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Risk factors associated with the antimicrobial resistance of staphylococci in canine pyoderma

Belén Huerta [a], Alfonso Maldonado [a], Pedro J. Ginel [b], Carmen Tarradas [a], Lidia Gómez-Gascón [a], Rafael J. Astorga [a], Inmaculada Luque [a,*]

[a] Departamento de Sanidad Animal, Facultad de Veterinaria, Campus Universitario de Rabanales, 14071 Córdoba, Spain
[b] Departamento de Medicina y Cirugía, Facultad de Veterinaria, Campus Universitario de Rabanales, 14071 Córdoba, Spain

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ABSTRACT

This study reports the susceptibility to antimicrobial agents of staphylococci ($n = 105$) isolated from dogs, and the factors associated with this resistance. The study animals were 23 healthy dogs (group A), 24 with first-time pyoderma (group B), and 27 with recurrent pyoderma that had undergone long-term antibiotic treatment (group C). Staphylococci were more commonly isolated from the pyoderma-affected than the healthy dogs ($p < 0.0001$).

Some 78% of the isolates were resistant to at least one antimicrobial agent. Resistance to amoxicillin–clavulanate, cephalosporins (OR 4.29, 95% CI [1.15, 16.3] respectively), enrofloxacin (OR 9.47, 95% CI [1.53, 58.5]) and ciprofloxacin (OR 79.7 95% CI [3.26, 1947.4]) was more common among group C isolates. Some 32% of all the isolates were multiresistant (MR) and 10.4% were methicillin-resistant (MRS). The probability of isolating MRS staphylococci in group C increased by a factor of four (95% CI [1.18, 17.9]) compared to A plus B. Multi-resistant (MR) isolates were obtained more commonly from urban than rural dogs (OR 3.79, 95% CI [1.09, 13.17]). All the MRS staphylococci encountered were obtained from urban dogs and more commonly from male dogs ($p = 0.07$).

This study shows that dogs bred in urban habitat, with a history of antibiotic therapy in the past year represents significant risk of being carriers of isolates resistant to methicillin (MRS) and other antimicrobials. These factors should be considered before applying an antimicrobial treatment in veterinary clinics.

1. Introduction

Staphylococcal skin infections are one of the most common reasons why animal owners seek the help of their veterinarians. The coagulase-positive staphylococci most commonly isolated in cases of canine pyoderma are *Staphylococcus pseudintermedius*, *S. intermedius* and *S. schleiferi* spp. *coagulans* (Shimizu et al., 2001; Morris et al., 2006; Fazakerley et al., 2009). The high degree of genetic similarity shown by the first two of these species has led to their reclassification as a single, genetically homogeneous group known as the *Staphylococcus* intermedius group (SIG) (Sasaki et al., 2007; Fitzgerald, 2009). Other coagulase-positive and coagulase-negative staphylococci (CoNS) have also been isolated from dogs with pyoderma (Zdovc et al., 2004; Hauschild and Wójcik, 2007).

The control of canine pyoderma is based on local or systemic antimicrobial therapy (Ganiere et al., 2005). However, recent years have seen a worldwide increase in the prevalence of resistance to commonly used antimicrobial agents (Petersen et al., 2002; Kadlec et al., 2010). Of

* Corresponding author. Tel.: +34 957 212 635; fax: +34 957 218 725.
E-mail address: sa1lumoi@uco.es (I. Luque).

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Table 1
Animals studied and staphylococcal species isolated in each group of animals.

Groups[a]	Positive animals N° (%)	Isolates N° (%)	Biochemical identification of the isolates[b]		
			SIG	*S. aureus*	CoNS
A (*n* = 23)	16 (69.5)	21 (20%)	13	2	6
B (*n* = 24)	24 (100)	40 (38%)	34	3	3
C (*n* = 27)	27 (100)	44 (42%)	36	5	3
Total (*n* = 74)	67 (90.5)	105 (100)	83 (79%)	10 (9.5%)	12 (11.4%)

[a] Groups of animals (number): group A (healthy dogs), group B (animals with first-time pyoderma), and group C (dogs presenting with recurrent pyoderma that received long-term antibiotic treatments).
[b] SIG: *Staphylococcus intermedius* group, *S. aureus*: *Staphylococcus aureus*, SCoN: coagulase negative staphylococci.

particular importance are methicillin-resistant strains (MRS) since they are resistant to all β-lactams antibiotics, commonly used in oral treatment of pyoderma. Also, animals can become reservoirs of such strains for humans, so they have a major impact on public health (Guardabassi et al., 2004; Loeffler et al., 2007; Fitzgerald, 2009). Resistance to methicillin is conferred by an altered penicillin-binding protein (PBP)2a, encoded by the *mecA* gene, which is located on a mobile genetic element designated staphylococcal cassette chromosome (SCC*mec*) (Matsuhashi et al., 1986).

A number of authors report differences in the resistance patterns between isolates (Holm et al., 2002; Hartmann et al., 2005; Futagawa-Saito et al., 2007); studies are therefore needed that determine the risk factors associated with resistance. The aim of the present work was to determine the antimicrobial susceptibility of staphylococci isolated from dogs presenting at the Clinical Veterinary Hospital of Cordoba University (Spain), and to determine the possible risk factors associated with resistance. Such knowledge should allow for the better control of canine pyoderma.

2. Material and methods

2.1. Animals and sample collection

The study animals were 74 dogs admitted to the Clinical Veterinary Hospital of Cordoba from October to December 2009 (Table 1). Three groups were established; group A with 23 healthy dogs, group B with 24 dogs with first-time pyoderma, and group C with 27 dogs presenting with recurrent pyoderma even though they had received long-term antibiotic treatments. The animals belonging to the first two groups had not received antibiotic therapy in the preceding year. Two reasons led us to select this time period: one year was a period of time that included all seasons, and also, all owners could confidently remember if their dogs had received any prior therapy during that time. The most common primary causes of chronic pyoderma in group C were atopic dermatitis, endocrine dermatoses and primary pyoderma. In this group, antibiotic treatment was ended at least two weeks before samples were taken. The following data were collected for each animal: sex, age, habitat, and details of cohabitation with other dogs, site of isolation and treatment history (Table 4).

Swabs for bacterial culture and transport (Culturette swabs with Amies Transport medium [EUROTUBO®], Deltalab) were taken from different body areas: the mouth mucosa and perineum in healthy animals, and the lesion zone and perineum in animals with pyoderma (Hartmann et al., 2005; Griffeth et al., 2008; Fazakerley et al., 2009). Swabs were rubbed vigorously against the sampling site for 5 s and processed immediately.

2.2. Bacterial isolation and identification

All swabs were grown on Blood Agar (Oxoid S.A., Spain) supplemented with 5% sterile, defibrinated sheep's blood (Oxoid S.A., Spain) and Mannitol Salt Agar (Oxoid S.A., Spain). All plates were incubated aerobically at 37 °C for 18–24 h. Isolates were identified on the basis of colony morphology, Gram staining, pigment production and haemolysis. All Gram-positive, catalase-positive cocci with colony morphology compatible with that of *Staphylococcus* species were selected for further analysis. Coagulase activity was determined via the tube coagulase test using rabbit serum (Difco S.A., Spain) and the clumping factor test (Oxoid S.A., Spain). Coagulase-positive isolates were further identified by conventional biochemical tests: acetoin production (Vogues Proskauer), acid production from lactose, the trehalose test, the beta-galactosidase test (ONPG test), and susceptibility to polymyxin B and furazolidone, as previously described (Zdovc et al., 2004; Sasaki et al., 2007). Coagulase-negative isolates were identified using the API 20 STAPH system (bioMerieux S.A., Spain) according to the manufacturer's recommendations.

2.3. Susceptibility tests

The antimicrobial susceptibility of the isolates was determined on Mueller–Hinton agar (Oxoid, Spain) using the disk diffusion method. Eight different groups of antimicrobial agents, widely used in companion animal clinical, were studied: beta lactams (represented by ampicillin [10 μg/disk]), amoxicillin–clavulanate (20 and 10 μg/disk), cephalothin (30 μg/disk), cephalexin (30 μg/disk), cephadroxyl (30 μg/disk) and ceftiofur (30 μg/disk). Fluoroquinolones were represented by ciprofloxacin (5 μg/disk) and enrofloxacin (5 μg/disk). Macrolides and lincosamides were represented by erythromycin (15 μg/disk) and clindamycin (2 μg/disk): erythromycin and clindamycin discs were placed approximately 15 mm apart to detect $MLSB_i$ resistance. Tetracycline (30 μg/disk), gentamicin (10 μg/disk), and rifampin (5 μg/disk) were also tested. All antimicrobial agents were purchased from