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PROFILE OF OTITIS EXTERNA ISOLATES IN DOGS AND CATS DIAGNOSED IN LABORATORY OF MICROBIOLOGY IN 2020

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Abstract: Otitis externa is one of the most frequent diseases in the clinical routine of dogs and cats, and they can be caused by several associated factors. Due to incorrect use of antimicrobial products, the treatment and control of otitis have become challenging. This study aims to analyze the results of otological exams at the Laboratory of Microbiology HV-ULBRA in 2020 and demonstrate the profile of patients and isolated bacteria. Staphylococcus was the main genus isolated, and 71,11% of samples showed multi-drug resistance to antimicrobial testing. These results indicate the need to use complementary examinations to control otitis externa.

Keywords: canines, felines, otological diseases, antimicrobial susceptibility test.

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PERFIL DOS ISOLADOS DE OTITES EXTERNAS EM CÃES E GATOS DIAGNOSTICADOS EM LABORATÓRIO DE MICROBIOLOGIA EM 2020

Resumo

A otite externa é um das enfermidades mais frequentes na rotina clínica de cães e gatos e pode ser causada por diversos fatores associados. Devido ao uso incorreto de antimicrobianos, o tratamento e o controle das otites se tornaram desafiadores. O objetivo desse estudo é analisar os resultados dos exames otológicos encaminhados ao Laboratório de Microbiologia HV-ULBRA em 2020 e, além disso, delinear o perfil dos pacientes e das bactérias isoladas. Staphylococcus foi o principal gênero isolado e 71,11% das amostras apresentou multirresistência aos antimicrobianos testados. Esses resultados evidenciam a necessidade do uso de exames complementares para controle das otites externas.

Palavras-chave: caninos, felinos, doenças otológicas, sensibilidade a antimicrobianos.

Introduction

Otological diseases are essential in the clinical routine of the company of animals, and they can be caused by several associated factors, such as primary, secondary, and perpetuating causes. Approximately 20% of animals treated in the clinical routine have otitis external (Garcia; De Freitas, 2019; Souza; Souza; Scott, 2015). Dogs with pendulous ears may be predisposed to ear infections because of a direct relation between ear canal conformation and otitis (Mcvey; Kennedy; Chengappa, 2016). Besides, agents involved are frequently present by endogen origin. These belong to the microbiome of the ear and may become opportunist pathogenic when there is an imbalance in this microenvironment (Santos et al., 2020; McVey; Kennedy; Chengappa, 2016). Common clinical signs of otitis externa are itching of the ear, malodorous ear, erythema, abnormal secretions, ear pain, and several causes, auricular hematoma and hearing loss (Garcia; De Freitas, 2019). Complementary exams, such as bacterial and fungal culture and antimicrobial susceptibility tests, support deciding the best treatment for the infection. Due to the generalized and frequently inappropriate use of antibiotics, the number of strains of resistant agents has increased by selective pressure, which reduces the effectiveness of treatment and causes chronic diseases (Garcia; De Freitas, 2019; Kohl; Pontarolo; Pedrassani, 2016). In this context, this study aims to analyze the results of otological examination at the Laboratory of Microbiology in 2020 and demonstrate the profile of patients and isolated bacteria.

Materials and methods

Otological examinations were obtained by sterile swabs and forwarded to the Laboratory of Microbiology at the Hospital Veterinário/Universidade Luterana do Brasil (HV-ULBRA), Canoas/Rio Grande do Sul. The examinations were performed in two steps. Firstly, samples were cultured and incubated aerobically at 35°C (± 2 °C) on blood agar with 5% sheep blood (Kasvi®) and MacConkey agar (Kasvi®) and swabs preserved in brain heart infusion agar (BHI; Kasvi®) for 24 hours. If there was no bacterial growth, the first step was repeated by the sample in BHI agar. When bacterial growth occurred, colonies were identified by morphological and biochemical tests Mac Faddin (2001). After phenotypic confirmation, isolates were performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Kasvi®) with 13 antibiotic discs (Table 1). Inhibition zones of all antibiotics were interpreted according to Clinical and Laboratory Standards Institute (NCCLS, 2003), and the descriptive statistical result was executed by Microsoft Excel® (2010) Analysis.

Table 1. Pharmacological classes and antibiotics tested otological samples at Laboratory of Microbiology HV-ULBRA in 2020.

Pharmacological class	Antibiotic
Beta-lactams	Amoxicillin 10 µg
	Amoxicillin + clavulanic acid 30 µg
	Cephalexin 30 µg
	Ceftiofur 30 µg
	Imipenem 10 µg
Fluroquinolones	Ciprofloxacin 05 µg
	Enrofloxacin 05 µg
	Norfloxacin 10 µg
Tetracyclin	Doxycycline 30 µg
Aminoglycosides	Gentamicin 10 µg
	Neomycin 30 µg
	Tobramycina 10 µg
Polypeptide	Polymyxin B 300 µg

Results

In 2020, 48 otological examinations were performed; 91,67% of the samples were from dogs (44/48) and 8,33% from cats (04/48). Mixed breed dogs were more frequent than other breeds (25%; 12/48), followed by Shih Tzu (22,92%; 11/48) and Yorkshire terrier (6,25%; 03/48). An other 14 breeds were observed. Three cats were mixed breed (75%; 03/04), and one cat was Persian (25%; 01/04). Most animals were female (66,67%; 32/48). Males represented 31,25% (15/49) and one animal had no information as to sex (2,08%; 01/48). The median age was eight years; the youngest animal was four months, and the oldest was 15 years old; both were dogs. Seven animals had no information regarding age.

Bacterial growth occurred in most samples in at least one of the ears (93,75%; 45/48). There was no bacterial growth in three samples (6,25%; 03/48). Staphylococcus spp. was identified in 75% of the samples (36/48) and Bacillus spp. in 41,67% (20/48). Pseudomonas spp. and Proteus spp. were the most observed Gram-negative bacteria, 12,5% (06/48) and 10,42% (05/48), respectively. Mixed infections represented 66,67% of those (32/48). An other six genus were identified (Table 2): Corynebacterium, Enterobacter, Enterococcus, Escherichia, Klebsiella, e Streptococcus. Besides, five unidentified isolates were performed only by morphological tests.

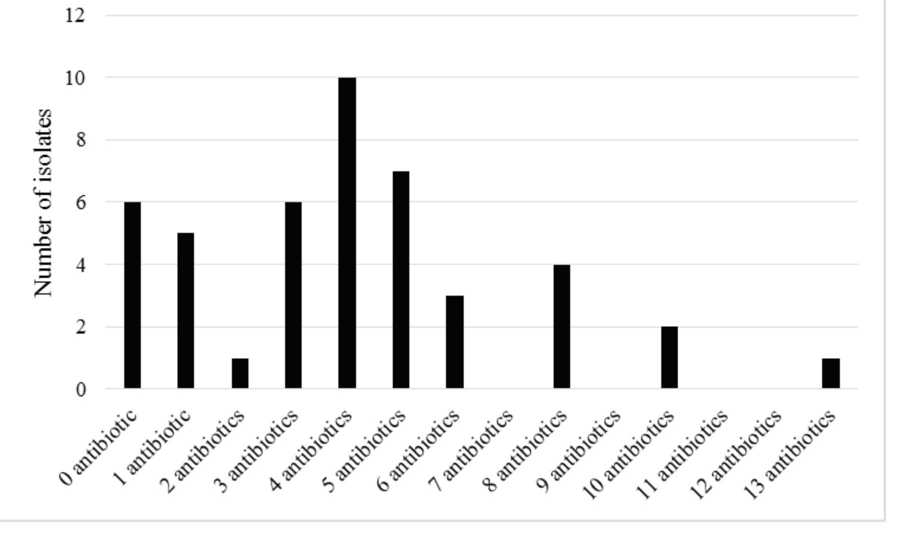
Table 2. Agents identified by means of otological samples at Laboratory of

Microbiology HV-ULBRA in 2020.

Agents	Number of isolates
Staphylococcus spp.	36
Bacillus spp.	20
Pseudomonas spp.	06
Proteus spp.	05
Corynebacterium spp.	04
Unidentified Gram-negative rods	04
Streptococcus spp.	03
Escherichia coli	02
Enterobacter spp.	01
Enterococcus spp.	01
Klebsiella spp.	01
Unidentified Gram-positive cocci	01

Antimicrobial susceptibility tests were performed on 45 positive samples. A once only assay was considered for all the isolates from the sample, even if the test had been performed in isolation. Most samples were resistant to at least one antibiotic (86,67%; 39/45), and multidrug-resistant (three or more pharmacological classes) isolates were detected in 73,33% of the samples (33/45) (Image 1). Six samples were sensitive to all antibiotics (13,33%; 06/45).

Image 1. Antimicrobial resistance of the isolates of otological samples at Laboratory of Microbiology HV-ULBRA in 2020.



Amoxicillin resistance was observed in 77,78% of the samples (35/45), followed by cephalexin (51,11%; 23/45), amoxicillin + clavulanic acid (42,22%; 19/45), and polymyxin (40%; 18/45) (Table 3). One sample was resistant to all antibiotics tested, including imipenem, used in several hospital infections. This isolate was obtained from a dog and Staphylococcus spp., Enterococcus spp., and E. coli. were identified.

Table 3. Profile of antimicrobial resistance of the isolates of otological samples at Laboratory of Microbiology HV-ULBRA in 2020.

Antibiotics	Profile of antimicrobial susceptibility		
	Resistant	Intermediate	Sensitive
Amoxicillin	77,78% (35/45)	0% (0/45)	22,22% (10/45)
Amoxicillin + clavulanic acid	42,22% (19/45)	0% (0/45)	57,78% (26/45)
Cephalexin	51,11% (23/45)	4,44% (02/45)	44,44% (20/45)
Ceftiofur	37,78% (17/45)	4,44% (02/45)	57,78% (26/45)
Ciprofloxacin	26,67% (12/45)	4,44% (02/45)	68,89% (31/45)
Doxycycline	26,67% (12/45)	4,44% (02/45)	68,89% (31/45)
Enrofloxacin	26,67% (12/45)	0% (0/45)	73,33% (33/45)
Gentamicin	13,33% (06/45)	4,44% (02/45)	82,22% (37/45)
Imipenem	2,22% (01/45)	0% (0/45)	97,78% (44/45)
Neomycin	20,00% (09/45)	15,56% (07/45)	64,44% (29/45)
Norfloxacin	31,11% (14/45)	4,44% (02/45)	64,44% (29/45)
Polymyxin B	40,00% (18/45)	6,67% (03/45)	53,33% (24/45)
Tobramycin	11,11% (05/45)	2,22% (01/45)	86,67% (39/45)

Discussion

Staphylococcus intermedius, Staphylococcus schleiferi spp. coagulans, Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus canis, E. coli, Klebsiella pneumoniae e Malassezia spp. were the most common identified agents in otitis externa. Frequently there were mixed infections (McVey; Kennedy; Chengappa, 2016; Biberstein, 2003). Staphylococcus spp. and Bacillus spp. were the primary isolates of otological samples in this study. Other studies found similar results (Almeida et al., 2016; Souza; Souza; Scott, 2015; Malayeri; Jamshidi; Salehi, 2010; Oliveira et al., 2006). Staphylococcus is one of the most isolated bacterial agents observed in acute infection (Santos et al., 2020). Bacillus spp. has a saprophytic characteristic, but it does not represent a pathogenic risk in the auricular microenvironment (Oliveira et al., 2006; Quinn et al., 2002). This may explain its presence in our study since the microbiome may have become an opportunist pathogenic when there is an imbalance (Santos et al., 2020).

P. aeruginosa is rarely the primary cause, but it is associated with complex elimination due to antibiotic resistance (McVey; Kennedy; Chengappa, 2016). Genus of Enterobacteriaceae, such as Proteus, Escherichia e Klebsiella, are considered opportunist agents, and some of them belong to the skin microbiome (McVey; Kennedy; Chengappa, 2016; Quinn et al., 2002). Corynebacterium spp. usually require previous tissue trauma to be a pathogenic bacteria (Quinn et al., 2002). Corynebacterium auriscanis is found in several canine diseases and is associated with otitis externa and dermatitis (McVey; Kennedy; Chengappa, 2016).

Our study showed resistant bacteria of otological samples in the specimens (samples), especially amoxicillin, cephalexin, amoxicillin + clavulanic acid, and polymyxin B. Most of the isolates were resistant to amoxicillin and cephalexin.

The Beta-lactams class acts as polypeptide synthesis blocker, which weakens the bacterial cell wall or activates enzymes of cell lysis (De Oliveira, 2012). Generally, this class has more activity against Gram-positive bacteria because it has more peptidoglycan in the cell wall (McVey; Kennedy; Chengappa, 2016). Resistance occurs mainly due to the presence of beta-lactamase enzymes. Gram-positive bacteria, especially coagulase-positive Staphylococcus, present these enzymes extracellularly; and are common in several genus of Gram-negative bacteria by plasmid dissemination (McVey; Kennedy; Chengappa, 2016). The association of beta-lactams with beta-lactamase inhibitors, such as clavulanic acid, increases the spectrum of action since they bind irreversibly to these enzymes (McVey; Kennedy; Chengappa, 2016).

Most of the studies found more than 25% of results resistant to beta-lactams (Garcia; De Freitas, 2019; Santos et al., 2019; Yamamoto et al., 2010; Malayeri; Jamshidi; Salehi, 2010; Oliveira et al., 2006), and 78,5% to penicillin in isolates of Staphylococcus epidermidis and up to 100% of Staphylococcus saprophyticus (Yamamoto et al., 2010). Unlike other beta-lactams, one of the samples was resistant to carbapenem imipenem, which showed excellent efficiency. This antibiotic is used to treat serious hospital infections, mainly caused by Gram-negative bacteria (Gales et al., 2002). Carbapenems are resistant to beta-lactamase and have extraordinary action against bacteria, mainly among Gram-negative pathogens (McVey; Kennedy; Chengappa, 2016). Similat to this study, Garcia and De Freitas (2019) found high sensitivity to imipenem.

Isolates showed resistance to polymyxin B, which was less effective when compared to other antibiotics. Polymyxins disrupt the outer cell membrane of bacteria because it binds to phospholipids (McVey; Kennedy; Chengappa, 2016; De Oliveira, 2012). These antibiotics are selectively toxic to Gram-negative bacteria (McVey; Kennedy; Chengappa, 2016), which may explain the high resistance of our study isolates since most of the isolates were Gram-positive bacteria.

Fluoroquinolones, tetracycline, and aminoglycosides showed similar results. Fluoroquinolones are effective against Gram-negative bacteria and act by inhibiting DNA gyrase and topoisomerase IV and, therefore, the bacterial DNA synthesis (McVey; Kennedy; Chengappa, 2016; De Oliveira, 2012). Tetracyclin inhibits bacterial protein synthesis by binding to the bacterial RNA messenger and ribosome (De Oliveira, 2012). This class has a broad spectrum of Gram-positive and negative bacteria (McVey; Kennedy; Chengappa, 2016). Aminoglycosides also act by misreading the genetic code of bacterial RNA messenger, which inhibits the protein synthesis; the activity is against Gram-negative bacteria (McVey; Kennedy; Chengappa, 2016; De Oliveira, 2012).

De Oliveira et al. (2012) found sensitivity similar to our study in otological samples of the different bacterial genus for ciprofloxacin and enrofloxacin. Likewise Oliveira et al. (2006) found results similar in resistance to tetracycline in samples of Staphylococcus intermedius. Tobramycin and gentamycin were the most effective antibiotics, which may be explained due to their having higher power, spectrum of action, and stability to resistance mechanism than other antibiotics of the same class (McVey; Kennedy; Chengappa, 2016). Ferraz et al. (2021) related results similar to our study on sensitivity to tobramycin and gentamycin to Staphylococcus spp. Silveira et al. (2009) and De Oliveira et al. (2012) also found similar sensitivity to gentamycin in isolates of Staphylococcus spp. and for different strains of otitis, respectively.

Conclusion

The presence of Staphylococcus spp. confirms its importance at infectious processes related to otological diseases in the company of animals, especially canines. Besides, the generalized and inappropriate use of antibiotics, frequently with no previous complementary examinations, cause the selection of pathogens with antibiotic resistance. Consequently, it is one of the primary responsibilities for treatment failure in several infectious diseases. Studies such as this may supply information as to the profile of antimicrobial resistance of strains, which may give support in deciding the best treatment for infections. However, it is essential to emphasize that complementary examinations, such as bacterial and fungal cultures and antimicrobial susceptibility tests, help establish the correct treatment and prevent the selection of resistant agents.

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