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IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCI ISOLATED FROM CANINE PYODERMA IN RIO DE JANEIRO, BRAZIL

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ABSTRACT

The study aimed to determine the etiology and antimicrobial resistance of staphylococci isolated from canine pyoderma. Samples were obtained from dogs with pyoderma and isolates were identified by biochemical reactions and tested for susceptibility to 15 antimicrobials. Thirty nine staphylococci isolates were obtained, and *S. pseudintermedius* was the most frequent (47.4%). All isolates showed resistance to at least one drug and 77.1% were multiresistant. The most effective drug was oxacillin. The study reports the alarming antimicrobial resistance of *Staphylococcus* isolated from canine pyoderma samples.

Key words: Staphylococci, resistance, antimicrobial agents, dog, pyoderma

Pyoderma is a common disease in dogs and its treatment usually involves antimicrobial therapy (3). Many studies have demonstrated that members of the *Staphylococcus* genus are commonly isolated from skin of healthy and diseased dogs (6), and that the coagulase-positive species *Staphylococcus pseudintermedius* (previously referred as *S. intermedius*), a common inhabitant of the skin of dogs, is the principal bacterial species responsible for canine pyodermas (3). The *S. intermedius* species entity was useful because it separated these staphylococci from *S. aureus,* avoiding in this way, an epidemiologically important source of confusion. Since this new species has been reported, strains from dogs identified by traditional means are to be reported as *S. pseudintermedius*, unless shown by genomic investigation to belong to other related species (2). Nevertheless, other staphylococcal species inhabiting skin may be involved, as *S. aureus, S. epidermidis, S. haemolyticus, S. simulans, S. saprophyticus* and *S. schleiferi* (6,8).

Knowledge about the resistance patterns of bacteria to antimicrobial drugs requires constant actualization. Although the impact of veterinary usage of the drugs is unknown, empiric treatments carried out by practitioners without assistance of bacteriological culture and antimicrobial susceptibility test (ATS) has contributed for the selection of multiresistant strains (6). The purpose of the present study was to determine the species distribution and antimicrobial

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resistance of *Staphylococcus* sp. isolates obtained from dogs with pyoderma in Rio de Janeiro, Brazil.

Specimens were obtained from samples of 39 adult dogs of both sexes submitted to the Bacteriology Laboratory of Universidade Federal Fluminense from veterinary clinics in Rio de Janeiro for identification and susceptibility testing between march of 2007 and march 2008. Although detailed historic of clinical cases were not provided, specimens were collected before treatment of the dogs. Animals presented lesions that indicated bacterial skin infections such as pustules, local pain, pruritus, erythema and desquamation of the epithelium. Samples were obtained using sterile swabs, which were immediately inoculated in Brain Heart Infusion broth (Merck, Rio de Janeiro, RJ - Brazil) and incubated at 37ºC. If growth occurred after 24 or 48h, smears were made, Gram-stained and examined microscopically. Samples with morphologies compatible with *Staphylococcus* sp. were transferred to tryptic soy agar (Merck, Rio de Janeiro, RJ - Brazil), 5% sheep blood agar (Newprov, Curitiba, PR – Brazil) and mannitol salt agar (Merck, Rio de Janeiro, RJ - Brazil). One sample of each dog was studied.

After growth, staphylococcal isolates were identified according to *Bergey's Manual of Determinative Bacteriology* (5) and tested for susceptibility to antibiotics by disc agar diffusion (1) for gentamicin, neomycin, amikacin, ciprofloxacin, enrofloxacin, amoxicillin-clavulanic acid combination - AMC, amoxicillin, oxacillin, cephalexin, cephadroxil, nitrofurantoin, erythromycin, mupirocin, bacitracin and chloramphenicol. After measuring the antimicrobial zone diameters and following the manufacturer's instructions (Oxoid, Hampshire, UK), the isolates were categorised as sensitive or resistant to the drug.

Thirty nine staphylococci isolates obtained from canine pyoderma were recovered. Among those 39 isolates of *Staphylococcus* genus, the coagulase-positive staphylococci (CoPS) were the most frequently isolated and were obtained from 25/39 (64.1%) samples, what agrees with other studies (3,4,9). Among the CoPS, *S. pseudintermedius* (19/39 – 48.7%) was the most common species, followed by *S. aureus* (5/39 – 12.8%) and *S. schleiferi coagulans* (1/39 – 2.6%). The predominance of *S. pseudintermedius* over *S. aureus* and *S.schleiferi coagulans* was not unexpected, since this species, previously referred as *S. intermedius*, is known as the major staphylococcal species involved in canine infections (3).

Coagulase negative staphylococci (CoNS) were isolated from 14 samples (35.9%), distributed among *S. schleiferi schleiferi* (7/39 – 17.9%), *S. epidermidis* (3/39 – 7.7%), *S. simulans* (3/39 – 7.7%), and *S. haemolyticus* (1/39 – 2.6%) (Table 1).CoNS constitutes a major component of the skin microflora of human beings, dogs and cats and can determine opportunistic infections (7,8). The species distribution of staphylococci isolates from pyoderma is depicted in table 1.

Regarding to the susceptibility pattern (table 1), all the isolates were resistant to at least one drug, and 94.9% of the isolates were multiresistant – here defined as resistance to three or more antimicrobial classes – what is a much higher proportion than that reported on older studies (3,9). One isolate of *S. pseudintermedius* was resistant to all the tested antimicrobial agents.

Some years ago it has been stated that this genus was presenting a rapid development and spread of antimicrobial resistance (3). Similar findings were observed in the present study, when compared to a previous study conducted by our group in a similar dog population in Rio de Janeiro, Brazil (6).

Resistance to the penicillin class was verified on 71.8% (28/39) of the isolates; although amoxicillin was the less effective penicillin (resistance of 71.8%), when combined to

clavulanic acid the resistance dropped to 20.5%. This was not surprising, since it has been recently demonstrated that up to 62% the isolates of the *S.pseudintermedius* (*S.intermedius*) strains of canine origin could be β-lactamase producers (3). When compared to a previous study conducted by our group (6), when resistance to that drug class in a similar population was 38.6% (17/44), a substantially grown in this resistance could be observed. Despite oxacillin was the most effective drug of the study, 17.9% (7/39) of the isolates were resistant to this drug, what is an alarming finding, since in 2000 our group reported such resistance on 4.6% of the isolates (2/44) (6).

Table 1. Resistance pattern of 39 staphylococci isolates obtained from dogs with pyoderma in Rio de Janeiro, Brazil.

N	Staphylococci isolates	Resistance Pattern
1	*S. aureus*	eri-neo-nit
2	*S. aureus*	ami-amo-bac-eri-gen-neo
3	*S. aureus*	ami-amo-bac-eri-gen-mup-neo-nit
4	*S. aureus*	ami-amo-cfd-cfe-eri-gen-mup-neo-nit
5	*S. aureus*	ami-amo-bac-clo-eri-gen-neo
6	*S.pseudintermedius*	ami-amo-neo
7	*S.pseudintermedius*	amo-bac-neo
8	*S.pseudintermedius*	amo-bac-eno-neo
9	*S.pseudintermedius*	eri-gen-mup-neo
10	*S.pseudintermedius*	ami-amo-bac-eri-neo
11	*S.pseudintermedius*	clo-eno-eri-gen-neo
12	*S.pseudintermedius*	ami-clo-eri-gen-mup-neo
13	*S.pseudintermedius*	ami-eri-gen-mup-neo-nit
14	*S.pseudintermedius*	ami-amo-eri-gen-neo-nit
15	*S.pseudintermedius*	amo-bac-eno-eri-gen-neo
16	*S.pseudintermedius*	amo-bac-cip-eno-eri-gen-neo
17	*S.pseudintermedius*	amo-bac-cfd-cfe-cip-clo-eno-neo
18	*S.pseudintermedius*	amc-ami-amo-eri-gen-neo-nit-oxa
19	*S.pseudintermedius*	ami-amo-cfd-cfe-clo-eri-gen-neo-nit
20	*S.pseudintermedius*	amc-ami-amo-cfd-cfe-eri-gen-neo-oxa
21	*S.pseudintermedius*	ami-amo-bac-cip-eno-eri-gen-mup-neo-nit
22	*S.pseudintermedius*	amc-amo-bac-cfd-cfe-clo-eri-neo-nit-oxa
23	*S.pseudintermedius*	amc-ami-amo-bac-cip-clo-eno-eri-gen-mup-neo-nit-oxa
24	*S.pseudintermedius*	All the 15 tested drugs
25	*S. schleiferi coagulans*	eri-neo
26	*S. schleiferi schleiferi*	bac-clo-eri-mup
27	*S. schleiferi schleiferi*	cip-eno-eri-gen-neo
28	*S. schleiferi schleiferi*	ami-amo-bac-eri-gen-neo-nit
29	*S. schleiferi schleiferi*	ami-amo-bac-eri-gen-mup-neo
30	*S. schleiferi schleiferi*	amo-cfd-cfe-cip-clo-eno-gen-neo
31	*S. schleiferi schleiferi*	ami-amo-bac-cip-clo-eno-gen-neo
32	*S. schleiferi schleiferi*	ami-amo-bac-cfd-cfe-clo-eno-gen-mup-neo-nit
33	*S. epidermidis*	ami-bac-cfe-cip-eno-eri-gen-neo
34	*S. epidermidis*	amo-cfd-cfe-clo-eri-gen-mup-neo-nit
35	*S. epidermidis*	amc-amo-bac-cfd-cfe-cip-clo-eno-eri-gen-mup-neo-oxa
36	*S. simulans*	ami-amo-cip-eno-eri-gen-neo-nit
37	*S. simulans*	ami-amo-bac-cfd-cfe-eri-gen-neo-nit
38	*S. simulans*	amc-ami-amo-bac-cfd-cfe-eri-gen-mup-neo-nit-oxa
39	*S. haemolyticus*	amc-ami-amo-bac-cfd-cfe-eri-gen-mup-neo

amc – amoxicillin + clavulanic acid; ami – amikacin; amo – amoxicillin; bac – bacitracin; cfd – cephadroxil; cfe – cefalexin; cip – ciprofloxacin; clo – chloramphenicol; eno – enrofloxacin; eri – erythromycin; gen – gentamicin; mup – mupirocin; neo – neomycin; nit – nitrofurantoin; oxa – oxacillin

Resistance to aminoglycosides was observed in 38 (97.4%) isolates; neomycin and gentamicin were the less effective drugs of this class, with 97.4% and 76.9% of the isolates being resistant, respectively; also amikacin did not present good efficiency rates, since 61.5% of the isolates were resistant to this drug. Gentamicin was reported as a very efficient drug for the treatment of canine otitis externa in 2000 (6), when resistance was 15.9% (7/44), what clearly contrasts to the 76.9% (30/39) frequency of resistant isolates observed in the present study. This fact is probably a consequence of the overusage of this drug in several topic phormulae indicated for the treatment of canine pyoderma, that became more common in the last decade. Similar findings were observed to other classes of drugs (Table 1) that, when compared to previous studies (3,7,8,9), constantly indicate an increasing resistance rate among the isolates.

In conclusion, antimicrobial resistance of staphylococci from canine pyoderma is a serious and increasing problem worldwide. The present study emphasizes the need for bacterial culture with species identification and antimicrobial susceptibility tests in order to choose the appropriate antimicrobial agent for each case.

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RESUMO

SUSCEPTIBILIDADE *IN VITRO* A ANTIMICROBIANOS DE STAPHYLOCOCCI ISOLADOS DE CÃES COM PIODERMATITE NO ESTADO DO RIO DE JANEIRO, BRASIL

O estudo se propõe a avaliar a etiologia e a susceptibilidade de estafilococos isolados de cães com dermatite. Os isolados foram identificados por provas bioquímicas e testados quanto a sua susceptibilidade a 15 antimicrobianos. Trinta e nove isolados de *Staphylococci* foram obtidos, e *S. pseudintermedius* foi mais freqüente (47,4%). Todos isolados apresentaram resistência a pelo menos uma droga e 77,1% foram multirresistentes. A oxacilina foi a droga mais eficaz. O estudo demonstra níveis alarmantes de resistência antimicrobiana nos *Staphylococcus* de pioderma canino.

Palavras-chave: Staphylococci, resistência, agentes antimicrobianos, cães, pioderma

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