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

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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.

Keywords

Staphylococci, pyoderma, dog, antimicrobial susceptibility, methicillin-resistance

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* (Quinn et al., 1998; 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 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.

Material and methods

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.

Bacterial isolation and identification

All swabs were grown on Blood Agar (Oxoid SA, 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 37ºC for 18 to 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 (Zdoc 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.

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 Oxoid (Oxoid, S.A., Spain). *Staphylococcus aureus* reference strain ATCC 25923 was used as a quality control. The measurement and interpretation of growth inhibition diameters was performed following the CLSI guidelines for veterinary antimicrobial susceptibility tests for pathogens of animal origin (CLSI, 2008). Quality control was performed for each day of testing or weekly if satisfactory performance was documented and whenever a new lot of media or lot of disk were used, as recommended the CLSI (2008). In this work, isolates with resistance to three or more classes of antimicrobial agents were considered multiresistant (MR), following the criteria of Holm et al (2002).

All 105 isolates were tested for β-lactamase production with nitrocefin disk (Oxoid, S.A., Spain) according to the manufacturer's instructions. Development of a red colour indicated positive results. Those beta-lactamase producing isolates were considered resistant to ampicillin.

Resistance to oxacillin was determined by the growth of blue colonies in the selective medium Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid S.A., Spain), supplemented with polymyxin B (50 IU/L) and oxacillin (2 mg/L), after 24-48 h aerobic incubation at 37ºC. Reference quality control strains of oxacillin-resistant *S. aureus* (ATCC 43300), oxacillin-susceptible *S. aureus* (ATCC 25923) were used for screening for methicillin-resistant isolates**.** Suspected MRS isolates were further confirmed by the latex agglutination assay (Oxoid, S.A., Spain), following the manufacturer's recommendations.

Epidemiological analysis

A total of 105 staphylococci was collected (table 1). This allowed the comparison of resistance to antimicrobial agents between the study groups (95% confidence and 80% power), with a minimum OR of 2, for an expected proportion of multiresistant isolates in pyoderma-affected dogs of 30% (Holm et al., 2002). Staphylococcal species distribution was examined by calculating the frequency of isolation of the different species in each group, which were then compared using the χ^2 test. For the study of the risk factors associated with antimicrobial resistance, multiresistance (MR) and methicillin resistance (MRS), the percentage of resistant isolates in each group and their 95% confidence intervals (95% CI) were determined and compared using Fisher's exact test and via the calculation of odds ratios (OR). Significance was set at $p<0.05$. All statistical analyses were performed using SPSS v.12.0 software for Windows.

Results

Staphylococcal species distribution

Table 1 shows that staphylococci were recovered from 67 of the 74 (90.5%) studied animals: from 16 (69.5%) healthy dogs (group A), from 24 (100%) dogs with first-time pyoderma (group B), and from 27 (100%) of the dogs with recurrent pyoderma (group C). Two corporal zones were studied from each animal; when various isolates belonged to the same species and similar susceptibility were obtained from an individual dog, only one isolate was considered to avoid duplicate results.

A total of 105 staphylococcus isolates were obtained (Table 1). Twenty-one (20%) isolates were obtained from group A animals, 40 (38%) from group B animals, and 44 (42%) from group C animals. The frequency of isolation of staphylococci was significantly higher ($p<0.0001$) in the pyoderma-affected dogs (groups B and C) than in the healthy dogs (group A).

Table 1 also shows that the majority of the isolates (83; 79%) were SIG members. Ten (9.5%) isolates were identified as *S. aureus* and 12 (11.4%) as different CoNS. The frequency of isolation of SIG members and CoNS was statistically higher in pyoderma-affected animals ($p<0.05$). No differences were observed among groups in terms of the frequency of isolation of *S. aureus* (data not shown).

Antimicrobial susceptibility

Table 2 summarises the frequency of antimicrobial resistance. Resistance was most commonly seen against ampicillin (68.6%), tetracycline (41%), erythromycin (35.2%) and clindamycin (28.6%).

The frequency of isolates resistant to erythromycin and clindamycin was very similar among groups, but the frequency of isolates resistant to tetracycline was significantly greater in the pyoderma-affected animals (groups B and C) (Table 2) (**tetracycline**: OR B/A groups 3.84, 95% CI [1.09-13.4]); OR C/A groups 3.54, 95% CI [1.02-12.2]). No differences were observed between groups B and C ($p>0.05$).

Large differences were also observed between the isolates obtained from group C animals compared to A plus B animals in terms of resistance to cephalosporins and amoxicillin-clavulanate (OR 4.29, 95% CI [1.15, 16.3]), enrofloxacin (OR 9.47, 95% CI [1.53, 58.5]) and ciprofloxacin (OR 79.7 95% CI [3.26, 1947.4]).

Table 3 shows the resistance pattern detected and the frequency of MSS and MRS isolates. A total of 82 of the staphylococci isolated (78.1%) were resistant to at least one antimicrobial agent, i.e., 14 of the 21 isolates (66.6%) obtained from animals of group A, 30 of the 40 isolates (75%) from the dogs in group B, and 38 of the 44 isolates (86.4%) of the group C animals. Statistical analysis (Table 4) showed no differences between treated and not-treated groups ($p = 0.08$), although a clear trend to resistance to one or more antimicrobials was observed among isolates obtained from animals with a history of antibiotic therapy.

Thirty-four (32.3%) of the 105 isolates were resistant to three or more antimicrobial agents (Table 3), and were considered multiresistant (6 isolates obtained from animals in group A, 13 isolates recovered from group B, and 15 from group C; however, these differences were not significant ($p>0.05$). Eleven (10.4%) isolates were resistant to methicillin (7/105 identified as SIG, 2/105 as *S. aureus*, 1/105 as *S. cohnii* subsp. *cohnii* and 1/105 as *S. capitis*). All isolates produced the protein PBP2a in the latex agglutination test. All the MRS isolates came from pyoderma-affected animals (Table 3). The Exact Fisher test showed a significant association between the use of

long-term treatments and the presence of MRS staphylococci (18.2% of MRS isolates in group C vs. 7.5% in A plus B animals; OR 4.3, 95% CI [1.15, 15.9]) (Table 4). Among the MRS isolates, 7 (63.6%) were also resistant to tetracycline, 3 (87.3%) to fluoroquinolones (ciprofloxacin and enrofloxacin), erythromycin and clindamycin, respectively, and 2 (18.2%) to gentamicin. One (9.1%) of the MR isolates was also resistant to rifampin.

Statistical analysis was performed to determine possible risk factors associated with the isolation of multidrug resistant (MR) and methicillin resistant (MRS), respectively (Table 4). MR isolates were obtained more commonly from urban than rural dogs (OR 3.79; 95% CI [1.09, 13.17]). However, no correlation was found between the multiresistance and sex, age, previous treatments of the animals (Table 4). All MRS staphylococci were obtained from urban dogs (11.2% versus 0%, p = 0.19) and a more frequently from males (14.9% versus 3.7%, p = 0.07)

The assessment of $MLSB_i$ resistance showed 29 (27.6%) of the 105 isolates to be resistant to erythromycin and clindamycin. Sixteen isolates showed an unusual pattern in the Kirby-Bauer test (ERY-resistant but CLI-susceptible). The results of the D-test showed three phenotypes: 2/16 isolates showed a clindamycin-inducible resistance phenotype (D-zone effect), 7/16 showed a negative phenotype (ERY-resistant but CLI-susceptible), and 7/16 showed a resistant phenotype (ERY-resistant and CLI-resistant).

Discussion

The present results highlight the large number of apparently healthy dogs that are carriers of staphylococci on their skin and mucosae (69.5%). This proportion increases significantly in animals with pyoderma (to 100%), as described by other authors (Hartmann et al., 2005; Fatagawa-Saito et al., 2007). The results of the biochemical tests showed the majority (83/105, 79%) of isolates to be SIG members (Table 1), with the frequency of isolation increasing significantly in pyoderma-affected animals ($p<0.05$). *S. aureus* and CoNS (9.5% and 11.4% respectively) were also isolated in all three groups of animals with a similar trend. *S. aureus* has traditionally been associated with different diseases in humans, but in dogs it is considered a

transient inhabitant that has the potential to cause disease; it is thought to be involved in <5% of skin infections (Holm et al., 2002; Hauschild and Wojcik, 2007; Fazakerley et al., 2009). However, in the present work, although its frequency was low, most isolates (70%) were obtained from areas of skin with lesions. The low frequency of isolation of CoNS should be noted, although recent studies indicate the emergence of these species' involvement in canine pyoderma and otitis (Hauschild and Wójcik, 2007).

The results of the *in vitro* susceptibility studies show the effectiveness of the different groups of antimicrobial agents examined (Table 2), and confirm the results of previous authors who report cephalosporins, fluoroquinolones, amoxicillin/clavulanic acid, gentamicin and rifampin to be first-line choices in staphylococcus-induced canine pyoderma (Morris et al., 2006; Vanni et al., 2009). In the present work, a high proportion of isolates were resistant to ampicillin (68.6%), tetracycline (41%), erythromycin (35.2%) and clindamycin (28.6%). Results from other countries show there to be wide variation in terms of bacterial resistance profiles, but in general the above agents are less effective for the empirical treatment of canine pyoderma (Holm et al., 2002; Ganiere et al., 2005; Hartmann et al., 2005; Fatagawa-Saito et al., 2007; Hauschild and Wójcik, 2007).

In the present study, 78% of the isolates were resistant to at least one antimicrobial agent. The isolates obtained from the group B and C animals were resistant to more antimicrobial agents than those obtained from the healthy dogs (group A) (Table 2), with significant differences for tetracycline. The frequency of isolates resistant to different antimicrobial agents increased when the animals had recurrent pyoderma and received long-term antibiotic treatments, significantly so for cephalosporins and amoxicillin-clavulanate (OR 4.29, 95% CI [1.15, 16.3]), enrofloxacin (OR 9.47, 95% CI [1.53, 58.5]) and ciprofloxacin (OR 79.7 95% CI [3.26, 1947.4]).

The acquisition of resistance to fluoroquinolones has been described in animals with recurrent pyoderma when treated with this group of antimicrobial agents (Ganière et al., 2001). In the present work, most of the group C animals had been treated with amoxicillin-clavulanate, cefalexin and ciprofloxacin (data not shown); significant differences were seen in the percentage of isolates resistant to these different agents.

These results suggest that a microbiological study is always advisable in clinical recurrent pyoderma despite the effectiveness generally shown by amoxicillin-clavulanate and cephalosporins (Rantala et al., 2004).

More than 30% of the isolates detected were multiresistant (Table 3), as described by other authors (Holm et al., 2002; Ganiere et al., 2005). However, in contrast to these studies, no significant difference (p>0.05) was seen (table 4) in the number of MR isolates in each group despite the larger number associated with group C (45.6%). A total of 23 resistance pattern were detected (data not shown), but no differences were observed among groups; these results agree with those of other studies (Shimizu et al., 2001; Vanni et al., 2009). Methicillin-resistant staphylococci (MRS) were isolated in the present study (10.4%) in a proportion higher than that recorded in other studies (Holm et al., 2002; Rantala et al., 2004; Vanni et al., 2009). Statistical analysis showed a notable association between exposure to long-term treatments and the presence of MRS staphylococci (OR 4.6, 95% CI [1.15, 15.9]). Among the MRS isolates, resistance to other antimicrobial agents have been detected. In veterinary medicine, recent publications have also demonstrated increased prevalence of MRS resistance to fluoroquinolones, macrolides, aminoglycosides and tetracyclines (Kadlec et al., 2010).

Statistical analysis to determine possible risk factors associated with the isolation of multidrug resistant (MR) and methicillin resistant (MRS), respectively (Table 4), shows that more MR staphylococci were isolated from urban than rural dogs (OR 3.79; 95% CI [1.09, 13.17]) and curiously all the MRS isolates were obtained from urban dogs. This is probably due to the greater prescription of antimicrobial agents for urban dogs and their greater use of veterinary clinics, hospitals and kennels. Other variables studied, such as sex or treatment history, were not found to be associated with the MR or MRS characteristics of the isolates.

Clindamycin has traditionally been the drug of choice for the empirical treatment of canine pyoderma, including the treatment of infections caused by MRS strains, given its good oral absorption, excellent penetration and scant secondary reactions (Faires et al., 2009). However, the high percentage of resistance detected in our study in all three groups of animals discourages this option without prior

microbiological analysis. In addition, there are many references referring to cross-resistance between erythromycin and clindamycin (Ganiere et al., 2005) as well as a form of inducible resistance to clindamycin. The latter is not detected in routine disc-plaque diffusion tests, and can lead to therapeutic failure. The identification of strains with inducible macrolide resistance can be achieved via double disc diffusion inhibition assays (the D-Test). A positive D-test suggests the presence of an *erm* gene that could result in a constitutive clindamycin resistance and potential clinical failure of this drug (Swenson et al., 2007; Yilmaz et al., 2007). If the D-test is negative, clindamycin can be used therapeutically. In the present study, 29 (27.6%) of the 105 isolates were resistant to both erythromycin and clindamycin (Fig. 1). The D-test results for 16 isolates that presented an unusual pattern in the Kirby-Bauer test (ERY-resistant but CLI-susceptible) showed two isolates (11.1%), both SIG members, to possess an inducible form of clindamycin resistance. Previous studies have shown the percentage of strains showing inducible resistance to range between 12.3% (Levin et al., 2005) and 37.5% (Rich et al., 2005) in both the human and veterinary medicine settings; this resistance has been associated with strains of *S. aureus* and CoNS (Faires et al., 2009). The present results suggest the D-test should be routinely performed concurrently with susceptibility testing to examine if the clindamycin can be used therapeutically.

Conclusions

Staphylococcal resistance to antimicrobial agents is more pronounced in urban animals with recurrent pyoderma that have undergone long-term, empirical, antimicrobial treatment. The susceptibility of staphylococci causing pyoderma should always be checked *in vitro* in order to select the best treatment. The present results suggest the routine use of the D-test to assess the effectiveness of clindamycin in the treatment of pyoderma, especially when caused by MRS strains.

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Conflict of interest statement

The authors have no financial or personal relationships with any persons or organizations that might inappropriately influence the content of this paper.

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

Groups[a]	Positive animals Nº (%)	Isolates Nº (%)	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

Table 2. ***In vitro*** **susceptibility (disk diffusion method) of the 105 staphylococci isolates.**

Antimicrobial (µg/disk)	No. of resistant isolates (%)			
	Total	**Group A[a]**	**Group B**	**Group C**
Ampicillin (10 µg/disk)	72 (68.6%)	12 (57.1%)	29 (72.5%)	31 (70.5%)
Amoxicillin-clavulanate (20 and 10 µg/disk)	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Cefadroxil (30 µg/disk)	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Ceftiofur (30 µg/disk).	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Cephalothin (30 µg/disk)	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Cephalexin (30 µg/disk)	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Oxacillin[b]	11 (10.5%)	-	3 (7.5%)	8 (18.2%)
Gentamicin (10 µg/disk)	4 (3.8%)	-	1 (2.5%)	3 (6.8%)
Tetracycline (30 µg/disk)	43 (41%)	4 (19%)	19 (47.5%)	20 (45.5%)
Ciprofloxacin (5 µg/disk)	5 (4.8%)	-	-	5 (11.4%)
Enrofloxacin (5 µg/disk)	7 (6.7%)	1 (4.8%)	-	6 (13.6%)
Clindamycin (2 µg/disk)	30 (28.6%)	7 (33.3%)	10 (25%)	13 (29.5%)
Erythromycin (15 µg/disk)	37 (35.2%)	8 (38.1%)	13 (32.5%)	16 (36.4%)
Rifampin (5 µg/disk)	2 (1.9%)	-	-	2 (4.5%)
TOTAL	**105**	**21**	**40**	**44**

[a] Groups of animals: 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] The oxacillin result is based on the latex agglutination test.

Table 3. Resistance pattern detected among the 105 staphylococci isolates analysed.

RESISTANCE PATTERN	No. OF ISOLATES (%)			
	Total n = 105	**Group A[a] n = 21**	**Group B n = 40**	**Group C n = 44**
Susceptible to all the antimicrobial agents	23 (21.9%)	7 (33.3%)	10 (27%)	6 (16.6%)
Resistant to 1 or more antimicrobial agents	82 (78.1%)	14 (66.6%)	30 (75%)	38 (86.4%)
Methicillin susceptible (MSS)	**94 (89.5%)**	**21**	**37**	**36**
Resistant to 1 antimicrobial agent	25 (26.5%)	5 (23.8%)	10 (27%)	10 (27.7%)
Resistant to 2 antimicrobial agents	17 (18.0%)	3 (14.2%)	5 (13.5%)	9 (25%)
Resistant to 3 or more antimicrobial agents	29 (30.8%)	6 (28.5%)	12 (32.4%)	11 (30.5%)
Methicillin resistant (MRS)	**11 (10.4%)**	**0**	**3**	**8**
Resistant only to oxacillin	3 (27.3%)	0	1 (33.3%)	2 (25%)
Resistant to 2 antimicrobial agents	3 (27.3%)	0	1 (33.3%)	2 (25%)
Resistant to 3 or plus antimicrobial agents	5 (45.4%)	0	1 (33.3%)	4 (50%)

[a] Groups of animals: 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]Antimicrobial abbreviations: Amp: Ampicillin; Cip: Ciprofloxacin; Enr: Enrofloxacin; E: Erythromycin; Da: Clindamycin; Te: Tetracycline; Cn: Gentamicin; Trimethoprim-sulphamethoxazole; Rd: Rifampin
[c]Isolates with resistance to three or more classes of antimicrobial agents were considered multiresistant (MR)

Table 4. Study of risk factors for multiresistance (MR) and methicillin-resistance (MRS) of staphylococci isolated.

Risk factors associated to multiresistance (MR)					
Variable	**Values**	**MR isolates**	***p***	**Odds ratio**	**95% IC (lower-upper)**
Sex	Male (n=47) Female (n=53) No items (n=5)	19 (40.4%) 14 (26.4%)	0.2	-	-
Age	< 5 years (n=50) >5 years (n=47) No item (n=8)	16 (32%) 15 (32%)	1	-	-
Habitat	Urban (n=80) Rural (n=21) No item (n=4)	31 (38.7%) 3 (14.3%)	**0.03**	**3.79**	**[1.09, 13.17]**
Cohabitation with other dogs	Yes (n=30) No (n=70) No item (n=5)	7 (23.3%) 26 (37.1%)	0.24	-	-
Treatment	Yes (n=44) No (n=61)	15 (34%) 19 (31.1%)	0.75	-	-
Risk factors associated to methicillin resistance (MRS)					
Variable	**Values**	**MRS isolates**	***p***	**Odds ratio**	**95% IC (lower-upper)**
Sex	Male (n=47) Female (n=53) No items (n=5)	7 (14.9%) 2 (3.7%)	**0.07**	-	-
Age	< 5 years (n=50) >5 years (n=47) No item (n=8)	4 (8%) 5 (10.6%)	0.74	-	-
Habitat	Urban (n=80) Rural (n=21) No item (n=4)	9 (11.2%) 0 (0%)	0.19	-	-
Cohabitation with other dogs	Yes (n=30) No (n=70) No item (n=5)	3 (10%) 6 (8.5%)	1	-	-
Treatment	Yes (n=44) No (n=61)	**8 (18.2%)** **3 (5%)**	**0.04**	**4.3**	**[1.15, 15.9]**