

Antimicrobial resistance in staphylococci isolated from canine pyoderma

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Abstract

This study aimed to determine the frequency as well as the antimicrobial susceptibility profile of staphylococci isolated from canine pyoderma. The isolated strains were identified by biochemical tests and characterized by their susceptibility to antibacterial agents. The coagulase positive specie *S. pseudintermedius* was the most prevalent (82.4%, 28/34), followed by coagulase negative species *S. chromogenes* (6.0%, 2/34), *S. epidermidis* (2.9%, 1/34), *S. warneri* (2.9%, 1/34), *S. sciuri* (2.9%, 1/34) and *S. haemolyticus* (2.9%, 1/34). High rates of resistance to lincomycin, ampicillin, penicillin G and tetracycline were observed, what is probably due to the frequent use of these antibiotics in veterinary practice. A 32.4% percentage of the strains has shown multi-drug resistance, including a *S. pseudintermedius* strain resistant to oxacillin. All strains were sensitive to gentamycin, amikacin, tobramycin, chloranphenicol, imipenem, cephalothin and vancomycin. These results indicate the occurrence of resistant staphylococci associated with canine pyoderma and point to the need of careful selection of antibiotics based on results of the susceptibility testing, in order to reduce the selection of multiresistant strains.

Key-words: Staphylococcus, drug resistance, pyoderma, dogs

Resistência aos antimicrobianos em estafilococos isolados de piodermite canina

Resumo

O presente estudo teve como objetivo determinar a ocorrência e o perfil de susceptibilidade aos antimicrobianos de cepas de estafilococos isoladas de piodermite canina. As linhagens isoladas foram identificadas através de provas bioquímicas e caracterizadas em relação a sua susceptibilidade aos agentes antibacterianos. A espécie coagulase positiva *Staphylococcus pseudintermedius* foi a mais prevalente com 82.4% (28/34), seguida das espécies coagulase negativas *S. chromogenes* com 6.0% (2/34), *S. epidermidis* com 2.9% (1/34), *S. warneri* com 2.9% (1/34), *S. sciuri* com 2.9% (1/34) e *S. haemolyticus* com 2.9% (1/34). Foram evidenciados elevados índices de resistência a lincomicina, a ampicilina, a penicilina G e a tetraciclina, o que provavelmente se deve ao uso frequente destes antibióticos na prática veterinária. Um percentual de 32.4% das cepas isoladas apresentou multiresistência, incluindo uma cepa de *S. pseudintermedius* resistente a oxacilina. Todas as cepas foram sensíveis a gentamicina, a amicacina, a tobramicina, ao cloranfenicol, ao imipenem, a cefalotina e a vancomicina. Estes resultados evidenciam a ocorrência de estafilococos resistentes associados a piodermite canina e alertam para a necessidade da escolha criteriosa da antibioticoterapia baseada nos resultados do antibiograma, visando reduzir a seleção de cepas multiresistentes.

Palavras-chave: *Staphylococcus*, resistência a fármacos, piodermite, cães

Introduction

Pyoderma is a common disease in domestic animals, especially dogs. The primary skin pathogens of dogs are the staphylococci species, of which the coagulase-positive species *Staphylococcus pseudintermedius*, previously referred as *S. intermedius* (Devriese et al., 2009), is most frequently isolated (Kruse et al., 1996). *S. pseudintermedius* is present in the healthy dog's skin and besides pyoderma, can also cause urinary infection, corneal ulcer, otitis externa, otitis media and endocarditis (Penna et al., 2010; Pereira et al., 2009; Oliveira et al., 2006; Prado et al., 2006; Smith et al., 2000). Moreover, the zoonotic transmission of *S. pseudintermedius* to humans has been demonstrated (Pottumarthy et al., 2004).

The intensive use of antimicrobial agents has promoted an increase in the number of multi-drug resistant staphylococci strains from animal sources (Werckenthin et al., 2001). The drug resistance mechanisms present in *S. pseudintermedius* are similar to those found in other staphylococci species. Resistance to chloramphenicol, for example, involves the production of the enzyme chloramphenicol acetyl transferase encoded by the *cat* gene located in plasmid pSCS1, which has a high similarity with the *S. aureus cat* gene (Schwarz, 1995). Other plasmids carrying genes for resistance to chloramphenicol, macrolides, lincosamides and tetracyclines have been isolated from *S. pseudintermedius* and have shown structural similarities to plasmids isolated from *S. aureus* of human origin (Greene & Schwarz, 1992).

It is known that the erythromycin resistance in *S. pseudintermedius*, *S. xylosus* and *S. hyicus* is mediated by ribosomal methylase encoded by *ermB* gene (Eady et al., 1993). Multi-drug resistance mediated by *mecA* gene was also demonstrated, which encodes for an altered penicillin-binding protein (PBP2a) with low affinity to beta-lactam antibiotics in *S. pseudintermedius*, *S. aureus* and coagulase negative staphylococci isolated from dogs (Rota et al., 2011; Cohn & Middleton, 2010; Bemis et al., 2009; Coelho et al., 2007).

Antibiotic susceptibility testing allows veterinarians to monitor the susceptibility of common bacteria to antimicrobials. However, many cases of staphylococcal infections are treated empirically, thus contributing to resistant strains selection. Moreover, susceptibility studies can help to guide veterinarians in choosing the most effective antibiotics in a particular geographic region. The purpose of this study

was to investigate the antimicrobial susceptibility pattern of staphylococci isolated from pyoderma canine.

Material and Methods

Samples from 34 adult dogs of both sexes were collected with sterile cotton swabs from the skin lesions at a private veterinary clinic in Natal City, Brazil. Swabs were streaked onto blood agar (Bióbrás, Brazil) supplemented with 5.0% sterile ovine blood and agar plates were incubated overnight at 37°C. Typical colonies were picked and bacteria were identified by standard laboratory procedures. The identification of the strains as *S. pseudintermedius* was based on the following characteristics: slide and tube coagulase, anaerobic utilization of mannitol and lack of acid production from D-mannitol, acid production from trehalose and sensitivity to polymixin B. Coagulase negative staphylococci were identified by API Staph identification system (BioMérieux, France) according to the manufacturer's recommendations.

The susceptibilities of the isolates to 16 antibiotics (Cecon, Brazil) commonly used in veterinary and human medicine (penicillin G 10 µg, ampicillin 10 µg, oxacillin 1 µg, vancomycin 30 µg, cephalothin 30 µg, imipenem 10 µg, erythromycin 15 µg, clindamycin 2 µg, lincomycin 2 µg, streptomycin 10 µg, tobramycin 10 µg, amikacin 30 µg, gentamicin 10 µg, tetracycline 30 µg, chloramphenicol 30 µg and rifampicin 30 µg) were performed by the disk diffusion method observing the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2007). The standard strain *S. aureus* ATCC 25923 was used as control in all tests.

Results and Discussion

In all pyoderma canine cases studied, it was possible to isolate staphylococci strains. The distribution percentage of isolated species is presented in Table 1. *S. pseudintermedius* was the predominant species, what agrees with other researchers (Scott et al., 2006; Pedersen & Wegener, 1995). Coagulase-negative staphylococci (*S. chromogenes*, *S. epidermidis*, *S. haemolyticus*, *S. warneri* and *S. sciuri*) were isolated with lower frequency, which is in agreement with other studies that have isolated these species from canine pyoderma (Penna et al., 2009; Hauschild & Wójcik, 2007; Medleau et al., 1986). Coagulase-negative staphylococci species are common inhabitants of the skin endogenous microflora of healthy dogs and can act as opportunistic pathogens (Malik et al., 2005).

Table 1. Distribution percentage of staphylococci isolates obtained from canine pyoderma.

Isolation	N	%
<i>Staphylococcus pseudintermedius</i>	28	82.4
<i>Staphylococcus chromogenes</i>	2	6.0
<i>Staphylococcus epidermidis</i>	1	2.9
<i>Staphylococcus warneri</i>	1	2.9
<i>Staphylococcus haemolyticus</i>	1	2.9
<i>Staphylococcus sciuri</i>	1	2.9
Total	34	100.0

The *S. pseudintermedius* strains showed a significant percentage of resistance to lincomycin, penicillin G, ampicillin and tetracycline (Table 2). Less significant levels of resistance were observed for streptomycin, erythromycin, rifampin, clindamycin and oxacillin. The resistance to lincomycin, penicillin G and ampicillin was also prevalent among coagulase-negative strains. The *S. warneri* strain has shown susceptibility to all antimicrobial tested.

Resistance to lincomycin, penicillins and tetracycline is often reported in staphylococci isolated from dogs, what is probably related to the frequent use of these antibiotics in veterinary practice, including the treatment of pyoderma in dogs (Hoekstra & Paulton, 1996; Harvey et al., 1993). Penna et al. (2009) have found that a percentage of 71.8% of strains isolated from canine pyoderma were resistant to penicillin.

Table 2. Antimicrobial susceptibility rate of staphylococci isolated from canine pyoderma*.

Antimicrobial	<i>S. pseudintermedius</i> (n = 28) %	<i>S. chromogenes</i> (n = 2) %	<i>S. epidermidis</i> (n = 1) %	<i>S. sciuri</i> (n = 1) %	<i>S. haemolyticus</i> (n = 1) %
Oxacillin	96.4	100.0	100.0	100.0	100.0
Rifampicin	92.9	100.0	100.0	100.0	100.0
Clindamycin	85.7	100.0	100.0	100.0	100.0
Erythromycin	78.6	100.0	100.0	100.0	-
Streptomycin	71.4	100.0	100.0	100.0	100.0
Tetracycline	53.6	100.0	100.0	100.0	100.0
Penicillin G	50.0	50.0	100.0	-	-
Ampicillin	46.4	50.0	100.0	-	-
Lincomycin	42.9	50.0	-	-	-

*The *S. warneri* strain was susceptible to all antimicrobial tested. (-) No susceptible strain recovery.

At the present study, the aminoglycosides gentamicin, tobramycin and amikacin were effective in inhibiting all isolated strains. The streptomycin was the only antibiotic in this class to which the occurrence of resistant strains has been verified. Gentamicin and other aminoglycoside antibiotics have been reported as highly effective against staphylococcal infections in dogs (Oliveira et al., 2006; Oliveira et al., 2005). Since aminoglycosides can induce nephrotoxicity, the low percentage of resistance observed for the antibiotics of this class, probably, is related to the limited use of these drugs in the treatment of canine pyoderma (Noli & Morris, 2011). However, high levels of resistance were observed

in aminoglycosides staphylococci isolated from canine pyoderma (Penna et al., 2009). These discrepant results may be related to the local habits of antibiotic therapy.

Regarding the resistance patterns (Table 3), it was found that 50.0% (17/34) of strains have shown resistance to one or two classes of antibiotics and 38.23% (13/34) were multi-drug resistant (have shown resistance to three or more classes of antibiotics). A similar percentage of multi-drug resistant strains from canine pyoderma was obtained by Ganiere et al. (2005). However, a more recent study has shown a higher rate (94.9%) of multi-drug resistant strains isolated from canine pyoderma (Penna et al., 2009).

Table 3. Resistance pattern of staphylococci resistant strains isolated from canine pyoderma.

Staphylococci isolates	Sensitive n (%)	Non multi-drug resistant n (%)	Multi-drug resistant* n (%)	Total n (%)
<i>S. pseudintermedius</i>	3 (10.7)	13 (46.4)	12 (42.9)	28 (82.4)
<i>S. chromogenes</i>	0 (0.0)	2 (100.0)	0 (0.0)	2 (6.0)
<i>S. epidermidis</i>	0 (0.0)	1 (100.0)	0 (0.0)	1 (2.9)
<i>S. warneri</i>	1 (100.0)	0 (0.0)	0 (0.0)	1 (2.9)
<i>S. haemolyticus</i>	0 (0.0)	0 (0.0)	1 (100.0)	1 (2.9)
<i>S. sciuri</i>	0 (0.0)	1 (100.0)	0 (0.0)	1 (2.9)
Total	4 (11.8)	17 (50.0)	13 (38.2)	34 (100.0)

*Resistant to three or more class of antibiotics.

The multi-drug resistant strains emergence is a phenomenon that is occurring worldwide and that has hindered the treatment of human and animal staphylococcal infections. Different mechanisms, such as efflux pumps capable of causing the extrusion of several types of antibiotics out of the bacterial cell, encoded by genes located on plasmids or the acquisition of mobile genetic elements, such as the SCCmec, which contains additional insertion sequences

that allow the incorporation of several resistance genes, contribute to the acquisition of multi-drug resistance phenotype (Cohn & Middleton, 2010). The antibiotic therapy not careful and empirical contributes to rising the prevalence of multi-drug resistant strains, since the intensive use of antibiotics acts by exerting a selective pressure on the resistant strains, which can progressively acquire new resistance genes (Morar & Wright, 2010). Since 2010, the Brazilian government has

started controlling the antibiotics sale, requiring the prescriptions retention by pharmacies, an important measure which has inhibited the self-medication practice (Brazil, 2010). The results obtained in this study indicate the necessity of implementing more effective control measures to reduce the use of antibiotics in veterinary practice.

Successful treatment of canine pyoderma depends on the proper choice of antimicrobial agent for which the etiologic agent presents susceptibility. This study has found that all *S. pseudintermedius* isolated were susceptible to amikacin, tobramycin, gentamicin, cephalothin, vancomycin, imipenem and chloramphenicol. oxacillin, clindamycin and rifampin have also shown high efficacy against these species. Coagulase negative strains, as well as susceptibility to antibiotics previously mentioned, were also susceptible to tetracycline and streptomycin.

Conclusions

The results indicate the occurrence of resistant staphylococci on canine pyoderma and that canine host may contribute to the maintenance and dissemination of drug resistant staphylococci in our midst. They also warn about the need of a careful selection of antibiotics based on results of susceptibility testing, to reduce the selection of multi-drug resistant staphylococci strains.

Acknowledgements

The authors are grateful to Dr. Euclides Leandro de Castro, owner of Prontocan Veterinary Clinic for providing clinical material and to M.Sc. Leonardo Souza Carvalho for his helpful suggestions and his revision of the English version of this manuscript.

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