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Microbiological investigation and determination of susceptibility to antimicrobial agents in feline otitis externa

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Abstract

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This study aimed to investigate the bacterial and yeast populations in cats with otitis externa, and to assess their susceptibility to antimicrobial agents. Fifty-six cats showing clinical signs of otitis externa were included in the study. The cats were of different ages, genders, and breeds. Sterile cotton swabs were used to collect samples from the external ear canal, and conventional microbiological techniques as well as biochemical testing were used to isolate and identify the microorganisms. Antimicrobial susceptibility testing was conducted using Kirby-Bauer's disc diffusion method. Of 56 swab samples analyzed, 8 (14.28%) showed no microbial growth, while *Malassezia pachydermatis* was found in 26.79% of the samples, followed by *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (12.5%), *Proteus mirabilis* (10.71%), and *Staphylococcus pseudintermedius* (5.36%). The examined bacterial isolates exhibited the highest sensitivity to ciprofloxacin, gentamicin, amikacin, and doxycycline.

Keywords: antimicrobial susceptibility; microbial agents; otitis externa; cats

Introduction

Feline otitis externa is a very common and aetiologically complex disease. The aetiology of otitis externa (OE) involves multiple causes. This condition is one of the most frequent and annoying issues in small animal practice. The primary causes of OE include congenital anatomical changes, allergic reactions, foreign objects, parasites, and viruses (Shokri et al., 2010; Kennis, 2013; Mascarenhas et al., 2019). The microflora of the normal ear canal, including *Staphylococcus pseudintermedius*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Corynebacterium* spp., *Escherichia coli*, and *Malassezia pachydermatis*, is the main secondary cause of OE in cats. Bacteria and yeasts are the opportunistic organisms that cause otitis externa, and they need a conducive environment to thrive when another cause is present (Rosser, 2004). In the skin and mucosa of domestic cats, as well as in the external ear canal of cats with and without otitis, many species of *Malassezia* have been confirmed to exist, in each of these investigations, at least one lipo-dependent species was discovered (Cafarchia et al., 2005; Nardoni et al., 2005). *Staphylococcus*, *Pseudomonas*, *Escherichia*, *Proteus*, *Enterococcus*, and *Corynebacterium* species have all been linked to bacterial otitis externa in cats (Kittl et al., 2018).

Otitis externa is more commonly seen in dogs than in cats (Baxter and Lawler, 1972; Hill et al., 2006; Topala et al., 2007). Headshaking, aural pruritus, pain and discomfort when the ear is palpated, and oedema, fibrosis, or calcification of the ear canal or pinna are typical clinical signs of otitis externa (Jacobson, 2002). Anti-inflammatory drugs, antifungal drugs, and chemotherapeutic agents are necessary for the successful treatment of otitis externa when bacterial and yeast agents are present, depending on the sensitivity of the etiological agents (Harvey et al., 2001).

Animal and public health are seriously threatened by the exceptional issue of antibiotic resistance. Due to the devel-

opment of resistance to the majority of popular antibiotics, antimicrobial-resistant strains of *Pseudomonas* and *Staphylococcus* have emerged as frustrating and challenging causes of otitis (Hariharan et al., 2006; Qekwana et al., 2017). Companion animals, specifically dogs and cats, have frequently been administered broad-spectrum antibiotics, and are considered a potential source of antimicrobial resistance (AMR) due to their close association with humans (Guardabassi et al., 2004; Lloyd, 2007). Studies on otitis externa in cats are scarce, and the multifactorial dermatological condition has not been completely understood (Bollez et al., 2018; Tyler et al., 2020).

The purpose of the present study was to investigate the microbial etiological agents of feline otitis externa and to determine their antimicrobial susceptibility.

Material and Methods

The current investigation was carried out from March 2023 to February 2024. In the Prizren and Ferizaj region, 56 unmedicated cats with clinical signs of otitis externa were assessed at the veterinary clinic, following a comprehensive physical examination and otoscopy. The cats were of different ages and genders. Sterile cotton swabs were used to collect samples from the external ear canal, and conventional microbiological techniques as well as biochemical testing were used to isolate and identify the microorganisms. Antimicrobial susceptibility testing was conducted using Kirby-Bauer's disc diffusion method. In order to isolate potential yeasts, samples were cultivated on Sabouraud dextrose agar plates supplemented with 0.5% cycloheximide and 0.5% chloramphenicol. For bacterial isolation, the samples were cultivated on blood agar containing 5% sheep blood, MacConkey agar, and Mannitol salt agar. Plates were incubated aerobically at 37°C for 24–48 hours. API bacterial identification strips (BioMérieux, Marcy L'Etoile, France) were also used for the identification of isolates.

Antimicrobial susceptibility testing

Bacterial isolates were tested for antimicrobial susceptibility on Mueller–Hinton agar (Oxoid, UK) using the disc diffusion method recommended by Clinical & Laboratory Standards Institute (CLSI, 2019). The following antibiotic discs were tested: penicillin (PEN 10 µg), ampicillin (AMP 10 µg), ciprofloxacin (CIP 5 µg), gentamicin (CN 30 µg), oxytetracycline (OT 3 µg), amikacin (AK 30 µg), doxycycline (DO 30 µg), and amoxicillin/clavulanic acid (AMC 20/10 µg). The bacterial suspension was inoculated onto Mueller – Hinton agar. The antibiotic discs were placed on the medium's surface, and the bacterial plate was incubated aerobically at 37°C for 18 hours. Interpretation was conducted according to the zone size inhibition for each disc according to CLSI standards (CLSI 2019; 2020).

Statistical analysis

In order to determine the significance of age, gender, and season characteristics of the cats, a chi-square test was performed. A P value of < 0.05 was considered significant. The determination of 95% confidence limits was performed.

Results and Discussion

The current study included 56 cats (aged <1 – >5 years), exhibiting symptoms of otitis externa. Microbiologic testing revealed that 21 (87.5%) and 27 (84.4%) of the 24 female and 32 male cats with external otitis were positive. While the frequency of otitis externa cases varied by age (more in cats aged 1–5 years and older than 5 years), the percentage of microbiologically positive samples in relation to the ages of the affected cats by category seems to be slightly higher in cats aged >5 years. The percentages of cats with microbial-induced external otitis were greater in the autumn and spring (Table 1). There were no significant variations in season, sex, or age ($p > 0.05$) (Table 1), and our results were consistent with those of other studies (Topala et al., 2007; Perego et al., 2014). Eight samples (8/56 or 14.28%) of the 56 swab samples that were analyzed showed no microbial growth (Table 2). Among the 48 positive samples, mono-infection was observed in 45 samples (80.36%). *Malassezia pachydermatis* was isolated as a single microbial pathogen with a prevalence of 26.79%

Table 1. Association of variables with presence of otitis externa in cats and positive samples

Data	No.[a]	Pos.[b] (%)	χ^2	df	p-value
Sex			0.1097	1	0.7404
Female	24	21 (87.5)			N.S.D.
Male	32	27 (84.4)			
Age			0.4666	2	0.7919
<1	12	10 (83.3)			N.S.D.
1–5	24	20 (83.3)			
>5	20	18 (90)			
Season			4.3463	3	0.2264
Spring	10	9 (90)			N.S.D.
Summer	8	5 (62.5)			
Autumn	23	21 (91.3)			
Winter	15	13 (86.7)			

No[a] – the number of cats with otitis externa examined

Pos[b] – positive microbial samples (bacteria and yeast) from the examined animals

N.S.D. – No Significant Difference at < 0.05

Source: Authors' own elaboration

(15/56), followed by *Staphylococcus aureus* (25%) (14/56), *Pseudomonas aeruginosa* 12.5% (7/56), *Proteus mirabilis* 10.71% (6/56), and *Staphylococcus pseudintermedius* 5.36% (3/56) among all the samples examined. Three samples showed co-infections between *Pseudomonas aeruginosa* and *Malassezia pachydermatis* (1/56 or 1.79%) and *Malassezia pachydermatis* and *Staphylococcus aureus* (2/56 cases or 3.57%). The frequency of *Malassezia pachydermatis* in the present study was lower than what was found in other studies (Cafarchia et al., 2005; Nardoni et al., 2005; Dizzoti and Coutihno, 2007; Shokri et al., 2010).

The percentage of the total of bacteria involved as positive samples in otitis externa in cats included the value of 58.93% (Table 2). These observations were comparable with the findings of Perego et al. (2014), who reported that bacterial causes were highly prevalent (>70%) in feline otitis externa cases. Our findings were comparable to those of Perego et al. (2014), who reported that bacterial causes were highly prevalent (>70%) in cases of feline otitis externa. Despite being in a smaller percentage, but with a nearly identical bacterial cause, our results were consistent with those of Hiblu et al. (2020), who found that the most common bacterial aetiology in cats with otitis externa was *Staphylococcus* species (75%), followed by *Proteus* species (16.6%) and *Pseudomonas* species (8.4%).

In the current investigation, *S. aureus* (25%) was the most common bacterial species found in cats with otitis externa, followed by *P. aeruginosa* (12.5%), *Proteus mirabilis* (10.71%), and *S. pseudointermedius* (5.36%). In contrast to our results, other authors, like Nardoni et al. (2014), found that *S. pseudintermedius* (6.3%) was the most frequently isolated staphylococcal species from cats with otitis externa. Previous studies (Degi et al., 2012) also reported that of the coagulase-positive staphylococci isolated from otitis externa in cats, *S. pseudintermedius* (43.75%) and *S. aureus* (15.6%) were the most dominant. Li et al. (2021) found that *Staphylococcus aureus* infections were significantly prevalent in both dogs and cats based on otitis specimens.

A total of 124 microbial isolates were recovered from the 48 samples that exhibited microbial growth (Table 3). Amikacin, doxycycline, gentamicin, and ciprofloxacin were more effective against isolates of *Staphylococcus aureus* and *Staphylococcus pseudointermedius* (Table 4).

The results also showed that amoxicillin/clavulanic acid, penicillin, and oxytetracycline were the least effective antibiotics against the bacterial isolates and were in agreement with the findings reported by Hiblu et al. (2020). Unlike our results, the findings reported by Hariharan et al. (2006) showed that the same bacterial isolates from cats with otitis externa showed high susceptibility to penicillin and amoxicillin.

Most of the *P. aeruginosa* isolates of feline origin in the current study were more sensitive to ciprofloxacin, amikacin, doxycycline, and gentamicin. Similarly, Hariharan et

Table 2. Microbial pathogens identified in feline otitis externa samples

Microbial pathogens (genus level)	No. of positive samples	Isolation rate (%)	95% confidence limits
Malassezia pachydermatis	15/56	26.79	18.11÷ 35.47
Malassezia pachydermatis+ Staphylococcus aureus	2/56	3.57	1.3÷8.4
Staphylococcus aureus	14/56	25.00	13.66÷36.34
Pseudomonas aeruginosa	7/56	12.5	3.84÷21.16
Pseudomonas aeruginosa+ Malassezia pachydermatis	1/56	1.79	1.6÷5.2
Proteus mirabilis	6/56	10.71	2.6÷18.8
Staphylococcus pseudointermedius	3/56	5.36	0.5÷11.2
No growth	8/56	14.28	5.1÷23.47
Total	48/56	100.00	

Source: Authors' own elaboration

Table 3. The frequency of microbiological pathogens isolated from cats' otitis externa ear swabs

Microbial pathogens	No. of isolates	Isolation rate (%)	95% confidence limits
M. pachydermatis	42	33.9	25.57÷42.23
Staphylococcus aureus	34	27.4	19.55÷35.25
Pseudomonas aeruginosa	20	16.1	9.63÷22.57
Proteus mirabilis	16	12.9	7.00÷18.80
Staphylococcus pseudointermedius	12	9.7	4.49÷14.91
Total	124	100.00	

Source: Authors' own elaboration

Table 4. Antimicrobial susceptibility rate of bacteria isolated from cats with otitis externa

Antibiotics	*S. aureus* (n = 34) (S)	Confidence limits	*S. pseud-intermedius* (n = 12) (S)	Confidence limits	*P. aeruginosa* (n = 20) (S)	Confidence limits	*P. mirabilis* (n = 16) (S)	Confidence limits
Penicillin PEN	5/34 14.7%	2.80÷26.60	3/12 25%	0.50÷49.50	2/20 10%	3.15÷23.15	0/16 0.0%	–
Ampicillin AMP	10/34 29.4%	14.09÷44.71	3/12 25%	0.50÷49.50	8/20 40%	28.09÷71.91	8/16 50%	25.50÷74.50
Gentamicin CN	20/34 58.8%	42.66÷75.34	8/12 66.7%	39.91÷93.29	16/20 80%	62.47÷97.53	10/16 62.5%	38.78-86.22
Amoxicillin/clavulanic acid AMC	6/34 17.6%	4.80÷30.40	0/12 0.0%	–	1/20 5%	4.55÷14.55	9/16 56.25%	31.94÷80.56
Amikacin AK	28/34 82.3%	69.47÷95.13	9/12 75%	50.50÷99.50	15/20 75%	56.02÷93.98	16/16 100%	–
Oxyetracycline OT	11/34 32.3%	16.58÷48.02	2/12 16.6%	0.44÷37.74	7/20 35%	14.10÷55.90	6/16 37.5%	13.78÷61.22
Doxycycline DO	30/34 94.1%	77.36÷99.04	8/12 66.7%	39.91÷93.29	16/20 80%	62.47÷97.53	9/16 56.25%	31.94÷80.56
Ciprofloxacin CIP	28/34 82.3%	69.47÷95.13	9/12 75%	50.50÷99.50	15/20 75%	56.02÷93.98	12/16 75%	53.78÷96.22

Source: Authors' own elaboration

al. (2006) observed that *P. aeruginosa* isolates were susceptible to both gentamicin and amikacin. *Proteus mirabilis* isolates in the current study were also more sensitive to amikacin and gentamicin and less susceptible to oxytetracycline, but resistant to penicillin. Hiblu et al. (2020) reported similar results, showing that *Proteus* isolates were more susceptible to gentamicin and oxytetracycline, but resistant to penicillin.

Conclusion

In conclusion, we can assume that our findings showed that the yeast *M. pachydermatis* and the bacteria *Staphylococcus aureus*, involved in both monoinfections and coinfections, were the most common microbial agents isolated from feline otitis externa. Finally, the data obtained in the present study from antibiotic susceptibility testing showed that bacterial isolates showed higher susceptibility to amikacin, doxycycline, ciprofloxacin, and gentamicin, so these data may be useful for local clinical veterinarians in treating such infections with adequate antibiotics, as well as the use of antifungals in the case of *M. pachydermatis* involvement.

Conflict of interest

The authors declare that there is no conflict of interest.

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