Antibiotic sensitivity of bacterial isolates from cases of canine dermatitis

Francesca Ghidini, Chiara Piancastelli, Simone Taddei, Emanuele Gandolfo, Sandro Cavirani, Clotilde Silvia Cabassi

Dipartimento di Salute Animale, Università di Parma, Italy

SUMMARY

Among 97 bacterial isolates, 74 strains of *Staphylococcus* spp developed from 95 swabs taken from skin lesions in dogs. Twenty-eight staphylococcal strains resistant to methicillin and/or oxacillin were identified and *mecA* expression was confirmed for 14 of these strains. *S. aureus* and *S. intermedius* group (SIG) strains were particularly relevant in our cases due to their antibiotic resistance leading to an increased veterinary and public health risk. We suggest a diagnostic protocol based on cytological examination, bacterial identification to species level, and antibiotic sensitivity testing prior to prescribing antibiotic treatment for canine skin diseases.

KEY WORDS: Dog, Dermatology, Staphylococcus, Antibiotic resistance, PBP2a, *mecA*

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Since dermatological diseases have assumed a prevalent role among dog pathologies, bacterial causes of dermatitis in dog have been investigated worldwide. Different bacteria have been involved in the course of dermatological diseases, among which *Staphylococcus* species, in particular *S. aureus*, *S. intermedius* and *S. pseudintermedius,* represent opportunistic pathogens in dogs (Ruscher *et al.*, 2009) and are the most frequently isolated bacteria in case of dermatological problems. Recently, *S. intermedius* and *S. pseudintermedius* have been included in the *S. intermedius* group (SIG) (Bannoehr *et al.,* 2007). The effects of therapy may include the development of major antimicrobial resistance profiles for *S. aureus* and *S. pseudintermedius* (Mason and Kietzmann, 1999; Jones *et al.,* 2007).
Consequently, it is important to obtain a correct evaluation of antibiotic sensitivity of bacteria within the genus *Staphylococcus* and particularly for SIG (Bemis *et al.,* 2006; Bemis *et al.,* 2009). Recently, criteria for oxacillin and methicillin activity have been reconsidered, redefining the inhibition diameter in the Kirby-Bauer susceptibility test (Bemis *et al.,* 2006). Methicillin resistant staphylococci are conventionally considered resistant to all beta-lactam antibiotics *in vivo*, so an accurate determination of methicillin resistance is of critical importance (Schissler *et al.,* 2009). Staphylococcal resistance to methicillin is linked to the expression of low affinity penicillin binding protein 2A (PBP2a), which is highly conserved among staphylococci and is encoded by the chromosomal gene *mecA* (Niemeyer *et al.,* 1996). On the basis of these considerations, it is important to determine the *mecA*-mediated resistance in bacteria isolated from dogs (Bemis *et al.,* 2009). Only few references can be found in Italian literature on the prevalence of bacteria found in canine skin lesions and their antibiotic sensitivities (Meucci *et al.,* 2010; Vanni *et al.,* 2009). The aim of this work was to determine the bacterial species involved in canine dermatitis and their antibiotic sensitivities, evaluated on the basis of the latest criteria employed by the Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical

Corresponding author
Clotilde Silvia Cabassi
Dipartimento di Salute Animale
Università di Parma
Via del Taglio, 10 - 43126 Parma
E-mail: clotildesilvia.cabassi@unipr.it

and Laboratory Standards (NCCLS) (CLSI 2008) and of those proposed by another researcher (Bemis *et al.,* 2006) with particular attention to methicillin/oxacillin resistant *Staphylococcu*s spp strains for their public health role (Faires *et al.,* 2010; Corrente *et al.,* 2009).

Samples were collected from dogs referred for dermatological problems to the Teaching Hospital of our Veterinary Faculty from September 2009 to September 2010. Ninety-five swabs were obtained from lesions of canine dermatitis and submitted to the laboratory within one hour. Swabs for cytological examination were taken from cases of superficial pyoderma and striked onto microscopy slides. Swab smears were air-dried, stained by a Romanovsky-type stain (Diff-Quick) and examined. Only swabs from lesions showing the presence of bacteria at cytological examination were cultured.

Each sample was streaked onto tryptose agar (Beckton Dickinson, Sparks, Maryland, USA) containing 5% bovine erythrocytes, Mc Conkey agar (Beckton Dickinson, Sparks, Maryland, USA) and Sabouraud agar (Beckton Dickinson, Sparks, Maryland, USA) and incubated aerobically for 24 and 48 hours at 37°C. Isolates were identified using standard microbiological procedures, as Gram staining, catalase and coagulase (Slidex Staph Plus, bioMérieux, Marcy-l'Etoile, France) tests, haemolysin production and other biochemical tests (API system, bioMérieux, Marcy-l'Etoile, France). Antibiotics were chosen on the basis of therapeutic use in dermatological clinics.

Antimicrobial susceptibility tests were done using the standard Kirby-Bauer disk-diffusion methods (Quinn *et al.,* 1994). Microbiological breakpoints for topical antimicrobials (fusidic acid and mupirocin) were defined as previously described by Fuchs *et al.* (1990). *Staphylococcus* spp strains were considered resistant to oxacillin with an inhibition diameter <10 mm (CLSI, 2008), and to methicillin with an inhibition diameter <9 mm (CLSI, 2005). For SIG strains we evaluated our data on the basis of the oxacillin breakpoint established by Bemis *et al.* (2009), who considered strains showing an inhibition diameter <17mm resistant. The expression of PBP2a was evaluated by a latex agglutination test (PBP2' Test kit - Oxoid, Hampshire, UK) following the manufacturer's instructions.

With the exception of two cases, all the cultured samples showed bacterial growth in pure culture, with a number of colonies >10 for each specimen. In the mixed cultures, *Escherichia coli* + *Pantoea* spp and *E. coli* + *Pseudomonas aeruginosa* developed. The number of isolates for each bacterial species and their susceptibilities to the tested antibiotics are reported in Table 1. The majority of isolates (n. 74/97) belonged to the genus *Staphylococcus*. Other isolates belonged to the *Enterobacteriaceae* family (n. 14/97), *Pseudomonas aeruginosa* (n. 6/97) and *Pasteurella multocida* (n. 3/97). Globally 28 *Staphylococcus* isolates (14 *S. aureus*, 7 SIG, 4 *S. xylosus*, 1 *S. hominis*, 2 *S. epidermidis* isolates) were resistant to one or both methicillin or oxacillin. On these strains *mecA* phenotypic expression was verified and confirmed in 14 cases (7 *S. aureus*, 3 SIG and 4 *S. xylosus* isolates) by PBP2a agglutination test. Discordance was noted between the activity of methicillin and oxacillin on 12 *Staphylococcal* isolates (Table 2). Almost all the *Staphylococcus* species were sensitive in over 50% of cases to Amoxicillin + Clavulanic Acid, Cefadroxil, Cefalexin, Cefovecin, Chloramphenicol, Cloxacillin, Doxiciclin, Imipenem, Mupirocin, Riphampicin and Vancomicin. For *Pseudomonas aeruginosa* isolates, we observed an overall sensitivity only to Amikacin, Ciprofloxacin, Imipenem and Marbofloxacin. Antimicrobial profile susceptibility regarding *Enterobacteriaceae* showed a very high level of resistance to Apramicin, Chlarithromycin, Clindamicin, Erythromicin, Fusidic Acid, Lyncomicin, Mupirocin, Tylosin, Vancomicin and, as expected, to the beta-lactams Cloxacillin, Methicillin and Oxacillin. Likewise, all the isolates of *Pasteurella multocida* were resistant to Amoxicillin + Clavulanic Acid, Chlarithromycin, Enrofloxacin, Rifaximin, Sulfametoxazole + Thrimetoprim and Vancomicin.

On the basis of the criteria expressed by Cox *et al.* (1988), who considered plates with 10 or more colonies indicative of resident bacteria, all the isolated bacteria should be considered residents. The heavy growth of pure cultures obtained from all our samples, except in two cases, could be indicative of the involvement of the isolated bacteria in the development of the lesions (May, 2006). The staphylococcal strains had a prevalence of 76.3% among all isolates. A risk factor for human

TABLE 1 - *Number (%) of bacterial isolates susceptible to the tested antibiotics.*

Antibiotic	*Staph. aureus n=39*	*Staph. intermedius group (SIG) n=20*	*Staph. xylosus n=7*	*Staph. sciuri n=2*	*Staph. hominis n=1*	*Staph. epidermidis n=5*	*Pseudomonas aeruginosa n=6*	*E. coli n=10*	*Pantoea spp n=1*	*Proteus mirabilis n=3*	*Pasteurella multocida n=3*
Amikacin (30 µg)	8 (20,51%)	6 (30%)	2 (28,57%)	1 (50%)	0 (0%)	1 (20%)	4(66,75)	1 (10%)	1 (100%)	0 (0%)	2 (66,6%)
Amox. + (30 µg) Clav. acid	25 (64,1%)	10 (50%)	5 (71,42%)	2 (100%)	1 (100%)	2 (40%)	0 (0%)	3 (30%)	1 (100%)	1 (33,3%)	0 (0%)
Apramicin (5 µg)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Cefadroxil (30 µg)	33 (84,61%)	17 (85%)	5 (71,42%)	2 (100%)	1 (100%)	3 (60%)	0 (0%)	1 (10%)	1 (100%)	0 (0%)	1 (33,3%)
Cefalexin (30 µg)	29 (74,36%)	14 (70%)	4 (57,14%)	2 (100%)	1 (100%)	3 (60%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)	1 (33,3%)
Cefovecin (30 µg)	31 (79,48%)	15 (75%)	3 (42,85%)	2 (100%)	1 (100%)	3 (60%)	1 (16,7%)	4 (40%)	1 (100%)	1 (33,3%)	1 (33,3%)
Ciprofloxacin (5 µg)	20 (51,28%)	8 (40%)	1 (14,28%)	2 (100%)	0 (0%)	1 (20%)	5 (83,3%)	4 (40%)	1 (100%)	1 (33,3%)	1 (33,3%)
Chlarithromycin (15 µg)	11 (28,2%)	8 (40%)	3 (42,85%)	2 (100%)	0 (0%)	4 (80%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Clindamicin (2 µg)	0 (0%)	1 (5%)	0 (0%)	1 (50%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Chloramphenicol (30 µg)	26 (66,66%)	16 (80%)	6 (85,71%)	2 (100%)	1 (100%)	4 (80%)	1 (16,7%)	6 (60%)	1 (100%)	1 (33,3%)	1 (33,3%)
Cloxacillin (5 µg)	25 (64,1%)	14 (70%)	4 (57,14%)	2 (100%)	1 (100%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Doxiciclin (30 µg)	31 (79,48%)	18 (90%)	5 (71, 42%)	2 (100%)	1 (100%)	4 (80%)	0 (0%)	4 (40%)	1 (100%)	0 (0%)	1 (33,3%)
Enrofloxacin (5 µg)	19 (48,71%)	14 (70%)	3 (42,85%)	2 (100%)	1 (100%)	1 (20%)	2 (33,3%)	6 (60%)	1 (100%)	1 (33,3%)	0 (0%)
Erythromicin (15 µg)	0 (0%)	1 (5%)	1 (14,28%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33,3%)
Fusidic Acid (10 µg)	10 (25,64%)	5 (25%)	2 (28,57%)	1 (50%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33,3%)
Gentamicin (10 µg)	10 (25,64%)	9 (45%)	3 (42,85%)	1 (50%)	0 (0%)	4 (80%)	1 (16,7%)	3 (30%)	1 (100%)	2(66,6%)	3 (100%)
Imipenem (10 µg)	39 (100%)	19 (95%)	7 (100%)	2 (100%)	1 (100%)	5 (100%)	6 (100%)	10 (100%)	1 (100%)	3 (100%)	1 (33,3%)
Lyncomicin (15 µg)	5 (12,82%)	9 (45%)	2 (28,57%)	1 (50%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Marbofloxacin (5 µg)	26 (66,66%)	14 (70%)	4 (57,14%)	2 (100%)	0 (0%)	2 (40%)	5 (83,3%)	6 (60%)	1 (100%)	0 (0%)	1 (33,3%)
Methicillin (5 µg)	27 (69,23%)	15 (75%)	3 (42,85%)	2 (100%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Mupirocin (5 µg)	26 (66,66%)	14 (70%)	5 (71,42%)	2 (100%)	1 (100%)	4 (80%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33,3%)
Oxacillin (1 µg)	25 (64,1%)	14 (70%)	3 (42,85%)	2 (100%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (66,6%)
Riphampicin (5 µg)	32 (82,05%)	14 (70%)	3 (42,85%)	2 (100%)	1 (100%)	4 (80%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	3 (100%)
Rifaximin (40 µg)	31 (79,48%)	14 (70%)	4 (57,14%)	2 (100%)	0 (0%)	4 (80%)	1 (16,7%)	0 (0%)	1 (100%)	1 (33,3%)	0 (0%)
Sulfam. + Thrim. (25 µg)	4 (10,25%)	4 (20%)	0 (0%)	1 (50%)	0 (0%)	1 (20%)	0 (0%)	2 (20%)	1 (100%)	0 (0%)	0 (0%)
Tylosin (30 µg)	1 (2,56%)	2 (10%)	1 (14,28%)	1 (50%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33,3%)
Vancomicin (30 µg)	32 (82,05%)	18 (90%)	6 (85,71%)	2 (100%)	1 (100%)	4 (80%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 2 - *Discordance between the activity of methicillin and oxacillin in 12 cases of staphylococcal isolates.*

Strain	*Methicillin inhibition zone diameter (mm)*	*Oxacillin inhibition zone diameter (mm)*	*PBP2a agglutination test*
Case 50. S. intermedius group	24 (S)	0 (R)	-
Case 53. S. intermedius group	0 (R)	22 (S)	-
Case 54 . S. intermedius group	22 (S)	0 (R)	-
Case 40. S. xylosus	14 (I)	26 (S)	+
Case 51. S. xylosus	20 (S)	0 (R)	+
Case 59. S. xylosus	15 (S)	0 (R)	-
Case 7. S. aureus	20 (S)	0 (R)	+
Case 14. S. aureus	14 (I)	0 (R)	+
Case 19. S. aureus	24 (S)	0 (R)	-
Case 32. S. aureus	0 (R)	11 (I)	-
Case 55. S. aureus	12 (I)	0 (R)	-
Case 56. S. aureus	12 (I)	0 (R)	-

Resistance profile abbreviations: I: intermedius; R: resistant; S: sensitive.

and animal health is the presence, in the bacterial genome, of genes encoding for multidrug, oxacillin/methicillin resistance, or simply the phenotypic resistance to oxacillin/methicillin evaluated through the disk diffusion method (Bemis *et al.,* 2009). So, the accurate determination of oxacillin/methicillin resistance is of critical importance for clinical, therapeutic and microbiological aspects. *S. pseudintermedius* is reported as the most common etiologic agent in canine pyoderma (Lloyd, 2007), so that Devriese e*t al.* (2009) proposed that canine strains identified by traditional means should be reported as *S. pseudintermedius* unless shown by genomic investigation to belong to other related species. However, since *S. intermedius* and *S. pseudintermedius* cannot be phenotypically discriminated with the biochemical tests used, we indicated them as SIG isolates. Bemis *et al.* (2009) demonstrated that the currently recommended CLSI interpretive criteria for oxacillin disk diffusion tests are not sufficient to detect all *mecA*-associated resistance in *S. pseudintermedius* while the former (2004-2008) CLSI breakpoints are in agreement with gradient diffusion and *mecA* gene detection. Oxacillin disk breakpoint of <= 17 mm is considered an accurate indicator of *mecA* resistance. In our cases, strains belonging to SIG were isolated in 20 cases, all showing an oxacillin inhibition zone diameter >19 mm (clearly sensitive) or =0 (totally resistant) and none methicillin resistant. Three strains belonging to the SIG were PBP2a positive, confirming previous findings worldwide (Jones *et al.,* 2007). In our cases, SIG strains showed less susceptibility to antibiotic activity compared to other data reported on strains isolated in Italy, where the percentage of susceptibility was higher than 85% (Vanni *et al.,* 2009) and resistant strains were few. Unlike results obtained for the Staphylococcal isolates, where in all species Cefadroxil, Cefalexin, Cloramphenicol, Cloxacillin, Doxiciclin, Mupirocin, Riphampicin and Vancomicin were effective on more than 50% of isolates, none of the antibiotics tested had a broad-spectrum efficacy on Gram negative bacteria isolated. Hence, it is difficult to suggest an

antibiotic therapy in case of a positive cytologic test to Gram negative bacteria without the results of the bacterial culture and the antibiotic susceptibility test. The high level of efficacy of Imipenem on isolated bacteria, with the exception of *P. multocida* isolates should be emphasized. This antibiotic is not routinely employed in veterinary medicine but commonly used in human medicine (Tejedor Junco and Martin Barrasa, 2002). In our opinion, the use of Imipenem should be limited, especially in veterinary medicine, to those cases which cannot be solved with alternative treatment, to minimize the occurrence of resistance in bacteria, particularly those with a zoonotic infection potential. The present study demonstrated resistance to various antimicrobial drugs that are commonly used as first choice in many veterinary dermatologic formulations. Awareness and monitoring of antimicrobial resistance in veterinary bacterial isolates are important since resistance in animal pathogens can lead to treatment failure in individual patients and may pose an increased zoonotic risk to owners. This has been shown for methicillin resistant *S. aureus* and *S. pseudintermedius* (Jones *et al.,* 2007; Manian, 2003). In fact, *S. pseudintermedius* is occasionally isolated from serious human infections, and the emergence and spread of methicillin-resistant *S. pseudintermedius* strains are major veterinary and public health issues (Bannoehr *et al.,* 2009).

In conclusion, we suggest the application of a strict diagnostic protocol based on a previous cytological examination, bacterial identification to species level, and antibiotic sensitivity testing prior to prescribing antibiotic treatment for canine skin diseases.

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