

ANTIBIOTIC RESISTANCE PROFILE OF BACTERIA ISOLATED FROM THE EXTERNAL EAR CANAL OF HEALTHY DOGS IN A KENNEL IN PELOTAS-RS, BRAZIL

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ABSTRACT

The proximity and sharing of spaces between dogs and their owners create a favorable scenario for zoonotic transmission of bacteria. The presence of bacteria resistant to antibiotics in healthy dogs indicates that they could be reservoirs of such microorganisms, representing a significant risk to animal and human health. The aim of this study was to establish the antimicrobial resistance profile of bacteria present in the external ear canal of healthy dogs. Samples were collected from 48 external ear canals of healthy mixed breed dogs. A total of six strains of *Staphylococcus sp.* were isolated, characterized and subjected to antibiogram. Two of them were identified as *S. intermedius* (Si) and four as coagulase-negative *Staphylococcus sp.* (CNS). All isolates were sensitive to amoxicillin with clavulanic acid, erythromycin, tetracycline, tobramycin, neomycin and cephalothin. All strains were resistant to at least one antibiotic. Five strains (2 Si and 3 CNS) were not sensible to amoxicillin. Four strains (2 Si and 2 CNS) were not sensible to penicillin. The two Si strains were also resistant to sulfamethoxazole/trimethoprim, demonstrating resistance to two different antibiotic classes. Further studies are needed to determine whether the isolated microorganisms could be multidrug-resistant.

Keywords: Antibiotics. *Staphylococcus sp.* Canine otitis externa.

INTRODUCTION

The interaction between humans and animals, especially dogs, has increased in recent years, mainly due to the inclusion of canines as family members (Atherton et al., 2023; Uccheddu et al., 2019). In the context of One Health, the proximity and sharing of spaces between dogs and their owners creates a favorable scenario for zoonotic transmission of bacteria (Monteiro et al., 2025). The increasing presence of multidrug-resistant bacteria (MDR) in humans who own dogs suggests this possibility (Beaulac et al., 2023). Although there has not yet been a systematic assessment of the risk of transmission of bacteria between humans and pets, there are a growing number of reports suggesting such transmission (Jin et al., 2023).

The presence of bacteria resistant to antibiotics in healthy, or asymptomatic, dogs facilitate the spread of resistance genes in the domestic environment (Frank et al., 2025). Recent studies indicate that healthy dogs could be reservoirs of such microorganisms, representing a significant risk to animal and human health. These bacteria are opportunistic pathogens that could cause dermatitis, including Canine Otitis Externa (COE), that are difficult to resolve clinically (Mihu et al., 2026). The complexity of treatment is aggravated by the bacteria antibiotic resistance profile, which limits the therapeutic options available to the veterinarian, often requiring the use of reserve drugs from human medicine (Patra et al., 2025).

COE is an inflammation of the external ear canal of dogs (Puigdemont et al., 2021) and is considered one of the most common diseases in small animal veterinary clinics (O'Neill et al., 2021). In the vast majority of cases, treatment of the disease is based on controlling the microorganisms involved in the etiology, in addition to the inflammatory process (Scherer et al., 2021). However, incorrect use of antibiotics in the treatment of the disease can lead to the development of antibiotic resistance (Tessin et al., 2023).

Several commensal microorganisms in the external ear canal of dogs, including bacteria, such as *Escherichia coli*, *Pseudomonas* sp., *Proteus* sp., *Staphylococcus* sp. and yeasts, such as *Malassezia* sp. and *Candida albicans*, have been associated with COE (Bajwa, 2019; Kasai et al., 2021; Saengchoowong et al., 2023). The presence of bacteria, including *Staphylococcus* sp., in canine skin without signs of active disease suggests an evolutionary adaptation that favors

long-term colonization and horizontal transmission of mobile genetic elements (Fàbregas et al., 2023).

The objective of this study was to determine the antibiotic resistance profile of bacteria isolated from the external ear of healthy dogs.

MATERIAL AND METHODS

This study was approved by the Ethics and Animal Experimentation Committee of the Federal University of Pelotas, process number 038194/2023-79.

Mixed breed dogs (n=24) from a public kennel in Pelotas – RS, Brazil, were evaluated, totaling 48 ears. This institution operated as a long-term shelter located on a peri-urban property. It had an estimated fluctuating population of 30 individuals, with a housing period exceeding 12 months. The animals are kept in collective kennels grouped by age, size and compatibility, and fed with standard commercial feed and water *ad libitum*.

The animals were identified and submitted to clinical evaluation. General clinical evaluation was performed according to Schaer et al. (2022). To verify the presence of COE, the external ears were examined by palpation and assessed for malodor. Erythema, mites, ulcer, stenosis and exudate in the external auditory canal were evaluated using an otoscope (Gowllands Limited, Croydon, England). Animals that presented pain, erythema, pruritus or cerumen were considered positive for COE (Tambella et al., 2020). Dogs with clinical signs or recovering from disease were removed from the study. Canines without clinical signs were considered healthy and kept in the research.

Samples were collected from the external auditory canal of healthy dogs with swabs. The samples were plated on blood agar medium containing 5% sheep blood and on MacConkey agar. The cultures were incubated at 37 °C for 48 hours, with visualization at 24 hours. The colonies obtained were classified according to Barrow and Feltham (1993).

To evaluate the antibiotic resistance profile, the disk diffusion method was used, according to the ANVISA (2005a). The isolated strains were suspended in saline solution (NaCl 0.85%), concentrated to 0.5 on the McFarland scale and were subsequently plated on Muller-Hinton

Agar. Antibiotic-impregnated disks (Laborclin®, Pinhais – PR, Brazil) were inserted into the medium and the plates were incubated at 37 °C for 24 hours. The inhibition zones were measured and interpreted according to the standards of the ANVISA (2005b). The antibiotics used in the test were: Penicillin 10 U (PEN), Amoxicillin 10 µg (AMO), Amoxicillin + Clavulanic Acid 30 µg (AMC), Ampicillin 10 µg (AMP), Cephalexin 30 µg (CFL), Erythromycin 15 µg (ERY), Neomycin 30 µg (NEO), Tobramycin 10 µg (TOB), Norfloxacin 10 µg (NOR), Sulfamethoxazole/Trimethoprim 25 µg (SUT) and Tetracycline 30 µg (TET).

Statistical analysis was performed by observing the resistant (R), intermediate (I) and susceptible (S) categorization of the isolates and the number corresponding to these results related to the evaluated molecule. For the statistical evaluation, the intermediate results were regarded as not susceptible. Each ear was treated as an independent sample. The results were evaluated by Fisher's exact test with a 2x2 contingency table using the BioEstat 5.0 program (Ayres et al., 2007).

RESULTS AND DISCUSSION

The antimicrobial sensitivity of bacteria isolated from the external ear canal of healthy dogs was evaluated. A total of 48 ears from 24 mixed-breed dogs were evaluated, of which 8 were males and 16 were females, aged between 2 and 8 years. The animals did not show clinical signs of COE or comorbidities. Furthermore, they were not undergoing treatment for diseases.

Of the 48 samples collected, six strains of *Staphylococcus* sp. were isolated, and no other bacterial genus was observed. This result differs from Bradley et al. (2020), who identified the genera *Staphylococcus*, *Streptococcus* and *Corynebacterium* among the most prevalent in the ear canal of healthy dogs. Saengchoowong et al. (2023) also observed more than one prevalent genus in the ear of healthy animals (*Pseudomonas* spp. and *Staphylococcus* spp.). It has been suggested that the microbiota of the ear canal of atopic dogs without COE is characterized by decreased diversity and increased abundance of *Staphylococcus* sp. (Ngo et al., 2018). This could indicate that, although the animals evaluated did not present clinical signs, they could be initiating a COE process, since bacteria can inhibit other prokaryotic cells present in their environments through competitive exclusion (Caballero-Flores et al., 2022).

Of the six strains isolated, two were characterized as *Staphylococcus intermedius*, recovered from both ears of a single dog, and four were coagulase-negative *Staphylococcus* sp. (CNS), isolated from four ears each of four different dogs. *S. intermedius* belongs to the *Staphylococcus intermedius* group (SIG), which includes three distinct species: *S. pseudintermedius*, *S. intermedius* and *S. delphini*; and it was considered an important cause of skin infection in dogs (Lynch; Helbig, 2021). The discovery of *S. pseudintermedius* in 2005 led to the reclassification of isolates (Sasaki et al., 2007). According to this new classification, *S. pseudintermedius*, and not *S. intermedius*, is the species that colonizes and causes infections in dogs and cats (Wang et al., 2013). This was corroborated by Lynch and Helbig (2021) who suggested that *S. pseudintermedius* would be the major cause of skin infections in dogs, including COE (Lee; Yang, 2020). The correct identification of SIG from animal samples is considered challenging because it can be misidentified, especially *S. intermedius*, according to the microbiological diagnostic method used (Elaadli et al., 2025). This suggests that it is possible that the two strains identified as *S. intermedius* in the present work could be another species.

The other four strains isolated from the animals were CNS, which was also identified in healthy animals, generally associated with other microorganisms, such as *Bacillus* sp., *Micrococcus* sp. and *Streptococcus* sp. (Almeida et al., 2016). CNS are part of the skin microbiota of dogs (Miszczak et al., 2022) and are considered rare pathogens (Morris et al., 2017).

Of the six isolates, all were resistant to at least one antibiotic, five were not susceptible to amoxicillin ($p=0.01$) and four to penicillin ($p=0.03$) (Table 1). Resistance to penicillins among *Staphylococci* is due to the production of β -lactamase, originated by the *blaZ* gene (Priyantha et al., 2016). In addition, mutations in penicillin-binding proteins and decreased expression of OprD proteins lead to reduced penetration of the drug (Valsamatzi-Panagiotou et al., 2020). Jeong et al. (2024) also observed resistance to amoxicillin in *S. pseudintermedius* isolated from dog skin, indicating that a combination of molecules should be considered.

Table 1 - Antibigram performed with *Staphylococcus* sp. isolated from the external ear canal of healthy dogs.

Antibiotic*	Isolates**					
	Si	Si	CNS	CNS	CNS	CNS
PEN 10 ^{ab}	I	I	S	S	I	I
AMO 10 ^a	R	R	R	S	R	I
AMC 30 ^c	S	S	S	S	S	S
AMP 10 ^{bc}	S	S	S	S	S	R
CFL 30 ^c	S	S	S	S	S	S
ERY 15 ^c	S	S	S	S	S	S
NEO 30 ^c	S	S	S	S	S	S
TOB 10 ^c	S	S	S	S	S	S
NOR 10 ^{bc}	S	S	S	R	S	S
SUT 25 ^{bc}	R	R	S	S	S	S
TET 30 ^c	S	S	S	S	S	S

PEN 10: penicillin; AMO 10: amoxicillin; AMC 30: amoxicillin + clavulanic acid; AMP 10: ampicillin; CFL 30: cephalothin; ERY 15: erythromycin; NEO 30: neomycin; TOB 10: tobramycin; NOR 10: norfloxacin; SUT 25: sulfamethoxazole/trimethoprim; TET 30: tetracycline. *Different letters indicate significant difference in the Fisher's exact test ($p < 0.05$). **Si: *S. intermedius*, CNS: Coagulase-negative *Staphylococcus*.

The finding of *Staphylococcus* sp. resistant to PEN, AMO and SUT reflects a global trend of spreading resistance in companion animals (Casemiro et al., 2025). Although the identification of penicillin-resistant bacteria in healthy dogs is frequent, ranging from 54% to 83% (Miszczak et al., 2025), the resistance to SUT in healthy animals is more variable, with prevalence between <10% (Priyantha et al., 2016) and up to 60% (Teixeira et al., 2023).

The presence of this profile in healthy animals is relevant to the One Health concept, since healthy dogs act as reservoirs of bacteria harboring antimicrobial resistance genes (such as *blaZ* and *dfrG*), which can be transmitted to other animals or humans (Nocera et al., 2025). Furthermore, although the strains isolated were not MDR (Magiorakos et al., 2012; Silva et al., 2025), the combination of resistance to β -lactams and sulfonamides is the first step to the emergence of methicillin-resistant *S. pseudintermedius*, which is a major clinical challenge (Priyantha et al., 2016).

Moreover, SUT is considered one of the few effective oral options for treating skin infections in dogs, especially those caused by *Staphylococcus* sp. in the ears (Zenatto et al., 2022),

particularly when first-tier antibiotics have failed or when the bacteria are suspected to be resistant to the first-choice molecules (Papich, 2023). The presence of SUT resistant *Staphylococcus* sp. in a healthy animal suggests that, should it develop an infection in the future, the first choice and the safety antibiotics options will already be reduced, compromising the treatment.

An alternative to the use of antibiotics, in this case, is to associate amoxicillin with clavulanic acid (AMC). In the present study, all isolates were sensitive to AMC (Table 1). Clavulanate is a β -lactamase inhibitor with minimal antimicrobial activity, ensuring that the mechanism of action of amoxicillin remains intact (Evans et al., 2024). However, there have been reports of *Staphylococcus* sp. isolated from sick dogs resistant to AMC in Czech Republic (Hisirová et al., 2025) and Germany (Moerer et al., 2023). A similar resistance profile was described in Bulgaria (Petrov et al., 2019), where the number of resistant strains increased significantly from 2022 to 2023 (Dinkova; Rusenova, 2024). However, in Brazil, this association showed a significantly lower frequency of resistance when compared to other antibiotics, as it would not be the most prescribed drug for treatments (Zenatto et al., 2022).

Variable prevalence of bacterial resistance has been observed over the years for clinically important antimicrobials (Papich, 2023), including some molecules evaluated in the present study. Bertelloni et al. (2021) observed high levels of resistance to erythromycin (62%) in *Staphylococcus* sp. isolated from sick dogs treated in Italy. Similarly, in the United States, a high prevalence of resistance of *S. pseudintermedius* isolated from the skin of dogs to β -lactams (>30%), tetracycline (>30%) and cephalosporins (40-50%) (Calabro et al., 2024). In contrast, we did not observe similar results. In addition to AMC, sensitivity of all isolated strains to erythromycin, tetracycline, tobramycin, neomycin, and cephalothin was also observed (Table 1).

It has been suggested that the extensive use of some of these molecules may be related to the selection of MDR bacteria (Viegas et al., 2022), due to the empirical and repeated use of antimicrobials (Zenatto et al., 2022). However, Del Fiol et al. (2023) demonstrated that the oral antibiotics most prescribed by veterinarians in Brazil, between 2014-2021, were cephalexin,

amoxicillin and metronidazole. This would explain the sensitivity profile of the strains isolated in the present study.

The main limitation of the present study lies in its single-center design, as sampling was restricted to a single shelter, which warrants caution when generalizing the findings to broader canine populations. However, evaluating 24 dogs (48 ear canals) from a total resident fluctuating population of 30 animals represents a high sampling coverage ($\approx 80\%$), offering a comprehensive, near-census profile of the micro-environment studied. Furthermore, the recovery of *S. intermedius* from both ear canals of a single dog represents a limitation regarding sample independence. Without molecular typing to confirm strain clones, autoinoculation between ears remains a likely scenario. Consequently, while the findings should be interpreted as an exploratory baseline, their primary value lies in the qualitative identification and awareness of circulating antimicrobial-resistant *Staphylococcus* sp. strains in asymptomatic dogs, highlighting the need for multi-center studies with molecular characterization in the future.

CONCLUSION

Staphylococcus sp. were isolated from the external ear canal of clinically healthy dogs. These microorganisms were not sensible to amoxicillin and penicillin. Furthermore, two strains identified as *S. intermedius* were also resistant to sulfamethoxazole/trimethoprim. However, all strains were sensitive to six molecules (amoxicillin/clavulanate, erythromycin, tetracycline, tobramycin, neomycin and cephalothin).

PERFIL DE RESISTÊNCIA A ANTIBIÓTICOS DE BACTÉRIAS ISOLADAS DA ORELHA EXTERNA DE CÃES SAUDÁVEIS

RESUMO

A proximidade e o compartilhamento de espaços entre cães e seus donos criaram um cenário favorável à transmissão zoonótica de bactérias. A presença de bactérias resistentes a antibióticos em cães saudáveis indica que eles podem ser reservatórios desses microrganismos, representando um risco significativo para a saúde animal e humana. O objetivo deste estudo foi estabelecer o perfil de resistência antimicrobiana de bactérias presentes no canal auditivo externo de cães saudáveis. Amostras foram coletadas de 48 canais auditivos externos de cães saudáveis de raças mistas. Um total de seis cepas de *Staphylococcus* sp. foram isoladas, caracterizadas e submetidas a antibiograma. Duas delas foram identificadas como *S. intermedius* (Si) e quatro como *Staphylococcus* sp. coagulase-negativo (CNS). Todos os isolados foram sensíveis à amoxicilina com ácido clavulânico, eritromicina, tetraciclina, tobramicina, neomicina e cefalotina. Todas as cepas foram resistentes a pelo menos um antibiótico. Cinco cepas (2 Si e 3 CNS) não foram sensíveis à amoxicilina. Quatro cepas (2 Si e 2 CNS) não foram sensíveis à penicilina. As duas cepas Si também foram resistentes ao sulfametoxazol/trimetoprima, demonstrando resistência a duas classes diferentes de antibióticos. Estudos adicionais são necessários para determinar se os microrganismos isolados podem ser multirresistentes a antibióticos.

Palavras-chave: Antibióticos. *Staphylococcus* sp. Otite Externa Canina.

PERFIL DE RESISTENCIA A ANTIBIÓTICOS DE BACTERIAS AISLADAS DEL OÍDO EXTERNO DE PERROS SANOS

RESUMEN

La proximidad y el uso compartido de espacios entre perros y humanos crean un escenario favorable para la transmisión zoonótica de bacterias. La presencia de bacterias resistentes a los antibióticos en perros sanos indica que podrían ser reservorios de dichos microorganismos, lo que representa un riesgo significativo para la salud animal y humana. El objetivo de este estudio fue establecer el perfil de resistencia antimicrobiana de bacterias presentes en el conducto auditivo externo de perros sanos. Fueron colectadas muestras de 48

conductos auditivos externos de perros sanos de raza mixta. Se aislaron, caracterizaron y sometieron a antibiograma un total de seis cepas de *Staphylococcus* sp. Dos de ellas se identificaron como *S. intermedius* (Si) y cuatro como *Staphylococcus* sp. coagulasa-negativo (CNS). Todos los aislados fueron sensibles a la amoxicilina con ácido clavulánico, eritromicina, tetraciclina, tobramicina, neomicina y cefalotina. Todas las cepas fueron resistentes a al menos un antibiótico. Cinco cepas (2 Si y 3 CNS) no fueron sensibles a la amoxicilina. Cuatro cepas (2 Si y 2 CNS) no fueron sensibles a la penicilina. Las dos cepas Si también mostraron resistencia a sulfametoxazol/trimetoprima, lo que demuestra resistencia a dos clases diferentes de antibióticos. Se necesitan más estudios para determinar si los microorganismos aislados podrían ser multirresistentes a antibióticos.

Palabras clave: Antibióticos. *Staphylococcus* sp. Otitis Externa Canina.

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