Multidrug-resistant *Staphylococcus pseudintermedius* isolated from dog: case report

Staphylococcus pseudintermedius *multirresistente isolado do cão: relato de caso*

Juliana AIZAWA[1]; Antonio Francisco de SOUZA-FILHO[1]; Adriana CORTEZ[2]; Carla Gasparotto Chande VASCONCELOS[3]; Janaina BIOTTO[4]; Marcos Bryan HEINEMANN[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo – SP, Brazil
[2] Universidade Santo Amaro, Curso de Veterinária, São Paulo – SP, Brazil
[3] Vida Vet Laboratório Veterinário, Botucatu – SP, Brazil
[4] Clínica Veterinária Vila Chico, Botucatu – SP, Brazil

Abstract

The zoonotic potential of multidrug resistant (MDR) bacteria is a worldwide concern and companion animals have been implicated in the spread of resistant bacteria. Therefore, surveillance is important, as there are reports of transmission of these bacteria from dog to men, as well as from men to dog. A 5-year-old mixed-breed male dog was admitted with obstructive struvite urolithiasis relapsing for over 18 months, in Botucatu, in the state of São Paulo, Brazil. The strain, biochemically identified as *Staphylococcus spp.*, was MDR and was treated off-label with vancomycin, which resulted in clinical cure. The strain was molecularly identified as *Staphylococcus pseudintermedius* and the *mecA* gene was identified. This is the main gene responsible for methicillin-resistant *S. pseudintermedius* (MRSP), which is often resistant to multiple antimicrobials. The hypotheses for this clinical case are the transmission from man to animal, since the tutor was an intensivist doctor, or the bacterium itself could be part of the animal's microbiota and due to other factors, such as stress or obstructive urinary disease, opened a doorway to infection by *S. pseudintermedius.* Further studies should elucidate the transmission of MDR bacteria between human and pets.
Keywords: Dog. Multidrug resistance. *Staphylococcus pseudintermedius*. Zoonosis.

Resumo

O potencial zoonótico de bactérias multirresistentes é uma preocupação global e os animais de companhia têm sido implicados na disseminação de bactérias resistentes; assim, é importante a vigilância, pois já existem relatos de transmissão destas bactérias do cão para o homem e vice-versa. Um cão, sem raça definida e de cinco anos de idade, foi atendido na cidade de Botucatu, São Paulo, Brasil, apresentando urolitíase obstrutiva de estruvita recorrente há um ano e meio. Na urocultura do animal foi isolada uma estirpe de *Staphylococcus spp.* multirresistente; o tratamento com vancomicina possibilitou acura clínica. A estirpe de *Staphylococcus spp.* isolada foi identificada molecularmente como *S. pseudintermedius* e nela foi identificada a presença do gene *mecA*, o principal responsável por *S. pseuidintermedius* resistente à meticilina (MRSP), e que é frequentemente resistente à múltiplos antimicrobianos. As hipóteses para este caso clínico são a transmissão do homem para o animal, pois o tutor era um médico intensivista, ou que a própria bactéria fazia parte da microbiota do animal e, devido a outros fatores como estresse e doença urinária obstrutiva, abriu-se uma porta de entrada para a infecção pelo *S. pseudintermedius*. Mais estudos são necessários para a elucidação da transmissão de bactérias multirresistentes entre animais de companhia e o ser humano.
Palavras-chave: Cão. Multirresistência. *Staphylococcus pseudintermedius*. Zoonose.

Correspondence to:
Marcos Bryan Heinemann
Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia
Av. Prof. Dr. Orlando Marques de Paiva, 87
CEP 05508-270, São Paulo, SP, Brazil
e-mail: marcosbryan@usp.br

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The development of antimicrobial resistance has been intensified by the overuse of antimicrobials, resulting in the emergence of multidrug-resistant (MDR) pathogens (CLARKE, 2006; THERETZBACHER, 2013). Resistance is defined as the acquired capability of bacteria to remain viable in the presence of concentrations of antimicrobials that are commonly lethal to the bacteria (WHO, 2015). MDR is defined as resistance to three or more classes of antimicrobials

(MAGIORAKOS et al., 2012). Antimicrobial resistance is a multidisciplinary problem that is directly related to the use of antimicrobials in human and veterinary medicine (RIZEK et al., 2011). The zoonotic potential of MDR bacteria, especially in food-producing animals, is a worldwide concern. Livestock and companion animals should be monitored, since they have also been implicated as one of the multifactorial causes of resistant bacteria spreading. Pet-to-human transmission of MDR bacteria such as methicillin-resistant staphylococci (MRS) is also a concern, and consequently risks for both humans and companion animals should be investigated (WIELER et al., 2011). Possible hypotheses for the clinical case described are that the bacterium was already present in the skin microbiota or a man-animal transmission, since the tutor was an intensivist physician.

A 5-year-old mixed-breed male dog was admitted with obstructive struvite urolithiasis relapsing over for 18 months in Botucatu, in the state of São Paulo, Brazil. Urine exams revealed the presence of bacteria and low urinary specific gravity. When treatment failed, urine culture was performed and *Staphylococcus spp.* was identified using standard biochemical procedures. Antibiogram results using Kirby-Bauer disk diffusion indicated resistance to penicillins, cephalosporins, cephamycins, carbapenems, quinolones, tetracyclines, lincosamides, phenicols, amynoglicosides, pholate pathway inhibitors, macrolides, fosfomycins, ansamycins, and monobactams. The strain presented susceptibility to glycopeptides (vancomycin, teicoplamin) and nitrofurans (nitrofurantoin). Off-label treatment with slow intravenous infusion of vancomycin twice daily for 7 days was necessary for clinical cure. The strain was molecularly identified as *Staphylococcus pseudintermedius*, in which the *mec*A gene was identified. PCR was performed after bacterial genomic DNA had been extracted from culture in mannitol salt agar that was incubated at 36°C for 24 hours. DNA extraction was performed using the boiling method. Species molecular identification was made via a conserved region of the thermonuclease gene (*nuc*) and the primers used were described by Sasaki et al. (2010). Methicillin resistance identification was made via the *mec*A gene and the primers used were described by Mehrotra et al. (2000).

The *mec*A gene is the main gene responsible for MRSP. Moreover, MRSP patients are often resistant to other classes of antimicrobials (KADLEC; SCHWARZ, 2012), agreeing with the observations found in the present case. *Staphylococcus pseudintermedius* is one of the most frequent colonizers of canine healthy skin (DEVRIESE et al., 2009) and MRSP can be a common member of the microbiota of healthy dogs (QUITOCO et al., 2013). Additionally, a breach in immune defenses might result in urinary tract infection caused by bacteria that is present in the skin, even though Gram-positive bacteria are less frequently isolated from urine cultures in dogs than Gram-negative bacteria (RUBIN; GAUNT, 2011; THOMPSON et al., 2011).

In addition, the *mec*A gene is located on a mobile genetic element, and the staphylococcal cassette chromosome *mec* (SCC*mec*) and transmission as part of the SCC*mec* between staphylococcal species are well known. However, the mechanism through which this inter-species transference occurs is still unknown (KATAYAMA et al., 2000; HIRAMATSU et al., 2013). Either the bacteria may be transferred from human to animal or resistance genes may be transferred between staphylococcus species. Nevertheless, the transfer of genes is least probable since the types of SCC*mec* elements present in MRSP seem to differ from those identified in MRSA (KADLEC; SCHWARZ, 2012).

The transmission of MRSP between the tutor and pet is a possibility because its owner was an intensive care doctor and MRS is one of the main causes of hospital acquired infections, especially MRSA (SIEVERT et al., 2013). However, cross-contamination of MRSP between a dog and the dog's owner may occur and has already been described (SOEDARMANTO et al., 2011). This was the first assumption that had to be investigated when the owner returned after successful treatment, and it was investigated by characterizing strains obtained from the dog and the owner and establishing genetic relatedness. Another theory is the possible human-dog transmission of MRSA or the dog-human transmission of MRSP and posterior transmission of the *mec*A gene between the *Staphylococcus* species.

In conclusion, control of MRSP requires the rational use of antimicrobials and prevention of transmission (GUARDABASSI, 2013). Thus, a national program guiding the use of antimicrobials in companion animals would be necessary for the first measure to be applied. Further studies must be conducted to elucidate the influence of the changing relationship between humans and pets, regarding interspecies transference of resistant bacteria and bacterial resistance encoding genes.

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