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# Antimicrobial Resistance Profile of Isolates Causing Otitis Externa in Sheltered Dogs (*Canis lupus familiaris*)

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**ABSTRACT**

***Background*:** Otitis externa in dogs is one of the most common diseases in veterinary dermatological care, characterized by inflammation of the epithelium of the external auditory canal, increased production of cerumen, itching, and pain. Bacteria and yeasts are often the causative agents. Laboratory diagnosis is essential for accurate identification of the infection. Bacterial multidrug resistance, including the *mecA* gene, has been described, but few studies have focused on dogs. The aim of this study was to isolate, identify, characterize, and diagnose bacterial resistance in otitis externa of dogs (*Canis lupus familiaris*) in a municipal shelter in the northwest region of the state of Paraná, Brazil.

***Materials, Methods & Results*:** One hundred swab samples were collected from the external auditory canal of 50 dogs (50 females and 50 males, both neutered). The samples were isolated and identified through macroscopic, microscopic, and biochemical tests. The samples were subjected to the disk diffusion test to evaluate antibacterial resistance and molecular identification of *Staphylococcus aureus* and detection of the *mecA* gene. Of the 100 samples, 90% (90 samples) showed Gram-positive bacteria, and 10% (10 samples) showed Gram-negative bacilli. Furthermore, 51% of the samples presented *Malassezia pachydermatis* on cytology. For enterobacteria, the highest prevalence was for *Obesumbacterium proteus* (3%) and *Kluyvera species* (2%). In molecular diagnosis, 48% of *Staphylococcus aureus* isolates were positive for the *mecA* gene. The highest resistance rate was for Metronidazole (99%), Enrofloxacin (85%), Tetracycline (72%), and Ceftriaxone (42%).

***Discussion*:** The results of this study indicate that otitis externa in dogs is frequently associated with bacterial and fungal infections, particularly *Staphylococcus aureus* and *Malassezia pachydermatis*. The high antimicrobial resistance observed, especially resistance to Metronidazole (99%), Enrofloxacin (85%), and Tetracycline (72%), and the presence of the *mecA* gene in *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp., are worrying and indicate an increase in resistance in shelter environments. These findings highlight the importance of accurate diagnosis and the rational use of antibiotics to prevent the development of chronic and antibiotic-resistant infections. The study underscores the importance of rigorous monitoring and more effective treatment protocols to manage antimicrobial resistance in shelter dogs.

**Keywords:** bacteria, One Health, otopathy, veterinary dermatology.

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## INTRODUCTION

The external auditory canal is lined by epithelial cells, compatible with the skin's epidermal tissue, with the presence of hair follicles, sebaceous glands, ceruminous glands, and is constituted by the skin microbiota [7]. Otitis externa may present with an abnormal increase in cerumen production, obstruction of the physiological airflow of the external auditory canal, changes in microbiota and/or iatrogenic irritations related to the animal's owner using ear hygiene products as cotton tips inserted into the auditory canal that lead to inflammation [21,26,33].

Factors capable of triggering an inflammatory response in the skin barrier include atopic dermatitis, hypersensitivity to food proteins, immunosuppressive diseases and high humidity in the ear canal due to changes that impede the physiological function of cleaning the ear canal, predispose individuals to the occurrence of otitis externa [7,19,29,30].

Antibiotic resistance is considered a global public health problem [6]. In recent years, microorganisms resistant to beta-lactam antibacterials such as amoxicillin, cephalexin and ceftriaxone have been increasing considerably as they are one of the first-choice drugs in dermatological treatments [5,34,36].

Clarification and understanding of predisposing factors, prevention, and therapeutic conduct are important for better adherence to care by pet owners, aiming to improve the well-being of affected dogs. For this reason, the objective of this work was to isolate, identify, characterize, and diagnose bacterial resistance in otitis externa in dogs (*Canis lupus familiaris*) from a municipal shelter in a city in the northwest region of the state of Paraná, Brazil.

## MATERIALS AND METHODS

### *Animals*

One hundred swabs were collected from the auditory canal of dogs (*Canis lupus familiaris*), all neutered, 50 males and 50 females, diagnosed with otitis externa. These dogs came from a municipal shelter affiliated with the government of a city in the northwest region of the state of Paraná, Brazil. These dogs were rescued by the environmental police, city hall, and/or the population and sent to the municipal shelter, where they remain until adoption.

### *Diagnosis of otitis externa*

The diagnosis of otitis externa was made through individual clinical analysis of the animals, which included the presence of erythema, pruritus, skin lignification, odor, and scaling of the auditory canal, without distinction of breed or age. Otoscopy and otologic cytology were used for a conclusive diagnosis. Microscope slides were evaluated for the detection of microorganisms and desquamation of the external auditory canal wall of dogs [10]. Cytology samples were collected using a sterile swab[1] (ABSORVE®), transferring the material by smear to an individual microscope slide for each sample, to obtain a thin layer of ear secretion for analysis. The staining used was Romanowsky[1] (Panótico®), according to the manufacturer's instructions. The slides were viewed under a microscope with 40x and 100x objective lenses for the diagnosis of *Malassezia pachydermatis*.

### *Bacterial isolation*

To perform bacterial isolations, samples were collected with a sterile transport swab[1] and immediately sent to the Preventive Veterinary Medicine Laboratory of the Universidade Paranaense (UNIPAR). The samples were introduced individually into tubes containing 3 mL of Brain Heart Infusion (BHI) medium and incubated at 37°C for 24 h. After this period, the cultures obtained were streaked onto plates containing Blood Agar and incubated at 37°C for 24 h to isolate Gram-positive and Gram-negative bacteria. Colonies on each plate were isolated and picked in BHI medium, incubated at 37°C for 24 h, and stored in BHI with 10% glycerol at -20°C.

### *Bacterial identification*

Each isolate was subjected to analysis of macroscopic and microscopic characteristics, Gram staining, and specific biochemical tests to define the bacterial genus. The identification of bacteria belonging to the Enterobacteriaceae family was performed using a set of biochemical tests from the “Kit for Enterobacteriaceae”[2] (NewProv®).

### *Antimicrobial susceptibility testing*

Antibacterial tests were performed using agar disc diffusion methodology, according to the criteria established by the Veterinary Microbiology Laboratory Standards [12]. Isolated colonies were plated on BHI medium for overnight growth. The bacterial inoculum was standardized according to the 0.5 McFarland scale, and the bacterial suspension was inoculated onto plates containing Mueller-Hinton agar using a swab. The antibacterial-impregnated discs were placed on the plates and incubated at 37°C for 24 h.

The antibiotics tested were those most commonly used in clinical practice at the municipal shelter: amoxicillin + clavulanic acid (30 µg), cephalothin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftiofur (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), metronidazole (4 µg), meropenem (10 µg), and tetracycline (30 µg).

The double-disc synergy test was performed by placing the discs containing cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), and aztreonam (30 µg) 20 mm away from a disc containing amoxicillin + clavulanate (30 µg). Any increase or distortion in the zone of inhibition of one of the antimicrobials towards the amoxicillin + clavulanic acid disk was considered suggestive of Extended Spectrum Beta-Lactamase (ESBL) production [1].

*Molecular identification of* Staphylococcus aureus

For identification of *Staphylococcus aureus* samples, DNA from CoPS samples was extracted using the Purelink™ Genomic DNA Kit[3] [Invitrogen™], according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed using primers Sa442-1 (5′-AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG-3′; positions 5–34) and Sa442-2 (5′-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3′; positions 83–112) [20]. Samples were visualized by electrophoresis on a 2% agarose gel stained [GelRed®][4] and detected as a single 241 bp band.

mecA *gene detection*

The DNA of all isolates positive for *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. was subjected to *mecA* gene screening. Deoxyribonucleic acid (DNA) was extracted for PCR using primers *mec*A1 (AAAATCGATGGTAAAGGTTGG) and *mec*A2 (AGTTCTGCAGTACCGGATTTG) at 5 µM [24]. Coagulase-negative *Staphylococcus aureus* and *Staphylococcus* spp. samples were visualized by electrophoresis on a 2% agarose gel stained [GelRed®][4] and detected as a single 533-bp band.

## RESULTS

In the auditory canal cytology, 51 (51%) samples presented *Malassezia pachydermatis*: 22 (22%) in males and 29 (29%) in females, with external auditory canal desquamation in all samples analyzed.

It was possible to isolate 100 bacteria, with individual isolation for 50 (50%) male dogs and 50 (50%) bitches. In 90 samples (90%), Gram-positive bacteria were isolated, and in 10 (10%), Gram-negative bacilli.

For Gram-positive samples, 8 (8%) were catalase negative, being 1 castrated male and 7 castrated bitches, and 50 (50%) samples were coagulase-positive cocci, being 22 castrated males and 28 castrated bitches, and 32 (32%) for coagulase-negative cocci, being 21 castrated males and 11 castrated bitches.

The highest resistance index identified for all isolates was for Metronidazole (99%), Enrofloxacin (85%), Tetracycline (72%), and Ceftriaxone (42%) [Table 2].

In the molecular diagnosis, of the 50 coagulase-positive *Staphylococcus* isolates, 24 (48%) were identified as *Staphylococcus aureus*, and among these samples, 2 (4%) were positive for the *mec*A resistance gene (Table 3).

Of the coagulase-negative *Staphylococcus* spp. samples subjected to PCR, 17 (53.12%) were identified as containing the *mec*A resistance gene (Table 4).

Among the 100 samples isolated from dogs, 10 (10%) were positive for enterobacteria, and the highest prevalence was for 3 *Obesumbacterium proteus* (3%) and 2 *Kluyvera* spp. (2%).

Related to bacterial resistance of enterobacteria, 2 (0.2%) samples showed higher resistance rates for *Proteus mirabilis* (92%) antibacterials (12/13) tested, being sensitive only to Amoxicillin + Clavulanate, and *Obesumbacterium proteus* was also resistant to (92%) of the antibacterials (12/13) tested, being sensitive only to Cefoxitin (Table 5). The presence of antibacterial resistance to Meropenem stood out in 4 (40%) isolates of enterobacteria, namely *Escherichia coli*, *Proteus mirabilis*, *Obesumbacterium proteus*, and *Serratia marcescens* (Table 5).

Of the 10 samples isolated for enterobacteria, 1 (0.1%) was positive for ESBL, showing distortion in the inhibition zone for Ceftriaxone towards Amoxicillin + Clavulanate.

**Table 1.** Enterobacteriaceae isolated in ear canal samples from neutered dogs diagnosed with otitis externa residing in a municipal shelter in a city in the northwest region of the state of Paraná, Brazil, 2022.

| Sample | Microorganisms | Nº | % |
|---|---|---|---|
| 34 | *Cedecea* sp. | 1/100 | 1.0% |
| 84 | *Escherichia coli* | 1/100 | 1.0% |
| 42 | *Obesumbacterium proteus* | 1/100 | 1.0% |
| 43 | *Obesumbacterium proteus* | 1/100 | 1.0% |
| 62 | *Obesumbacterium proteus* | 1/100 | 1.0% |
| 65 | *Proteus mirabilis* | 1/100 | 1.0% |
| 38 | *Serratia marcescens* | 1/100 | 1.0% |
| 57 | *Kluyvera* sp. | 1/100 | 1.0% |
| 60 | *Kluyvera* sp. | 1/100 | 1.0% |
| 61 | *Yersinia pestis* | 1/100 | 1.0% |
| | TOTAL | 10 | |

**Table 2.** Bacterial resistance profile of isolates from samples collected from the ear canal of neutered dogs diagnosed with otitis externa residing in a municipal shelter in a city in the northwest region of the state of Paraná, Brazil, 2022.

| Antibiotics | Males | % | Females | % |
|---|---|---|---|---|
| Amoxicillin + Clavulanate | 17/50 | 34% | 18/50 | 36% |
| Cephalothin | 7/50 | 14% | 25/50 | 50% |
| Ceftriaxone | 21/50 | 42% | 29/50 | 58% |
| Cefotaxime | 18/50 | 36% | 24/50 | 48% |
| Cefoxitin | 17/50 | 34% | 22/50 | 44% |
| Ceftiofur | 16/50 | 32% | 18/50 | 36% |
| Chloramphenicol | 17/50 | 34% | 17/50 | 34% |
| Doxycycline | 20/50 | 40% | 19/50 | 38% |
| Enrofloxacin | 41/50 | 82% | 44/50 | 88% |
| Gentamicin | 21/50 | 42% | 27/50 | 54% |
| Metronidazole | 49/50 | 98% | 50/50 | 100% |
| Meropenem | 14/50 | 28% | 17/50 | 34% |
| Tetracycline | 36/50 | 72% | 36/50 | 72% |
| TOTAL | 294 | | 346 | |

**Table 3.** Bacterial resistance profile of *Staphylococcus aureus* isolates and presence of the *mec*A gene in samples collected from the ear canal of neutered dogs diagnosed with otitis externa residing in a municipal shelter in a city in the northwest region of the state of Paraná, Brazil, 2022.

| Sample | Number | Gender | Antibiotics and Resistance | | | | | | | | | | | | | Gene *mec*A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AMC | CFL | CRO | CTX | CFO | CTF | CLO | DOX | ENO | GEN | MTZ | MER | TET | |
| 1 | 1 | Female | R | S | S | S | S | S | S | S | R | S | R | S | R | N |
| 3 | 2 | Female | S | S | S | S | S | S | S | S | R | S | R | S | S | N |
| 4 | 3 | Female | S | R | R | R | R | R | S | S | R | S | R | S | S | N |
| 8 | 4 | Female | S | S | S | S | R | S | S | S | R | S | R | S | S | N |
| 9 | 5 | Female | S | R | S | S | S | S | R | R | R | R | R | S | S | N |
| 36 | 6 | Female | S | S | S | S | S | S | R | S | R | R | R | S | R | N |
| 44 | 7 | Female | R | R | R | R | R | R | S | R | R | R | R | R | R | N |
| 47 | 8 | Female | S | S | S | S | S | S | R | S | R | R | R | S | R | N |
| 66 | 9 | Female | S | R | R | S | S | S | S | S | R | R | R | R | R | P |
| 72 | 10 | Female | S | S | S | S | S | S | S | S | S | S | R | S | R | P |
| 77 | 11 | Female | S | S | S | S | S | S | S | S | R | R | R | S | R | N |
| 79 | 12 | Male | S | S | R | S | S | S | S | R | R | S | R | S | R | N |
| 10 | 13 | Male | S | R | S | R | R | R | R | R | R | R | R | S | R | N |
| 15 | 14 | Male | R | S | R | S | S | S | R | R | R | S | R | R | R | N |
| 23 | 15 | Male | S | S | S | S | S | S | S | S | R | S | R | S | R | N |
| 24 | 16 | Male | S | R | R | R | R | R | S | R | R | R | R | R | R | N |
| 31 | 17 | Male | S | S | S | S | S | S | S | S | S | S | R | S | R | N |
| 32 | 18 | Male | S | S | S | S | S | S | S | S | R | S | R | S | S | N |
| 35 | 19 | Male | S | S | S | S | S | S | R | S | R | S | R | S | R | N |
| 45 | 20 | Male | S | R | R | R | S | S | S | S | R | R | R | S | R | N |
| 53 | 21 | Male | S | S | S | S | S | S | S | S | R | S | R | S | S | N |
| 56 | 22 | Male | S | S | R | S | R | S | S | S | R | R | R | S | R | N |
| 78 | 23 | Male | S | S | R | S | S | S | S | S | R | R | R | S | R | N |
| 93 | 24 | Female | S | S | R | R | S | R | S | S | R | S | R | S | R | N |

R: Resistant; S: Susceptible; P: Positive; N: Negative. AMC- Amoxicillin + Clavulanate; CFL - Cephalothin; CRO - Ceftriaxone; CTX - Cefotaxime; CFO - Cefoxitin; CTF - CEFTIOFUR; CLO - Chloramphenicol; DOX - Doxycycline; ENO - Enrofloxacin; GEN - Gentamicin; MER - Meropenem; MTZ - Metronidazole; TET - Tetracycline.

**Table 4.** Bacterial resistance profile of coagulase-negative *Staphylococcus* spp. isolates and presence of the *mec*A gene in samples collected from the ear canal of neutered dogs diagnosed with otitis externa residing in a municipal shelter in a city in the northwest region of the state of Paraná, Brazil, 2022.

| Sample | Number | Gender | Antibiotics and Resistance | | | | | | | | | | | | | Gene *mec*A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AMC | CFL | CRO | CTX | CFO | CTF | CLO | DOX | ENO | GEN | MTZ | MER | TET | |
| 46 | 1 | Female | S | S | S | S | S | S | S | R | S | R | S | R | R | P |
| 49 | 2 | Female | S | S | S | R | S | S | S | S | R | R | S | R | R | P |
| 63 | 3 | Female | R | S | R | R | R | R | R | R | R | R | R | R | R | P |
| 69 | 4 | Female | R | R | R | R | R | R | S | R | R | R | R | R | R | P |
| 70 | 5 | Female | R | R | R | R | R | R | S | R | R | R | R | R | R | P |
| 81 | 6 | Female | R | S | R | R | R | R | R | R | R | R | R | R | R | P |
| 87 | 7 | Female | S | S | S | R | S | S | S | S | R | R | S | R | R | P |
| 90 | 8 | Female | R | S | R | R | R | R | R | R | R | R | R | R | R | P |
| 93 | 9 | Male | S | S | S | R | R | S | R | S | S | R | S | R | R | P |
| 94 | 10 | Male | S | S | S | S | S | S | S | S | S | S | R | S | R | P |
| 98 | 11 | Male | R | R | R | R | R | R | R | R | R | R | R | R | R | P |
| 99 | 12 | Male | S | S | S | R | S | S | S | R | R | R | R | S | R | P |
| 100 | 13 | Male | R | S | R | R | R | R | R | S | R | R | R | R | R | P |
| 13 | 14 | Male | S | S | S | S | S | S | S | S | S | S | S | S | R | P |
| 39 | 15 | Male | R | R | R | R | R | R | R | R | R | R | R | R | R | P |
| 67 | 16 | Male | R | S | R | R | S | S | S | S | R | R | R | S | R | P |
| 76 | 17 | Female | R | R | R | R | R | R | R | R | R | R | R | R | R | P |

R: Resistant; S: Susceptible; P: Positive. AMC- Amoxicillin + Clavulanate; CFL - Cephalothin; CRO - Ceftriaxone; CTX - Cefotaxime; CFO - Cefoxitin; CTF - CEFTIOFUR; CLO - Chloramphenicol; DOX - Doxycycline; ENO - Enrofloxacin; GEN - Gentamicin; MER - Meropenem; MTZ - Metronidazole; TET - Tetracycline.

**Table 5.** Bacterial resistance profile of enterobacteria isolates in samples collected from the ear canal of neutered dogs diagnosed with otitis externa residing in a municipal shelter in a city in the northwest region of the state of Paraná, Brazil, 2022.

| Microorganism | Sample | Number | Gender | Antibiotics and Resistance | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AMC | CFL | CRO | CTX | CFO | CTF | CLO | DOX | ENO | GEN | MTZ | MER | TET |
| *Escherichia coli* | 84 | 1 | Female | R | R | R | R | R | S | S | S | R | R | S | R | S |
| *Obesumbacterium proteus* | 42 | 2 | Female | S | R | S | R | S | S | S | S | S | S | R | S | S |
| *Proteus mirabilis* | 65 | 3 | Female | S | R | R | R | R | R | R | R | R | R | R | R | R |
| *Kluyvera* sp. | 57 | 4 | Female | S | R | R | R | R | R | S | S | R | R | R | S | R |
| *Yersinia pestis* | 61 | 5 | Female | S | S | S | S | S | S | S | S | S | S | R | S | S |
| *Cedecea* sp. | 34 | 6 | Male | S | S | R | S | S | S | R | R | R | S | R | S | R |
| *Obesumbacterium proteus* | 43 | 7 | Male | R | R | R | R | S | R | R | R | R | R | R | R | R |
| *Obesumbacterium proteus* | 62 | 8 | Male | S | S | S | S | S | S | S | S | S | S | R | S | S |
| *Kluyvera* sp. | 60 | 9 | Male | S | S | S | S | S | S | S | S | S | S | R | S | R |
| *Serratia marcescens* | 38 | 10 | Female | S | R | R | R | R | R | S | S | R | R | R | R | R |

R: Resistant; S: Susceptible; P: Positive. AMC- Amoxicillin + Clavulanate; CFL - Cephalothin; CRO - Ceftriaxone; CTX - Cefotaxime; CFO - Cefoxitin; CTF - CEFTIOFUR; CLO - Chloramphenicol; DOX - Doxycycline; ENO - Enrofloxacin; GEN - Gentamicin; MER - Meropenem; MTZ - Metronidazole; TET - Tetracycline.

## DISCUSSION

In this research, biological samples were collected homogeneously from male (50%) and female (50%) dogs, both neutered, and 100% of the animals were diagnosed with otitis externa. Different authors report a higher rate of otitis externa in female dogs than in males; however, no consensus defines the existence of possible risk factors regarding sexual predisposition, age, or physiological changes for the presence of otopathies [11,19,38,40]. Another situation that must be considered is the anatomical differentiation of the auditory canal of dogs, which varies widely between breeds, suggesting that breed should be considered as an important predisposing factor for the presentation of otitis externa [7]. Although little is reported about predisposing breeds, studies indicate that breeds such as Pug, Labrador Retriever, Golden Retriever, Fox Terrier, Belgian Shepherd, Lhasa Apso, Shih-tzu, and Rottweiler are susceptible to ear canal infections due to their auricular anatomy or excessive hair growth in the region [9].

The cytological examination made it possible to accurately identify the presence of microorganisms, confirming the results of the cytological examination, an important tool for the diagnosis of otitis externa in dogs [37]. In this research, auditory canal cytology identified 51 (51%) samples presenting *Malassezia pachydermatis*, 22 (22%) in males, and 29 (29%) in females. This result is similar to a study carried out in 2023, which analyzed 100 dog samples and identified *Malassezia pachydermatis* in 48% of these samples [39].

*Malassezia pachydermatis* is a fungus naturally found on healthy skin, and its uncontrolled growth can occur due to some alteration in the skin barrier, altering the skin microbiota, favoring its growth and the appearance of infections [4]. Samples analyzed from a Pet Shop in the city of Pimenta Bueno and Cacoal in Rondônia identified *Malassezia pachydermatis* disseminated throughout the pet shop environment and on animal objects, such as side tables, combs, and general utensils, allowing for possible cross-contamination, with the possibility of zoonotic potential being reported in the literature [17,22,39]. In this research, the animals were housed in shared environments with constant movement of staff, which may favor cross-contamination in the municipal shelter where the study dogs were housed.

Regarding the gender of the animals in this research, the difference occurred in the greater detection of fungi in samples from the external auditory canal of bitches associated with a coccus or an enterobacterium, and with greater identification of antibacterial resistance. According to a recent study, this situation can be explained by changes in the animals' environments that can generate stress, changes in temperature, pH, humidity levels, imbalance in the skin microbiota, and the sharing of environments and materials by the animals may have played an important role in the dissemination and increase of these microorganisms [39].

Studies conducted by different researchers showed greater detection of Gram-positive bacteria in samples from the ear canal of dogs with otitis externa compared to Gram-negative bacteria [3]. Skin characterization studies in dogs demonstrate a greater predominance of Gram-positive bacteria, presenting different species of *Staphylococcus* spp., mainly *Staphylococcus aureus*, both in healthy skin and in skin with some dermatological disorder [25]. Other authors have shown that otitis can be associated with the presence of *Staphylococcus* spp., *Staphylococcus aureus*, *Malassezia pachydermatis* and *Escherichia coli* classified according to its location in the auditory canal as external, middle, and internal [4,13,35,41]. In this work, Gram-positive bacteria were isolated from 90 samples (90%) and Gram-negative bacteria from ten (10%).

Related to the clinical signs of this work, the most frequent were erythema, localized seborrhea, unpleasant body odor, and skin lichenification, which were also identified in dogs through physical evaluation [8]. These clinical signs are possibly due to the proliferation of bacteria and may begin with mild itching and progress to head shaking, ear discharge, and odor.

Researchers obtained the highest number of isolations for the genus *Staphylococcus* from samples from the ear canal of dogs with otitis, both external, middle, and internal. In Minas Gerais (Brazil) 52.18% of the samples were identified as *Staphylococcus* sp. [38]. In Rio Grande do Norte (Brazil) 40.6% were isolated as *Staphylococcus* sp. [23]. The presence of *Staphylococcus* in this research was detected in 90% of the isolated samples, being compatible with those found in the literature.

In the state of São Paulo (Brazil), ear infections caused by the genus *Staphylococcus* spp. were described in isolation and/or associated with other microorganisms in 43.3% of cases [27]. Of the samples isolated and identified in this research as *Staphylococcus*, these were associated (51%) or not with another microorganism, such as *Malassezia pachydermatis*, which was also identified in cytological microscope slides in this research.

The *mec*A gene is frequently found in strains of methicillin-resistant *Staphylococcus aureus* (MRSA), being an important marker in microbiology for the identification of MRSA, and the presence of this gene confers resistance to beta-lactam antibiotics, such as methicillin and penicillin, complicating the treatment of infections [2]. Samples of coagulase-negative *Staphylococcus* spp. that were positive for the *mec*A 17 gene (5.44%) were identified. Similar results were found in 122 samples of bacterial isolates of *Staphylococcus* spp. from external otitis in dogs: 14 were positive for the *mec*A gene, 8 for coagulase-positive *Staphylococcus* spp., and 6 for coagulase-negative [11]. These findings imply a possible health risk due to the difficulty in treatment, significantly impacting healthcare costs, since infections resistant to multiple drugs often require prolonged and complex treatments.

In this study, the number of *Staphylococcus aureus* samples positive for the *mec*A gene presented similar results for several authors. Five *Staphylococcus aureus* isolates were positive for the *mec*A gene in studies of dog otitis samples among the total of 76 collections performed and identified through PCR [31]. Other similar results were also found in 33 samples isolated from *Staphylococcus aureus* of external otitis in dogs, 4 of which were positive for the *mec*A gene [28]. Recent studies indicate an increase in infections among companion animals by methicillin-resistant *Staphylococcus aureus* (MRSA), which may have implications for public health, as these animals can become reservoirs for MRSA and potentially transmit it to humans [15]. According to the report of the Global Antimicrobial Resistance and Use Surveillance System (GLASS), alarming rates stand out in more than 120 countries studied, including Brazil, where 35% of *Staphylococcus aureus* were positive for the methicillin-resistant *mec*A gene, making medical treatments difficult [43].

Related to enterobacteria, a study conducted in dogs reported the presence of *Obesumbacterium proteus* in samples collected from the vaginal and preputial mucosa of castrated dogs, presenting multiresistance to more than 5 antimicrobials tested, including amoxicillin + clavulanate, cephalothin, metronidazole, enrofloxacin, and others [32]. This result is compatible with those found in this research, with 1 of the *Obesumbacterium proteus* samples (1%) being positive for Extended Spectrum Beta-Lactamase (ESBL). According to the cutaneous characterization of dogs, the presence of enteric bacteria may be associated with exposure to bacteria from the normal microbiota of the intestines of humans and animals, as well as from contaminated soil or water, a situation that may occur in the municipal shelter, where dogs are housed together. There may be, at some point during the day, direct exposure to feces from these animals, as well as contamination of utensils and materials (feeding bowls, toys, blankets, among others) used by the animals [25]. ESBL-producing bacteria are those that can spread rapidly in hospital environments, contributing to the complication of treatments, and are generally associated with serious infections, which limit therapeutic alternatives and increase the toxicity of medications [16]. The use of broad-spectrum antibiotics in the respective municipal shelter to treat diseases can favor the selection of these bacteria, making them multi-resistant, putting the health of the animals there, as well as veterinarians and the environment, at risk.

Another important enterobacteria detected in this work is *Kluyvera* spp., described in the literature as a bacterium naturally found in contaminated soil, water, sewage, hospital environments, and foods of animal origin [42]. The presence of this bacterium in samples of external otitis in dogs may be related to environmental contamination and poor hygiene in the animals' housing.

The presence of enterobacteria in the ear canal is already an indication of otitis externa, according to a study carried out by the University of Ljubljana, due to the unusual presence of these microorganisms among the microbiota [18]. Inadequate manipulation of the ear canal, such as the insertion of cotton swabs and chronic topical treatment with antibacterials, antifungals, and anti-inflammatories, can favor the growth of these bacteria, triggering a pathogenic condition. These data highlight the importance of microbiological analyses of the external auditory canal of dogs for bacterial identification.

According to the literature, the identification of Gram-negative bacteria in the auditory canal of dogs is uncommon [11,14,27]. Gram-negative bacteria, when present, tend to be associated with secondary infections or chronic otitis externa. Research shows that the low prevalence of these bacteria may be attributed to factors such as the pH of the ear canal, the presence of cerumen, and the specific characteristics of the normal bacterial microbiota in dogs' ears [11,14]. Of the microorganisms isolated, 10% of the samples were Gram-negative bacteria in dogs diagnosed with otitis externa, highlighting the need for better investigation to understand the presence of these bacteria in samples from dogs at the municipal shelter.

Safety measures must be analyzed to better understand ways to prevent, manage, and treat diseases caused by these multidrug-resistant microorganisms and reservoirs for MRSA. It is important to highlight that these microorganisms identified in the municipal shelter, combined with the number of animals and different species present there, could become an environment conducive to cross-contamination.

## CONCLUSIONS

The present study identified that ear diseases in dogs at this municipal shelter are associated with bacterial and fungal infections, with a higher prevalence of *Staphylococcus* spp. and *Malassezia pachydermatis*.

The analysis of the collected samples showed high antimicrobial resistance (100%), with high rates in all samples for Metronidazole (99%), Enrofloxacin (85%), Tetracycline (75%), and Ceftriaxone (42%), demonstrating the urgent need for more effective and targeted management strategies when treating animals.

The identification of ESBL among enterobacteria and *Staphylococcus* resistance to methicillin reinforces the importance of performing antimicrobial sensitivity tests for effective treatment, given the difficulty in resolving infections caused by these microorganisms. The indiscriminate use of broad-spectrum antibiotics should be avoided to prevent the development of resistant strains and ensure the effectiveness of treatments. Regular monitoring and appropriate management protocols are crucial to reducing the incidence of otitis externa and improving the hearing health of dogs in shelters.

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## REFERENCES

1 **Abayneh M., Tesfaw G. & Abdissa A. 2018.** Isolation of Extended-Spectrum β-lactamase- (ESBL-) Producing *Escherichia coli* and *Klebsiella pneumoniae* from patients with community-onset urinary tract infections in Jimma University Specialized Hospital, Southwest Ethiopia. *The Canadian Journal of Infectious Diseases & Medical Microbiology*. 18: 8. DOI: 10.1155/2018/4846159.

2 **Algammal A.M., Hetta H.F., Elkelish A., Alkhalifah D.H.H., Hozzein W.N., Batiha G.E.-S., El Nahhas N. & Mabrok M.A. 2020.** Methicillin-resistant *Staphylococcus aureus* (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and Drug Resistance*. 13: 3255-3265. DOI: 10.2147/idr.s272733.

3 **Barros A.M.T., Torquarto N.R.V., Santos M.M., Carmo A.B.P.C.M.T., Silva R.R.F.D. & Matos R.A.T. 2021.** Ocorrência de otite externa em cães e gatos atendidos em uma clínica escola de medicina veterinária. *Revista Multidisciplinar em Saúde*. 2(3): 74. DOI: 10.51161/rems/1893.

4 **Bond R., Morris D.O., Guillot J., Bensignor E.J., Robson D., Mason K.V., Kano R. & Hill P.B. 2020.** Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. *Veterinary Dermatology*. 31(1): 73-77. DOI: 10.1111/vde.12809.

5 **Botoni L.S., Reis Filho N.P., Scherer C.B., Braga L., Leme F.O.P. & Bicalho A.P.C.V. 2014.** Piodermite superficial canina por *Staphylococcus pseudintermedius* resistente à meticilina (MRSP) - Revisão de literatura. *Medvep Dermato - Revista de Educação Continuada em Dermatologia e Alergologia Veterinária*. 3(10): 270-277.

6 **Brasil. 2022.** Agência Nacional de Vigilância Sanitária - Anvisa. Resistência antimicrobiana e ameaça mundial. (RDC 17). 40p.

7 **Bradley C.W., Lee F.F., Rankin S.C., Kalan L.R., Horwinski J., Morris D.O., Grice E.A. & Cain C.L. 2020.** The Otic Microbiota and Mycobiota in a Referral Population of Dogs in Eastern USA with Otitis Externa. *Veterinary Dermatology*. 31(3): 225-e49. DOI: 10.1111/vde.12826.

8 **Brito R.S.A., Santin R., Nobre M.O. & Mueller E.N. 2018.** *Malassezia* e Malasseziose em cães e gatos. *Medvep - Revista Científica de Medicina Veterinária - Pequenos Animais e Animais de Estimação*. 15(47): 67-72.

9 **Bugden D.L. 2013.** Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Australian Veterinary Journal*. 91(1-2): 43-46. DOI: 10.1111/avj.12007.

10 **Cabañes F.J. 2021.** Diagnosis of *Malassezia dermatitis* and otitis in dogs and cats, is it just a matter of counting? *Revista Iberoamericana de Micología*. 38(1): 3-4. DOI: 10.1016/j.riam.2020.03.001.

11 **Carvalho L.C.A., Cidral T.A., Melo M.C.N.D., Porto W.J.N. & Motta Neto R. 2020.** Ocorrência de *Staphylococcus* spp. resistentes à meticilina em otite externa canina. *Revista Brasileira de Análises Clínicas*. 51(4): 263-269. DOI: 10.21877/2448-3877.201900891.

12 **CLSI. 2023.** Performance Standards for Antimicrobial Susceptibility Testing. 33rd Report. (CLSI supplement, M100). 34p.

13 **Ez A., Hidalgo F., Morales P., Silva V., Thomson P. & Castro R. 2022.** Antifungal susceptibility of *Malassezia pachydermatis* isolated from the external auditory canal of dogs in central Chile. *Open Veterinary Journal*. 12(1): 99. DOI: 10.5455/ovj.2022.v12.i1.12.

14 **Ferraz C.M., Morais J.N.S., Loureiro B., Rodrigues J.A., Vilela V.L.R., Bicalho A.C.V., Horta R.S., Langoni H., Braga F.R. & Tobias F.L. 2021.** Etiologia microbiana e perfil de resistência bacteriana *in vitro* em otites externas de cães: estudo retrospectivo em animais atendidos na rotina de Hospital Veterinário (2013 a 2020). *Veterinária e Zootecnia*. 28: 10-12. DOI: 10.35172/rvz.2021.v28.578.

15 **Gulaydin O., Gurturk K., Ekin I. H., Ilhan Z. & Arabaci C. 2023.** Phenotypic and genotypic characterization of macrolide-lincosamide-streptogramin resistance in isolates from bovine and human. *Acta Veterinaria-Beograd*. 73(1): 102-118. DOI: 10.2478/acve-2023-0008.

16 **Husna A., Rahman M.M., Badruzzaman A.T.M., Sikder M.H., Islam M.R., Rahman M.T., Alam J. & Ashour H.M. 2023.** Extended-spectrum β-lactamases (ESBL): challenges and opportunities. *Biomedicines*. 11(11): 2937. DOI: 10.3390/biomedicines11112937.

17 **Hobi S., Cafarchia C., Romano V. & Barrs V.R. 2022.** Malassezia: zoonotic implications, parallels and differences in colonization and disease in humans and animals. *Journal of Fungi (Basel)*. 8(7): 708. DOI: 10.3390/jof8070708.

18 **Kušar D., Šrimpf K., Isaković P., Kalšek L., Hosseini J., Zdovc I., Kotnik T., Vengušt M. & Tavčar-Kalcher G. 2016.** Determination of N-acylhomoserine lactones of *Pseudomonas aeruginosa* in clinical samples from dogs with otitis externa. *BMC Veterinary Research*. 12(1): 233. DOI: 10.1186/s12917-0

19 **Lucas R., Correa S., Calabria K.C. & Palumbo M.I.P. 2016.** Otites. In: Larsson C.E. & Lucas R. (Eds). *Tratado de Medicina Externa - Dermatologia Veterinária*. Cap.59. 2.ed. São Caetano do Sul: Interbook, pp.977-952.

20 **Martineau F., Picard F.J., Roy P.H., Ouellette M. & Bergeron M.G. 1998.** Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *Journal of Clinical Microbiology*. 36(3): 618-623. DOI: 10.1128/JCM.36.3.618-623.1998.

21 **Miller W., Griffin C. & Campbell K.L. 2013.** Diseases of eyelids, claws, anal sacs, and ears. In: Miller W.H., Griffin C.E. & Campbell K.L. (Eds). *Small Animal Dermatology*. 7th edn. St. Louis: Elsevier Mosby, pp.724-773.

22 **Morris D.O., O'Shea K., Shofer F.S. & Rankin S. 2005.** *Malassezia pachydermatis* carriage in dog owners. *Emerging Infectious Diseases*. 11(1): 83-88. DOI: 10.3201/eid1101.040882.

23 **Moura E.S.R., Souza Fonseca Z.A.A., Feijó F.M.C., Filgueira K.D. & Silva J.B.A. 2010.** Isolamento e identificação de microrganismos causadores de otites em cães. *Pubvet*. 4(2): 83. DOI: 10.3201/eid1101.040882.

24 **Murakami K., Minamide W., Wada K., Nakamura E., Teraoka H. & Watanabe S. 1991.** Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *Journal of Clinical Microbiology*. 29(10): 2240-2244. DOI: 10.1128/jcm.29.10.2240-2244.1991.

25 **Older C.E., Rodrigues Hoffmann A., Hoover K. & Banovic F. 2020.** Characterization of cutaneous bacterial microbiota from superficial pyoderma forms in atopic dogs. *Pathogens*. 9(8): 638. DOI: 10.3390/pathogens9080638.

26 **Oliveira D.M.N.M., Carieli E.P.O., Fagundes A.K.F., Costa R.M.F., Oliveira K. P., Lima E.R. & Silva L.B.G. 2004.** Avaliação da atividade antimicrobiana do óleo essencial de aroeira (*Schinus terebinthifolius*, Raddi), em cães com otite externa. *Revista Brasileira de Medicina Veterinária*. 26: 79-82. DOI: 10.33448/rsd-v11i10.23450.

27 **Oliveira V.B. de, Ribeiro M.G., Almeida A.C.S., Paes A.C., Condas L.A.Z., Lara H.B., Franco M.M.J., Fernandes M.C. & Listoni F.J.P. 2012.** Etiologia, perfil de sensibilidade aos antimicrobianos e aspectos epidemiológicos na otite canina: estudo retrospectivo de 616 casos. *Semina: Ciências Agrárias*. 33(6): 2367-2374. DOI: 10.5433/1679-0359.2012v33n6p2367.

28 **Öztürk D., Avkî S., Türütoglu H., Yiğitarslan K. & Sağnak S. 2010.** Methicillin resistance among coagulase-positive staphylococci isolated from dogs with otitis externa, skin wounds and pyoderma. *Veterinary Science Development*. 16(4): 651-656. DOI: 10.9775/kvfd.2009.1423.

29 **Pegram C., Wonham K., Brodbelt D.C., Church D.B. & O'Neill D.G. 2020.** Staffordshire Bull Terriers in the UK: their disorder predispositions and protections. *Canine Medicine and Genetics*. 7(1): 1-11. DOI: 10.1186/s40575-020-00092-w.

30 **Penna B., Varges R., Medeiros L., Martins G.M., Martins R.R. & Lilenbaum W. 2009.** *In vitro* antimicrobial susceptibility of staphylococci isolated from canine pyoderma in Rio de Janeiro, Brazil. *Brazilian Journal of Microbiology*. 40: 490-494. DOI: 10.1590/S1517-83822009000300011.

31 **Prošić I., Milčić-Matić N., Milić N., Radalj A., Aksentijević K., Ilić M., Nišavić J., Radojičić M., Gajdov V. & Krnjaić D. 2024.** Molecular prevalence of *Mec*A and *Mec*C genes in coagulase-positive staphylococci isolated from dogs with dermatitis and otitis in Belgrade, Serbia: A one year study. *Acta Veterinaria*. 74(1): 117-132. DOI: 10.2478/acve-2024-0009.

32 **Rey L.M.R., Guaitolini C.R.D.F., Fazoli K.G.Z., Silva L.L., Fendrigo T.T., Santos I.C., Zaniólo M.M., Martins L.D.A. & Gonçalves D.D. 2020.** Microbiome and antimicrobial resistance in members of the Enterobacteriaceae family from vaginal and preputial mucous isolates of stray dogs. *Acta Scientiae Veterinariae*. 48: 1-7. DOI: 10.22456/1679-9216.104699.

33 **Ribeiro D.S.F., Supptitz J.S. & Ribeiro R.M. 2022.** Avaliação da prevalência infecciosa e sensibilidade aos antimicrobianos em otite externa de cães em Mineiros, região Centro-Oeste do Brasil. *Research, Society and Development*. 11(13): 1-8. DOI: 10.33448/rsd-v11i13.34092.

34 **Chicuti M.M., Paier G.G.S. & Senhorello I.L.S. 2018.** Avaliação do uso de antimicrobianos e suas interações medicamentosas em cães e gatos hospitalizados. *Ars Veterinaria*. 38(3): 139-148. DOI: 10.15361/2175-0106.2022v38n3p139-148.

35 **Rosenfeld R.M., Schwartz S.R., Cannon C.R., Roland P.S., Simon G.R., Kumar K.A., Huang W.W., Haskell H.W. & Robertson P.J. 2014.** Clinical practice guideline: acute otitis externa. *Otolaryngology - Head and Neck Surgery: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery*. 150(1): 1-24. DOI: 10.1177/0194599813517083.

36 **Saman A., Chaudhry M., Ijaz M., Shaukat W., Zaheer M.U., Mateus A. & Rehman A. 2023.** Assessment of knowledge, perception, practices and drivers of antimicrobial resistance and antimicrobial usage among veterinarians in Pakistan. *Preventive Veterinary Medicine*. 212: 1-8. DOI 10.1016/j.prevetmed.2022.105836.

37 **Sousa J.G.C. & Gomes R.A. 2024.** Diagnóstico citológico em otite externa em cães frequentadores de banho e tosa. Revista Ibero-Americana de Humanidades. *Ciências e Educação*. 10(5): 4216-4225. DOI: 10.51891/rease.v10i5.14220.

38 **Silveira A.C.P. 2008.** Flora bacteriana aeróbica em otites caninas. *Revista Portuguesa de Ciências Veterinárias*. 103(567): 171-175.

39 **Simini M.G., Violato T.C. & Sérvio C.S. 2023.** Pesquisa de *Malassezia pachydermatis* em cães e gatos de pet shop nos municípios de Pimenta Bueno e Cacoal. *Revista Ibero-Americana de Humanidades, Ciências e Educação*. 9(11): 387-395. DOI: 10.51891/rease.v9i11.12293.

40 **Tuleski G.L.R., Warth J.F.G. & Montiani-Ferreira F. 2008.** Prevalência infecciosa em otites de cães e padrão de sensibilidade *in vitro* aos antibacterianos. *A Hora Veterinária*. 162: 22-27.

41 **Voget M., Lorenz D., Lieber-Tenorio E., Hauck R., Meyer M. & Cieslicki M. 2015.** Is transmission electron microscopy (TEM) a promising approach for qualitative and quantitative investigations of polymyxin B and miconazole interactions with cellular and subcellular structures of *Staphylococcus pseudintermedius*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Malassezia pachydermatis*? *Veterinary Microbiology*. 181(4): 261-270. DOI: 10.1016/j.vetmic.2015.10.002.

42 **Widodo A., Effendi M.F. & Khairullah A.R. 2020.** Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. *Systematic Reviews in Pharmacy*. 11(7): 382-392. DOI: 10.1016/j.vetmic.2015.10.002.

43 **World Health Organization. 2023.** GLASS manual for antimicrobial resistance surveillance in common bacteria causing human infection. Geneva, Switzerland. (World Health Organization). 70p.