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Predictors of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* infections at a veterinary hospital in Malaysia

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ABSTRACT

Aims: This study was aimed to identify the risk factors associated with the spread of *Staphylococcus aureus* and *S. pseudintermedius* isolated from dogs, cats and humans who visited a veterinary hospital.
Methodology and results: A questionnaire was employed to address potential risk factors among dogs, cats and their owners, with a total of 375 samples collected. The data from the questionnaire was analysed using Fisher's exact test and multivariable logistic regression. Six (4.8%) owners, 7 (9.3%) pet dogs and 4 (8%) pet cats were positive for *S. pseudintermedius* and 2 (4%) pet cats and 2 (1.6%) owners were positive for *S. aureus.* Keeping other pets in a house where humans also live and cats that lick the face of owners were among the significant risk factors in the carriage of *S. pseudintermedius.*
Conclusion, significance and impact of study: It was found that the presence of other pets in the home and the habit of cats licking the faces of their owners were among the risk factors that resulted in a significant association with *S. pseudintermedius* in pet cats. Research findings suggest that the close contact between pets and their owners resulted in a higher rate of colonisation of *S. aureus* and *S. pseudintermedius*.

Keywords: Close contact, pet owners, pets, questionnaire, risk factors

INTRODUCTION

Colonising germs can operate as endogenous reservoirs, such as overt clinical diseases or spread to others. Previous or present antibiotic therapy, hospital admission, surgery, contact with healthcare workers (HCWs), needle injections and persistent skin lesions can all be risk factors in hospital settings (Halablab *et al.*, 2010; Cuny *et al.*, 2022).

There are several factors that are responsible for the increased risk factor of getting colonised with hospital-associated-MRSA (methicillin-resistant *Staphylococcus aureus*), such as long-term hospitalisation, overcrowding, understaffing, intensive care unit (ICU) admission, nursing home residency, antibiotic exposure (particularly cephalosporins and fluoroquinolones, which cause antibiotic selection pressure), surgery, haemodialysis, chronic wounds, advanced age, use of topical corticosteroids, having contact with an MRSA carrier or indwelling invasive devices (Epstein *et al.*, 2016; Abad *et al.*, 2020; Rampal *et al.*, 2020). Understanding colonisation dynamics, transmission routes, risk factors for disease and situations that favour resistance evolution will improve *S. aureus* control measures (Lee *et al.*, 2018).

Dog and cat risk factors are highly comparable to human risk factors (Magalhães *et al.*, 2010); the number of antimicrobial courses completed, recent veterinary hospitalisation days and surgical implants received are among the risk factors in dogs and cats (Loncaric *et al.*, 2019). Furthermore, one of the significant risk factors for MRSA infection in pets is interaction with humans who have been sick and admitted to the hospital (Magalhães *et al.*, 2010; Hogan *et al.*, 2019).

The conditions and situations for being considered a risk factor for *S. pseudintermedius* are similar to *S. aureus* (Magalhães *et al.*, 2010). Antimicrobial therapy, surgical procedures and wound infections in hospitalized animals are among the suspected risk factors for MRSP (methicillin-resistant *S. pseudintermedius*) infection in pets. Additionally, antimicrobial medications administered within 30 days before sampling, regular visits to veterinary

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clinics, pets receiving topical medicines and chronic diseases have also been implicated. These findings pointed to a link between MRSP infection and veterinary clinic/hospital environments (Lehner *et al.*, 2014; Lai *et al.*, 2022).

Besides, MRSP has public health consequences since it can be transmitted through direct and indirect contact. Although MRSP infections in humans have been reported on rare occasions, its zoonotic transmission has increased in recent years as pet ownership and close contact between humans and pets increased (Campanile *et al.*, 2007; Moses *et al.*, 2023).

In Malaysia, there are limited studies regarding the risk factors associated with MRSA and MRSP (Al-Talib *et al.*, 2010; Hamzah *et al.*, 2019; Afshar *et al.*, 2023); finding these risk factors will definitely help veterinarians, pet owners and policymakers to reduce the occurrence and transmission of MRSA and MRSP. Therefore, this study aims to address predictors related to the carriage of *S. aureus* and *S. pseudintermedius* among pet dogs, pet cats and their owners who visited a university veterinary hospital in Malaysia.

MATERIALS AND METHODS

Data source and inclusion criteria

The animal and human studies were approved by the Institutional Ethics Committee of the Institutional Animal Care and Use (AUP 101) and the Ethics Committee for Research Involving Human Subjects (approval number: JKEUPM-2020-191), respectively.

One hundred and fifty oral swabs were collected from the pet, and stray dogs (75 samples from pet dogs and 75 samples from stray dogs), 100 oral swab samples were collected from the pet and stray cats (50 samples from pet cats and 50 samples from stray cats) and 125 nasal samples were obtained from the nasal cavity of pet owners.

Animals included in this study had not received any antibiotics for at least 14 days before sampling with no visible signs of pyoderma, sneezing and nasal discharge, except for stray cats and dogs with no history of antimicrobial use.

One hundred and twenty-five pet owners consented to participate in the study and therefore were given the questionnaire. The pet owners were also guided for any inquiries they had regarding the questions.

Isolation and identification of MRSA and MRSP

For isolation of bacteria, the oral and nasal swabs were placed into brain heart infusion (BHI) broth (Oxoid™, UK) supplemented with 6.5% NaCl, followed by 24 h of incubation at 37 °C. A loopful of this suspension was transferred onto an oxacillin-resistant staphylococcal agar base (ORSAB, Oxoid™, UK) and incubated for 24 h at 37 °C. Gram staining reaction, coagulase test, catalase test and DNase test were used to identify Gram-positive, coagulase-positive Staphylococci (Gómez-Sanz *et al.*, 2011; Mohamed *et al.*, 2017).

The *nuc*A gene was utilised to confirm the presumptive *S. aureus* and *S. pseudintermedius*. The reaction was performed in a thermal cycler (Eppendorf® pro S, Germany) under the PCR (polymerase chain reaction) cycling condition described earlier (Baron *et al.*, 2004; Sasaki *et al.*, 2010). The *mec*A gene was used to confirm methicillin resistance under the thermal conditions described by Strommenger *et al.* (2003). *S. aureus* ATCC 25923 and *S. pseudintermedius* CCUG 49543 were exploited as positive controls.

Questionnaire

The questionnaire was adapted from Boost *et al.* (2008) and Loeffler *et al.* (2010) with some minor alterations. In August 2020, the sampling process and distribution of the questionnaires to 125 pet owners began.

Data analysis

The data obtained from participants were entered into Microsoft Excel 2019 and coded for further analysis in other software. Next, the data was imported into the statistical software [SPSS version 27.0 (SPSS Inc., Chicago, IL, USA)]. The close-ended questions contained categorical data; therefore, Fisher's Exact test was used to find the association between *Staphylococcus* carriage and risk factors. In two steps, univariable and multivariable models were employed to explore the association between the predictors of *Staphylococcus* spp. infections. A univariable logistic regression model was fitted in the first stage to determine the relationships between the health condition of owners in the last six months, pet age, breed and the outcome variable, *Staphylococcus* carriage. A relaxed *p*-value of 0.20 was used to find the potential predictors of *Staphylococcus spp*. infections at this stage. As a result, in the second phase, variables with a *p*-value of 0.20 in the univariable model were taken into consideration for inclusion in the multivariable model. Since the significance level was chosen at a *p*-value of 0.05, the second stage involved fitting a multivariable logistic regression model using the enter approach. For factors included in the final model, odds ratios (O.R.s) and their 95% confidence intervals were calculated (Qekwana *et al.*, 2017).

RESULTS

The results of this study revealed that dogs and pet owners carried *S. pseudintermedius* more than *S. aureus* while pet cats carried both of these organisms (Table 1).

The results of the analysis of putative risk factors for a total of 125 pets and 125 owners (50 pet cat owners, 75 pet dog owners, 50 pet cats and 75 pet dogs) were performed. Due to not having any history, stray dogs and cats were excluded from the risk factor analysis.

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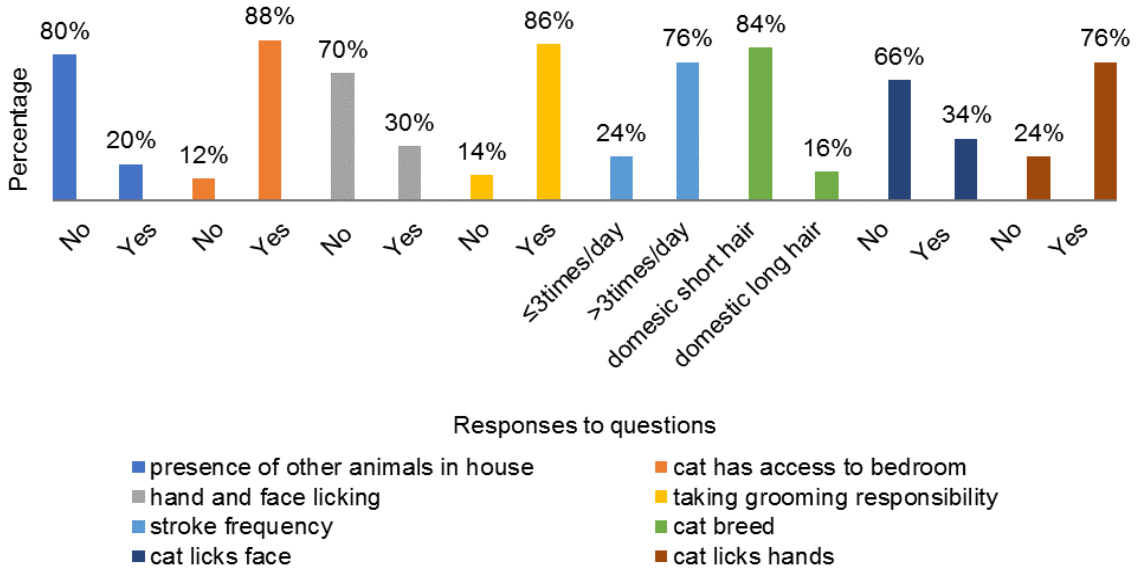

Figure 1: Percentage of factors that resulted in identifying more *S. pseudintermedius* isolates in 50 cats.

Table 1: Confirmed *S. aureus* and *S. pseudintermedius* isolates from different hosts.

Sample source (n)	Confirmed SA isolates n (%)	Confirmed SP isolates n (%)	Methicillin resistance	
			MRSA	MRSP
Pet dogs (75)	0 (0)	7 (9.3)	0 (0)	1 (1.3)
Pet cats (50)	2 (4)	4 (8)	1(2)	1 (2)
Pet owners (125)	2 (1.6)	6 (4.8)	0 (0)	1 (0.8)
Stray cats (50)	3 (6)	0 (0)	0 (0)	0 (0)
Stray dogs (75)	0 (0)	0 (0)	0 (0)	0 (0)

SA: *Staphylococcus aureus*; SP: *Staphylococcus pseudintermedius*; MRSA: Methicillin-resistant *Staphylococcus aureus*; MRSP: Methicillin-resistant *Staphylococcus pseudintermedius*.

There was no significant association between *S. aureus* isolates of pet cats and risk factors in the questionnaire.

Although being a domestic short-haired breed [2/32 (6%)], having more than one dog/cat at home [2/32 (6%)], licking face [2/17 (11%)] and hand [2/38 (5.2%)] resulted in detecting more *S. aureus* isolates, none of these factors were significantly associated with carriage of *S. aureus* isolated from pet cats (p-value>0.05). It should be noted that no cat owner was detected to be an *S. aureus* carrier.

Interestingly, having other animals in the house, as well as cats that used to lick the faces of owners, was significantly (0.05>p-value) associated with *S. pseudintermedius* carriage in pet cats.

Domestic short-haired pet cats had more frequency of carrying *S. pseudintermedius* [3/42 (7%)]; in addition, cats that accessed the bedroom [3/45 (6%)], cats that were stroked more than three times a day [3/38 (7%)], cats that used to lick hands [4/38 (10%)] and cats that their owner took the responsibility of their grooming [4/44 (9%)] had a higher prevalence of carrying *S. pseudintermedius* than the other categories (Figure 1 and Table 2). In contrast, no cat owners were detected to carry *S. pseudintermedius*.

There were only two *S. aureus* isolated from pet dog owners, but no pet dogs were detected to be carriers of *S. aureus.* Therefore, no association between risk factors and carriage of *S. aureus* was recorded (p-value>0.05).

Thirteen *S. pseudintermedius* were isolated from dogs and their owners (six dog owners and seven pet dog isolates). However, there was no significant association between the risk factors mentioned in the questionnaire and *S. pseudintermedius* carriage (p-value<0.05) in pet dogs and their owners.

There were; however, a few factors that resulted in higher prevalence of *S. pseudintermedius* in owners and pet dogs, such as having more than one dog/cat in the house was also a sign of higher carriage [9/38 (23%)], allowing a pet to roam outside the home [9/49 (18%)], access to the bedroom [9/40 (22.5%)], stroking more than three times a day [9/60 (15%)], taking the grooming responsibility [11/7 (15.7%)] and hand licking [13/65 (20%)].

Finally, logistic regression was performed to determine the health condition of owners in the last six

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Table 2: Distribution of Staphylococci isolates and results of the Fisher's exact test showing predictors of *S. pseudintermedius* infection among cats tested at the Veterinary Teaching Hospital, UPM.

Variables		*S. pseudintermedius* (%)		*p*-value*
		Negative	Positive	
Animal-related questions				
Cat gender	Male	24(52.2)	1(25)	0.60
	Female	22(47.8)	3(75)	
Cat breed	Domestic short hair	39(84.8)	3(75)	0.51
	Domestic long hair	7(15.2)	1(25)	
Number of dogs/cats in the house	One	17(37)	2(50)	0.62
	More than one	29(63)	2(50)	
Other animals in the house	No	39(84.8)	1(25)	0.02
	Yes	7(15.2)	3(75)	
Allowing outdoors	No	30(65.2)	2(50)	0.61
	Yes	16(34.8)	2(50)	
Daily location	Inside	39(84.8)	2(50)	0.14
	Outside	7(15.2)	2(50)	
Cat long-term illness	No	44(95.7)	3(75)	0.23
	Yes	2(4.3)	1(25)	
Having used antibiotics in the last six months	No	42(91.3)	3(75)	0.35
	Yes	4(8.7)	1(25)	
Cat travel in the last two months	No	41(89.1)	4(100)	1.00
	Yes	5(10.9)	0(0.00)	
Vaccination status up to date	No	4(8.7)	0(0.00)	1.00
	Yes	42(91.3)	4(100)	
Cat had wound	No	35(76.1)	3(75)	1.00
	Yes	11(23.9)	1(25)	
Nature of human-pet contact				
Cat access to the bedroom	No	4(8.7)	1(25)	0.35
	Yes	42(91.3)	3(75)	
Stroke frequency	Less than three times/day	11(23.9)	1(25)	1.00
	More than three times/day	35(76.1)	3(75)	
Type of food	Dry processed pet food	38(82.6)	2(50)	0.17
	Raw meat/animal product	8(17.4)	2(50)	
Washing cat dish	No	1(2.2)	0(0.0)	1.00
	Yes	45(97.8)	4(100)	
Washing hand after touching cat	No	0(0.0)	0(0.0)	-
	Yes	46(100)	4(100)	
Taking responsibility for grooming	No	6(13)	0(0.0)	1.00
	Yes	40(87)	4(100)	
Cat licks hands	No	12(26.1)	0(0.0)	0.56
	Yes	34(73.9)	4(100)	
Cat licks face	No	33(71.7)	0(0.00)	0.01
	Yes	13(28.3)	4(100)	
Kiss your cat	No	10(21.7)	0(0.00)	0.57
	Yes	36(78.3)	4(100)	

*The predictor variables with *p*-values<0.05 were considered to be statistically significant based on the Fisher's exact test.

months, pet age and breed. As a result, none of the above factors were significantly associated with the carriage of *S. aureus* and *S. pseudintermedius* in pet cats, pet dogs or their owners.

DISCUSSION

Staphylococcus is a genus of bacteria that naturally resides on the skin of animals and humans. *Staphylococcus* can cause severe infections in various tissues, including the skin, due to their ability to infect a wide range of hosts as well as humans and pets. Although companion animals are often in close contact with humans, our understanding of their significance remains limited as potential sources of human infections (Loncaric *et al.*, 2019).

In our study, licking the face and hand of pet owners show a higher prevalence of *S. aureus* among pet cats, which is in line with another study that revealed close contact with pets was associated with 20% carriage of *S. aureus* in pets; interestingly, the pets whose primary caretaker was an MRSA carrier were more likely to carry

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MRSA (Hogan *et al.*, 2019). Nevertheless, contrasting findings were reported by Bierowiec *et al.* (2016), who noted that a significant association (0.02) existed between having close contact (touching, kissing) with a pet and *S. aureus* carriage. These results, which agreed with our findings, disclosed that close contact between pet owners and pets can be a way of transmitting *S. aureus* from owners to pets or vice versa.

Having more than one dog/cat at home [2/32 (6%)] resulted in a higher prevalence of *S. aureus* in pet cats in our study. This is in line with other reports that found keeping other animals in the house associated with *S. aureus* carriage; for instance, a recent report from Pakistan concluded that keeping more than three dogs in a house was a potential risk of getting MRSA, but there was not a significant association, which is consistent with our findings (Shoaib *et al.*, 2020). however, keeping dogs in the house was significantly (p-value=0.007) associated with S. aureus carriage (Bierowiec *et al.*, 2016). These findings indicated that having numerous pets at home could increase the risk of getting infected with *S. aureus.* The increased risk could be due to crowdedness, which eventually led to more contact and, therefore, more transmission of bacteria among humans and their pets.

It is worth mentioning that one of the *S. aureus* isolates from pet cats in our study was methicillin-resistant. Our previous study (Afshar *et al.*, 2023) revealed that this isolate was sequence type (ST)789 by multilocus sequence typing (MLST), which was reported from humans of other countries like Switzerland, Myanmar, United Kingdom and Thailand as well (PubMLST, n.d.). Thus, these findings suggest the zoonotic transmission of this sequence type from humans to pets.

While *S. aureus* had a higher prevalence in domestic short-haired cats, it was not significantly associated with *S. aureus* carriage in pet cats or their owners. Similar results were obtained in an experiment conducted by Bierowiec *et al.* (2016), where there was no significant association between the breed of cats and their likelihood of carrying *S. aureus*; in addition, these results were supported by another study that stated a lack of association between the breed of cat and *S. aureus* carriage (Magalhães *et al.*, 2010). The higher prevalence of S. aureus in our study may be attributed to the more significant number of short-haired cats than long-haired cats, which was also recorded in other studies in the United States and Australia with non-significant associations (Ma *et al.*, 2019; Cotter *et al.*, 2023).

Remarkably, having other animals in the house is significantly associated with the carriage of *S. pseudintermedius* in pet cats. Having other animals in the house mostly leads to crowding, which eventually leads to more contact among dogs and cats, and more contact results in the dissemination of organisms; in addition, crowding can result in reduced hygiene and has a reputation for having an increased susceptibility to bacterial infections. Therefore, the greater concentration of people and pets could lead to more frequent physical contact among individuals, thereby promoting the spread of *S. pseudintermedius* within the population (Rynhoud *et al.*, 2021).

Even though *S. pseudintermedius* is more common in dogs, it has also been reported in other companion animals (Somayaji *et al.*, 2016b). *S. pseudintermedius* has been reported in dogs, rabbits, horses and cattle (Nielsen *et al.*, 2022; Morais *et al.*, 2023). This means that other companion animals can also carry *S. pseudintermedius*, which subsequently facilitates the transmission of *S. pseudintermedius* to humans and pet cats/dogs. Yet, other studies found no significant association between Staphylococci carriage and having other animals in the house (Boost *et al.*, 2008; Han *et al.*, 2016). The difference between our findings and others could be due to different geographic areas of study or transient localisation of Staphylococci in pets.

It is not a surprise that bacteria transfer from owners to pets or conversely, during contact with pets, as the extensive contact of pets with owners is common (Schwarz *et al.*, 2017). Our results show that there was a significant association between carriage of *S. pseudintermedius* in pet cats that used to lick the faces of their owners. Close contact with pets, which is now considered a potential risk factor for Staphylococci transmission, includes but is not limited to licking, kissing, biting, and allowing a pet to sleep in the bedroom (Morris *et al.*, 2017). Similar findings have been found by Cocca *et al.* (2021), who reported a high prevalence of *S. pseudintermedius* from pet dogs and cats, as *S. pseudintermedius* colonises typically in the skin and mucous membranes of pets. However, one thing that is not clear is whether pets transfer the bacteria to owners or vice versa. Since no cat owner was found to carry *S. pseudintermedius* in our study, the pet cats may have become colonised by other means, such as their surroundings or interactions with other animals.

Our findings revealed that other types of close contact, such as close contact (pet access to bedroom, hand, kissing of pet, grooming, stroking) with a pet in both dogs and cats resulted in a higher prevalence of *S. pseudintermedius*, which is also agreed with Han *et al.* (2016), who reported that spending time with a pet was not significantly associated with *Staphylococci* carriage (*p* value=0.14); moreover, having access to bedroom, having contact more than three times with a pet, and licking hands were reported by Lai *et al.* (2022), which were not significantly associated with *S. pseudintermedius* carriage in pets. As proximity between humans and animals has made it easier for *Staphylococcus aureus* to switch hosts (Hogan *et al.*, 2019), the same could be true for *Staphylococcus pseudintermedius*; therefore, it is essential to monitor and control the spread of resistant phenotypes at both local and global levels in order to identify and restrict the development of new resistant strains that can easily transfer between animals and humans (Smith *et al.*, 2020).

Our findings show that no pet cat owners carried *S. pseudintermedius*. It has recently been reported that close contact with pets, such as access to the bedroom

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and licking the hand or face of owners, were not significantly associated with MRSP carriage in humans (Ference *et al.*, 2019). Furthermore, this finding was also supported by the fact that colonisation of humans with *S. pseudintermedius* was not common even with frequent contact (Weese and Van Duijkeren, 2010; Somayaji *et al.*, 2016a; Solanki *et al.*, 2023).

Our results depict that no pet dogs carried *S. aureus* and only two pet dog owners carried this species. These results are aligned with the widely prevailing belief that humans are the primary source of transmitting *S. aureus* to pets (Mork *et al.*, 2020); moreover, only two pet dog owners in our study carried *S. aureus*, which may further explain why there was no pet dog carrier. It has been reported that *S. aureus* did not transmit easily in apparently healthy dogs, and exposure on its own might not result in the acquisition of *S. aureus* by dogs unless other contributing factors are present (Loeffler *et al.*, 2010). These findings are consistent with another study in Nigeria that reported a low carriage rate (5%) of *Staphylococcus* in apparently healthy dogs (Bata *et al.*, 2020). Another explanation for the presence of *S. aureus* in the pet dog owners and the absence of *S. aureus* in the oral cavity of the pet dogs in our study could be due to our sampling technique; we gathered oral samples from pet dogs and nasal samples from pet owners. It is suspected that the carriage rate in the mouth is lower than nares because the digestive enzymes could have inhibited *S. aureus* (Patterson *et al.*, 2017).

The use of antibiotics is considered a potential risk factor for *S. aureus* colonisation in dogs (Rana *et al.*, 2022); in contrast, the included dogs in our study were apparently healthy and had not received antibiotics in the last 14 days. Hence, another reason for the absence of *S. aureus* could be due to the presence of commensals that inhibited the growth of *S. aureus.*

Although *S. pseudintermedius* was detected in pet dogs and their owners, no significant risk factors were associated with the of carriage *S. pseudintermedius* in pet dogs or their owners. Our findings show that pet dog owners carrying *S. pseudintermedius* had close contact with their pet dogs. It has been reported that those in close contact with pet dogs are prone to being colonised by S. pseudintermedius (Zukancic *et al.*, 2020); moreover, it has also been reported that human patients colonised with *S. pseudintermedius* had diabetes, were hospitalised, and had a history of having close contact with dogs (Chandak *et al.*, 2019; Carroll *et al.*, 2021). Our records also show that three of the *S. pseudintermedius* carrier owners had diabetes or were recently hospitalised; remarkably, one of these owners carried MRSP, as found in our previous study (Afshar *et al.*, 2023), was found to be ST2297 and related to clonal complex 45 (CC). Clonal complex 45 has a worldwide spread. Based on our previous MLST database search, the members of CC45 have been reported from healthy humans in Thailand and Brazil, a dog with otitis from the Netherlands and a cat with pyoderma in Israel (PubMLST, n.d.). These reports also depict the potential of *S. pseudintermedius* as a zoonotic agent. Furthermore, pet dog owners in our study carrying *S. pseudintermedius* were living in households with more than two people. The significance of crowded conditions in facilitating the spread of zoonotic agents should not be overlooked. Therefore, an increase in population density among humans and their associated dogs could lead to more frequent skin contact between individuals, promoting the spread of MRSP throughout the population (Rynhoud *et al.*, 2021).

Our records show that in addition to having close contact with owners, pet dogs that carried *S. pseudintermedius* were allowed to roam outside of the house, which may be the reason why these pets carried *S. pseudintermedius.* In addition, one of these isolates was MRSP. Similar findings have been reported by Mohamed *et al*. (2020), who reported a higher prevalence (6.5%) of *S. pseudintermedius* in dogs roaming freely. The high prevalence of S. pseudintermedius in freely roaming dogs may be attributed to their increased exposure to the environment and other animals.

CONCLUSION

In the current study, having other animals in the house and cats licking their owner's face were among the significant factors that helped the carriage of *S. pseudintermedius* in pet cats*.* As this study targeted apparently healthy dogs and cats, a small number of targeted bacteria were detected; hence, most of the risk factors included in the questionnaire were not significantly associated with the carriage of these organisms, and further studies are necessary.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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