Case Report

A case of conjunctivitis due to MDR-MR *Staphylococcus pseudintermedius* in a dog

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Abstract

A young male Dachshund dog who came to the animal hospital was diagnosed with conjunctivitis on clinical examination. Methicillin-resistant *Staphylococcus pseudintermedius* was identified as a result of culture and bacteriological examination of the conjunctival swab, and then antimicrobial susceptibility test. Methicillin resistant *S. pseudintermedius* identified with the VITEK-II Compact device was also confirmed by molecular methods so that susceptible antibiotics were selected for the treatment of the patient and reported. In accordance with the antimicrobial susceptibility results, the patient was treated with an eye drop preparation containing neomycin. This is the first report of a canine conjunctivitis caused by multidrug-methicillin resistant *Staphylococcus pseudintermedius* in Cyprus.

Keywords: *Staphylococcus pseudintermedius,* methicillin resistant, multidrug resistant, dog

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Introduction

Staphylococcus pseudintermedius is a member of the *Staphylococcus intermedius* group (SIG). This opportunistic agent colonizes many parts of body in cats and dogs, especially the mucocutaneous flora and is associated with pyoderma and otitis externa infections in pet animals (Kang *et al*., 2014). In recent years, *S. pseudintermedius* has begun to be isolated from dogs with keratoconjunctivitis sicca, septic keratitis and conjunctivitis (Auten *et al*., 2020; Soimala *et al*., 2020). Antibacterial enzymes of the conjunctiva begin to decrease in traumatized eyes, a superficial inflammation occurs and epithelial integrity is impaired. Depending on these results, the colonization of opportunistic bacteria and fungi to the eye increases (Perreira *et al*., 2019). The most isolated agents from the eye are coagulase positive and coagulase negative *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp. and *Escherichia coli* (Suter *et al*., 2018; Auten *et al*., 2020). *S. pseudintermedius* can participate in ocular infections as an opportunistic pathogen and is also important because it is a zoonotic microorganism (Hamed *et al*., 2017; Perreira *et al*., 2019). *S. pseudintermedius* does not typically colonize humans but transmission can occur between dogs and their owners/guardians and small animal veterinarians. If people come into close contact with pet animals colonized and/or infected with *S. pseudintermedius*, they can become temporary carriers and various infections can occur in humans (Bannoehr and Guardabassi, 2012). The first report in humans was *S. pseudintermedius*, which was isolated from pus and tissue in a 60-year-old patient's infected implantable cardioverter defibrillator in 2006 (Van Hoovels *et al*., 2006). Since 2006, *S. pseudintermedius* cases in humans have been increasing steadily. It is difficult to distinguish *S. pseudintermedius* from other staphylococci (*S. aureus*, other members of SIG) by conventional methods and it may be misidentified as *S. aureus* in some medical laboratories. Therefore, its prevalence in humans is not known exactly (Moses *et al*., 2023).

Staphylococci have many virulence factors. One of the most significant virulence factors in staphylococci is thought to be biofilm formation. Bacterial biofilms are therapeutically important because they all give resistance to phagocytosis, the host immune system, antibiotics and disinfectants, which are factors that induce chronic infections (Pompilio *et al*., 2015). Additionally, the development of biofilms can assist *Staphylococcus* to survive in the presence of antimicrobial agents and aid the bacteria in evading the host immune system, which can result in prolonged infection (Resch *et al*., 2005). *S. pseudintermedius* conjunctivitis-associated canine strains have also shown enhanced biofilm formation (Hamed *et al*., 2017).

Antimicrobial resistance has become a worldwide problem in both human and veterinary medicine (Kang *et al*., 2014; Soimala *et al*., 2020). Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) can show resistance not only to methicillin but also to many antibiotics (tetracycline, fluoroquinolone) used in ophthalmic infections (Kang *et al*., 2014; Perreira *et al*., 2019). Methicillin resistance is encoded by the *mec*A gene carried on a mobile genetic element (Staphylococcal Cassette Chromosome – SCC*mec*) (Kang *et al*., 2014). The transfer and exchange of SCC*mec* components among various staphylococcal species is an important concern in both veterinary and human medicine because staphylococcal strains have the potential to evolve into superbugs that cause infections that are difficult to successfully treat medically (Moses *et al*., 2023). Due to the indiscriminate use of antimicrobial agents, antimicrobial resistance has developed and multi-drug resistant (MDR) *S. pseudintermedius* strains have emerged (Little *et al*., 2019). A study investigating MDR in dogs with ulcerative keratitis found incidence increasing from 5% (2016) to 34% (2020) (including 33% of all *Staphylococcus* species) during the period studied (Hewitt *et al*., 2020). In particular *S. pseudintermedius*, which has both methicillin resistance and multi-drug resistance, creates difficulties in the treatment of patients, prolongs the duration of treatment or worsens the prognosis of the disease.

Materials and Methods

Conjunctivitis was diagnosed after routine ophthalmological examination in a 3 year old male Dachshund breed that came to an animal hospital in Cyprus with the complaints of red eye and mucopurulent tear discharge. No previous treatment had been applied to the patient's eye before. As a result of the clinical examinations, no other systemic disease was diagnosed in the patient.

Conjunctival samples were taken from both eyes (right and left) with a swab containing liquid Amies medium (Copan-493CE03, MRSA system) under aseptic conditions. Samples were taken by carefully separating the palpebrae from the conjunctiva and inserting the swab into the conjunctival sac. After the samples were taken, they were transported to the microbiology diagnostic laboratory in the cold chain.

For bacteriological examination, the swabs were inoculated on 5% Columbia sheep blood agar (Biomerieux, 43041), MacConkey agar (Merck, 105465), Eosin Methylene Blue agar (Merck, 103858) and Baird Parker agar (Merck, 105406). All agar plates were incubated in an aerobic conditions at 37°C for 24-48h and the formed bacterial colonies were evaluated. Bacterial colonies of approximately 1-2 mm in diameter were observed in the blood agar, on which the right eye swab was inoculated, in grey color and with double haemolysis zones. Gram staining method (Biomerieux, 55542) was applied to pure bacterial colonies and colonies showing Gram positive cocci microscopic morphology were examined for catalase (Biomerieux, 55561), coagulase tests (slide and tube) (Merck, 113306) and inoculated on Dnase agar (Merck, 110449). The Congo red agar (Oxoid, UK) method applied by Freeman *et al*., (1989) was used to investigate the slime (biofilm) forming ability.

The antibiotic susceptibility profile of *S. pseudintermedius* was determined by microdilution method using VITEK-II Compact device and AST-GP80 (Biomerieux, 421826) card. MIC values were evaluated according to Clinical Laboratory Standards

Institute (CLSI, 2021) guidelines (Table 1). The GP-AST card also included an inducible Clindamycin resistance test and a Cefoxitin screening for methicillin resistance.

For molecular diagnosis, nucleic acid extraction was performed from the pure bacterial colony by the boiling method. The *pta* gene of the isolate was revealed by the previously published RFLP-PCR (Restriction Fragment Length Polymorphism - Polymerase Chain Reaction) method and primers (Bannoehr *et al*, 2009). In order to determine methicillin resistance, the *mec*A gene was detected with the previously applied PCR procedure and primers (Choi *et al*., 2003). PCR and RFLP-PCR products were run in 1.5% (wt/vol) agarose and visualized under UV light. *Staphylococcus pseudintemedius* ED99 (GenBank Accession NC_017568.1) and MRSA ATCC33591 were used as positive controls in the detection of *pta* and *mec*A genes.

Table 1 Antimicrobial susceptibility/resistance profile of *S. pseudintermedius* (right eye).

Drug	Minimum inhibitory concentration (µg/ml)	Susceptible(S) / Resistance (R)
Clindamycin	0.5	S
Nitrofurantoin	<16	S
Neomycin	<2	S
Gentamicin	>16	R
Kanamycin	>64	R
Marbofloxacin	>4	R
Pradofloxacin	2	R
Erythromycin	>8	R
Enrofloxacin	>4	R
Doxycycline	>16	R
Tetracycline	>16	R
Chloramphenicol	>64	R
Trimethoprim/Sulphamethoxazole	>320	R
Sefoxitin Screening	POS	
Inducible Clindamycin Resistance	NEG	

Results and Discussion

Staphylococci are among the microorganisms isolated from ocular infections (Auten *et al*., 2020; Soimala *et al*., 2020). In this case, *S. pseudintermedius* was identified from a right eye conjunctival swab from a dog with conjunctivitis in clinical examination. Similarly, in a study by Hamed *et al*., (2017), *S. pseudintermedius* was identified from both of two cases of keratoconjunctivitis sicca. In another study, 88 (66.7%) *S. pseudintermedius* were found in samples taken from 65 canine keratoconjunctivitis cases (Perreira *et al*., 2019). The ability to form biofilm (slime) is one of the important virulence factors of staphylococci (Bertelloni *et al*., 2021). Some strains of *S. pseudintermedius* are capable of forming biofilms (Hamed *et al*., 2017; Seo *et al*., 2021). *S. pseudintermedius* produces a biofilm that is crucial to the pathophysiology of the infection and potential colonization. This virulence character is likely to have a role in the rapid global emergence of MRSP (Perreten *et al*., 2010). The disadvantage in terms of ocular infections is that when topical antibiotics are used, it delays the penetration of the antibiotic and complicates the treatment (Pompilio *et al*., 2015). A total of 29 *S. pseudintermedius* were isolated from 119 conjunctival swab from healthy dogs and dogs with keratitis, and biofilm formation of all isolates was evaluated as positive (Wang *et al*., 2022). In this case, the biofilm forming ability of the strain was evaluated as positive due to the presence of black colonies on Congo red agar (Fig 1).

Figure 1 *S. pseudintermedius* forms black colonies on Congo red agar (biofilm formation)

Conjunctivitis can be accompanied by a number of other diseases. Especially atopic individuals often experience various ocular problems such as atopic keratoconjunctivitis, keratoconus, cataract and retinal detachment. Canine atopic dermatitis is associated with conjunctivitis with a prevalence ranging from 29% to 50% (Griffin and DeBoer, 2001; DeBoer and Hillier, 2001). In the study of Furiani *et al*., (2011), it was shown that bacterial colonization in the conjunctival sac in atopic dogs was more frequent and wider than in healthy dogs and *S. pseudintermedius* was the most isolated species. However, there was no concomitant skin disorder in this case.

In recent years, MDR-MR *S. pseudintermedius* has been widely isolated from pet animals (Kang *et al*., 2014; Perreira *et al*., 2019). *S. pseudintermedius* strains with MDR properties are not more virulent than antibiotic-susceptible isolates but they constitute a problem in treatment (Saputra *et al*., 2017). According to the results of microdilution applied in this case, *S. pseudintermedius* strain was found resistant to macrolide group (erythromycin), tetracycline group (tetracycline, doxycycline), amphenicol group (chloramphenicol), aminoglycoside group (gentamicin, kanamycin), fluoroquinolone group (marbofloxacin, pradofloxacin, enrofloxacin)and trimethoprim/sulfamethoxazole. The AST-GP80 card of the VITEK-II device also has Cefoxitin Screening property. As a result, the strain Cefoxitin Screening was also positive. After VITEK-II microdilution results, methicillin resistance was also confirmed by PCR and strain was evaluated as *mec*A positive (Fig 2).

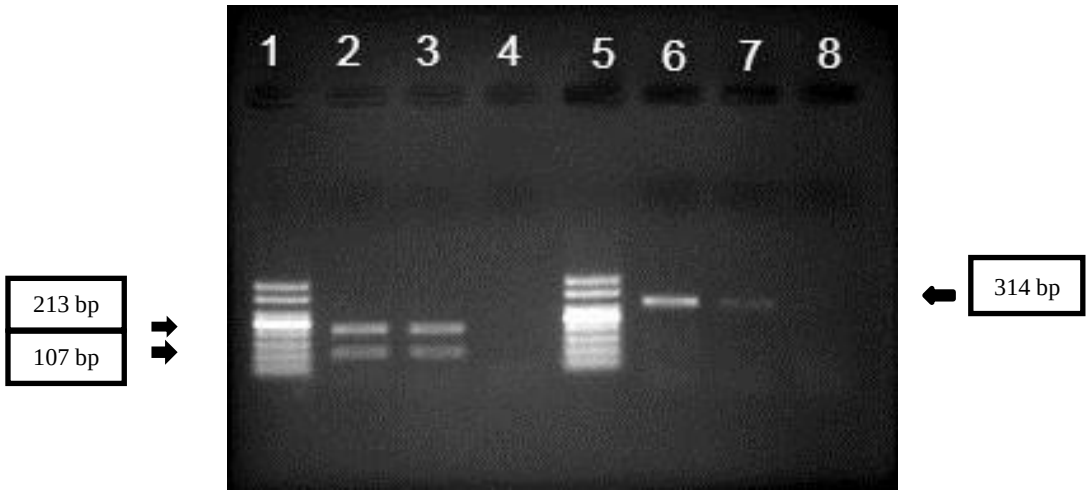

Figure 2 Agarose gel electrophoresis showing the result of RFLP-PCR of 107bp and 213bp for the *pta* gene and PCR of 314bp for *mec*A gene *Staphylococcus pseudintermedius* isolate. Lane 1 and 5: Ladder, Lane 2 and 6: *S. pseudintermedius* strain, Lane 3: *S. pseudintermedius* ED99 strain (positive control for *pta* gene), Lane 7: MRSA ATCC33591 strain (positive control for *mec*A gene), Lane 4 and 8: negative control

In accordance with the antimicrobial susceptibility results, the patient was treated with an eye drop preparation containing neomycin. In a study, 18 of 50 *S. pseudintermedius* isolated from ophthalmic specimens of dogs were identified as MDR-MR (Kang *et al*., 2014). Contrary to the results of Kang *et al*., (2014), Soimala *et al*., (2020) found a very low rate of MRSP from various ophthalmic infections of cats and dogs. Soimala *et al*. (2020); 38 (39.6%) *S. pseudintermedius* were isolated from conjunctival swabs taken from 72 dogs and 24 cats, and 3 (7.9%) of them were found to be MRSP. All MRSP isolates were obtained from dog samples and 2 from conjunctivitis cases (Soimala *et al*., 2020). Suter *et al*. (2018), identified 11 *S. pseudintermedius* and 3 MRSPs from 113 dog eye isolates in their study with cats, dogs and horses with septic keratitis. Based on these studies, we can see increasing rates of *S. pseudintermedius* in canine ophthalmic infections.

In conclusion, this is the first report of a canine conjunctivitis caused by MDR-MRSP in Cyprus. MDR-MRSP zoonotic transmission could have a serious impact on public health. When used carelessly, broad-spectrum systemic or topical antibiotics might enhance bacterial resistance and zoonotic potential while treating and preventing ocular diseases. The results of this multidrug resistance profile show that empirical treatment without antibiotic susceptibility tests should be avoided in cases of bacterial conjunctivitis caused by *S. pseudintermedius*.

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