REVISTA DE CIÊNCIAS AGRÁRIAS
Amazonian Journal
of Agricultural and Environmental Sciences
www.ajaes.ufra.edu.br

http://dx.doi.org/10.22491/rca.2018.2794

COMUNICAÇÃO CIENTÍFICA

Detection of the mecA gene in *Staphylococcus* sp. isolated from dog skin with atopic dermatitis and recurrent pyoderma

Detecção do gene mecA em Staphylococcus *sp. isolados da pele de cão com dermatite atópica e piodermite de repetição*

Sinerey Karla Salim Aragão de Sousa[1]
Alexandre do Rosário Casseb[1]
Sandro Patroca da Silva[2]
Samir Mansur Casseb[2]
Andre Marcelo Conceição Meneses[1*]

[1] Universidade Federal Rural da Amazônia – UFRA, Avenida Presidente Tancredo Neves, 2501, Terra Firme, 66077-830, Belém, PA, Brasil
[2] Instituto Evandro Chagas – Rodovia BR 316, Km 7, s/n, Levilândia, 67030-000, Ananindeua, PA, Brasil
***Corresponding author:**
E-mail: andre.meneses1974@icloud.com

KEYWORDS
CAD
mecA gene
Staphylococcus
PCR

PALAVRAS-CHAVE
DAC
Gene mecA
Staphylococcus
PCR

ABSTRACT: Canine atopic dermatitis (CAD) is a multifactorial, allergic and inflammatory skin disorder with a genetic predisposition manifested by clinical signs, and it is generally associated with the production of specific IgE antibodies, mainly against environmental allergens. The objective of this study was to report the occurrence of mecA gene in an isolate of *Staphylococcus* sp. from a dog with canine atopic dermatitis and recurrent pyoderma. Clinical specimen was collected for culture and antimicrobial susceptibility analysis. The isolate of *Staphylococcus* was then submitted to PCR for the detection of the mecA gene. The isolation of *Staphylococcus* sp. from this patient's skin is worrisome, since the presence of such multi-resistant bacterium in such circumstance is a potentially grave risk for public health.

RESUMO: *A dermatite atópica canina (DAC) é uma afecção cutânea multifatorial, alérgica e inflamatória, com predisposição genética e manifestada por sinais clínicos característicos, e está geralmente associada à produção de anticorpos IgE específicos, principalmente contra alérgenos ambientais. O objetivo deste estudo foi relatar a ocorrência do gene mecA em um isolado de* Staphylococcus *sp. de um cão com dermatite atópica canina e pioderma recorrente. A amostra clínica foi coletada para cultura e análise de susceptibilidade antimicrobiana, submetendo-se o* Staphylococcus *isolado à PCR para a detecção do gene mecA. O isolamento de* Staphylococcus *sp. da pele deste paciente é uma situação preocupante, uma vez que a presença de bactérias multirresistentes é um fato de grande perigo para a saúde pública.*

Received: 29 jan. 2018
Accepted: 6 jun. 2018

1 Introduction

Skin is the largest and most visible organ of the body. It works as an anatomical and physiological barrier between the animal and the environment. Its interactions with the internal organs will often be indicative of pathological events that are occurring in such organs and their associated tissues (Miller et al., 2013).

Canine atopic dematitis (CAD) is a multifactorial, allergic and inflammatory skin disease that is associated with genetic predisposition and manifests itself with characteristic clinical signs, associated with the production of specific IgE antibodies, mainly against environmental allergens (Dethioux, 2006).

Methicillin-resistant *Staphylococcus pseudointermedius* (MRSP) has been described since the 1980s, and it is common in recurrent pyoderma, making it difficult to treat. The prevalence of methicillin-resistant strains of *Staphylococcus* has recently increased, and, in some areas of the United States of America, more than 50% of dog's skin cultures are composed of methicillin-resistant *Staphylococcus* (Guardabassi et al., 2010).

Staphylococcus sp. is a very frequent bacterium in nosocomial infections, and since dogs are increasingly prevalent in Brazilian homes, and CAD is being more frequently diagnosed, the recovery of this bacterial pathogen from a dog skin it is a case of extreme importance.

The objective of this study was to report the presence of the mecA gene in *Staphylococcus* sp. isolated from the skin of a dog with CAD and recurrent pyoderma.

2 Material and Methods

The paper is registered in Ethics Committee of Federal Rural University of Amazonia under protocol nº. 004/2014 (CEUA) -23084.001959 / 2014-99.

A female Poodle, two years old, presented a chronic / recurrent condition, for almost a year, of intense pruritus associated with pyoderma that subsided with the use of systemic antibiotic therapy.

Erythematous, dry, scaly skin with pustular lesions and numerous epidermal collars and pruritus were observed during clinical examination.

Skin scrapings were negative for mites and fungi. Cytology revealed frequent Gram-positive cocci and *Malassezia* sp. Following exam results, a clinical diagnosis of CAD was determined, using the criteria of Favrot (2010).

The microbial species were identified based on colony morphology, Gram staining, pigment production and biochemical tests (Ikram, 2005).

The culture confirmed the presence of coagulase positive *Staphylococcus* sp., and the antimicrobial susceptibility indicated resistance to oxacillin.

The bacterium was submitted to polymerase chain reaction (PCR) to detect the mecA gene.

DNA was extracted with kit Purelink Genomic DNA Mini Kit (Invitrogen), commercial kit.

After extraction, conventional PCR was performed, according to the established protocol for mecA gene detection. The primer were mecA-F1 (5'-AAAATCGATGGTAAAGGTTGGC-3') and mecA-R (5'-AGTTCTGCAGTACCGGATTTGC-3') (Maluta, 2008), with conventional PCR performed following to the protocol used by Guardabassi (2010).

The generated amplicon was analyzed by electrophoresis on a 2% agarose gel, at a voltage of 100 v / 40min and viewed under ultraviolet (UV) light, with a 533 base pairs fragment.

Purification of amplified PCR-DNA in PCR was performed with the Quick Gel extraction kit (Promega, USA).

Purified DNA was sequenced in cycles with the ABI PRISM Dye Terminator Version 3.0 Kit, which employs the dideoxyribonucleotide chain termination method labeled with different fluorophores for each nucleotide at the 3 'end. The mixture for each sequencing reaction contained 4 μL of Terminator ready reaction mix, 50 ng PCR product, 3.5 pmol oligonucleotides used for PCR (mecA-F1 and mecA-R primers), and sufficient amount of water for one final volume of 20 μL.

Amplification was performed using a 25 cycle program, each cycle consisting of denaturation steps at 96°C for 10 seconds, primer hybridization at 52°C for 5 seconds and extension at 60°C for 4 min. Following this procedure, the sequencing product was purified using a commercial X-terminator kit (Applied Biosystems, USA). Assembly and curation of the obtained readings were made using the Geneious v.9.1.6 program. The nucleotide sequences obtained from sequencing were aligned and compared against each other and against other sequences from various *Staphylococcus* spp. available at Genbank (http://www.ncbi.nlm.nih.gov) using the Mafft v.7 program.

3 Results and Discussion

Because it is a cutaneous, multifactorial condition with allergic and inflammatory characteristics, CAD causes a lot of discomfort for the animal according to Dethioux (2006). The patient showed pruritus throughout the consultation. One of the characteristics of this disease is the loss of the integrity of the epidermal barrier, which, according to Olivry et al. (2010) is one of the key factors triggering the clinical signs. In the current study this was manifested by the patient in the form of erythema, desquamation and secondary bacterial infection. As noted by Maluta (2008), bacterial pyoderma has been widely reported as a baseline cause of such dermal reactions as allergies and endocrinopathies. Furthermore, as shown experimentally by Pianta et al. (2006), inflamed skin with pyoderma is associated with the decrease of antimicrobial peptides, which favors colonization by coagulase-positive staphylococci more rapidly than on healthy skin.

A cytology test found Gram positive cocci. This is significant as this method is regarded by Hillier (2002), as highly conclusive for the confirmation of bacterial colonization, who emphasize the use of cultures with antibiogram.

The presence of *Staphylococcus* sp. in the patient sample of the present report is according Pereira et al. (2009), who detected resistance in 48.6% of the isolates of *Staphylococcus* sp. of different infectious sites of companion animals. Tunon et al. (2008) found strains of *Staphylococcus* sp. in canine otitis and found 83% of strains resistant to at least one of the antibiotics used in research. In this study, the detection of the mecA gene in Staphylococcus sp. with resistance to oxacillin showed that the disc diffusion test may be an indication for the

use of PCR, not according to Penteado Filho (2007), which says that diffusion test on disk does not have sufficient sensitivity to detect resistance.

4 Conclusions

CAD causes loss of the epidermal barrier, which may facilitate the colonization of the skin by multi-resistant microorganisms. Consequently, treatment should be directed towards the restoration of this protection and subsequent reduction of these microorganisms, thereby diminishing the possibility of cross-species infection between animal and human.

References

DETHIOUX, F. Dermatite atópica canina, um desafio para o clínico. *Focus Royal Canin*, v. 1, p. 7-56, 2006. Suplemento.

FAVROT, C.; STEFFAN, J.; SEEWALD, W.; PICCO, F. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Veterinary Dermatology*, v. 21, n. 1, p. 23-31, 2010. doi: 10.1111/j.1365-3164.2009.00758.x.

GUARDABASSI, L.; HOUSER, G. A.; FRANK, L. A.; PAPICH, M. G. Orientações para o uso de antimicrobianos em cães e gatos. In: GUARDABASSI, L.; JENSEN, L. B.; KRUSE, H. (Ed.). *Guia de antimicrobianos em veterinária*. Porto Alegre: Artmed, 2010. p. 224-246.

HILLIER, A. Definitively diagnosing atopic dermatitis in dogs. *Veterinary Medicine*, v. 97, n. 3, p. 198-208, 2002.

IKRAM, M. Microbiologia diagnóstica. In: HENDRIX, C. M. *Procedimentos laboratoriais para técnicos veterinários*. 4. ed. São Paulo: Roca, 2005. p. 109-225.

MALUTA, R. P. *Isolamento e perfil de suscetibilidade a antimicrobianos de cepas de estafilococos resistentes à em um hospital veterinário de ensino no Brasil*. 2008. 44 f. Dissertação (Mestrado em Microbiologia Agropecuária) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, 2008.

MILLER, W. H.; GRIFFIN, C. E.; CAMPBELL, K. L. Atopic disease. In: MILLER, W. H.; GRIFFIN, C. E.; CAMPBELL, K. L. *Muller and Kirk's small animal dermatology*. 7. ed. St. Louis: Saunders, 2013. p. 364-388.

OLIVRY, T.; DEBOER, D. J.; FAVROT, C.; JACKSON, H. A.; MUELLER, R. S.; NUTTALL, T.; PRÉLAUD, P. Clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Veterinary Dermatology*, v. 21, n. 3, p. 233-248, 2010. doi: 10.1111/j.1365-3164.2010.00889.x.

PENTEADO FILHO, S. R.; LOPES, H. V.; LEVI, G. C. Antibióticos clássicos: principais características e uso terapêutico. In: NETO, V. A.; NICODEMO, A. C.; NICODEMO, A. C.; LOPES, H. V. *Antibióticos na prática médica*. 6. ed. São Paulo: Sarvier, 2007. p. 87-156.

PEREIRA, I. A.; SOARES, L. C.; COELHO, S. M. O.; PRIBUL, B. R.; SOUZA, M. M. S. Suscetibilidade à azitromicina de isolados bacterianos de processos infecciosos em cães e gatos. *Pesquisa Veterinária Brasileira*, v. 29, n. 2, p. 153-156, 2009. doi: 10.1590/S0100-736X2009000200012.

PIANTA, C.; OLIVEIRA, S. J.; FALLAVENA, L. C. B.; ESMERALDINO, A. T.; SILVA JÚNIOR, V. B. Pioderma estafilocócico canino: identificação das espécies e sensibilidade aos antimicrobianos. *Revista de Ciências Agroveterinárias*, v. 5, n. 1, p. 60-63, 2006.

TUNON, G. I. L.; SILVA, E. P.; FAIERSTEIN, C. C. Isolamento de estafilococos multirresistentes de otites em cães e sua importância para a saúde pública. *Boletim Epidemiológico Paulista*, v. 58, n. 5, p. 4-7, 2008.

Authors' contribution: Sinerey Karla participated in the collection of materials for the microbiological and PCR exams. Alexandre Casseb coordinated all the microbiological exams. Sandro Patroca and Samir Casseb coordinated the PCR examinations, and Andre Meneses coordinated the entire research. All authors contributed and participated in the implementation of this article.

Acknowledgements: To University Federal Rural of Amazon and Evandro Chagas Institute.

Funding source: Not applicable.

Conflicts of interest: The authors declare no conflicts of interest. The authors are the sole responsible for the study content and writing.