

Original Research Paper

# Detection of *Staphylococcus pseudintermedius* in Dogs and Cats in Selangor, Malaysia

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**Abstract:** *Staphylococcus pseudintermedius* is an emerging coagulase gram-positive staphylococci in dogs and cats. It has now become a threat to animal health due to its multidrug resistance leading to very limited therapeutic options particularly in the treatment of small animals and therefore, requires urgent action to control its spread. The organism is currently recognised as a significant pathogen in veterinary medicine causing skin, ear and wound infections in dogs and cats. So far, there is limited coverage in relation to research studies concerning on *S. pseudintermedius* in dogs and cats in Malaysia. Therefore, this study was conducted to determine the presence of *S. pseudintermedius* in dogs and cats as well as to evaluate the best site for isolation of the organism. A total of two hundred dogs (n = 100) and cats (n = 100) were randomly sampled. Swab samples were taken from the nasal cavity, buccal cavity, rectum and perineal skin aseptically from both pet and stray dogs and cats. The samples were cultured and isolates were identified by biochemical tests and confirmed using a species-specific PCR assay. Our results revealed that the proportion of detection of *S. pseudintermedius* was higher in stray dogs and cats than pet animals. *Staphylococcus pseudintermedius* was detected in 6.5% (13/200) stray dogs, 6% (12/200) stray cats, 1.5% (3/200) pet dogs and 0% in pet cats. Our findings also demonstrated that nasal cavity was the ideal site for isolation of the organism (5.5%), followed by buccal cavity (3.5%), skin (2.5%) and rectum (2.5%), but statistically, the difference was not significant ( $p = 0.174$ ). In conclusion, this study provided an empirical evidence in relation to the detection of *S. pseudintermedius* in stray and pet dogs and cats in Selangor, Malaysia. Future research is urgently needed to better understand the epidemiology of *S. pseudintermedius* in field settings.

**Keywords:** *Staphylococcus pseudintermedius*, Prevalence, Dogs, Cats, Malaysia

## Introduction

*Staphylococcus pseudintermedius* (*S. pseudintermedius*) is an emerging opportunistic coagulase gram-positive pathogen in dogs and cats. The organisms are commensals on the skin and mucous membranes of humans and a wide range of mammalian species. In most cases, the infection is manifested as topical infections and otitis externa in pet animals (Weese and van Duinkerken, 2010).

The clinical significance of *Staphylococcus* species is quite variable, with some being important causes of diseases and other minimally pathogenic organisms. Among the important species of *Staphylococcus* in animals are *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus pseudintermedius*.

Until recently, *Staphylococcus pseudintermedius* was described as a new coagulase-positive *Staphylococcus* species based on 16S rRNA gene sequence analysis of

isolates from a wide range of animals that include cats, dogs, horses and parrots (Devriese *et al.*, 2005; Sasaki *et al.*, 2007a). The organism was grouped and classified as a member of a genetically heterogeneous group of bacteria called *Staphylococcus Intermedius* Group (SIG) which consists of three related species: *S. intermedius*, *S. pseudintermedius* and *S. delphini* (Sasaki *et al.*, 2007b). Since then, *S. pseudintermedius* is recognized as the species which colonizes and causes infections in pet dogs and cats more than the other staphylococcal species (Perreten *et al.*, 2010). A number of animal body sites can be colonized by the organism, especially the nasal passages, oral cavity, skin and perineal mucosa (Hartmann *et al.*, 2005; Fazakerley *et al.*, 2010). In Malaysia, there is limited coverage in relation to research studies concerning on *S. pseudintermedius* in dogs and cats. Therefore, this study was undertaken with the objectives of determining the prevalence of *S. pseudintermedius* along with evaluating the ideal sites for the isolation of the organism in pet and stray dogs and cats.

## Materials and Methods

### Samples Collection

A total of 200 animals consisting of 100 pet and stray dogs and 100 pet and stray cats were sampled. The stray dogs and cats were from a municipality council animal pound and an animal shelter. The pet dogs and cats were from a University Veterinary Hospital (UVH) and private clinics. For pet animals, owners were individually approached with a consent form to take samples from their pets. A total of 50 stray dogs were sampled from animal pound and 50 pet dogs were from private clinics. A total of 50 stray cats were sampled from an animal shelter and 50 pet cats were from the hospital. In each animal, samples were taken from the skin, nasal cavity, buccal cavity and rectum using different sterile cotton swabs moistened with normal saline for each of the site on/in the animal. A cotton swab was rubbed gently on the skin at the perineal part of the body. Each nasal swab was taken by rubbing the swab against the nasal cavity for at least 5 sec. Rectal swab was taken by inserting a swab into the rectum by touching and rubbing the mucous membrane. The sample from oral cavity was done by inserting a swab into the buccal cavity and rubbing the mucous membranes. Each swab was placed in a Bijou bottle containing Tryptone Soya broth (Oxoid) as enrichment media and kept cool during transportation to the laboratory. All the enrichment broth containing the swabs were incubated at 37°C overnight.

### Bacterial Isolation and Identification

Of 10 µL of enriched culture was streaked onto Mannitol salt agar plate (Oxoid) and incubated

overnight at 37°C for 24 h. The suspected colonies of *Staphylococcus* on the agar plates were examined by observing the colonial morphology and colour (yellow colonies). Presumptive *Staphylococcus* colonies were sub-cultured onto Columbia blood agar (Oxoid) with 5% horse blood added and were incubated at 37°C for 24 h. Suspected *S. pseudintermedius* isolates were identified on the basis of colony characteristics, pigment production, Gram-stained appearance and hemolysis. All suspected *S. pseudintermedius* isolates were further tested for catalase, coagulase, DNase, O-Nitrophenyl-beta-D-Galactopyranoside (ONPG), Arginine Dihydrolase (ADH) and acetoin production. All the suspected isolates were stored in skim milk powder broth (Oxoid) at -80°C until used.

### Genotypic Confirmation of Isolates

#### DNA Extraction

Stored frozen isolates were thawed and each was streaked onto Columbia Blood agar plate with 5% horse blood added and incubated for 24 h at 37°C. DNA was extracted by using boiling method. In brief, 1 mL of sterile distilled water was transferred to a sterile 1.5 mL Eppendorf tube and a loopfull of the isolate was picked from a plate and transferred into the Eppendorf tube. The mixture was vortexed for 1 min and then heated in a dry bath at 96°C for 10 min. The mixture was cooled down for 5-10 min to room temperature and centrifuged at 13000×g for 3 min. The pellet was used as DNA template for multiplex PCR (mPCR) assay.

#### Multiplex PCR Assay Procedure

The mPCR assay was performed as described by Sasaki *et al.* (2010). The reaction mixture for PCR consisted of 5 µL DNA extract, 25 µL MyTaq Red Reaction Buffer (Bioline), 18 µL deionized water and 2 µL of the primers (forward and reverse) in a total volume of 50 µL. The following PCR conditions were used: heat denaturation at 95°C for 2 min, 30 cycles with denaturation at 95°C for 30 s, annealing at 56°C for 35 s, extension at 72°C for 1 min and a final extension at 72°C for 2 min. the PCR amplification was done as described earlier by Sasaki *et al.* (2010), with the following sequences: NucA1, 5'-TRGGCAGTAGGATTCGTAA-3' and NucA2, 5'-CTTTTGTGCTYCMTTTTGG-3'. The reference strain *S. pseudintermedius* (CCUG 49543) was used as a positive control. In addition, 5 µL of sterile deionized distilled water was used as a negative control. DNA fragments were analysed by electrophoresis in 1\_ Tris-acetate-EDTA on a 1% agarose gel stained with ethidium bromide.

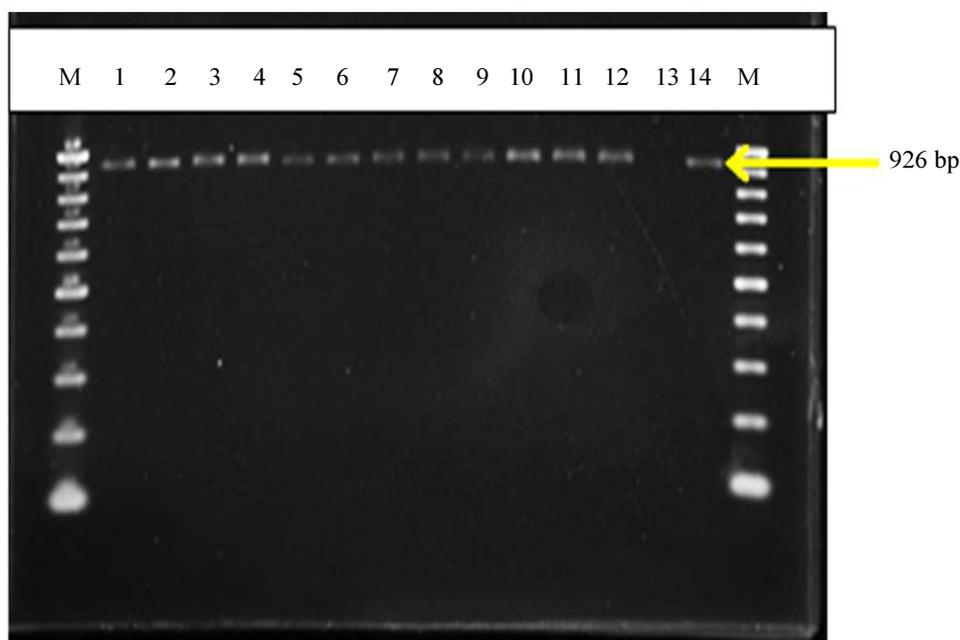
### Data Analysis

Data was analysed using statistical package SPSS software 20 ((IBM SPSS Version 20) and Openepi software (<http://www.openepi.com/>). The differences in the proportion of positive samples between stray and pet animals and also between the different sites of isolation were analysed using Chi-square test. The results were considered statistically significant at  $p \leq 0.05$  on 95%CI.

### Results

From a total of 100 dogs and 100 cats sampled, 28 (3.5%) *S. pseudintermedius* were isolated; of these, 13

(6.5%) isolates were from stray dogs, 12 (6%) isolates from stray cats, 3 (1.5%) isolates from pet dogs and none (0%) from pet cats as shown in Table 1. There was a statistical significant difference between stray and pet animals ( $p = 0.001$ ). A total of 11 (5.5%), 7(3.5%), 6(3%) and 5(2.5%) isolates from dogs and cats were exclusively from nasal cavity, buccal cavity, skin and rectum, respectively as shown Table 2. The overall isolation rates of *S. pseudintermedius* from these sites did not show any significant difference ( $\chi^2 = 4.973$ ,  $p = 0.174$ ). In the m-PCR assay, all the suspected isolates were confirmed as *S. pseudintermedius*, showing bands at 926 bp similar to the reference strain *S. pseudintermedius* (CCUG 49543) (Fig. 1).



**Fig. 1:** m-PCR on *S. pseudintermedius* Lane M: marker 100 bp ladder, Lane 1 to 12: *S. pseudintermedius* isolates, Lane 13: negative control, Lane 14: *S. pseudintermedius* CCUG 49543 as positive control

**Table 1:** Detection of *S. pseudintermedius* in dogs and cats

Animals (%)	No. of animals	Total no. of samples collected	Positive for <i>S. pseudintermedius</i> No.
Stray dogs	50	200	13(6.5)
Stray cats	50	200	12(6)
Pet dogs	50	200	3(1.5)
Pet cats	50	200	0(0)
Total	200	800	28(3.5)

**Table 2:** Occurrence of *S. pseudintermedius* at each sites on/in dogs and cats

Sites of isolation	No. (%) of <i>S. pseudintermedius</i> isolated			
	Stray dogs N = 13	Stray cats N = 12	Pet dogs N = 3	Pet cats N = 0
Nasal cavity	3(6%)	6(12%)	2(4%)	0(0%)
Buccal cavity	3(6%)	3(6%)	1(2%)	0(0%)
Rectal swab	4(8%)	1(2%)	0(0%)	0(0%)
Skin swab	3(6%)	2(4%)	0(0%)	0(0%)

## Discussion

To our knowledge, this is the first study carried out on *S. pseudintermedius* in dogs and cats in Malaysia. A total of 28 (3.5%) *S. pseudintermedius* isolates were identified from 200 dogs and cats sampled. The result showed a high number of stray animals were colonized with *S. pseudintermedius* compared to pet animals, 6.5% in stray dogs, 6% in stray cats, 1.5% in pet dogs and 0% in pet cats. This is probably because the stray animals are more exposed to open environment and thus frequently in contact with other animals from many sources including animal shelter that suffer from high density, suboptimal cleaning and disinfection of its facilities as well as the stress of the environment (Miller, 2004).

The colonization of *S. pseudintermedius* in companion animals, especially dogs varies greatly on the parts of the body (Hanselman *et al.*, 2009). According to Fazakerley *et al.* (2010) and Hartmann *et al.* (2005), various body sites can be colonized, particularly the nasal passages, oral cavity, skin and perineal mucosa. Up to date, only few studies, if any, determined the *S. pseudintermedius* colonization pattern on four parts of the animal body. Sampling from different sites on the same animal is time consuming and resource demanding particularly if large numbers of animals are involved. This study showed that the nasal cavity (5.5%) and buccal cavity (3.5%) were the most frequently colonized body sites compared to perineal skin (2.5%) and rectum (2.5%). The occurrence of *S. pseudintermedius* in nasal cavity in this study was less than that reported in Spain (23%) (Gómez-Sanz *et al.*, 2013), Germany (13.9%) (Walther *et al.*, 2012), but more than that reported in healthy dogs in Canada (0.7%) (Rubin and Chirino-Trejo, 2011). The prevalence of *S. pseudintermedius* in the buccal cavity was less than that in the study of Paul *et al.* (2012) which was 65%. The prevalence on the perineal skin was considered very low compared to that reported by Fazakerley *et al.* (2010) in both dogs having atopic dermatitis (87%) and the healthy ones (37%). However, it should be considered that *S. pseudintermedius* causes skin infections such as pyoderma mostly in dogs and cats. The prevalence of *S. pseudintermedius* in rectum is very low compared to the work of Rubin and Chirino-Trejo (2011) who reported at 11.1%. The difference in the reported prevalence in this and other studies were due to different geographical location, sampling and isolation procedures and animal breed. The knowledge of common sites of colonization is essential for conducting surveillance; this is because failing to sample relevant sites may result in false-negatives and thus underestimation of its prevalence. Conversely, the increased expense and time required for excessive sampling are undesirable.

Although the prevalence was low compared to other studies, the presence of *S. pseudintermedius* in dogs and cats could be a potential source of zoonotic infection in humans as evidenced by recent outbreaks in which veterinary personnel and pet owners involved (Paul *et al.*, 2011). Therefore, we strongly recommend strict sanitary measures with the highest level of international excellency to be maintained when contacting infected animals.

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## Authors' Contributions

**Mohamed Abdelrahman Mohamed:** Conducted the study, data analysis and drafted the manuscript.

**Saleha Abd Aziz, Gurmeet Kaur Dhaliwal and Siti Khairani Bejo:** Designed, supervised and coordinated the research.

**Muhammad Luqman Nordin, Rumaizi Shaari, Sharifo Ali Elmi, Abubakar Abdulkarim Kanamma, Mohammed Dauda Goni and Abdinasir Yusuf Osman:** Provided valuable input in data analysis and interpreted the data, revised the manuscript, commented on and approved of the final version.

## Ethics

Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Universiti Putra Malaysia.

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