

Staphylococcus sp., antimicrobial treatment and resistance in canine superficial bacterial pyoderma

Meneses, M.L.^{1,2}; Martin, P.L.²; Manzuc, P.³; Arauz, M.S.²; Pardo, A.G.¹

¹Lab. Micología Molec., Depto. Ciencia & Tecnol. Univ. Nac. Quilmes, Bernal, Buenos Aires, Argentina.

²Lab. Centr. Hosp. Escuela, Univ. Nac. La Plata. ³Cát. Patol. Gral. Fac. Cs. Vet. Univ. Nac. La Plata, Argentina. E-mail: meneses.laura@gmail.com

Abstract

Meneses, M.L.; Martin, P.L.; Manzuc, P.; Arauz, M.S.; Pardo, A.G.: *Staphylococcus sp.*, antimicrobial treatment and resistance in canine superficial bacterial pyoderma. *Rev. vet.* 29: 2, 88-92, 2018. In Buenos Aires, Argentina, few studies are available regarding the frequency of the antimicrobial treatment for canine pyoderma and the level of antimicrobial resistance of *Staphylococcus sp.* The main objectives of this study were to analyze the antimicrobial resistance of *Staphylococcus sp.* and the frequency of antimicrobial treatment of canines with pyoderma and their relapses. A total of 39 canines with clinical diagnosis of pyoderma from private veterinary clinics in Buenos Aires area, were analyzed. Skin lesions swabs for both bacterial culture and mass spectrometry analysis were collected at the time of active pyoderma. Additionally, breed, sex, pyoderma classification, antimicrobial treatment and relapses of disease were recorded. Seventy-six percent of the studied animals received oral cephalexin after the clinical checkup, and within this percentage 31.6% also received other types of antimicrobial agents due to relapses. The remaining 24% had only antimicrobial treatment with lincomycin, minocycline and/or doxycycline. In sixty percent of the animals, pyoderma was related to allergy, which in turn was in concordance with relapses and was similar to the percentage of methicillin resistance (51%) of the different isolates of *Staphylococcus sp.* Eighty percent of the methicillin resistances were previously treated with cephalexin. Surprisingly, the highest percentages of resistance were to erythromycin, clindamycin (demonstrating constitutive MLSB phenotype), and sulfatrimethoprim.

Key words: canine, *Staphylococcus sp.*, pyoderma, antimicrobial resistance.

Resumen

Meneses, M.L.; Martin, P.L.; Manzuc, P.; Arauz, M.S.; Pardo, A.G.: *Staphylococcus sp.*, tratamiento antimicrobiano y resistencia en pioderma bacteriana superficial canina. *Rev. vet.* 29: 2, 88-92, 2018. En la Provincia de Buenos Aires, Argentina, existen pocos estudios sobre la frecuencia del tratamiento antimicrobiano en pioderma canina y el nivel de resistencia antimicrobiana de *Staphylococcus sp.* Los principales objetivos de este estudio fueron analizar la resistencia antimicrobiana de *Staphylococcus sp.*, la frecuencia del tratamiento antimicrobiano y sus recaídas en caninos con pioderma. Se analizaron 39 caninos con diagnóstico clínico de pioderma, procedentes de clínicas veterinarias privadas del área de Buenos Aires. Se recogieron, en el momento de la pioderma activa, hisopados de lesiones cutáneas para cultivo bacteriano y análisis por espectrometría de masas. Además, se registró raza, sexo, clasificación clínica de la pioderma, tratamiento antimicrobiano y recaídas de la enfermedad. El 76% de los animales estudiados recibieron cefalexina por vía oral después del examen clínico, y dentro de este porcentaje, el 31,6% también recibió otros tipos de agentes antimicrobianos debido a recaídas. El 24% restante recibió como tratamiento antimicrobiano lincomicina, minociclina y/o doxiciclina. En el 60% de los animales estudiados, la pioderma diagnosticada tenía una causa alérgica, que a su vez coincidió con las recaídas y fue similar al porcentaje de resistencia a la meticilina (51%) de los diferentes aislamientos de *Staphylococcus sp.* El 80% de las resistencias a meticilina observadas se trataron previamente con cefalexina. Inesperadamente, el mayor porcentaje de resistencia observada fue a eritromicina, clindamicina (demostrando fenotipo constitutivo MLSB) y sulfatrimetoprima.

Palabras clave: canino, *Staphylococcus sp.*, pioderma, resistencia antimicrobiana.

INTRODUCTION

Superficial bacterial folliculitis or superficial pyoderma, is a bacterial infection confined to the superficial portion of the hair follicle²². It is commonly accepted that canine pyoderma is usually associated with underlying causes such as allergies or endocrinopathies that either break down the epidermal barrier and/or produce alterations in the immune system. Identification and control of the underlying causes is critical for effective treatment and prevention of recurrence¹².

*Staphylococcus pseudointermedius*²⁴ (SPI), formerly *Staphylococcus intermedius* (SI), is considered the most frequently isolated bacterial pathogen in canine pyoderma²⁵. Less commonly, dogs may also be colonized and infected by other *Staphylococcus* species and other bacteria²².

Recently, guidelines for antimicrobial therapy for canine superficial bacterial folliculitis were published, although these recommendations were for diagnosis and based mainly on clinical signs emphasizing the need for demonstration of cocci in lesional skin by cytology as a powerful adjunctive diagnostic test¹⁵.

It is good practice to perform impression cytology as an in-clinic test while diagnosing skin infections. Hence, appropriate techniques have been described for both specimen collection and examination to optimize the value of this diagnostic procedure¹⁵.

Bacterial culture for canine pyoderma is never contraindicated and is, in fact, encouraged in patients with chronic or recurrent pyoderma due to the increased frequency of isolation of antibiotic resistant staphylococci in veterinary medicine over the past decade¹³.

At present, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of nine bacterial pathogens of particular public health concern declared by the World Health Organization in its 2014 antimicrobial resistance surveillance report. This report describes resistance as a problem so serious that it jeopardizes the achievements of modern medicine. In the post-antibiotic era, where common infections and minor injuries can kill, far from being an apocalyptic fantasy, it is instead a very real possibility in the 21st century³⁰. Likewise, this threat also applies to staphylococcal infections in veterinary medicine.

The first signs of change in the veterinary sector were in the late 1990s with the emergence of clinical infections in companion animals, initially with MRSA and then with methicillin-resistant SPI (MRSP)¹⁸.

MRSP strains are characterized by the presence of the *mecA* gene that encodes for a low affinity penicillin binding protein (PBP2a); this altered protein confers resistance to all β -lactam antibiotics, including penicillins, cephalosporins and carbapenems¹⁶. MRSP are frequently resistant to multiple classes of antibiotics, including fluoroquinolones, tetracyclines, macrolides and others¹⁰.

Such infections, mainly of dermal origin, led to an increase in the prevalence of MRSP, initially attract-

ing attention in the United States and soon after in Europe^{17,23}. This threat has been strongly reinforced by a recent report from China that almost 50% of *S. pseudointermedius* isolates from dogs were MRSP¹⁹.

At the moment there are few studies indicating the prevalence of this microorganism in our region. Therefore, the aims of our study were to evaluate this prevalence, to analyze the antimicrobial sensitivity and to consider the antimicrobial treatment applied and its evolution in canines with a diagnosis of pyoderma.

MATERIAL AND METHODS

Sampling. We analyzed 39 cases of canines with superficial bacterial pyoderma from private veterinary clinics in the Province of Buenos Aires, diagnosed by clinical symptomatology and with their respective cytological confirmation. They were sampled from dermal lesions and tested for bacteriological identification and evaluation of antimicrobial susceptibility. At the same time, the implemented treatments and their clinical evolution were followed up.

Isolates identification. The samples were analyzed at the Central Laboratory of the Veterinary Sciences School Hospital of the National University of La Plata, Argentina. The following biochemical tests were performed: saline mannitol agar, catalase, coagulase, pyr (pyrrolidonyl-beta-naphthylamide) and acetoin. Subsequently, the samples were analyzed by mass spectrometry at the Special Bacteriology Service of the National Institute of Infectious Diseases (Dr. Carlos G. Malbrán) in the autonomous city of Buenos Aires, Argentina.

Antimicrobial susceptibility. Isolates were evaluated by the agar diffusion method according to the M2-A11 standards described in the guidelines from CLSI (Clinical & Laboratory Standards Institute) and those described in *CLSI Approved Standard Supplement Vet 01-S2*^{6,7}. We used oxacillin 1 μ g, erythromycin 15 μ g, clindamycin 2 μ g, ciprofloxacin 5 μ g, chloramphenicol 30 μ g, trimethoprim-sulfamethoxazole 25 μ g, rifampicin 5 μ g, gentamicin 10 μ g and minocycline 30 μ g (Britania Laboratory, Buenos Aires, Argentina).

Statistical analysis. The frequency of each isolated *Staphylococcus* species and resistance to the antibiotics used were expressed as a percentage with a confidence interval of 95%¹.

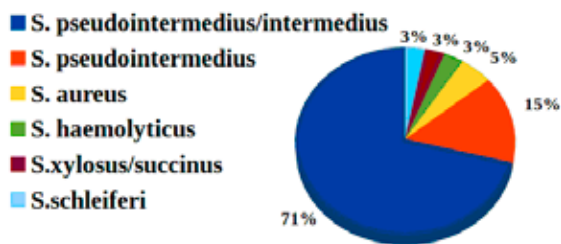
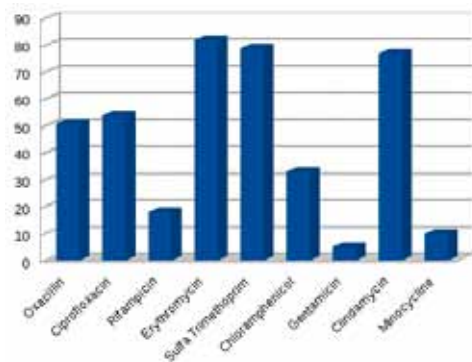
RESULTS

The *Staphylococcus* species identified by mass spectrometry (MALDI-TOF) were *S. pseudointermedius*-*S. intermedius* group 72%, *S. pseudointermedius* 15%, *S. aureus* 5% and *S. haemolyticus*, *xylosus/succinus*, *Schleiferi* 3% (Figure 1).

It is important to note that the *S. pseudointermedius*-*S. intermedius* and *S. xylosus/succinus* species were

Table 1. Frequency of antimicrobial treatments.

antimicrobial treatment	%	Nº
cephalexin	51	20
cefadroxil / minocycline	5	2
cephalexin / lincomycin / minocycline	5	2
cephalexin / doxycycline	15	6
lincomycin	5	2
minocycline	8	3
doxycycline	11	4

**Figure 1.** Percentage of isolated *Staphylococcus* species (n=39).**Figure 2.** Percentage (%) of antimicrobial resistance (n=39).

not able to be confirmed by sequencing the *dnaJ*, *tuf* or *rpoB* genes as indicated by CLSI *mM18A*⁵ since access to the proper equipment could not be acquired.

A high percentage of multidrug resistances were observed in antimicrobial susceptibility tests, showing in 10 samples susceptibility for only one or two antimicrobials. Figure 2, shows the percentage of antimicrobial resistances for all isolates of staphylococcal species.

Likewise, in Table 1 the frequency of the antimicrobial systemic treatment is observed. It should be noted that the animals with recurrences received several different antimicrobial treatments.

Seventy-six percent of the animals studied received cefalexin or cefadroxil orally after the clinical visit, and among these, 32% received in addition other types of antimicrobials due to relapses. The remaining 24% received lincomycin, minocycline and/or doxycycline as the sole antimicrobial treatment. In turn, 96% of the animals also received topical treatments with chlorhexidine baths.

In Table 2 the frequency of breeds studied is showed with their respective diagnostics. In 64% of the studied animals, pyoderma was due to an aller-

Table 2. Frequency of canine breeds and their respective diagnoses.

canine breed	%	Nº	diagnosis
french bulldog	8	3	SPDAR
beagle	5	2	"
boxer	5	2	"
cocker spaniel	5	2	"
crossbreed	13	5	SPWUPC
dogue de bordeaux	5	2	"
english bulldog	5	2	SPDAR
german shepherd	8	3	"
labrador retriever	5	2	"
pitbull	13	5	"
schnauzer	5	2	"
shar pei	5	2	"
terrier	5	2	"
toy poodle	8	3	"
white swiss shepherd	5	2	SPIC

SPDAR: superficial pyoderma due to allergic reaction; SPWUPC: superficial pyoderma with unknown primary cause; SPIC: superficial pyoderma in calluses.

gic cause. In turn, this percentage coincides with the relapses observed and is similar to the percentage of methicillin resistance (51%) of the different isolates of *Staphylococcus*.

Regarding the frequency discriminated by sex we found 22 females (56%) and 17 males (43%). It should be stressed that 80% of the methicillin-resistant observed were previously treated with cephalexin and the remaining 20% had no previous treatment records. However, the highest percentage of resistance observed were to erythromycin, clindamycin and sulfatrimethoprim.

DISCUSSION

As described in the epidemiological study of others authors, it was also observed in our study that canine pyoderma is more frequent in purebred dogs²⁶. Additionally, agrees the frequency of incidence discriminated by sex mentioned by others authors^{2,27}.

Staphylococcus intermedius was first described in pigeon, dog, mink and horse isolates¹⁴. For more than 30 years, *S. intermedius* was considered the most common causal pathogen in skin and soft tissue infections in dogs²⁰. However, high levels of genotypic and phenotypic diversity have been observed by several researchers suggesting the existence of more than one species¹⁴.

Recent studies have shown that the isolates phenotypically identified as *S. intermedius* differ, in fact, in three different species specifically known as *S. intermedius*, *S. pseudointermedius*¹¹ and *S. delphini*, which are collectively referred to as the *S. intermedius* group³.

S. delphini was isolated from purulent skin lesions in dolphins and has rarely been reported since²⁸. The recently described *S. pseudointermedius* species, not *S.*

intermedius, is the most common cause of cutaneous infections in canines⁴.

Proteomic mass spectrometry (MALDI-TOF MS or laser assisted array of ionization desorption) is a fast and highly accurate technique that has been introduced in human and animal diagnostic laboratories⁴. Databases of mass spectra are initially classified by identifications based on 16S and 18S rDNA sequences⁴.

Recently, MALDI-TOF MS has been applied for species differentiation within the SIG group⁸. The estimated sensitivity and specificity for *S. pseudointermedius* were 78 and 97%, respectively, indicating that this approach might be useful for rapid and accurate identification at the species level⁸.

S. pseudointermedius is the major cause of canine pyoderma²². Coinciding with other authors^{4,21}, our study observed a high frequency of *S. pseudointermedius* as causal to canine pyoderma. Bacteria can cause infection following the effects of local trauma, scratching, lack of grooming, seborrhea, parasitic infestation, hormonal factors, local irritants, or allergens²¹.

In our case, 60% of the animals studied had an allergic reaction as the primary cause. As expected, these animals were observed as recurrent, that is, all animals with a history of allergies had subsequent relapses after a period of improvement by antimicrobial treatment. These animals, in their relapses, presented a high level of antimicrobial resistance.

In our region, there are few studies indicating the frequency of antimicrobial resistance of *S. pseudointermedius*. In this sense we can mention the study who registered a prevalence of 9.8% (n=207) of *S. pseudointermedius* methicillin resistant (SPMR) isolated from skin infections in dogs in the Province of Buenos Aires⁹.

Likewise, in other study analyzed the antimicrobial resistance of 28 isolates of *S. pseudointermedius* from different clinical samples of canines where 10.8% were SPMR. Highest levels of resistance were observed for erythromycin (39.3%), clindamycin (42.8%), ciprofloxacin (42.8%) and sulfatrimethoprim (42.8%), which are similar to our profiles of susceptibility²⁹.

The high percentage of resistance to erythromycin, clindamycin and sulfatrimethoprim observed in our study is striking, taking into account that it does not coincide with the frequency of the antimicrobial treatments implemented. However, it is not ruled out whether the animals received previous treatments with these antimicrobials.

We must take into account the constitutive MLSB phenotype in which an enzyme methylates the 23s rRNA, altering the receptor affinities of not only macrolides, but also lincomycins and streptogramins²¹. Therefore, we observed roughly the same level of resistance for both erythromycin and clindamycin.

The misuse of these three types of antimicrobials is currently evident in clinical practice in our region. Also, it should be noticed that most of the animals are from specific canine breeds coming from different ca-

nine breeding centers where the indiscriminate use of sulfas for "prevention" of *coccidia* diarrhea is common.

On the other hand, although elevated, this level of methicillin-resistance is expected since we had 60% of animals with relapses due to allergic causes and especially since cefalexin was the first antimicrobial choice in these dermatopathies.

Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases¹⁵ recommend clindamycin or lincomisinas, first-generation cephalosporins, amoxicillin-clavulanic acid and sulfa-trimethoprim as a first choice in empirical treatments for pyodermias. Of second choice, and whenever the isolates are susceptible, they recommend doxycycline, minocycline, chloramphenicol, fluoroquinolones, rifampicin and aminoglycosides (gentamicin and amikacin).

Finally, as a third option, they recommend linezolid, teicoplanine and vancomycin. However, regardless of the fact that most (or all) MRSP are susceptible, the use of these last three antimicrobials is totally discouraged. These drugs should be considered "reserved for the treatment of serious infections by methicillin-resistant *Staphylococcus aureus* in humans"¹⁵.

In conclusion, it is noteworthy that the results of our work showed a high level of resistance to the recommended as the first option: oxacillin 51% (susceptibility marker for beta-lactams), clindamycin 77%, trimethoprim-sulfadiazine 79%. Antimicrobials recommended as a second option showed intermediate susceptibility with 54% for fluoroquinolones, 33% for chloramphenicol, 18% for rifampicin, 10% for minocycline and 5% for gentamicin. That is, the high level of resistance observed in this study for recommended antimicrobials as the first and/or second option generates an alert to professionals. These results are mainly due to two causes: firstly, the indiscriminate use of antimicrobials due to underdiagnosis of the causative agent of pyoderma, and secondly, the misuse of the same by breeders of purebred dogs.

Acknowledgements. We would like to thank at Central Laboratory of the Veterinary Sciences School Hospital of the National University of La Plata, Argentina, for be source of funding.

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