

Identification and antimicrobial susceptibility of microbial agents of otitis externa in dogs¹⁾

SERPIL KAHYA DEMIRBILEK, ÖZGE YILMAZ

Uludag University, Faculty of Veterinary Medicine, Department of Microbiology, 16.059, Görükle-Bursa, Turkey

Received 18.06.2018

Accepted 05.09.2018

Kahya Demirbilek S., Yılmaz Ö.

Identification and antimicrobial susceptibility of microbial agents of otitis externa in dogs

Summary

In this study, a total of 277 unmedicated dogs with otitis externa were used. Overall, 413 agents were isolated from 277 ear swab samples; 52.7% of the cases were mono-infections (146 cases), and 37.1% of the cases were poly-infections (103 cases). In 10.1% (28) of the cases, neither bacteria nor yeasts were isolated. Coagulase-positive *Staphylococcus* spp. were the most frequently isolated bacteria and were found in 90 (21.8%) of the samples. Fifty-eight samples, (14%) were positive for *Staphylococcus aureus*, 51 (12.3%) for *Pseudomonas aeruginosa*, 27 (6.5%) for *Proteus mirabilis*, 27 (6.5%) for *Malassezia pachydermatis*, 21 (5%) for *Corynebacterium* spp., 21 (5%) for β -haemolytic *Streptococcus* spp., 15 (3.6%) for *Staphylococcus pseudointermedius*, 12 (2.9%) for *Proteus* spp., 12 (2.9%) for *Escherichia coli*, 9 (2.1%) for *Acinetobacter calcoaceticus*, 7 (1.6%) for *Trichophyton mentagrophytes*, 5 (1.2%) for *Staphylococcus auricularis*, and 46 (11.1%) for different bacteria and yeasts. A total of 14 different bacteria and yeasts were isolated and identified. Kirby-Bauer antibiotic susceptibility testing was carried out for 10 different antibiotics. The bacterial isolates were found to be resistant to amoxicillin-clavulanic acid (45%), gentamycin (28%), ampicillin/cloxacillin (69%), tobramycin (28%), amikacin (23%), enrofloxacin (47%), chloramphenicol (58%), doxycycline (65%), lincomycin/spectinomycin (58%) and polymyxin B (62%). In conclusion, it is important to test the antimicrobial sensitivity of aetiological agents of otitis externa before treatment so as to prevent the development of antibiotic resistance in bacteria and yeasts.

Keywords: otitis externa, dogs, bacterial isolation, yeast, antimicrobial susceptibility

Otitis externa (OE) is defined as an acute or chronic inflammation of the epithelium of the external ear (15). The disease is more frequent in dogs than in cats. Although OE is not a life-threatening disease, it can be frustrating for both the patient and the owner, because of the risk of zoonotic infection and antibiotic resistance development (1, 15). The external ear canal is similar in structure to the interfollicular epidermis of the skin. It is a stratified cornifying epithelium with adnexal organs, such as hair follicles and their associated sebaceous and ceruminous glands. Therefore, any disease that affects the skin can also affect the external ear canals. The predisposing factors for OE are ear conformation, excessive moisture, obstructive ear disease, systemic disease and treatment effects (11).

The most recent classification for the aetiology of otitis externa has been proposed by Griffin (4). In this system, the aetiology of otitis externa can be divided into primary and secondary causes, which are,

respectively, diseases or infections that directly cause inflammation in the ear, and perpetuating or predisposing factors that contribute to ear disease. The latter do not cause ear disease in their own right, but may prevent resolution of the disease and lead to recurrence if they are not dealt with adequately. Significant primary causes of otitis externa are allergy, endocrine dysfunction, autoimmune/immune-mediated disorders, keratinisation disorders, ectoparasites, foreign bodies and idiopathic causes. Significant secondary causes of otitis externa are acute bacterial disease, chronic bacterial disease and yeast infection (11).

A number of predisposing factors have been identified, such as high humidity, foreign bodies in the ear canal, obstructive diseases, immunosuppression, hair in the ear canal, hair on the concave ear surface, and pendulous ears. Other causes include progressive pathological alterations and opportunistic infection by the normal ear canal microflora, which consists of coagulase-positive and coagulase-negative *Staphylococcus* spp., *Proteus mirabilis* (*P. mirabilis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*),

¹⁾ The article is part of the research project (V-2017/36) entitled "Identification of bacteria from otitis externa in dogs" supported by the Scientific Research Fund of Uludag University.

etc. It should be remembered, however, that bacteria and yeasts are opportunists and not primary pathogens that cause only otitis externa, and they can find favourable conditions for growth provided that another cause is present (13). The condition may progress to otitis media, which serves as a source of infectious agents, resulting in chronic hyperplastic changes and obstruction of the ear canal (6).

It is well known that microbial drug resistance is a growing global problem. In developing countries, this problem is particularly acute due to the misuse of antibiotics and inadequate antibiotics. Antibiotics are used to treat infections, but most organisms acquire resistance. Reports of the continuous emergence of resistant pathogens and the changing pattern of antibiotic effectiveness in the treatment of OE necessitated this study. Therefore, antibiotic susceptibility tests were performed to evaluate treatments for chronic OE.

The aim of this study was to provide updated information on the bacterial and fungal flora involved in canine otitis externa and on the antimicrobial susceptibility patterns of some antibiotics that are most commonly used for the treatment of this disease.

Material and methods

Samples. A total of 277 dogs with otitis externa were used in this study. These dogs did not get antibiotic treatment before the ear swabs were obtained. The ear swabs were collected from the horizontal portion of the external ear canal with swabs that were placed in Stuart's transport medium and sent to the laboratory of the Microbiology Department at the Faculty of Veterinary Medicine at Uludag University within 18 hours of collection.

Bacteriological examinations. Ear swab samples were inoculated onto sheep blood agar (Columbia agar with 5% sheep blood) and MacConkey agar (Oxoid, cat. no. CM0007R, UK) plates. Mycological tests included aerobic cultivation of the samples on Sabouraud Dextrose Agar (SDA) (Oxoid, cat. no. CM0041B, UK) for 4-14 days at 25°C and 37°C. Plates were incubated at 37°C under aerobic conditions and examined daily for 5 days for the presence of bacterial growth. The isolated bacteria were identified on the basis of their cultural, morphological, and biochemical characteristics (14). A species was regarded as significant in causing the condition if there was a substantial growth of the species in

a mixed culture or a moderate growth of the species in a pure culture (Clinical and Laboratory Standards Institute (CLSI) (3). Fungal species were identified by various methods, such as assessment of colony morphology, texture and surface pigmentation, microscopic examination and culture (on chrome agar). Gram staining was performed for the identification of yeasts and yeast-like fungi.

Antibiotic sensitivity test. The response of the isolates to antimicrobial drugs was tested by the disk agar diffusion method according to the CLSI guidelines (3). The 10 antimicrobial agents tested and their respective concentrations were as follows: amoxicillin-clavulanic acid (30 µg), gentamicin (120 µg), ampicillin/cloxacillin (25 µg), tobramycin (10 µg), amikacin (30 µg), enrofloxacin (5 µg), chloramphenicol (30 µg), doxycycline (30 µg), lincomycin/spectinomycin (9/100 µg), and polymyxin B (10 µg). The reference bacterial strains *E. coli* (ATCC 25922) and *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) were used as quality control strains according to the CLSI recommendations.

Results and discussion

Bacteriological examination. The distribution of the microbial agents involved in co-infections (100 cases) in dogs with bacterial otitis externa by species is summarized in Table 1. A total of 413 agents were isolated from 277 ear swab samples; 52.7% of the cases were mono-infections (146 cases), and 37.1% of the cases were poly-infections (103 cases). In 10.1% (28) of the cases, neither bacteria nor yeasts were isolated. At least two species were isolated from 103 samples: 76 samples had 2 microbial species, 24 had 3 species, and 3 had 5 species (Tab. 1). The most frequent microbial species involved in poly-infections were coagulase-positive *Staphylococcus* spp., β -haemolytic *Streptococcus* spp. (16/277, 5.7%),

Tab. 1. Distribution of microbial agents by species involved in co-infections (100 cases) in dogs with bacterial otitis externa

	Bacterial/yeast associations	Number and frequency (%)
2 Species		
Coagulase-positive <i>Staphylococcus</i> spp.	β -haemolytic <i>Streptococcus</i> spp.	16 (5.7%)
	<i>Pseudomonas aeruginosa</i>	13 (4.6%)
	<i>Escherichia coli</i>	9 (3.2%)
	<i>Corynebacterium</i> spp.	7 (2.5%)
<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	7 (2.5%)
β -haemolytic <i>Streptococcus</i> spp.	β -haemolytic <i>Streptococcus</i> spp.	12 (4.3%)
	<i>Escherichia coli</i>	9 (3.2%)
	<i>Corynebacterium</i> spp.	6 (2.1%)
<i>Acinetobacter calcoaceticus</i>	<i>Escherichia coli</i>	5 (1.8%)
	<i>Proteus mirabilis</i>	4 (1.4%)
3 species		
Coagulase positive <i>Staphylococcus</i> spp.	<i>Proteus mirabilis</i> / <i>Escherichia coli</i>	4 (1.4%)
	β -haemolytic <i>Streptococcus</i> spp./ <i>Pseudomonas aeruginosa</i>	3 (1%)
β -haemolytic <i>Streptococcus</i> spp.	<i>Corynebacterium</i> spp./ <i>Acinetobacter calcoaceticus</i>	3 (1%)
<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i> / β -haemolytic <i>Streptococcus</i> spp.	2 (0.7%)

P. aeruginosa (13/277, 4.6%), *E. coli* (9/277, 3.2%) and *Corynebacterium* spp. (7/277, 2.5%). Other species involved in co-infections are listed in Table 1. Although 3 samples were positive for 5 microbial species, these 3 samples were most probably contaminated with normal microflora or environmental microorganisms.

Coagulase-positive *Staphylococcus* spp. were the most frequently isolated bacteria, found in 90 (21.8%) of the samples. Fifty-eight samples (14%) were positive for *S. aureus*, 51 (12.3%) for *P. aeruginosa*, 27 (6.5%) for *P. mirabilis*, 27 (6.5%) for *Malassezia pachydermatis* (*M. pachydermatis*), 21 (5%) for *Corynebacterium* spp., 21 (5%) for β -haemolytic *Streptococcus* spp., 15 (3.6%) for *Staphylococcus pseudointermedius*, 12 (2.9%) for *Proteus* spp., 12 (2.9%) for *E. coli*, 9 (2.1%) for *Acinetobacter calcoaceticus* (*A. calcoaceticus*), 7 (1.6%) for *Trichophyton mentagrophytes*, 5 (1.2%) for *Staphylococcus auricularis*, and 46 (11.1%) for different bacteria and yeasts. On top of all this, we isolated and identified 5 *Flavobacterium* spp., 5 *Mucor* spp., 4 *Alcaligenes* spp., 4 *Enterobacter* spp., 4 *Microsporium canis*, 4 *Penicillium* spp., 3 *Pseudomonas fluorescens*, 3 *Branhamella* spp., 3 *Providencia stuartii*, 3 *Penicillium* spp., 2 *Corynebacterium urealyticum*, 2 *Neisseria* spp., 2 *Enterococcus* spp. and 2 *Candida* spp.

These findings were similar to those in other parts of the world, where 21.8% of all microbial isolates belonged to the coagulase-positive staphylococci (6, 7, 10, 12, 13, 15, 18). The presence of *Pseudomonas* spp. and *Corynebacterium* spp. in dogs often indicates a chronic disease. Yeasts are commensal flora in the external ear canal of dogs that are predisposed to initial opportunistic infection by damp environmental conditions and frequent antimicrobial therapy. For example; *M. pachydermatis* is a common fungal pathogen of otitis externa in dogs; this fungus is also commensal in the ear. As is common with other types of skin infection, ear infections usually occur secondary to an underlying cause. These underlying factors can be divided into 3 categories. In reality, bacteria are secondary causes of otitis externa because infection with either yeasts or bacteria does not occur in a normal ear.

Among 257 ear swab samples from Bulgarian dogs with otitis externa, bacterial and yeast species were isolated in 93.77% of cases; the

mono-infections (109 cases) involved mainly coagulase-positive staphylococci (60 strains), *M. pachydermatis* (97 strains) and *P. aeruginosa* (42 strains), and these organisms were found even more often in poly-infections (132 cases) (13). A total of 234 unmedicated dogs with otitis externa were used. A total of 18 different microbial genera (eleven bacterial genera and seven fungal genera) were identified. Two microorganisms were isolated in 26 cases, and in 84 samples, three or more agents were isolated. In addition, no bacterial and fungal growth was seen in 48 samples. Sarierler et al. (17) studied 81 clinical cases of otitis externa and isolated a *Staphylococcus* sp. (48.6%), a *Pseudomonas* sp. (21.5%), a *Proteus* sp. (14.95%), *E. coli* (8.41%) and a *Klebsiella* sp. (6.54%). Mande et al. (6) examined 78 dogs with clinical signs of otitis externa, and the microbial isolates recovered were *S. aureus* (40/78, 51.3%), *Streptococcus* and *Pseudomonas* spp. (11/78, 14.1%), *Proteus* spp. (8/78, 10.2%), *E. coli* (2/78, 2.6%), *Corynebacterium* spp. (1/78, 1.3%) and yeasts (3/78, 3.8%), which were infrequently isolated by Murugan et al. (8). Öztürk et al. (10) studied 58 ear swab samples from 41 dogs with otitis externa. As far as mycotic infections are concerned, Kamaljiyoti et al. (5) evaluated 115 dogs with history and clinical signs of otitis externa and found 22 infections with *Malassezia*, 4 with *Aspergillus*, 3 with *Candida* and 1 with *Cryptococcus*. Nardoni et al. (9) collected auricular cerumen from 115 cats and 203 dogs and isolated *M. pachydermatis* in pure culture from 28.3% of cats and from 87.1% of dogs.

Antibiotic sensitivity test: The response of the bacterial isolates to antimicrobial drugs is summarized in Table 2. The isolates were resistant to amoxicillin-clavulanic acid (45%), gentamycin (28%), ampicillin/

Tab. 2. Antibiotic resistance (%) of microbial agents isolated from dogs with bacterial otitis externa

Antibiotics	Coagulase-positive <i>Staphylococcus</i> spp. (n = 90) %	<i>Pseudomonas</i> <i>aeruginosa</i> (n = 51) %	<i>Proteus mirabilis</i> (n = 27) %	<i>Corynebacterium</i> spp. (n = 21) %	β -haemolytic <i>Streptococcus</i> spp. (n = 21) %	<i>Proteus</i> spp. (n = 12) %	<i>Escherichia coli</i> (n = 12) %	<i>Acinetobacter</i> <i>calcoaceticus</i> (n = 9) %	Total
Amoxicillin-clavulanic acid	5	98	28	97	19	38	25	52	45
Gentamycin	22	1	23	50	17	26	26	56	28
Ampicillin/cloxacillin	13	96	100	97	19	100	88	43	69
Tobramycin	23	23	2	65	33	7	4	67	28
Amikacin	11	3	0	76	23	5	9	56	23
Enrofloxacin	27	35	17	100	87	19	23	67	47
Chloramphenicol	37	100	68	97	1	78	7	78	58
Doxycycline	39	100	85	86	34	77	15	86	65
Lincomycin/spectinomycin	63	36	66	89	46	67	8	93	58
Polymyxin B	61	1	65	94	97	87	1	89	62
Total	241	493	454	851	376	504	206	687	

cloxacillin (69%), tobramycin (28%), amikacin (23%), enrofloxacin (47%), chloramphenicol (58%), doxycycline (65%), lincomycin/spectinomycin (58%) and polymyxin B (62%). The analysis of the strain response to antibiotics showed a preserved high resistance to tetracyclines (65%), penicillins (57%), quinolones (47%), lincosamides (58%) and polypeptides (62%).

Development of antibiotic resistance is of great concern. Since the analysis of the strain response to antibiotics showed a preserved resistance to tetracyclines, penicillins and quinolones, these drugs may not be effective in treating bacterial otitis externa in dogs. Additionally, a high sensitivity to aminoglycosides was seen.

The susceptibility profiles of the bacteria provide evidence for bacterial resistance to many antimicrobial agents in the form of multiple drug resistance (MDR). This can be clearly seen from the susceptibility patterns of *Corynebacterium* spp. and *A. calcoaceticus*, as well as other bacteria, in Table 2. The results of our study showed the following average resistance values for bacteria: *Corynebacterium* spp. (77.3%), *A. calcoaceticus* (62.4%), *Proteus* spp. (45.8%), *P. aeruginosa* (44.8%), *P. mirabilis* (41.2%), β -haemolytic *Streptococcus* spp. (34.1%), coagulase-positive *Staphylococcus* spp. (21.9%), and *E. coli* (18.7%). These values differ from those obtained in other studies (13, 15, 16), and this difference in the susceptibility profiles might be due to the different frequency of use of these agents in different geographic locations for the treatment of ear infections.

In conclusion, the isolation and identification of microorganisms are important for the correct diagnosis and correct treatment of otitis externa in dogs. The bacterial agents most commonly isolated from canine otitis externa were coagulase-positive staphylococci. It should be remembered, however, that yeasts are also frequently involved in the aetiology of ear canal inflammations, and further research is required to determine the importance of yeasts in the pathogenesis of otitis externa. All data from this study showed that further research is required to determine the importance of antibiotics in the treatment of otitis externa in dogs. These results highlight the need to combine antimicrobial susceptibility tests with bacterial isolation. In addition, susceptibility data would be useful in determining the most suitable approaches/strategies for medical management.

References

1. August J. R.: Otitis externa. A disease of multifactorial etiology. Vet. Clin. North Am. Small Anim. Pract. 1988, 18, 731-742.
2. Borum A. E., Çeçen G., Demir G., Çetin C., Şentürk S.: Determination and antimicrobial susceptibility of microorganisms isolated from otitis externa with dogs. Kocatepe Vet. Derg. 2014, 7, 27-31.
3. CLSI (Clinical Laboratory Standard Institute): National committee for clinical and laboratory standards and methods for antimicrobial susceptibility testing of anaerobic bacteria, Approved standard. 6th edition, NCCLS, Wayne, PA, Document M11-A6, 2004.
4. Griffin C. E.: Classifying cases of otitis externa the PPSP System. Pro. ESVD Workshop on Otitis. St Helens 2010.
5. Kamaljiyoti A. K. S., Sood N. K., Sharma S., Folia G.: Epidemiology and diagnosis of mycotic infections in canine otitis externa. Intas Polivet 2016, 17, 302-303.
6. Mande J. D., Kitaa J. M. A.: Microbial profile and antimicrobial susceptibility of isolates from dogs with otitis externa in Kenya. J. Kenya Vet. Assoc. 2005, 29, 40-44.
7. Moraes L. A., Pereira J. M. M., Silva S. P., Moreira V. M. T., Casseb A.: Microbiological diagnosis and bacterial multiresistance in vitro of external otitis externa of dogs. Veterinaria Zootecnia 2014, 21, 98-101.
8. Murugan M. S., Parthiban S., Malmarugan S., Rajeshwar J. J.: Antibigram and therapeutic management of bacterial otitis externa: A clinical study of 81 dogs. Intas Polivet 2016, 17, 292-294.
9. Nardoni S., Ebani V. V., Fratini F., Mannella R., Pinferi G., Mancianti F., Finotello R., Perrucci S.: Malessezia, mites and bacteria in the external ear canal of dogs and cats with otitis externa. Slov. Vet. Res. 2014, 51, 113-118.
10. Öztürk D., Pehlivanoglu F., Türütoglu H., Şirin Y. S., Şababoğlu E.: Otitis externalı köpeklerden izole edilen mikroorganizmalar ve antibiyotik duyarlılıkları. Euro. J. Vet. Sci. 2016, 32, 84-88.
11. Paterson S.: Discovering the causes of otitis externa. In Practice, Focus 2016. <http://dx.doi.org/10.1136/inp.i470>.
12. Perry L. R., MacLennan B., Korven R., Rawlings T. A.: Epidemiological study of dogs with otitis externa in Cape Breton, Nova Scotia. Can. Vet. J. 58, 168-174.
13. Petrov V., Mihaylov G., Tsachev I., Zhelev G., Marutsov P., Koev K.: Otitis externa in dogs: microbiology and antimicrobial susceptibility. Rev. Med. Vet. 2013, 164, 18-22.
14. Quinn P. J., Carter M. E., Markey B.: Section: 2 Bacteriology, [in:] Clinical Veterinary Microbiology. Harcourt Publishers Limited, USA 1999, p. 118-254.
15. Rosser E. J.: Causes of otitis externa. Vet. Clinics Small Animal. 2004, 34, 459-468.
16. Rosychuk R. A. W.: Management of otitis externa. Vet. Clin. North Am. Small Anim. Pract. 1994, 24, 921-952.
17. Sarierler M., Kirkan Ş.: Microbial diagnosis and therapy of canine otitis externa. Veteriner Cerrahi Dergisi 2004, 10, 11-15.
18. Selvakumar S., Mohamed A. M. G., Potheappan P.: A clinical study of otitis externa with bacterial isolation, antimicrobial susceptibility and therapeutic management in 44 dogs. Intas Polivet 16, 332-333.
19. Scott D. W., Miller W. H., Griffin C. E.: Otitis, [in:] Muller and Kirk's Small Animal Dermatology. Saunders, Philadelphia 2001, p. 143-148.

Corresponding author: Assoc. Prof. Serpil Kahya Demirbilek, Uludag University, Faculty of Veterinary Medicine, Department of Microbiology, 16.000, Görükle-Bursa, Turkey; e-mail: serpilkahya@uludag.edu.tr