DOI: 10.1590/1089-6891v20e-52164
MEDICINA VETERINÁRIA

EFFECTIVENESS OF TOBRAMYCIN AND CIPROFLOXACIN AGAINST BACTERIAL ISOLATES IN CANINE OTITIS EXTERNA IN UBERABA, MINAS GERAIS

EFICÁCIA DE TOBRAMICINA E CIPROFLOXACINA CONTRA ISOLADOS BACTERIANOS DE OTITE EXTERNA CANINA EM UBERABA, MINAS GERAIS

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Abstract
Canine otitis externa is a common and recurrent disease in domestic dogs. Although not being life threatening, it can result in the overuse of antibiotics, increased bacterial resistance and reduction of options for treatment. This study aimed to determine: the principal characteristics (age, race, and gender) of dogs affected by otitis externa; the most frequently bacteria identified as being associated with it and their antimicrobial resistance profiles. A total of 221 bacterial isolates were identified by morphological staining and biochemical processes and tested against eleven antibiotics. Among the 148 dogs diagnosed with otitis externa, 51.4% (76/148) were males and 48.6% (72/148) females. The cases were detected mainly in dogs with age above 7 years (54.7%; 81/148. Among the races examined, poodles were the most numerous (14.84%; 22/148). The most common bacteria found were: coagulase-negative *Staphylococcus* (StCN), 33.9% (75/221); coagulase-positive *Staphylococcus* (StCP), 19% (42/221); *Proteus* spp., 17.2% (38/221) and *Pseudomonas aeruginosa*, 9.5% (21/221). Simultaneous resistance to more than three classes of antimicrobials were most frequently detected in *P. aeruginosa* (61.9%; 13/21) and *Proteus* spp (39.47%; 15/38). According to data obtained from this study, two veterinary medications found to be most effective for topical treatment were tobramycin and ciprofloxacin.

Keywords: antimicrobial, bacteria infection, dogs, ear, resistance

Resumo
A otite externa canina é uma doença comum e recorrente em cães domésticos. Embora não seja uma ameaça à vida, pode resultar no uso excessivo de antibióticos, aumento da resistência bacteriana e redução das opções de tratamento. Este estudo teve como objetivo determinar: as principais características (idade, raça e gênero) de cães afetados por otite externa; as bactérias mais frequentemente identificadas como associadas a enfermidade e seus perfis de resistência. Um total de 221 isolados bacterianos foram identificados por características morfo-tintoriais e testes bioquímicos, e posteriormente testados contra onze antibióticos. Entre os 148 cães com diagnóstico de otite externa,

51,4% (76/148) eram machos e 48,6% (72/148) fêmeas. Os casos foram detectados principalmente em cães com idade acima de 7 anos (54,7%; 81/148). Entre as raças examinadas, poodles foram os mais acometidos (14,84%; 22/148). As bactérias mais encontradas foram: *Staphylococcus* coagulase-negativo. (StCN), 33,9% (75/221), *Staphylococcus* coagulase-positiva (StCP), 19% (42/221), *Proteus* spp., 17,2% (38/221) e *Pseudomonas aeruginosa*, 9,5% (21/221) Resistência simultânea a mais de três classes de antimicrobianos foi mais frequentemente detectada em *P. aeruginosa* (61,9%; 13/21) e *Proteus* spp (39,47%; 15/38). Segundo os dados obtidos neste estudo, os antibióticos com maior capacidade de inibição do crescimento bacteriano in vitro foram tobramicina e ciprofloxacina.

Palavras-chave: antimicrobiano, infecção bacteriana, cães, orelhas, resistência

Received on: April, 13th, 2018.
Accepted on: June 5th, 2019.

Introduction

Otitis externa is a common problem of dogs taken to veterinary centers[1] for treatment. The result is often related to excessive use of antibiotics. The clinical signs include ear inflammation and may be associated with a more complex disease process including a combination of primary causes: atopic dermatitis, foreign bodies, or simply conformational abnormalities or excess hair in the ear canal and perpetuated by bacteria and fungi[2].

Microbiology laboratories should periodically and systematically conduct etiological identification and antimicrobial susceptibility testing to help ensure appropriate treatment and monitor the epidemiological profiles of isolated bacteria[3]. Positive coagulase *Staphylococcus* (StCP) and *Pseudomonas aeruginosa* are bacteria often isolated in canine otitis externa[4].

The treatment of this disease is based on the use of antibiotics, including aminoglycosides, quinolones, amoxicillin/clavulanate and cephalosporins, nevertheless, the incorrect and/or prolonged use of these drugs can result in bacterial resistance to antibiotics[5]. Otitis treatment using erythromycin, penicillin, neomycin or sulfa drugs[6,7], in the face of Gram positive and Gram negative bacteria, has been described as having low efficiency, while the drugs, ciprofloxacin and tobramycin has shown higher ability to inhibit the bacterial growth[4].

This study aimed to determine the main characteristics (race, age and gender) of dogs affected with otitis externa, characterize frequently isolated bacteria and describe the antimicrobial resistance profiles of diagnosed bacterial isolates.

Material and methods

An observational and descriptive study was conducted, using clinical and microbiological data on dogs attended in the Uberaba Veterinary Hospital, in Uberaba, Minas Gerais, Brazil, between 2009 and 2013. The criteria for inclusion in the study were: (i) clinical diagnosis of otitis externa; (ii) bacterial identification; (iii) antimicrobial susceptibility testing and (iv) at least 15 days without antibiotics before the collection of material, since the reliability of the cultures obtained can be reduced by the use of antimicrobial agents. The clinical signs of otitis externa considered in this study were: (i) the

presence of secretion in the ear; (ii) unpleasant odor; (iii) auditory canal hyperemia and (iv) pain upon palpation. Bilateral samples were collected separately from 93 dogs, while 55 animals had only one sample collected. The total was, thus, 148 dogs and 241 auditory canal samples.

Samples of ear discharge were collected using sterile swabs after cleaning the excess ear secretion with 70% alcohol. Samples of bilateral otitis were collected and cultured separately. The time between collection and the cultivation was less than 30 minutes. The samples were cultivated in blood agar (Oxoid®) and MacConkey (Difco®), by aerobic incubation at 37 °C for 24 to 72 hours. The bacterial colonies were identified by their morphological, staining and biochemical characteristics[8].

The Antimicrobial Susceptibility Testing (TSA) was conducted using the Kirby-Bauer disk diffusion method on Mueller Hinton Agar (Difco®), and interpretation of the results performed according to the Clinical and Laboratory Standards Institute[9]. An intermediate pattern of susceptibility was classified as resistant. The following antibiotics were tested: amikacin (30µg); ampicillin (10mg); azithromycin (15µg); amoxycillin/clavulanic acid (30µg); cephalexin (30µg); ceftiofur (30µg); ciprofloxacin (5µg); enrofloxacin (5µg), gentamicin (10mg); neomycin (30µg) and tobramycin (10mg). Strains of *Staphylococcus* aureus (ATCC: 25923); *Escherichia coli* (ATCC: 25922) and *Pseudomonas aeruginosa* (ATCC: 27853) were used as controls. Multi drug resistance was defined as resistance of the bacterial isolate on a combined basis, to three or more distinct classes of antibiotics[7].

Results

Altogether, there were identified 148 dogs showing clinical signs of otitis externa during the period from 2009 to 2013. Among these animals 51.4% (76/148) were males and 48.6% (72/148) were female. The majority of the dogs were above seven years old (54.7%; 81/148). There was a predominance of purebred dogs (68.8%; 102/148) and fewer mongrel dogs (31.2%; 46/148). Poodles 14.84% (22/148) followed by Cocker Spaniels 12.15% (18/148) and Shih Tzu 7.4% (11/148) were the most affected (Figure 1).

The microorganisms growth was verified in 87.1% (210/241) of the samples, while 12.9% (31/241) did not develop any colonies. Among the plates that showed growth, 73.8% (155/210) had only one type of colony of bacteria and 15.7% (33/210) had two visually different types. The growth of yeasts, probably Malassezia spp., occurred in 10.5% (22/210) of the cultures with agar blood, as the only culture.

There was a predominance of Gram-positive bacteria (65.6%; 145/221), with coagulase negative *Staphylococcus* (StCN) and coagulase positive *Staphylococcus* (StCP) as the most frequent. *Proteus* spp. and *Pseudomonas aeruginosa* were the prevailing Gram negative bacteria. Other bacteria with lower occurrence were also isolated (Table 1).

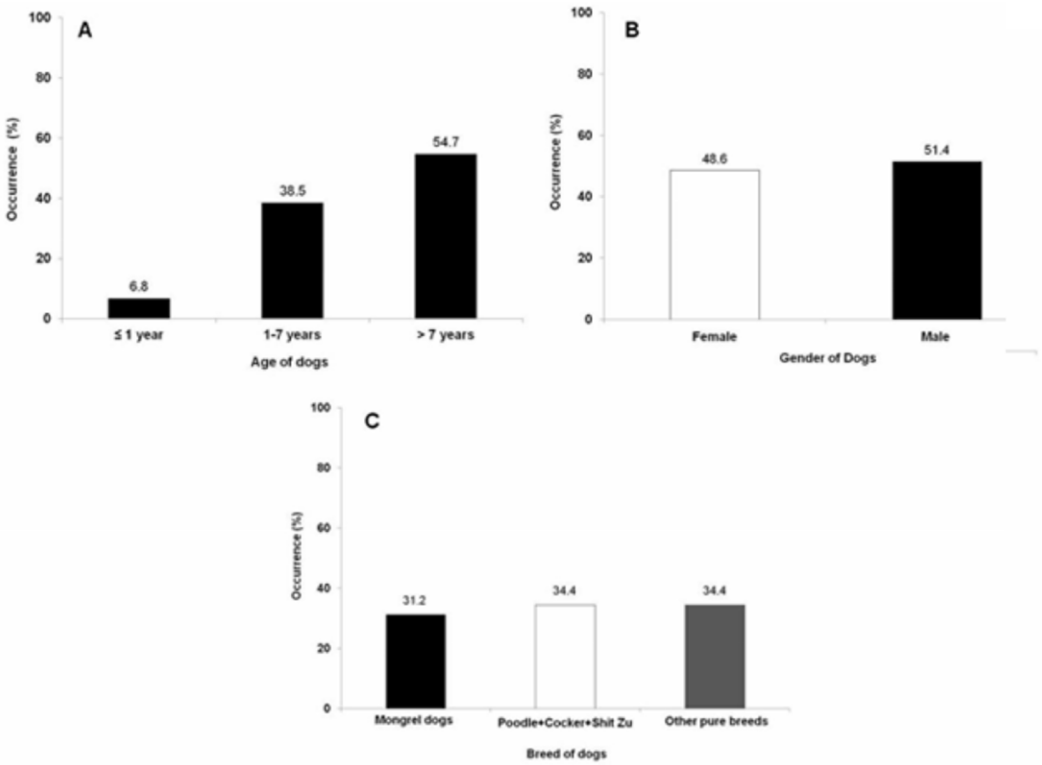

Figure 1 The characterization of 148 dogs diagnosed with otitis externa according to: (A) age; (B) sex and (C) breed in Uberaba, Minas Gerais, Brazil.

Table 1 Identification of 221 bacteria samples taken from the ear of dogs with otitis externa attended in Uberaba, Minas Gerais, Brazil.

Genera	**% (*n*)**
Gram Positive Bacteria	
StCN	51.7 (75/145)
StCP	29.0 (42/145)
Corynebacterium spp.	7.6 (11/145)
Streptococcus spp.	6.9 (10/145)
Enterococcus spp.	4.8 (7/145)
Gram Negative Bacteria	
Proteus spp.	50.0 (38/76)
Pseudomonas aeruginosa	27.6 (21/76)
Enterobacter spp.	10,5 (8/76)
Escherichia coli	6,6 (5/76)
Klebsiella spp.	5,3 (4/76)

StCN (coagulase negative *Staphylococcus*); StCP (coagulase positive *Staphylococcus*)

StCN and StCP colonies showed lower resistance levels to amicacina, ceftiofur and the association of amoxicilina/clavulanate (Table 2). *Proteus* spp. and *P. aeruginosa* demonstrated higher percentages of colonies resistant to neomycin, azithromycin and cephalexin. The percentage of colonies resistant to gentamicin was similar among the four genera of bacteria (Table 2).

Table 2 Resistance *in vitro* of the most common bacterial isolates found in ears of dogs with otitis externa attended in Uberaba, Minas Gerais, Brazil.

Antibiotics	Frequence of resistant (%)			
	Proteus spp.	*Pseudomonas aeruginosa*	StCN	StCP
Aminoglycosides				
Amikacin	16	19	3	0
Gentamicin	29	29	35	32
Neomycin	69	92	26	25
Tobramycin	33	0	29	0
Cephalosporins				
Cephalexin	81	82	16	10
Ceftiofur	28	79	9	6
Macrolides				
Azithromycin	92	58	39	21
Penicillins				
Amoxycillin/clavulanic acid	50	94	3	0
Ampicillin	53	100	46	68
Quinolones				
Ciprofloxacin	8	0	23	5
Enrofloxacin	34	80	36	40

StCN (coagulase negative *Staphylococcus*); StCP (coagulase positive *Staphylococcus*)

Among *P. aeruginosa* colonies, 61.9% showed simultaneous resistance to more than three classes of antimicrobials, while *Proteus* spp. revealed 39.47% of its colonies with the same profile (Table 3).

Table 3 Resistance *in vitro* to three or more classes of antibiotics, by the bacterial isolates that most occur in ears of dogs with otitis externa attended in Uberaba, Minas Gerais, Brazil.

Bacteria	*Proteus* spp. %(*n*)	*Pseudomonas aeruginosa* %(*n*)	StCN %(*n*)	StCP %(*n*)
Multi resistence	39.47(15/38)	61.9 (13/21)	33.33 (25/75)	16.66 (7/42)

StCN (coagulase negative *Staphylococcus*); StCP (coagulase positive *Staphylococcus*)

Discussion

The characterization of the canine population studied revealed agreement with other authors, in respect to the most common breeds of dogs with otitis externa (OE)[4,11]. The sex of the animals was not decisive for the occurrence of this condition, as reported in other studies[4,10]. Dog breeds with the ear canal lined with hair and excessive production of ear wax and/or in high humidity locations, showed predisposition to some inflammation of the externa ear[2,11].

In the present study, there was a higher number of inflammatory processes in the ears of dogs over seven years old, suggesting that age may influence the occurrence of OE. This apparent predisposition may be associated with endocrine diseases or immune mediated disorders in older dogs[2,11].

The absence of bacterial growth, observed in 12.9% of the analyses, does not imply the absence of inflammation in the auditive canal[12]. Not all canine OE processes are caused by bacteria. Other microorganisms may be involved such as mites and yeast or filamentous fungi[2]. Fungi require different culture conditions than those for bacteria[8]. The growth of yeasts, probably *Malassezia* spp., was observed in 10.5% of the cultures in blood agar.

Lesions in the epithelium of the ear canal can create a suitable environment for excessive multiplication of microbes, resident or transient, leading to inflammation[13]. In canine OE, the identification of Gram-positive bacteria is more often found than Gram-negative[13], with bacteria of the genus *Staphylococcus* spp. the most commonly isolated[2,4,12,14]. Positive coagulase *Staphylococcus* (StCP) is the most frequent inflammation of the canine ear canal[13,14]. However, in this study coagulase negative *Staphylococcus* (StCN) was the most more frequently found, corroborating other studies[4,15].

In the public health context the biology and epidemiology of coagulase negative *Staphylococcus* (StCN) must be accompanied systematically[16,17]. The transmission of these bacteria among animals and humans has been described[5,18]. StCN has the potential to produce severe infection in immune suppressed individuals, including humans[19,20] and transfer genes for multi resistance genes to other bacteria[21]. In the present study 33.3% of the StCN isolates were resistant to three or more classes of antibiotics.

P. aeruginosa and *Proteus* spp. are among the most common enterobacteria isolated from canine ear

canal infections[4,22]. The elevated number of observations of these microorganisms among dogs with OE, can be understood as a complication of the inflammation[13,14].

The aminoglycosides are presently considered the drugs of choice for the treatment of otitis because of their efficiency[23]. Tobramycin also demonstrated effectiveness in inhibiting colonies of StCP and *P. aeruginosa* colonies[4]. Gentamicin and neomycin, routinely used in antibiotic formulations for topical treatment of ear diseases[24], was found to be of low efficiency. This finding reinforces the need for testing of sensibility[7]. In spite of the requirement to test its high efficacy, amikacin is not available in veterinary medicines but may be used in specific cases[25]. Perhaps the great variation observed in the effectiveness of the aminoglycosides tested was due to the lack of criteria for choosing these antibiotics.

In terms of the quinolones, although *P. aeruginosa* has been found to be resistant to enrofloxacin, as described in other works[13,26,27], the same phenomenon was not observed for ciprofloxacin in this study. Excessive use of quinolones in the antibiotic therapy procedures with dogs may explain the resistance to enrofloxacin and perhaps in the future, ciprofloxacin[26,27].

Other antibiotics, such as penicillins, cephalosporins, and macrolides have formulations for systemic treatment of various diseases in animals, among them, cases of otitis. This route of administration is usually restricted to cases in which the otitis externa is severe, there is the presence of a medium level of otitis infection and/or the owner is unable to carry out the topical treatment[28].

In this study, StCN and StCP were susceptible to amoxicillin/clavulanate, probably due to the presence of the inhibitor of beta-lactamase (clavulanate), which may have enhanced the bactericidal effect of this antibiotic[29]. Gram-negative bacteria were resistant to the first-generation cephalosporins, possibly as a result of the narrower spectrum of action of these antibiotics[23] The multi resistance intrinsic of the *Pseudomonas aeruginosa*[8,30] and the formation of biofilm[31,32] are factors that justify the few options for treatment. Azithromycin has shown variable efficiency against StCP, StCN, *Proteus* spp. and *P. aeruginosa*, however, there are reports of bacterial resistance to this antibiotic[33]. Bacteria resistance is its ability to evade from the bactericidal or bacteriostatic mechanisms of a particular antibiotic, which reflects their structural or functional characteristics[34]. In this context, the identification of antibiotic bacteria resistance is relevant for an efficient antibiotic choice and successful treatment[35] .

The results of this research show that breed and age are determining factors in the diagnosis of OE, regardless of the sex of the dogs. It was found that Gram-positive bacteria predominate in cases of canine OE, StCN being the microorganism most frequently isolated. Topical medications are most commonly used for otitis. In this context, tobramycin and ciprofloxacin are the most effective antibiotics, in accordance with the data in vitro obtained in this study. The data also revealed a large variation in the sensitivity to antibiotics and elevated resistance to the medications commonly used in canine therapy for otitis externa.

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