of *S. aureus* and *S. intermedius* in 325 dogs, considering gender and age. Their findings aligned with the isolation rates of *Staphylococcus* spp. in our study. They reported that male dogs had a higher isolation rate at 54.46% compared to female dogs at 45.54%. Additionally, the age group under one year old had a lower isolation rate at 32.92% compared to the group over one year old at 67.08%. On the other hand, Magalhães et al. (2010) did not observe any significant influence of breed or sex on the colonization of *Staphylococcus* in their study. In our study, we also observed differences in the occurrence of *S. pseudintermedius* among different age groups of animals.

In our study, approximately 50% of the isolates were identified as *S. pseudintermedius*. This finding is consistent with the research conducted by Suepaul et al. (2021) in Trinidad, where they detected 440 strains of *Staphylococcus* from 112 dogs. Among CoPS species, the SIG group (consisting of *S. pseudintermedius*, *S. intermedius*, and *S. delphini*) was the most frequently detected (38.7%), followed by *S. aureus* (2.1%). However, the higher detection rate of *S. pseudintermedius* in our study could be attributed to geographical and temporal differences in the study. These results are in line with the findings of Bertelloni et al. (2021), who identified 50 strains of *Staphylococcus* from sick dogs and found that *S. pseudintermedius* was the predominant species (52%). Similarly, Ruiz-Ripa et al. (2021) conducted a study on 28 sick dogs from 2009 to 2011 at a veterinary hospital in Spain, and their results showed that *S. pseudintermedius* was frequently isolated among the SIG species. Besides, our results are consistent with the results of (Boost et al., 2008) and (Tarazi et al., 2015) which reported *S. aureus* isolation rates of 8.8% in dogs from Hong Kong and 12.7% in dogs from Jordan.

Research results about the coagulase test of Staphylococci in our respiratory swab sample in contrast to some previous studies. It has been observed that dogs can carry both CoPS and CoNS species (Gandolfi et al., 2013). CoPS is known to be an important pathogen in both humans and animals, while CoNS can act as opportunistic or commensal agents (Heilmann et al., 2019). *Staphylococcus* is a diverse genus comprising at least 71 species, categorized into coagulase-positive Staphylococci and coagulase-negative Staphylococci (Parte et al., 2020). In Australia, Ma et al. (2020) isolated three CoPS species (*S. pseudintermedius*, *S. aureus*, *S. intermedius*) and 20 species of CoNS from mucocutaneous sites of dogs. In the UK, Loeffler et al. (2005) conducted a study on 270 nasal and oral swab samples and found 118 CoPS and 152 CoNS. Elnageh et al. (2020) identified 38 strains, including 24 species of CoNS and 14 species of CoPS. In contrast to our findings, Suepaul et al. (2021) reported that most of the *Staphylococcus* species isolated from dogs were CoNS (50.2%), followed by CoPS (41.7%), with the lowest occurrence observed in coagulase variable Staphylococci (CoVS). These variations in isolation results of *Staphylococcus* spp. could be attributed to differences in research sites and surveyed subjects, highlighting the potential influence of location and study population on the prevalence and distribution of *Staphylococcus* species.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

Nguyen Thi Lan Anh, Lam Thanh Nguyen: Conceptualization and design the experiment, investigation, supervision, editing and finalization. **Nguyen Vu Thuy Hong Loan, Bui Nguyen Thanh Vy, Dao Huyen Tran, Nguyen Thuy Y Vi, To My Quyen:** Investigation, methodology, formal analysis, manuscript preparation.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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How to cite this article;

Nguyen Thi Lan Anh, Nguyen Vu Thuy Hong Loan, Bui Nguyen Thanh Vy, Dao Huyen Tran, Nguyen Thuy Y Vi, To My Quyen, Lam Thanh Nguyen. Detection of Staphylococci from nasal samples from healthy and sick dogs in Ho Chi Minh City, Vietnam. Veterinary Integrative Sciences. 2024; 22(3): 871 - 881