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RESEARCH ARTICLE

Methicillin Resistance in *Staphylococcus pseudintermedius* Isolated from Shelter Dogs in Turkey

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Summary

Staphylococcus pseudintermedius is a recently described species of staphylococci that is frequently isolated from dogs. The increase in antimicrobial resistance in staphylococci and transfer of *S. pseudintermedius* from infected pets to humans threaten the public health worldwide. In this study, it was aimed to determine the prevalence of *S. pseudintermedius* from skin infections of dogs and to determine the prevalence of methicillin-resistance in *S. pseudintermedius* isolates by phenotypic and genotypic methods. A total of 41 staphylococci were isolated from 53 dogs. Thirty three (80.4%) staphylococci were identified as *S. pseudintermedius* by PCR-RFLP analysis of *pta* gene. Methicillin resistance was also identified in 11 (33.3%) of these isolates by inoculation on chromogenic chromID MRSA agar, oxacillin disc diffusion method and the determination of *mec*A gene by PCR. This is the sole report in Turkey that described the situation of methicillin resistant *S. pseudintermedius*.

Keywords: *Dog, Staphylococcus pseudintermedius, Methicillin resistance*

Türkiye'deki Barınak Köpeklerinden İzole Edilen *Staphylococcus pseudintermedius*'larda Metisilin Direnci

Özet

Staphylococcus pseudintermedius, stafilokoklar içerisinde yeni tanımlanmış bir tür olup köpeklerden sıklıkla izole edilmektedir. Stafilokoklarda artan antimikrobiyal direnç ve *S. pseudintermedius*'un enfekte petlerden insanlara geçmesi, halk sağlığını tehdit etmektedir. Bu çalışmada, köpek deri enfeksiyonlarından izole edilen *S. pseudintermedius*'ların ve metisilin direncinin prevalansının fenotipik ve genotipik metotlar ile belirlenmesi amaçlanmıştır. Toplam 53 köpekten 41 stafilokok izole edildi. Stafilokok izolatlarının 33'ü (%80.4) *pta* geninin PCR-RFLP ile analiziyle *S. pseudintermedius* olarak belirlendi. Metisilin direnci kromogenik chromID MRSA agar, oxacillin disk difüzyon metodu ve *mec*A geninin belirlendiği PCR yöntemi ile bu izolatların 11'inde (%33.3) tespit edildi. Bu çalışma, Türkiye'de metisilin dirençli *S. pseudintermedius*'ların durumunu gösteren ilk bilimsel rapordur.

Anahtar sözcükler: *Köpek, Staphylococcus pseudintermedius, Metisilin direnci*

INTRODUCTION

Isolates phenotypically identified as *Staphylococcus intermedius* consist of three distinct species, including *S. intermedius*, *S. pseudintermedius*, and *S. delphini*, which together represent the *S. intermedius* group (SIG). *Staphylococcus pseudintermedius* rather than *S. intermedius* is the opportunistic species of the SIG that colonizes and causes infections in dogs and cats frequently. Beside this, *S. pseudintermedius* could be transferred from infected and/or colonized pets to humans, which causes an increasing public health concern [1]. *Staphylococcus pseudintermedius* is occasionally isolated from serious human infections, particularly from people who are in close contact with pets, such as small animal veterinarians and pet owners [2-5].

Recently, methicillin-resistant *S. pseudintermedius* (MRSP) has emerged worldwide and spread of strains has been reported increasingly in Europe [6-10]. Methicillin resistance determinant, *mec*A gene, carried on a mobile genetic element called Staphylococcal Cassette Chromosome (SCC), also exists on *S. pseudintermedius* similarly to *S. aureus* [11].

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This gene encoding the penicillin-binding protein 2a which mediates methicillin resistance in *S. aureus* and coagulase negative staphylococci by lowering affinity to all β-lactam antimicrobials [2].

In order to detect methicillin resistant staphylococci (MRS), oxacillin is used and oxacillin resistant strains are referred as methicillin resistant. Besides this, any staphylococci resistant to oxacillin or methicillin should be considered resistant to other β-lactam antimicrobials such as amoxillin-clavulanic acid, cephalosporins, etc. Clinical Laboratory Standards Institute (CLSI) asked from laboratories to report *mec*A positive MRS as resistant to all other β-lactam antimicrobials regardless of antibiotic susceptibility tests [12]. In 2008, CLSI published a new document M31-A3, which determines the *in vitro* antimicrobial susceptibility of MRSP. According to this guideline, new interpretation criterion for MRSP isolates for oxacillin is ≥4 mg/L for agar and broth dilution, and ≤10 mm for disc diffusion tests similar to *S. aureus* clinical susceptibility breakpoints [13,14].

In Turkey, there are few reports on methicillin resistance in coagulase negative staphylococci isolated from companion animals and pets [15,16]. Nevertheless, the prevalence of MRSP is not known in particular. The aim of this study was to determine the prevalence of *S. pseudintermedius* among staphylococcal isolates from skin infections of dogs and to determine the prevalence of methicillin-resistance in *S. pseudintermedius* isolates.

MATERIAL and METHODS

Bacteriological Identification

A total of 41 isolates from 53 shelter dogs with dermatitis in Ankara region were used in the study. These isolates were obtained by inoculation of swap samples taken from skins of dogs on blood agar containing 5-7% ovine blood and incubated aerobically at 37°C for 24 h. After incubation, suspected colonies were Gram stained and treated with catalase, tube coagulase and DNAse tests. Further phenotypic identification was performed with the Microbact™ Staph 12S system (Staphylococcal 12S Identification System, MB1561, Oxoid).

Molecular Identification of Staphylococcus pseudintermedius

Staphylococci identified as *S. intermedius* by Microbact™ Staph 12S system were molecularly investigated for *S. pseudintermedius* by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the *pta* gene encoding the enzyme phosphoacetyltransferase [17]. *Mbo*I enzyme was used in RFLP to detect *S. pseudintermedius* isolates harboring this restriction site. Briefly; 1.5 mM $MgCl_2$, 0.5 U *Taq* DNA Polymerase (Fermentas, Lithuania), 200 µM each dNTPs, 5 µl PCR reaction buffer (1×), 4 µl template DNA and 0.2 µM of each primer (pta_f1, AAA GAC AAA CTT TCA GGT AA, and pta_r1, GCA TAA ACA AGC ATT GTA CCG) were used to amplify a 320 bp fragment of *pta* gene. Thermal cycling conditions were as follows: initial denaturation at 95°C for 2 min followed by 30 cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min with final extension at 72°C for 7 min. Two fragments (213 bp and 107 bp) of *pta* gene confirming *S. pseudintermedius* identification were detected after the digestion of amplified products with 5 U *Mbo*I for 2h at 37°C. A *S. pseudintermedius* strain obtained from Associate Professor Dr. Ken Kikuchi (Department of Infection Control Science, Faculty of Medicine Juntendo University, Tokyo, Japan) was served as positive control in all phenotypic and genotypic tests.

Antimicrobial Susceptibility

All phenotypically and genotypically identified *S. pseudintermedius* strains were inoculated on chromogenic chromID MRSA agar (bioMérieux) for investigating methicillin resistance. After incubation at 37°C for 24 h, blue-green colonies were picked up and assigned as MRSP. Methicillin resistance was also investigated by disk diffusion method with oxacillin standard disks according to the guidelines of CLSI [14]. The zone diameter ≤10 mm for oxacillin is referred as resistance threshold. *Staphylococcus aureus* ATCC29213 was served as quality control strain in all tests.

Molecular Detection of Methicillin Resistance

For all isolates identified as *S. pseudintermedius*, methicillin resistance was investigated by a modified PCR method of Choi et al.[18] for detection of *mec*A gene. Primers (F: CCT AGT AAA GCT CCG GAA; and R: CTA GTC CAT TCG GTC CA) were used to amplify a 314 bp fragment of *mec*A gene in a total volume of 25 µl PCR mixture consists of 0.2 µM each primer, 200 µM each dNTPs, 5 µl PCR reaction buffer, 1.5 mM $MgCl_2$, 0.5 U Taq DNA Polymerase and 2 µl template DNA. The thermal cycling conditions were as follows: 1 min of preliminary denaturation at 95°C followed by 30 cycles of 2 min denaturation at 95°C, 1 min of annealing at 54°C, 1 min of extension at 72°C and then a final extension at 72°C for 7 min. *Staphylococcus aureus* SR27 strain was served as the positive control for the *mec*A gene.

RESULTS

Bacterial Identification

Out of 41 Staphylococcal isolates from skin infections of dogs, 35 (85.4%) isolates were phenotypically identified as *S. intermedius,* 4 (9.8%) as *S. aureus* and 2 (4.8%) as different species (*S. chromogenes* and *S. capitis*). According to the PCR-RFLP results 33/35 (94.2%) *S. intermedius* strains were molecularly identified as *S. pseudintermedius (Table 1).*

Table 1. *Phenotypic and genotypic identification test results*
Tablo 1. *Fenotipik ve genotipik testlere ait identifikasyon sonuçları*

Staphylococci	Microbact Staph 12S Identification	PCR-RFLP	MRSA Agar	*mec*A PCR
S. intermedius	35/41 (85.4%)	0/41	N	N
S. pseudintermedius	0/41	33/41 (80.4%)	11/33 (33.3%)	11/33 (33.3%)
S. aureus	4/41 (9.8%)	4/41*	N	N
S. capitis	1/41 (2.4%)	0/41	N	N
S. chromogenes	1/41 (2.4%)	0/41	N	N

** S. aureus isolates contained a unique MboI site, resulting in restriction fragments of 156 bp and 164 bp that appeared as a single band after agarose electrophoresis; this band was readily distinguishable from the S. pseudintermedius restriction fragment profile [17], **N:** not applicated*

Identification of Methicillin Resistant Staphylococcus pseudintermedius

Methicillin resistance was observed in 11/33 (33.3%) *S. pseudintermedius* isolates after incubation on chromogenic chromID MRSA agar. According to disc diffusion method, 11 (33.3%) *S. pseudintermedius* isolates were found to be resistant to oxacillin. The *mec*A gene was also detected in 11/33 (33.3%) *S. pseudintermedius* isolates *(Table 1)*.

DISCUSSION

Staphylococcal isolates from dogs with pyoderma identified conventionally as *S. intermedius* should be re-investigated with appropriate methods, since they could in fact be *S. pseudintermedius*. Results of the present study confirmed that *S. pseudintermedius* 33/41 (80.4%) is the predominant pathogenic *Staphylococcus* species isolated from dermatitis cases in dogs.

The prevalence and occurrence of MRSP has been described in companion animals [19,20]. Former studies revealed the prevalence rates of MRSP in dogs approximately between 0%-7% [21-24]. Unfortunately, there are several recent reports of MRSP isolates that exhibited high rates of prevalence in FarEastern parts of the world. In China, 69 of 144 (47.9%) *S. pseudintermedius* were identified as MRSP [25]. In Japan, 27 MRSP characterized by the growth on chromID MRSA agar and confirmed by the presence of *mec*A gene, in 200 cats and dogs, most of which were isolated from dogs (n: 25) [26]. Another study from Japan revealed 66.5% (113/170) as the prevalence of MRSP isolated from dogs based on the detection of *mec*A gene [27]. The prevalence of MRSP in Europe seems to be low as reported in Spain 4.6% (9/196) [6], in Germany 7.4% (60/814) [7] and in Italy 2% (10/590) [8]. In the present study, high prevalence of MRSP (33.3%) was detected in dogs with dermatitis. This prevalence was found to be higher when compared to the results of studies in Europe and similar to the results of studies in Far Eastern countries. Nevertheless, high prevalence could have been resulted from transmission of a resistant strain or strains between dogs held in close contact in shelter conditions.

In Turkey, there is limited data on MRS. Öztürk et al.[16], investigated 96 dogs with otitis externa, skin wounds and pyoderma for the presence of MRS. Of all isolated 54 coagulase-positive staphylococci, 4/33 (12.1%) and 1/21 (4.8%) were found to be methicillin resistant *S. aureus* and *S. pseudintermedius* respectively. Beside this, Aslantaş et al.[15], reported the prevalence of MRS in dogs as 15.4% (25/162) all of which were coagulase negative. However, none of the isolates were found to be MRSA or MRSP [15]. Authors concluded their report as methicillin resistant coagulase negative staphylococci were common in dogs in Turkey. In comparison with these studies in this report the isolation rate was found to be 33/41 (80.4%) that indicates the *S. pseudintermedius* is the leading agent among staphylococci that infects and/or colonizes the skins of dogs in Turkey. The prevalence of MRSP (33.3%) also exhibits that MRSP is an emerging problem that threatens the dog population and public health in Turkey. Although rare MRSP infections in humans have been described, MRSP has implications for public health as it can spread between people and pets via direct and indirect contact [13,28]. Methicillin-resistant staphylococci are known as important zoonotic pathogens that cause serious public health problems by increasing both the failure rate of antibiotic therapy and mortality rates among human beings and animals.

This is the first report that demonstrates the situation of MRSP isolated from dogs with dermatitis in Turkey. In conclusion, the high rate (i.e. one third of the isolates) of methicillin resistance of *S. pseudintermedius* isolates from dogs constitutes a significant risk for public health considering the pathogen's zoonotic potential and the risk of transmission from these companion animals to their owners. More and comprehensive studies should be performed to reveal the epidemiology of MRSP infections in companion animals in Turkey, and their role in transmission of antibiotic resistant bacteria between different species.

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