

High-level Mupirocin Resistance in a *Staphylococcus pseudintermedius* Strain from Canine Origin ^[1]

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Abstract

Mupirocin is an important antibacterial agent used in both humans and animals topically, especially against nasal carriage and decolonization of methicillin-resistant *Staphylococcus pseudintermedius*. In this report, a *S. pseudintermedius* strain was isolated from a dog with pyoderma. Molecular identification of this strain was performed by digestion of *pta* gene with *Mbol* enzyme. High-level mupirocin resistance was determined by agar dilution method and amplification of *ileS-2* gene. Sequence analysis of 16S rRNA and *ileS-2* gene of *S. pseudintermedius* was also performed and both sequences were accessed in GenBank. This is the first report of a high-level mupirocin resistant *S. pseudintermedius* strain from a dog with pyoderma in Turkey.

Keywords: Antibiotic resistance, Dog, Mupirocin, *Staphylococcus pseudintermedius*

Köpek Kökenli Bir *Staphylococcus pseudintermedius* Suşunda Yüksek Seviye Mupirosin Direnci Olgusu

Özet

Mupirosin insan ve hayvanlarda özellikle metisilin dirençli *Staphylococcus pseudintermedius*'un dekolonizasyonuna ve burun taşıyıcılığına karşı kullanılan önemli bir antibakteriyel ajandır. Bu çalışmada, piyodermalı bir köpekten *S. pseudintermedius* izole edildi. Bu suşun moleküler teşhisi için *pta* geni *Mbol* enzimi ile kesildi. Yüksek seviye mupirosin direnci agar dilüsyon metodu ve *ileS-2* geninin çoğaltılmasıyla ortaya kondu. *S. pseudintermedius*'un 16S rRNA ve *ileS-2* genlerinin dizi analizi yapılarak her iki genin sekansları GenBank'a kayıt ettirildi. Bu rapor, Türkiye'de piyodermalı bir köpekten izole edilen ilk yüksek seviye mupirosin dirençli *S. pseudintermedius* olgusunu bildirmektedir.

Anahtar sözcükler: Antibiyotik direnci, Köpek, Mupirosin, *Staphylococcus pseudintermedius*

INTRODUCTION

Staphylococcus pseudintermedius is an important, opportunistic, coagulase-positive staphylococcus species that is frequently isolated from skins of healthy dogs and dogs with cutaneous infections particularly canine pyoderma. Canine pyoderma is generally treated with antibacterial shampoos and topical or systemic antibiotics. Nevertheless, inaccurate or ampic misuse of antibiotics are the leading factors in development of bacterial antibiotic resistance. Recently, multidrug-resistant *S. pseudintermedius* (MDRSP) to different antibiotic classes and methicillin-resistant *S. pseudintermedius* (MRSP) is

described in detail ^[1,2]. Acquired antibiotic resistance in *S. pseudintermedius*, challenges the treatment of infected animals and concerns public health closely, since it is thought to have a zoonotic potential. In 2006, Van Hoovels et al. ^[3] demonstrated the first zoonotic description of *S. pseudintermedius*.

Penicillins, cephalosporins, tetracyclines, macrolides, fusidic acid, chloramphenicol, aminoglycosides and fluoroquinolones are used in the treatment of canine pyoderma frequently. Beside this, mupirocin is also an



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important agent used in both humans and animals topically, especially against nasal carriage and decolonization of methicillin-resistant *Staphylococcus aureus* (MRSA) and MRSP [4]. Mupirocin resistance in *S. aureus* is classified in categories according to MIC values. Low-level mupirocin resistance MICs are between 8-256 mg/L, whereas MICs above or equal to 512 mg/L referred to high-level mupirocin resistance. High-level resistance to mupirocin in *Staphylococci* generally acquired by horizontal gene transfer, especially those carry *ileS-2* gene on conjugative plasmids [5]. We here report a high-level mupirocin resistant *S. pseudintermedius* strain from a dog with pyoderma for the first time in Turkey.

CASE HISTORY

Swap and skin samples taken from a 5 year old, female Rottweiler, clinically diagnosed as pyoderma was accepted for microbiological investigation. Skin swap samples, skin scrapings and hair from the edge of lesions were taken for bacteriological and fungal examination. Fungal culture was found to be negative.

Swap samples were cultured 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, coagulase and DNase tests. Microbact Staphylococcal 12S Identification System (Oxoid MB 1561) was used to identify coagulase-positive staphylococci. For further phenotypic identification, β -galactosidase, resistance to polymyxin B and acetoin production were also tested.

Molecular identification of *S. pseudintermedius* was carried out by PCR-restriction fragment length polymorphism method of Bannoehr et al. [6], and genomic DNA was extracted by a modified phenol-chloroform extraction method as described previously by Ardic et al. [7]. A 320 bp fragment of *pta* gene was amplified in a 50 μ l total volume with 1.5 mM MgCl₂, 0.5 U *Taq* DNA Polymerase (Fermentas, Lithuania), 200 μ M each dNTPs, 5 μ l PCR reaction buffer (1 \times), 4 μ l template DNA and 0.2 μ M of each primer (*pta_f1*, AAA GAC AAA CTT TCA GGT AA, and *pta_r1*, GCA TAA ACA AGC ATT GTA CCG). DNA amplification was performed with the following thermal cycling conditions: an 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. Amplified products were digested enzymatically with 5 U *Mbol* for 2 h at 37°C. Two fragments (213 bp and 107 bp) of *pta* gene confirming *S. pseudintermedius* identification were detected after the digested products were resolved by 2% agarose gel electrophoresis.

Agar dilution method (MIC value) and PCR assay (*ileS-2* gene) was used to determine the high-level mupirocin resistance in *S. pseudintermedius*. Serial dilution of

mupirocin beginning with a concentration of 1024 mg/L in Mueller-Hinton agar was prepared according to Clinical and Laboratory Standards Institute guidelines [8]. Mupirocin MIC value of *S. pseudintermedius* isolate was found as 512 mg/L. *Staphylococcus aureus* ATCC 29213 was served as quality control strain in all tests.

In order to verify high-level mupirocin resistance genotypically, *ileS-2* gene was amplified according to the PCR method of Anthony et al. [9]. Primers (*mupA*, TAT ATT ATG CGA TGG AAG GTT GG, and *mupB*, AAT AAA ATC AGC TGG AAA GTG TTG) each with a concentration of 50 pmol were used in a 50 μ l PCR mixture that contains; reaction buffer (1 \times) (50 mmol/L KCl, 10 mmol/L Tris-HCl; pH 9.0), 2.5 mM MgCl₂, 200 μ M each dNTPs, 1.5 U of *Taq* DNA Polymerase (Fermentas, Lithuania) and 5 μ l template DNA. Same amplification conditions were used as mentioned above in identification of *S. pseudintermedius* by PCR-RFLP. After agarose gel electrophoresis, an amplicon with a size of 458 bp was visualized by UV transilluminator. A high-level mupirocin resistant, *ileS-2* gene positive *S. aureus* strain obtained from culture collection of Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey was used as positive control. Sequence analysis of 16S rRNA and *ileS-2* gene of *S. pseudintermedius* was performed by Sanger sequencing method on an Applied Biosystems 3130 Genetic Analyzer, using standard protocols. Both sequences were included in GenBank with the corresponding accession numbers KC561085 for *ileS-2* and KC561086 for 16S rRNA, respectively.

Staphylococcus pseudintermedius was identified by both phenotypic and molecular methods as described above. Beside this high-level mupirocin-resistant *S. pseudintermedius* with a MIC value of 512 mg/L was found. High-level mupirocin resistance was also confirmed by the amplification of *ileS-2* gene genotypically.

DISCUSSION

Zoonotic potential and acquisition of resistance to different antibiotics increased the interest in *S. pseudintermedius* recently. Although new studies are being published on MDRSP and MRSP, there are few reports about mupirocin resistance in *S. pseudintermedius* isolated from dogs. Loeffler et al. [10], investigated mupirocin and fucidic acid resistance in coagulase-positive staphylococcal isolates from dogs and cats. High-level mupirocin-resistant *S. pseudintermedius* strain could not be detected and the results (89.7% of all MICs \leq 0.25 mg/L) were found to be compatible with previous studies. Authors concluded their research that both mupirocin and fucidic acid could be used in treatment of superficial staphylococcal infections and decolonization of multi-resistant strains of *S. aureus* and *S. pseudintermedius*.

High-level mupirocin-resistance in a *S. pseudintermedius* isolate from a dog with canine pyoderma was reported for the first time with this study in Turkey. This is an important result, since, mupirocin is known as an important antimicrobial agent used in eliminating nasal carriage and treatment of MRSA, MRSP, and MDRSP strains in animals and humans. Hurdle et al.^[11], reported that conjugative transfer of mupirocin resistance between different *Staphylococcus* species and acquisition of high-level mupirocin resistance could occur during treatment with mupirocin. Regarding zoonotic potential of the agent and likelihood of transfer of antibiotic resistance amongst staphylococci and/or other competent microorganisms, it can be concluded that comprehensive research and studies should be performed on prevalence of mupirocin resistance in staphylococci of animal origin.

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