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Genetic Characterization of Methicillin Resistant *Staphylococcus pseudintermedius* in Dogs and Cats in Cyprus: Comparison of MRSP and MRSA Results

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ABSTRACT

The aim of this research was to investigate the existence and frequency of *Staphylococcus pseudintermedius* in cats and dogs with pyoderma and otitis externa by conventional culture methods, automated identification system and PCR-RFLP. This is the first study to research the presence of MRSP in cats and dogs in Cyprus. The PCR-RFLP method based on the *pta* gene was confirmed to *S. pseudintermedius.* Methicillin resistance results were revealed with VITEK 2 and *mec*A gene detection was performed by PCR. A total of 100 samples including 50 cats and 50 dogs were examined from 6 veterinary clinics. *S. pseudintermedius* (4%) and *S. aureus* (8%) were isolated in cat samples; *S. pseudintermedius* (56%) *S. aureus* (6%) and *S. schleiferi* (2%) were isolated in dog samples, and 3 MRSP strains were identified. Methicillin resistance of these isolates was found to be statistically significant (χ^2= 90.013, p<0.0001). As in MRSA, the increasing presence of both *S. pseudintermedius* and methicillin resistance has started to become a global problem, and with this study, awareness will be raised in both patient owners and veterinary clinics so that they can take the necessary precautions.

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HT and HG designed the study, conducted the experiment and wrote the manuscript.

Key words
Cat, Dog, *Staphylococcus pseudintermedius*, MRSP, MRSA

INTRODUCTION

Staphylococcus pseudintermedius was first described as a new species in 2005 by using 16S rRNA gene sequence analysis from samples taken from cats, dogs, horses and parrots (Devriese *et al*., 2005). Recent studies has revealed that isolates phenotypically recognized as *S. intermedius* can be classified into three species: *S. intermedius*, *S. pseudintermedius*, and *S. delphini* (groups A and B), together known as the *Staphylococcus intermedius* group (SIG) (Sasaki *et al*., 2007). *Staphylococcus cornubiensis,* which was isolated from human skin in 2018, is also included in this group (Murray *et al*., 2018).

Staphylococcus pseudintermedius colonizes the skin, hair, and mucocutaneous sites in healthy dogs and cats as part of the normal cutaneous flora (Rubin *et al*., 2011). *S. pseudintermedius* may cause opportunistic infections such as pyoderma, otitis externa, urinary tract infections, wound and surgical infections, and abscesses (Scherer *et al*., 2018; Loeffler and Lloyd, 2018; Diribe *et al*., 2015; Wettstein *et al*., 2008). An underlying immunosuppressive condition or break in host barriers is present in the majority of infections (Chrobak-Chmiel *et al*., 2018). *S. pseudintermedius* is an adapted species to the Canidae family, which includes dogs and foxes, according to epidemiological research (Bannoehr *et al*., 2009). Both wild and domesticated cats had lower carriage rates than dogs, implying that cats are not a natural host of *S. pseudintermedius* (Hanselman *et al*., 2009; Hariharan *et al*., 2011). However, *S. pseudintermedius* can be clinically isolated from feline cases with pyoderma, particularly from inflammatory skin lesions (Abraham *et al*., 2007; Loeffler *et al*., 2007; Chandak *et al*., 2019). As in *S. pseudintermedius*, *S. aureus* has been reported to cause pyoderma and otitis in cats and dogs (Faires, 2008). In humans, *S. pseudintermedius* has been found in sporadic diseases such as pyoderma, sinonasal infections, and wounds from dog bites (Chandak *et al*., 2019; Ference *et al*., 2019; Börjesson *et al*., 2015).

Methicillin resistance is a serious worldwide problem in *S. pseudintermedius* strains isolated from both diseased and healthy cats and dogs (Walther *et al*., 2017). Methicillin resistance occurs due to the low affinity of PBP2a, which is encoded by the *mec*A gene, to beta-lactam antibiotics. In staphylococci, the *mec*A gene is located on the mobile genetic element, Staphylococcal Cassette

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Chromosom (SCC*mec*) (Duim *et al*., 2018). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) was first reported in Europe in 2006 (Moodley *et al*., 2013). Many *S. pseudintermedius* isolates are not only resistant to methicillin, but also to many classes of antibiotics (MDR) (Wegener *et al*., 2018). In recent years, the rate of methicillin and multidrug-resistant *S. pseudintermedius* (MDR-SP) has increased in clinical samples of pet animals. It has been demonstrated that MRSP is a multi-drug resistant pathogen against almost all antimicrobial agents applicable in veterinary medicine. Methicillin-resistant *Staphylococcus aureus* (MRSA) in pets, like MRSP, has the potential to have serious consequences for both veterinary and human medicine (Worthing *et al*., 2018; Somayaji *et al*., 2016; Van Duijkeren *et al*., 2004; Loeffler *et al*., 2005).

The aim of this research was to use bacteriological and molecular techniques to determine the presence and frequency of *S. pseudintermedius* in cats and dogs with pyoderma and otitis externa. However, this is the first study to investigate the *mec*A gene and MRSP in cats and dogs in Cyprus in order to establish which strains are resistant to methicillin.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ethics Committee of Near East University (No: 2019/87; 27.09.2019).

Animal sampling

A total of 100 cats and dogs were randomly selected from 6 veterinary clinics. The samples were obtained from animals brought to Near East Animal Hospital and 5 veterinary clinics in Morphou, Famagusta, Nicosia and Kyrenia districts of Cyprus. Skin and ear examinations of cats and dogs of different ages, races and genders were performed, and skin and ear swabs were taken from animals thought to have pyoderma and otitis externa. A total of 100 animals, including 50 (25 ear and 25 skin samples) cats and 50 (25 ear and 25 skin samples) dogs were sampled. The samples were collected with swabs containing liquid Amies medium (Copan-493CE03, MRSA system).

Identification of Staphylococcus *species*

Columbia blood agar with 5% sheep blood (Biomerieux, 43041), MacConkey agar (Merck, 105465), and Baird Parker agar (Merck, 105406) were used to culture the materials. Under aerobic conditions, all agar plates were incubated for 24 h at 37°C. After incubation, the isolates were determined by colony morphology, microscopic morphology, and growth parameters. The Gram staining (Biomerieux, 55542), colony morphology, haemolysis, and tests for catalase (Biomerieux, 55561), clumping factor (Merck, 113306), tube coagulase (Merck, 113306), and DNase test (Merck, 110449) were all used to identify staphylococci. A VITEK 2 Compact automated system device (Biomerieux, France) was used to perform confirmatory phenotypic identification.

Antibiotic susceptibility testing

VITEK GP-AST80 cards (Biomerieux, 421826) and a VITEK 2 Compact system device were used to determine antibiotic susceptibility. VITEK 2 Compact analyzes and interprets MIC values for a total of 14 antibiotics using Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). These antibiotics are cefoxitin screening, gentamicin, kanamycin, marbofloxacin, pradofloxacin, erythromycin, enrofloxacin, neomycin, doxycycline, clindamycin, tetracycline, nitrofurantoin, chloramphenicol and trimethoprim/sulfamethoxazole. The GP-AST card also includes cefoxitin screening to determine methicillin resistance and inducible clindamycin resistance test. The isolates identified as *S. pseudintermedius* and *S. aureus* as a result of VITEK 2 were inoculated on ChromID MRSA SMART agar (Biomerieux, 413050) and incubated at 37˚C for 24 h. The isolates that formed pink/red colonies at the conclusion of the incubation period were considered positive.

PCR-RFLP of S. pseudintermedius

The boiling method (Kocatepe, 2015) was used for DNA extraction of the isolated *S. pseudintermedius* strains. A loopful of pure colonies was suspended with deionized water in a 500 µl DNase-RNase free Eppendorf tube and then boiled at 100˚C for 10 min. The supernatant (300 µl) was kept at -20˚C to be used as target DNA in PCR amplification. The *pta* gene, which encodes the phosphoacetyl-transferase enzyme, was detected in the *S. pseudintermedius* isolates using PCR-RFLP (Bannoehr *et al*., 2009). A 320-bp fragment of the *pta* gene was amplified by PCR in a 25 µl volume that included 12.5 µl PCR master mix (2X) (Thermo Scientific, K0171), 1.25 µl of each oligonucleotide primer (forward: 5'-AAA GAC AAA CTT TCA GGT-3', reverse: 5'-GCA TAA ACA AGC ATT GTA CCG-3'), 6 µl of nuclease-free water, and 4 µl of template DNA. The amplification process as follows: initial denaturation at 95˚C for 2 min followed by 30 cycles of denaturation at 95˚C for 1 min, annealing 53˚C for 1 min, extension at 72˚C for 1 min and a final extension at 72˚C for 7 min.

To detect two fragments of 213 bp and 107 bp of the *pta* gene specified for *S. pseudintermedius*, 10 µl of amplified product was digested with a total volume of 20 µl FastDigest *Mbo*I (Thermo Scientific, FD0814), 10X FastDigest Green buffer (Thermo Scientific, B72) and

nuclease-free water for 5 min at 37˚C. The enzyme was then inactivated for 15 min at 65˚C and the digestion products were resolved in 1.5% (wt/vol) agarose by electrophoresis. In all phenotypic and genotypic analyses, the positive control was the *Staphylococcus pseudintermedius* ED99 (GenBank Accession NC_017568.1) strain obtained from Ross Fitzgerald (The Roslin Institute of the Edinburgh University, Scotland).

Detection of mecA *gene of* Staphylococcus *spp.*

In order to determine methicillin resistance of isolates defined as *S. pseudintermedius* and *S. aureus*, the *mec*A gene was investigated using the method previously used by (Choi *et al*., 2003). A 314-bp fragment of the *mec*A gene was amplified by PCR in a 25 µl volume that included 12.5 µl PCR master mix (2X) (Thermo Scientific, K0171), 1.25 µl of each oligonucleotide primer (forward: 5'-CCT AGT AAA GCT CCG GAA-3', reverse: 5'-CTA GTC CAT TCG GTC CA-3'), 6 µl of nuclease-free water, and 4 µl of template DNA. The amplification process as follows: initial denaturation at 95˚C for 2 min followed by 30 cycles of denaturation at 95˚C for 1 min, annealing 54˚C for 1 min, extension at 72˚C for 1 min and a final extension at 72˚C for 7 min. The amplicons were separated on a 2.5% agarose gel, stained with ethidium bromide, and visualised using UV light. *Staphylococcus aureus* 33591 (*mec*A positive) strain obtained from the Bursa Uludag University (Department of Microbiology, Faculty of Veterinary Medicine) was used as the positive control of the *mec*A gene.

Statistical analysis

Statistical tests were used to compare the data in the tables: following cross tabulation, statistical significance was determined using Pearson's chi-square test. The IBM SPSS Statistics 18 program was used to conduct the analyses. P values less than 0.05 were considered statistically significant. Statistical data are indicated in the Tables I, II and III.

RESULTS

Bacteriological identifications

From 50 (25 ear and 25 skin samples) cat samples of 100 samples collected from veterinary clinics, 6 (12%) coagulase positive staphylococci (CoPS) and 19 (38%) coagulase negative staphylococci (CoNS) were isolated. Of the CoPS, 2 isolates (4%) were identified as *S. pseudintermedius* and 4 isolates (8%) as *S. aureus* (Table I). There was no growth in 25 (50%) cat samples. From 50 (25 ear and 25 skin samples) dog samples of the 100 samples collected from veterinary clinics, 32 (64%) CoPS and 3 (6%) CoNS were isolated. Of the CoPS, 28 (56%) were identified as *S. pseudintermedius*, 3 (6%) as *S. aureus* and 1 (2%) as *S. schleiferi* (Table I). There was no growth in 15 (30%) dog samples. The difference between bacterial species isolated according to animal species was statistically significant (χ^2=31.926, p<0.0001). According to the sample type, 11 (22%) CoPS and 17 (34%) CoNS were isolated from ear swabs, whereas 27 (54%) CoPS and 5 (10%) CoNS were isolated from skin swabs. There was no growth in 22 (44%) ear swabs and 18 (36%) skin swabs. The difference between bacterial species isolated according to the sample types was statistically significant (χ^2=13.682, p=0.001).

Staphylococcus pseudintermedius was isolated from 2 (4%) cat and 28 (56%) dog samples. A comparison of the sample types (Table I) reveals that *S. pseudintermedius* was isolated from 21 (42%) pyoderma and 9 (18%) otitis externa cases (Table II). As a result of the Pearson chi-square test, the difference between the *S. pseudintermedius* identification rate according to sample type (χ^2= 6.857, p=0.009) and animal species (χ^2= 32.190, p<0.0001) was determined to be statistically significant. Furthermore, 7 (7%) *S. aureus* isolates were identified with the VITEK 2 compact system. The distribution rate of *S. aureus* isolates according to animal species (p=1) and sample type (p=0.436) in the VITEK 2 method was identified to be homogeneous. It was statistically insignificant (Table I).

PCR-RFLP of S. pseudintermedius

According to the VITEK 2 system and PCR-RFLP (*pta* gene) analysis, 30 (30%) *S. pseudintermedius* were identified from a total of 100 swab samples. As a result of the Pearson chi-square test, the difference between the PCR-RFLP analysis results of the isolates identified with the VITEK 2 system and *S. pseudintermedius* was determined to be statistically significant (χ^2=100.00, p<0.0001). PCR-RFLP electrophoresis results of *S. pseudintermedius* strains are shown in Figure 1.

Table I. According to animal species and sample types *Staphylococcus* species isolation rates.

	Coagulase positive staphylococci			Coagulase negative staphylococci	p-value and χ^2
	S. pseudintermedius	*S. aureus*	*S. schleiferi*		
Cat	2 (4%)	4 (8%)	-	19 (38%)	p<0.0001
Dog	28 (56%)	3 (6%)	1 (2%)	3 (6%)	χ^2=31.926

Table II. *S. pseudintermedius* isolation rates by sample type.

Sample type	*S. pseudintermedius*			
	Positive samples	**Negative samples**	**Total**	**p-value and χ^2**
Pyoderma (skin swap)	21 (42%)	29 (58%)	50 (100%)	p<0.0001 χ^2=100.00
Otitis externa (ear swap)	9 (18%)	41 (82%)	50 (100%)	
Total	30 (30%)	70 (70%)	100 (100%)	

Table III. Methicillin resistance rates of *S. pseudintermedius* and *S. aureus* isolates according to VITEK 2, MRSA agar and PCR analyzes.

Staphylococcus species	VITEK 2 compact system	p-value and χ^2	MRSA agar	p-value and χ^2	PCR (*mec*A gene)	p-value and χ^2
S. pseudintermedius	2/30 (6.7%)	p<0.0001 χ^2=88.556	3/30 (10%)	p<0.0001 χ^2=90.013	3/30 (10%)	p<0.0001 χ^2=90.013
S. aureus	2/7 (28.6%)	p<0.0001 χ^2=17.111	3/7 (42.9%)	p<0.0001 χ^2=18.568	3/7 (42.9%)	p<0.0001 χ^2=18.568

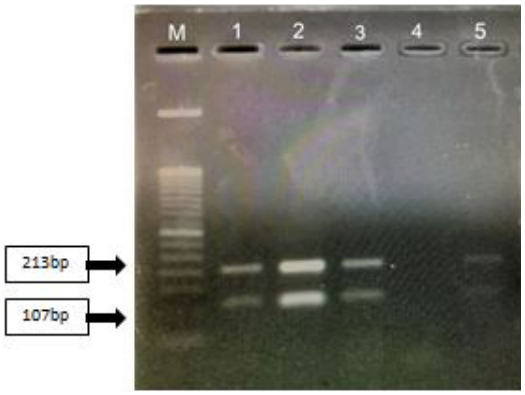

Fig. 1. Agarose gel electrophoresis showing the results of PCR-RFLP of 107bp and 213bp for the *pta* gene *Staphylococcus pseudintermedius* isolates. M: Marker (50 bp), Lane 1, 2, 3: *S. pseudintermedius* strains, Lane 4: negative control, Lane 5: *S. pseudintermedius* positive control.

Methicillin resistance profiles

According to the antibiotic susceptibility test performed with the VITEK 2 system, *S. pseudintermedius* isolates (6.7%) were detected to be resistant to methicillin (χ^2=88.556, p<0.0001). After incubation on MRSA agar, methicillin resistance was found in 10% of *S. pseudintermedius* isolates (χ^2=90.013, p<0.0001). In 10% of *S. pseudintermedius* isolates, the *mec*A gene was also detected (χ^2=90.013, p<0.0001). The methicillin resistance of these isolates was found to be statistically significant (Table III). Of the 30 *S. pseudintermedius*, 3 (10%) strains were identified as MRSP. Of the MRSP strains, 2 (6.7%) were isolated from dogs with pyoderma and 1 (3.3%) from a cat with pyoderma. According to the antibiotic susceptibility test performed with VITEK 2 system, *S. aureus* isolates (28.6%) were detected to be resistant to methicillin (χ^2=17.111, p<0.0001). After incubation on MRSA agar, methicillin resistance was found in 42.9% of *S. aureus* isolates (χ^2=18.568, p<0.0001). In 42.9% *S. aureus* isolates, the *mec*A gene was also detected (χ^2=18.568, p<0.0001). Methicillin resistance of these isolates was found to be statistically significant (Table III). Of the MRSA strains, 2 (28.6%) were isolated from cats with pyoderma and 1 (14.3%) from a cat with otitis externa. The *mec*A gene electrophoresis results of *S. pseudintermedius* strains are shown in Figure 2.

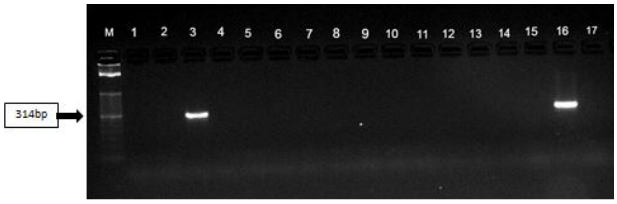

Fig. 2. Agarose gel electrophoresis showing the results of PCR amplified product of 314 bp for the *mec*A gene of *Staphylococcus pseudintermedius* isolates. M: Marker (50 bp), Lane 3: MRSP strain, Lane 1, 2, 4-15: MSSP strains, Lane 16: positive control, Lane 17: negative control.

DISCUSSION

Staphylococci can cause many infections in pet animals (Haag *et al*., 2019; Lynch and Helbig, 2021). In particular, *S. pseudintermedius* and *Malassezia* species, two commensal species of the skin, are known

as secondary causes of otitis externa (Bugden, 2013). *S. pseudintermedius* is known to be the primary cause of pyoderma in dogs (Loeffler and Lloyd, 2018). The rate of *S. pseudintermedius* in canine pyoderma can reach up to 92%, (Bugden, 2013; Szewczuk *et al*., 2020) and it can vary between 20 to 94.3% in otitis externa (Bugden, 2013; Sim *et al*., 2019). The prevalence of *S. pseudintermedius* in dogs has been found to be higher than in cats (Rusenova *et al*., 2020; Göçmen *et al*., 2020). In a study in Poland, 255 skin and 219 ear samples were collected from dogs with pyoderma and otitis externa. Szewczuk *et al*. (2020) identified *Staphylococcus* species in 82.4% of pyoderma cases and 44.8% of otitis externa. *S. pseudintermedius*, the most dominant species, and 1 *S. aureus* have been identified as *Staphylococcus* isolates. A total of 24 *S. pseudintermedius* have been isolated from 18 strains of skin and 6 strains of ear swabs and all isolates were resistant to methicillin (Szewczuk *et al*., 2020). Scherer and colleagues collected 104 ear swabs from both ears of 52 dogs with otitis externa, and 44 *S. pseudintermedius* strains were identified from 31 dogs in their investigation (Scherer *et al*., 2018). The rate of *S. pseudintermedius* isolation was 60% in dogs with otitis externa. In this study, *S. pseudintermedius* was identified in 21 (42%) pyoderma specimens and 9 (18%) otitis externa specimens. When these two studies were compared, a similarly high rate of *S. pseudintermedius* was identified depending on the number of samples. The rate of *S. pseudintermedius* identification according to sample type was detected to be statistically significant (χ^2= 6.857, p=0.009).

In the present study, 6 (12%) CoPS and 19 (38%) CoNS were isolated from cats. Of the CoPS, 2 (4%) *S. pseudintermedius* and 4 (8%) *S. aureus* were identified. In a study that investigated *Staphylococcus* species in healthy and sick cats with similar results, the frequency of CoNS was found to be higher. Statistical analysis, unlike this work, confirmed that animals in the sick cat group were more frequently colonized with *S. pseudintermedius* and *S. haemolyticus* (Bierowiec *et al*., 2019). In another study that described *S. pseudintermedius* by conventional and molecular methods, no *S. pseudintermedius* isolates were detected in cat samples (Rusenova *et al*., 2020). Contrary to this study, our study revealed that *S. pseudintermedius* and MRSP were also isolated from cat samples. In this study, 32 (64%) CoPS and 3 (6%) CoNS were isolated from dogs. Of the CoPS, 28 (56%) *S. pseudintermedius*, 3 (6%) *S. aureus* and 1 (2%) *S. schleiferi* were identified. Sarreyyüpoğlu and colleagues revealed that the rate of *S. psedintermedius* detected in dogs with skin infections was 80.4% (Sareyyüpoğlu *et al*., 2014). In another study conducted in Turkey, 61 *S. pseudintermedius* strains, 18 of which were MRSP, were isolated from 77 dogs with skin infections (Müştak *et al*., 2020). Similar to the results of these studies, the most common strain isolated among CoPS in dog samples was *S. pseudintermedius*. Based on the statistical analyses conducted in this study, the difference between bacterial species isolated according to animal species was statistically significant (χ^2=31.926, p<0.0001). *S. aureus*, a human and animal pathogen, was detected in 7 (7%) isolates from dog and cat samples. These isolates were from 4 cat samples and 3 dog samples. Cats and dogs are not considered a typical reservoir of *S. aureus*, as confirmed by our data (Haag *et al*., 2019). Just as *S. pseudintermedius* is transmitted from pets to humans, *S. aureus* can also be transmitted from humans to animals and cause serious infections (Somayaji *et al*., 2016; Van Duijkeren *et al*., 2004; Loeffler *et al*., 2005).

It is difficult to differentiate between bacterial species of SIG by phenotypic tests (Sasaki *et al*., 2007). In a study investigating the *pta* gene, phosphoacetyl-transferase enzyme, using the PCR-RFLP method, a total of 37 strains, which were from 27 patient and healthy dogs as well as 10 from their owners, were identified as *S. pseudintermedius* (29 strains) (Alcalá *et al*., 2015). However, both phenotypic and genotypic techiques were used to identify *S. pseudintermedius* in the current study. The VITEK 2 device was primarily used for the identification of *S. pseudintermedius* and these isolates were confirmed by PCR-RFLP. A total of 30 (30%) *S. pseudintermedius* strains were detected with both methods. As a result of the Pearson chi-square test, the difference between the PCR-RFLP analysis and VITEK 2 results identified as *S. pseudintermedius* was revealed to be statistically significant (χ^2=100.00, p<0.0001). These results also supported other studies that used VITEK 2 and MALDI-TOF to identify *S. pseudintermedius* (Chandak *et al*., 2019; Alcalá *et al*., 2015; Yarbrough *et al*., 2018).

The resistance developed to methicillin and other antimicrobials by staphylococci has become a global problem in the chemotherapy of staphylococcal infections (Walther *et al*., 2017). MRSP isolates have been detected at rates of up to 59% in canine pyoderma cases (Yoo *et al*., 2010; Wang *et al*., 2012) and in 10 to 48.1% of otitis externa cases (Sim *et al*., 2019; Chan *et al*., 2018). In a study in which 771 clinical specimens were collected from a total of 556 dogs, MRSP isolates were identified at a rate of 22% from the skin and 7% from ear swabs (Saab *et al*., 2018). In these two studies on dogs with pyoderma in China and Japan, the prevalence of MRSP was 48% and 66%, respectively (Kawakami *et al*., 2010; Feng *et al*., 2012). On the other hand, in a study conducted in China, MRSP was identified in dogs at a rate of 44% (Wang *et al*., 2012). Jayalakshmi and colleagues identified 5 (35.7%) MRSP and 14 (56%) *S. pseudintermedius* from 25 swabs collected

from healthy dogs and dogs with otitis externa (Jayalakshmi *et al*., 2019). When we investigated methicillin resistance with VITEK 2 in *S. pseudintermedius* strains, 2/30 (6.7%) MRSP strains were detected, but when the *mec*A gene was examined by PCR, 3/30 (10%) MRSP strains were detected. Of the MRSPs, 2 (6.7%) isolates were identified from pyoderma samples of dogs and 1 (3.3%) isolate was identified from the pyoderma sample of a cat. Contrary to other studies, the rate of MRSP was found to be low in our study and the number of infection cases in the sample was also low. However, this was thought to be due to the relatively infrequent use of beta-lactam antibiotics or the conscious use of antibiotics in cats and dogs. While most of the *S. pseudintermedius* strains were detected in dog samples in other studies, an MRSP isolate was detected in a cat sample in this study. On the other hand, studies reporting low MRSP rates supporting our results are also available in the literature (Maluping *et al*., 2014; Dziva *et al*., 2015). Likewise, the methicillin resistance rate of *S. aureus* in VITEK 2 was 2/7 (28.6%) and the *mec*A gene rate was 3/7 (42.9%). Of the MRSA strains in cats, (28.6%) isolates were identified from pyoderma samples and 1 (14.3%) isolate was identified from an otitis externa sample. MRSA was not identified in dogs with pyoderma and otitis externa samples. The differences in the results of PCR with VITEK 2, which was applied to investigate methicillin resistance, once again emphasized the need for genotypic tests.

CONCLUSIONS

In conclusion, this was the first investigation to reveal the existence of *S. pseudintermedius* and methicillin-resistant *Staphylococcus* isolates in veterinary clinics in Cyprus. As a result of this, the correct diagnosis of *S. pseudintermedius*, which is accepted as an important micro-organism in terms of human and animal health, was achieved by genotypic methods. In infections caused by bacteria resistant to antibiotics, the selection of sensitive antibiotics and the implementation of the correct treatment protocols for this purpose, as well as the increase in studies on the patients' response to treatment, reduction of hospital visits and treatment costs, are important and thought to be beneficial. It is positive for our country that the MRSP ratio obtained did not reach high values. Contrary to the low MRSP rate, the high rate of *S. pseudintermedius* in dogs and the zoonotic importance of this bacterium are likely to have potential public health effects.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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