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1 **Risk factors associated with the antimicrobial resistance of staphylococci in canine**
2 **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*,

1 *S. intermedius* and *S. schleiferi* spp. *coagulans* (Quinn et al., 1998; Shimizu et al., 2001;
2 Morris et al., 2006; Fazakerley et al., 2009). The high degree of genetic similarity
3 shown by the first two of these species has led to their reclassification as a single,
4 genetically homogeneous group known as the *Staphylococcus intermedius* group (SIG).
5 (Sasaki et al., 2007; Fitzgerald, 2009). Other coagulase-positive and coagulase-negative
6 staphylococci (CoNS) have also been isolated from dogs with pyoderma (Zdovc et al.,
7 2004; Hauschild and Wójcik, 2007).

8

9 The control of canine pyoderma is based on local or systemic antimicrobial
10 therapy (Ganiere et al., 2005). However, recent years have seen a worldwide increase in
11 the prevalence of resistance to commonly-used antimicrobial agents (Petersen et al.,
12 2002; Kadlec et al., 2010). Of particular importance are methicillin-resistant strains
13 (MRS) since they are resistant to all β -lactams antibiotics, commonly used in oral
14 treatment of pyoderma. Also, animals can become reservoirs of such strains for humans,
15 so they have a major impact on public health (Guardabassi et al., 2004; Loeffler et al.,
16 2007; Fitzgerald, 2009). Resistance to methicillin is conferred by an altered penicillin-
17 binding protein (PBP)2a, encoded by the *mecA* gene, which is located on a mobile
18 genetic element designated staphylococcal cassette chromosome (*SCCmec*) (Matsuhashi
19 et al., 1986).

20

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

28

29 **Material and methods**

30

31 *Animals and sample collection*

32 The study animals were 74 dogs admitted to the Clinical Veterinary Hospital of
33 Cordoba from October to December 2009 (Table 1). Three groups were established;
34 group A with 23 healthy dogs, group B with 24 dogs with first-time pyoderma, and

1 group C with 27 dogs presenting with recurrent pyoderma even though they had
2 received long-term antibiotic treatments. The animals belonging to the first two groups
3 had not received antibiotic therapy in the preceding year. Two reasons led us to select
4 this time period: One year was a period of time that included all seasons, and also, all
5 owners could confidently remember if their dogs had received any prior therapy during
6 that time. The most common primary causes of chronic pyoderma in group C were
7 atopic dermatitis, endocrine dermatoses and primary pyoderma. In this group, antibiotic
8 treatment was ended at least two weeks before samples were taken. The following data
9 were collected for each animal: sex, age, habitat, and details of cohabitation with other
10 dogs, site of isolation and treatment history (Table 4).

11

12 Swabs for bacterial culture and transport (Culturette swabs with Amies
13 Transport medium [EUROTUBO[®]], Deltalab) were taken from different body areas: the
14 mouth mucosa and perineum in healthy animals, and the lesion zone and perineum in
15 animals with pyoderma (Hartmann et al., 2005; Griffeth et al., 2008; Fazakerley et al.,
16 2009). Swabs were rubbed vigorously against the sampling site for 5 s and processed
17 immediately.

18

19 *Bacterial isolation and identification*

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

34

1 *Susceptibility tests*

2 The antimicrobial susceptibility of the isolates was determined on Mueller-Hinton
3 agar (Oxoid, Spain) using the disk diffusion method. Eight different groups of
4 antimicrobial agents, widely used in companion animal clinical, were studied: beta
5 lactams (represented by ampicillin [10 µg/disk]), amoxicillin-clavulanate (20 and 10
6 µg/disk), cephalothin (30 µg/disk), cephalexin (30 µg/disk), cephadroxyil (30 µg/disk)
7 and ceftiofur (30 µg/disk). Fluoroquinolones were represented by ciprofloxacin (5
8 µg/disk) and enrofloxacin (5 µg/disk). Macrolides and lincosamides were represented
9 by erythromycin (15 µg/disk) and clindamycin (2 µg/disk): erythromycin and
10 clindamycin discs were placed approximately 15 mm apart to detect MLSB_i resistance.
11 Tetracycline (30 µg/disk), gentamicin (10 µg/disk), and rifampin (5 µg/disk) were also
12 tested. All antimicrobial agents were purchased from Oxoid (Oxoid, S.A., Spain).
13 *Staphylococcus aureus* reference strain ATCC 25923 was used as a quality control. The
14 measurement and interpretation of growth inhibition diameters was performed
15 following the CLSI guidelines for veterinary antimicrobial susceptibility tests for
16 pathogens of animal origin (CLSI, 2008). Quality control was performed for each day of
17 testing or weekly if satisfactory performance was documented and whenever a new lot
18 of media or lot of disk were used, as recommended the CLSI (2008). In this work,
19 isolates with resistance to three or more classes of antimicrobial agents were considered
20 multiresistant (MR), following the criteria of Holm et al (2002).

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

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

33 *Epidemiological analysis*

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

13

14 **Results**

15

16 *Staphylococcal species distribution*

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

23

24 A total of 105 staphylococcus isolates were obtained (Table 1). Twenty-one
25 (20%) isolates were obtained from group A animals, 40 (38%) from group B animals,
26 and 44 (42%) from group C animals. The frequency of isolation of staphylococci was
27 significantly higher ($p < 0.0001$) in the pyoderma-affected dogs (groups B and C) than in
28 the healthy dogs (group A).

29

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

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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

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

7
8 Statistical analysis was performed to determine possible risk factors associated
9 with the isolation of multidrug resistant (MR) and methicillin resistant (MRS),
10 respectively (Table 4). MR isolates were obtained more commonly from urban than
11 rural dogs (OR 3.79; 95% CI [1.09, 13.17]). However, no correlation was found
12 between the multiresistance and sex, age, previous treatments of the animals (Table 4).
13 All MRS staphylococci were obtained from urban dogs (11.2% versus 0%, $p = 0.19$)
14 and a more frequently from males (14.9% versus 3.7%, $p = 0.07$)

15
16 The assessment of MLSB₁ resistance showed 29 (27.6%) of the 105 isolates to
17 be resistant to erythromycin and clindamycin. Sixteen isolates showed an unusual
18 pattern in the Kirby-Bauer test (ERY-resistant but CLI-susceptible). The results of the
19 D-test showed three phenotypes: 2/16 isolates showed a clindamycin-inducible
20 resistance phenotype (D-zone effect), 7/16 showed a negative phenotype (ERY-resistant
21 but CLI-susceptible), and 7/16 showed a resistant phenotype (ERY-resistant and CLI-
22 resistant).

23 24 **Discussion**

25
26 The present results highlight the large number of apparently healthy dogs that
27 are carriers of staphylococci on their skin and mucosae (69.5%). This proportion
28 increases significantly in animals with pyoderma (to 100%), as described by other
29 authors (Hartmann et al., 2005; Fatagawa-Saito et al., 2007). The results of the
30 biochemical tests showed the majority (83/105, 79%) of isolates to be SIG members
31 (Table 1), with the frequency of isolation increasing significantly in pyoderma-affected
32 animals ($p < 0.05$). *S. aureus* and CoNS (9.5% and 11.4% respectively) were also
33 isolated in all three groups of animals with a similar trend. *S. aureus* has traditionally
34 been associated with different diseases in humans, but in dogs it is considered a

1 transient inhabitant that has the potential to cause disease; it is thought to be involved in
2 <5% of skin infections (Holm et al., 2002; Hauschild and Wojcik, 2007; Fazakerley et
3 al., 2009). However, in the present work, although its frequency was low, most isolates
4 (70%) were obtained from areas of skin with lesions. The low frequency of isolation of
5 CoNS should be noted, although recent studies indicate the emergence of these species'
6 involvement in canine pyoderma and otitis (Hauschild and Wójcik, 2007).

7
8 The results of the *in vitro* susceptibility studies show the effectiveness of the
9 different groups of antimicrobial agents examined (Table 2), and confirm the results of
10 previous authors who report cephalosporins, fluoroquinolones, amoxicillin/clavulanic
11 acid, gentamicin and rifampin to be first-line choices in staphylococcus-induced canine
12 pyoderma (Morris et al., 2006; Vanni et al., 2009). In the present work, a high
13 proportion of isolates were resistant to ampicillin (68.6%), tetracycline (41%),
14 erythromycin (35.2%) and clindamycin (28.6%). Results from other countries show
15 there to be wide variation in terms of bacterial resistance profiles, but in general the
16 above agents are less effective for the empirical treatment of canine pyoderma (Holm et
17 al., 2002; Ganiere et al., 2005; Hartmann et al., 2005; Fatagawa-Saito et al., 2007;
18 Hauschild and Wójcik, 2007).

19
20 In the present study, 78% of the isolates were resistant to at least one
21 antimicrobial agent. The isolates obtained from the group B and C animals were
22 resistant to more antimicrobial agents than those obtained from the healthy dogs (group
23 A) (Table 2), with significant differences for tetracycline. The frequency of isolates
24 resistant to different antimicrobial agents increased when the animals had recurrent
25 pyoderma and received long-term antibiotic treatments, significantly so for
26 cephalosporins and amoxicillin-clavulanate (OR 4.29, 95% CI [1.15, 16.3]),
27 enrofloxacin (OR 9.47, 95% CI [1.53, 58.5]) and ciprofloxacin (OR 79.7 95% CI [3.26,
28 1947.4]).

29
30 The acquisition of resistance to fluoroquinolones has been described in animals
31 with recurrent pyoderma when treated with this group of antimicrobial agents (Ganière
32 et al., 2001). In the present work, most of the group C animals had been treated with
33 amoxicillin-clavulanate, cefalexin and ciprofloxacin (data not shown); significant
34 differences were seen in the percentage of isolates resistant to these different agents.

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

4

5 More than 30% of the isolates detected were multiresistant (Table 3), as
6 described by other authors (Holm et al., 2002; Ganiere et al., 2005). However, in
7 contrast to these studies, no significant difference ($p>0.05$) was seen (table 4) in the
8 number of MR isolates in each group despite the larger number associated with group C
9 (45.6%). A total of 23 resistance pattern were detected (data not shown), but no
10 differences were observed among groups; these results agree with those of other studies
11 (Shimizu et al., 2001; Vanni et al., 2009). Methicillin-resistant staphylococci (MRS)
12 were isolated in the present study (10.4%) in a proportion higher than that recorded in
13 other studies (Holm et al., 2002; Rantala et al., 2004; Vanni et al., 2009). Statistical
14 analysis showed a notable association between exposure to long-term treatments and the
15 presence of MRS staphylococci (OR 4.6, 95% CI [1.15, 15.9]). Among the MRS
16 isolates, resistance to other antimicrobial agents have been detected. In veterinary
17 medicine, recent publications have also demonstrated increased prevalence of MRS
18 resistance to fluoroquinolones, macrolides, aminoglycosides and tetracyclines (Kadlec
19 et al., 2010).

20

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

29

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

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

19

20 **Conclusions**

21

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

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31

32 **Conflict of interest statement**

1 The authors have no financial or personal relationships with any persons or
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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	<i>S. aureus</i>	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).

^bSIG: *Staphylococcus intermedius* group, *S. aureus*: *Staphylococcus aureus*, SCoN: Coagulase negative staphylococci

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

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

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3 ^a Groups of animals: Group A (healthy dogs), Group B (animals with first-time pyoderma), and group C
4 (dogs presenting with recurrent pyoderma that received long-term antibiotic treatments).5 ^b The oxacillin result is based on the latex agglutination test.

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1 **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
<i>Susceptible to all the antimicrobial agents</i>	23 (21.9%)	7 (33.3%)	10 (27%)	6 (16.6%)
<i>Resistant to 1 or more antimicrobial agents</i>	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%)

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3 ^a Groups of animals: Group A (healthy dogs), Group B (animals with first-time pyoderma), and group C
4 (dogs presenting with recurrent pyoderma that received long-term antibiotic treatments).

5 ^bAntimicrobial abbreviations: Amp: Ampicillin; Cip: Ciprofloxacin; Enr: Enrofloxacin; E: Erythromycin;
6 Da: Clindamycin; Te: Tetracycline; Cn: Gentamicin; Trimethoprim-sulphamethoxazole; Rd: Rifampin

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

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