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Polish Journal of Veterinary Sciences Vol. 26, No. 3 (2023), 449–459

DOI 10.24425/pjvs.2023.145052

Original article

Prevalence of the microbiological causes of canine otitis externa and the antibiotic susceptibility of the isolated bacterial strains

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Abstract

Otitis externa is a canine disease of multifactorial etiology in which bacteria plays a significant role. Due to the predominant bacterial etiology otitis is usually treated with antibiotics. However, non-prudent use of antibiotics promotes the emergence of antibiotic-resistant bacteria thus compromising the therapy effectiveness. Currently, the increase of antimicrobial resistance (AMR) is one of the biggest threats to global health. For this reason, the aim of the study was to investigate prevalence of the microbiological causes of canine otitis externa and the antibiotic susceptibility of the isolated bacterial strains. The research and sampling were conducted at Veterinary Clinics for small pets in Serbia. Samples were sent to laboratory for bacteriological and mycological testing. Additionally, the sensitivity of the isolated bacteria to antibiotics was evaluated using disc diffusion method. Sixty dogs with otitis externa clinical symptoms were included in the study. Out of a total of 53 positive samples for pathogen presence, bacteria were present in 40. The most prevalent bacteria was *Staphylococcus pseudintermedius,* followed by *Pseudomonas aeruginosa* and *Proteus* spp., while *Malassezia pachydermatis* was the only isolated yeast pathogen occurring in 36 samples. Generally, the lowest resistance against all bacteria showed enrofloxacin. On the contrary, high resistance to penicillin and amoxicillin was a common finding for G+ and G- bacteria. These results indicate the need for laboratory testing in terms of isolation, identification and antibiotic susceptibility testing, not only in the case of otitis externa in dogs, but in all diseases when it is possible, in order to enhance antimicrobial stewardship and consequently to contribute AMR reduction.

Keywords: otitis externa, bacteria, antimicrobial resistance, dog

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Introduction

Antimicrobial resistance (AMR) is one of the major health treats since it is estimated that until 2050 deaths attributable to AMR will reach 10 million people on annual basis (O'Neill 2014). Moreover, recent data from 2019 have shown that deaths of 1.27 million people were directly ascribable to AMR, as well as that the 4.95 million deaths were related to drug-resistant bacterial infections (Lancet 2022, Laxminarayan 2022).

As a serious global concern nowadays in human, as well as in veterinary medicine, AMR problem could be provoked by excessive antimicrobial use (AMU) and prolonged treatments (Silva et al. 2013, Jasovský et al. 2016). Furthermore, AMR bacteria can be transmitted from animals to humans through direct or indirect contact with contaminated food, and their shared environment (Dierikx et al. 2013, Silva et al. 2013). Interestingly, the possibility of AMRtransmission from companion animals to humans has been the subject of recent research, where dogs and cats could be both, reservoirs and transmission vectors (EMA/CVMP 2015, Marques et al. 2016, Walther et al. 2017, Kaspar et al. 2018, Hong et al. 2019, Gwenzi et al. 2021, Glavind et al. 2022). Furthermore, microbiological hazards of concern may directly or indirectly cause adverse health effects in humans. In addition, direct hazards for human health are defined as AMR bacteria that are transmitted from animals to humans and cause disease in humans (zoonoses), while indirect hazards are the resistance genes that may be transmitted from companion animals to humans with consequences for public health (Pomba et al. 2017).

Based on the global nature of AMR and considering that AMR does not recognize barriers, solving this problem is urgent. Nowadays, implementation of antimicrobial stewardship (AMS) principles trough the One Health concept represents one of the possible solutions in both, human and veterinary medicine (Hernandez-Santiago et al. 2019, WHO 2019, Hubbuch et al. 2020, Pinto Ferreira et al. 2022). Although the antimicrobial susceptibility testing is recommended as a part of AMS programs, doctors and veterinarians sometimes use antibiotics on their own, without prior analysis (Sahoo et al. 2010), while the reason usually could be that owners explicitly requested antibiotics from a veterinarian in order to obtain fast results (Scarborough et al. 2021).

As a frequent disease with bacteriological etiology, otitis externa involves inflammation of the external ear canal (Rosser 2004), being very common in dogs, with prevalence rate from 5 to 20% (Fernández et al. 2006, Lyskova et al. 2007, Terziev and Urumova 2018). Furthermore, as a highly prevalent multifactorial skin disease, it represents up to 20% of small-animal counseling cases (August 1988). The most common clinical symptoms of otitis externa are inflammation, ear scratching, head shaking, pain and ear discharge (Terziev and Urumova 2018), while prolonged disease could cause symptoms such as offensive smell, oedema, erythema and pruritus (De Martino et al. 2016). In addition, factors that can cause otitis can be divided into predisposing (increase the risk of otitis) and perpetuating (from inflammation and pathology in ear, prevent resolution of otitis) (Scott et al. 2001, Jacobson et al. 2002). Moreover, factors that induce otitis are known as primary, while secondary factors contribute to otitis only in an abnormal ear or in conjunction with predisposing factors. Besides, among secondary causing factors the most important are bacteria and yeasts (Saridomichelakis et al. 2007). Furthermore, regarding to the otitis externa bacteria causing agents in dogs, the most frequent species are *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, and *Proteus* spp. (Zamankhan Malayeri et al. 2010). The major role in otitis externa among Gram-negative bacteria plays *Pseudomonas aeruginosa*, especially because of the increasing number of multiresistant strains (Penna et al. 2011). Besides, *Staphylococcus pseudintermedius* and the other coagulase-positive staphylococcal species are predominant among Gram-positive bacteria (Rubin and Chirino-Trejo 2011, Petrov et al. 2013).

The major role in the treatment of all infectious bacterial diseases in the companion animals (in otitis externa also) is attributed to antimicrobial drugs (Morris 2004, Paterson 2016, Nuttall 2016). Indiscriminate and non-prudent AMU in the treatment of this disease could cause loss of efficacy of antimicrobial substances and consequently cause AMR (Mateus et al. 2011). Furthermore, this can seriously compromise animal health and welfare and consequently people health.

Hence, the aim of the present study was to determine the prevalence of the microbiological causes of canine otitis externa and the antibiotic susceptibility patterns of the isolated strains at the Veterinary Clinics for Small animals at the Department of Veterinary Medicine, University of Novi Sad in Serbia.

Materials and Methods

Collecting of the samples

The study was conducted in the time period from October 2020 to October 2022 at Veterinary Clinic for Small Animals at the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia. From a total of 60 dogs of different breeds, sex and age that showed clinical symptoms of the otitis

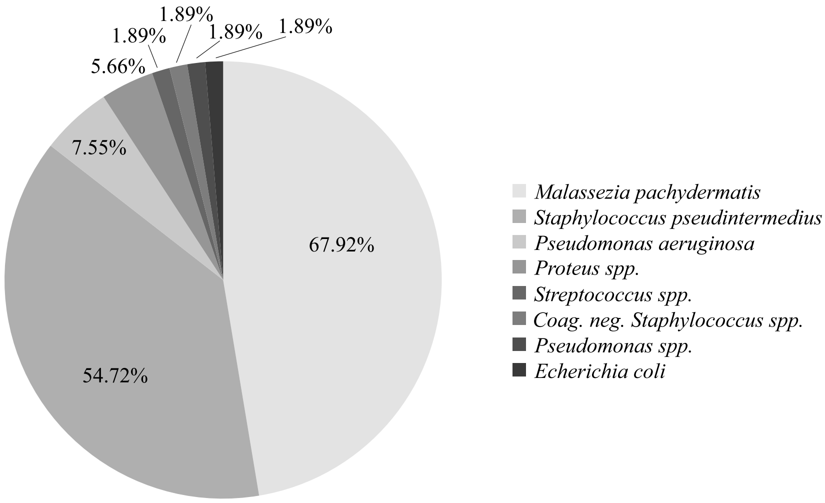

Fig. 1. The incidence of otitis externa causing pathogens in dogs from collected positive samples.

externa, samples were taken for further bacteriological and mycological examination. Clinical symptoms of the otitis externa were determined by the clinical and otoscopic examination of the dogs and were present in minimum one ear. Furthermore, the samples were taken from the external ear canal using sterile cotton swabs and transferred to tube with transport media (Eurotubo®, Deltalab, Spain). Afterwards, the collected samples were maintained at 4°C and transported on the same day to the Veterinary laboratory "Vetlab" Belgrade, Serbia for further testing.

Isolation and identification of pathogens

Isolation of the yeast was carried out by a conventional method under aerobic conditions (SDA agar, Promedia, Serbia). To confirm the identification and determine the concentration of *Malassezia pachydermatis* in the case of otitis externa microscopic method was used. Additionally, on the cytological preparation based on the average number of yeast per one epithelial cell, the concentration was evaluated.

The bacteria were isolated by conventional microbiological method under aerobic conditions (Columbia agar, Biomerieux, France), whereas commercial biochemical tests were used for identification.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were done using disc diffusion method based on the European Society of Clinical Microbiology and Infectious Diseases (EUCAST) guidelines.

Data analysis

The obtained results were summarized by application of Microsoft Office Excel (v2019) and further statistically analyzed by TibcoStatistica (v13.5.). Regarding the generally nominal nature of data, they were represented as frequencies of occurrence, while for comparison of frequency distributions among different groups and comparisons including the age of evaluated animals X^2 and Mann Whitney U test were used, respectively. The differences were considered significant if $p<0.05$. Furthermore, in order to better evaluate the obtained dataset patterns of variability multivariate correspondence analysis was applied. The specified analysis is a dimensional reduction technique which represents the original dataset by a lower number of dimensions called correspondent axes which are mutually orthogonal. This results in reduction of dataset variability (which is in case of this analysis application termed inertia), but, on the other hand, simplification of grouping patterns of recorded data.

Results

Bacteriological testing

A total of 60 collected samples were examined for the presence of bacteria and yeasts. Based on the laboratory results, 53 (88.33%) of these samples were positive for bacteria and/or yeast presence, while in the remaining 7 (11.67%) samples the causative agents were not detected. Actually, they could be present as "viable but nonculturable" (VBNC) state where cells have been defined as cells which, induced by some stress, become nonculturable on media that would normally support their growth but which can be demonstrated by various methods to be alive and capable of returning to a metabolically active and culturable state (Ayrapetyan et al. 2018). Among the positive samples, 76 microbial agents were isolated and their frequency is shown in Fig. 1. Besides, *Malassezia*

Table 1. The incidence of antibiotic resistance of isolated bacteria strains.

Antibiotics	*S. pseudintermedius* (%)	Total number of G+ bacteria (%)	*P. aeruginosa* (%)	*Proteus* spp. (%)	Total number of G- bacteria (%)
Amoxicillin + clavulanic acid	10	13	100	0	56
Amikacin	NT	NT	50	33	33
Amoxicillin	69	77	100	67	89
Gentamicin	31	32	25	0	11
Neomycin	24	26	0	33	11
Penicillin	76	74	100	67	89
Sulfamethoxazole-Trimethoprim	41	45	100	33	67
Ceftriaxone	14	19	25	0	22
Ciprofloxacin	21	19	25	0	11
Enrofloxacin	14	13	25	0	11
Tobramycin	52	52	25	33	22
Doxycycline	52	52	75	100	89
Clindamycin	66	65	NT	NT	NT
Azithromycin	62	61	75	100	89
Tetracycline	52	52	100	100	100
Erythromycin	55	55	NT	NT	NT
Levofloxacin	24	29	100	0	56
Ampicillin	NT	NT	100	67	89
Vancomycin	31	29	100	100	100
Colistin	NT	NT	75	67	78
Pradofloxacin	24	23	25	0	22
Cefoxitin	3	6	100	0	56
Nitrofurantoin	45	45	100	67	89
Rifampicin	NT	NT	100	67	78
Cefquinome	31	35	100	67	78
Marbofloxacin	21	19	25	33	33
Lincomycin	62	61	NT	NT	NT
Fusidic acid	28	32	NT	NT	NT

* NT – not tested

pachydermatis was the only isolated yeast pathogen, and occurred in 36 (67.92%) samples. Furthermore, it was the most frequent isolated pathogen (with incidence of 47.37%) among all pathogens recognized as causative agents of otitis externa. Bacteria were isolated from 40 samples (75.47%), whereas 31 isolates contained Gram positive bacteria. Specifically, *Staphylococcus pseudintermedius* was the most common bacterial pathogen found in 54.72% (29 cases) of samples, representing 38.16% of the total number of isolated pathogens. Furthermore, other bacterial agents were represented by significantly lower number of cases. Actually, *Pseudomonas aeruginosa* and *Proteus* spp. were present in 4 (7.55%) and 3 (5.66%) cases, respectively, while *Echerichia coli*, coagulase-negative *Staphylococcus* spp., *Streptococcus* spp. and other species of the genus *Pseudomonas* were present in only 1 (1.89%) case, each.

Antibiotic susceptibility testing of otitis externa associated bacteria

According to the results of antimicrobial susceptibility testing of the isolated bacteria strains (Table 1). *S. pseudintermedius* was the most resistant to the penicillin (76%) and amoxicillin (69%). In addition, high resistance to clindamycin (66%), as well as lincomycin and azithromycin, both represented by 62%, has also been shown. On contrary, these bacteria have shown low resistance against cefoxitin (3%), amoxicillin + clavulanic acid (10%), ceftriaxone (14%) and enrofloxacin (14%). The percentage of G- bacteria resistant to antimicrobial drugs was higher than to G+ bacteria. Moreover, *P. aeruginosa* manifested the greatest resistance against combination of amoxicillin and clavulanic acid, amoxicillin, penicillin, sulfamethoxazole-trimethoprim, tetracycline, levofloxacin, ampicillin, vancomycin, cefoxitin, nitrofurantoin, rifampicin and cefquinome (100%), while neomycin was the only antibiotic without recorded resistance (0%). *Proteus* spp. have shown 100% resistance to doxycycline, tetracycline, azithromycin and vancomycin, while the most active antimicrobial agents against these bacteria were combination of amoxicillin and clavulanic acid, gentamicin, ceftriaxone, ciprofloxacin, enrofloxacin, levofloxacin, pradofloxacin and cefoxitin.

Overall, all G- isolated bacteria strains showed full resistance against tetracycline and vancomycin (100%), followed by amoxicillin, penicillin, doxycycline, azithromycin, ampicillin and nitrofurantoin (89%), while the lowest observed resistance was against gentamicin, neomycin, enrofloxacin and ciprofloxacin (11%). Generally, the highest susceptibility of G+ and G- bacteria was recorded in case of enrofloxacin, whereas the lowest was against penicillin and amoxicillin.

Out of a total number of dogs diagnosed with bacterial otitis externa the most frequent breed types were Maltese and Mixed breed, both represented by 5 (12.5%) cases, followed by Bichon Frise with 4 cases (10%), as well as Labrador Retriever, German Shepherd Dog and Pug with 3 cases each (7,5%) (Table 2). In relation to the ear form, from pendulous eared dog's bacteria were present in 26 number of samples (65%), while 14 (35%) were isolated from dogs with erect ears. As for gender of the dogs, 25 (62.5%) cases were males, whereas 15 (37.5%) were females. Dogs up to one year of age had 7 (17.5%) positive samples, from two to five years had 21 (52.5%), six to nine had 4 (10%) and dogs older than 10 years had 8 positive samples (20%). Mixed infection with yeast was present in 23 cases (57.5%), while bacteria-only infection was found in 17 (42.5%) cases. However, the age of animals did not statistically affect the presence of yeast co-infection ($U=168$, $z=0.74$, $p=0.46$). Regarding to the season of the year the largest number of cases was recorded in spring (14 cases, 35%) and autumn (13 cases, 32.5%), followed by summer (9 cases, 22.5%) and winter with only 4 (10%) cases.

The application of multivariate correspondence analysis on dataset describing the breed, gender, type of ears (pendulous or erect), presence of mixed infection with yeast, causative agent of bacterial infections and season of year when the clinical condition of otitis externa occured shows that the first two correspondent axes (CA1 and CA2) describe around 17% of samples variability (inertia). The position of the evaluated variables in the space defined by the first two correspondent axes (Fig. 2) indicates that during winter season (W) *Proteus* spp. and *E. coli* were the main pathogens causing otitis externa in Labrador Retriever and Akita breeds (positive part of CA1 and CA2). Furthermore, during autumn season (A) the infections caused by *Streptococcus* spp. and *Pseudomonas* spp. are usually not accompanied by yeast infection. On the other side the grouping in the positive part of CA1 and negative part of CA2 indicates that *Pseudomonas aeruginosa* infections occur mostly in Bichon Frise, Shih Tzu and Mixed breeds with pendulous ears. The negative side of CA1 correlates with the otitis externa cases reported in summer (SU). Especially in the space defined by the negative part of CA1 and CA2 can be observed that *S. pseudintermedius* infections are usually accompanied by yeast infection in Golden Retriever, Chow chow, Dogo Argentino, Staffordshire Bull Terrier, West Highland White Terrier, Boston Terrier and Dachshund breeds.

Discussion

Prevalence of the microbiological causes of canine otitis externa and the antibiotic susceptibility of the isolated strains were the main subject of many studies (Lyskova et al. 2007, Zamankhan Malayeri et al. 2010, Bugden 2012, Petrov et al. 2013), while in Serbia no one has focused on this issue yet. Insight into these data could help to mitigate AMR in companion animals, since this represents huge problem nowadays (Guardabassi 2004, So et al. 2012, Schmiedel et al. 2014).

Our research results have shown that among isolated bacteriological otitis externa agents, the most common was *Staphylococcus pseudintermedius* with incidence of 54.72%. This is similar to the results of Lyskova et al. (2007) and Forster et al. (2018) where these bacteria were present in 58.8% and 46% of cases, respectively. On the contrary, our results show higher values than those of Kiss et al. (1997) who isolated *S. pseudintermedius* in 39.22%, as well as from Bornard (1992) where these bacteria were present in only 23% of canine otitis externa cases. However, Lilenbaum et al. (2000) got unexpected results. They confirmed that *Staphylococcus* spp. are the most frequent pathogens isolated in otitis externa of dogs, but in their study the most prevalent were coagulase-negative species, representing 27 of the 44 isolates, whereas *S. pseudintermedius* was present in only 6 samples. Different research results regarding the presence of these bacteria could be provoked with origin of data influenced by the geographical area where the study was conducted.

Table 2. Occurrence of bacterial pathogen in dogs with diagnosed otitis externa in relation to the breed, ear type, gender, age, type of infection and year season.

Dog breed	Type of the ear	Gender	Age	Mixed infection with yeast	Type of the bacteria	Season of the year
POM	E	M	5m	no	*S. pseudintermedius*	A
MLT	P	F	3y	yes	*S. pseudintermedius*	SP
SHIH	P	F	2 y	no	*S. pseudintermedius*	A
MIXED	P	F	4y	yes	*S. pseudintermedius*	A
AK	E	M	12y	no	*S. pseudintermedius*	W
MIXED	P	F	2y	yes	*S. pseudintermedius*	SP
MLT	P	F	1y	yes	*S. pseudintermedius*	SP
PUG	P	F	1y	yes	*S. pseudintermedius*	SP
BF	P	M	11y	no	*Streptococcus spp.*	A
CHOW	E	M	5y	no	*S. pseudintermedius*	SP
BT	E	M	3y	yes	*S. pseudintermedius*	SP
MLT	P	F	3y	no	*S. pseudintermedius*	SP
BF	P	F	4y	no	*S. pseudintermedius*	SP
BF	P	F	5y	no	*S. pseudintermedius*	SP
POM	E	M	4y	no	*S. pseudintermedius*	SP
DH	P	F	1y	yes	*S. pseudintermedius*	SU
DOGO	P	M	3y	yes	*S. pseudintermedius*	SU
SBT	E	F	4y	yes	*S. pseudintermedius*	SU
GR	P	M	1y	no	*S. pseudintermedius*	SU
BF	P	M	11y	yes	*S. pseudintermedius*	A
LAGR	P	M	2y	yes	*S. pseudintermedius*	A
APBT	E	M	5y	yes	*S. pseudintermedius*	A
MIXED	P	F	4y	no	*S. pseudintermedius*	A
MLT	P	M	3m	no	*S. pseudintermedius*	A
PULI	P	M	10y	yes	*S. pseudintermedius*	SP
MLT	P	M	1y	yes	*S. pseudintermedius*	SP
WT	E	M	3y	yes	*Coag. neg. Staphylococcus* spp.	SU
WHWT	E	F	5y	yes	*S. pseudintermedius*	SU
GR	P	M	13y	yes	*S. pseudintermedius*	SU
GSD	E	M	7y	yes	*S. pseudintermedius*	SU
APBT	E	M	5y	yes	*S. pseudintermedius*	A
LR	P	M	10y	no	*Escherichia coli*	A
PUG	P	M	3y	yes	*P. aeruginosa*	SP
MIXED	E	M	5y	no	*Pseudomonas* spp.	A
LR	P	M	10y	yes	*Proteus* spp.	W
GSD	E	F	9y	no	*P. aeruginosa*	A
MIXED	P	F	8y	no	*P. aeruginosa*	W
PUG	P	M	3y	yes	*P. aeruginosa*	SP
LR	P	M	10y	no	*Proteus* spp.	W
GSD	E	M	7y	yes	*Proteus* spp.	SU

* POM-Pomeranian, MLT – Maltese, SHIH – Shih Tzu, MIXED – Mixed Breed, AK – Akita, PUG – Pug, BF – Bichon Frise, CHOW – Chow chow, BT – Boston Terrier, DH – Dachshund (Miniature, Standard), DOGO – Dogo Argentino, SBT – Staffordshire Bull Terrier, GR – Golden Retriever, LAGR – Lagotto Romagnolo, APBT – American Pit Bull Terrier, PULI – Puli, WT – Welsh Terrier, WHWT – West Highland White Terrier, GSD – German Shepherd Dog, LR – Labrador Retriever, P – Pendulous, Erect, F – Female, M – Male, m – month, y – years, W – Winter, A – Autumn, SU – Summer, SP – Spring

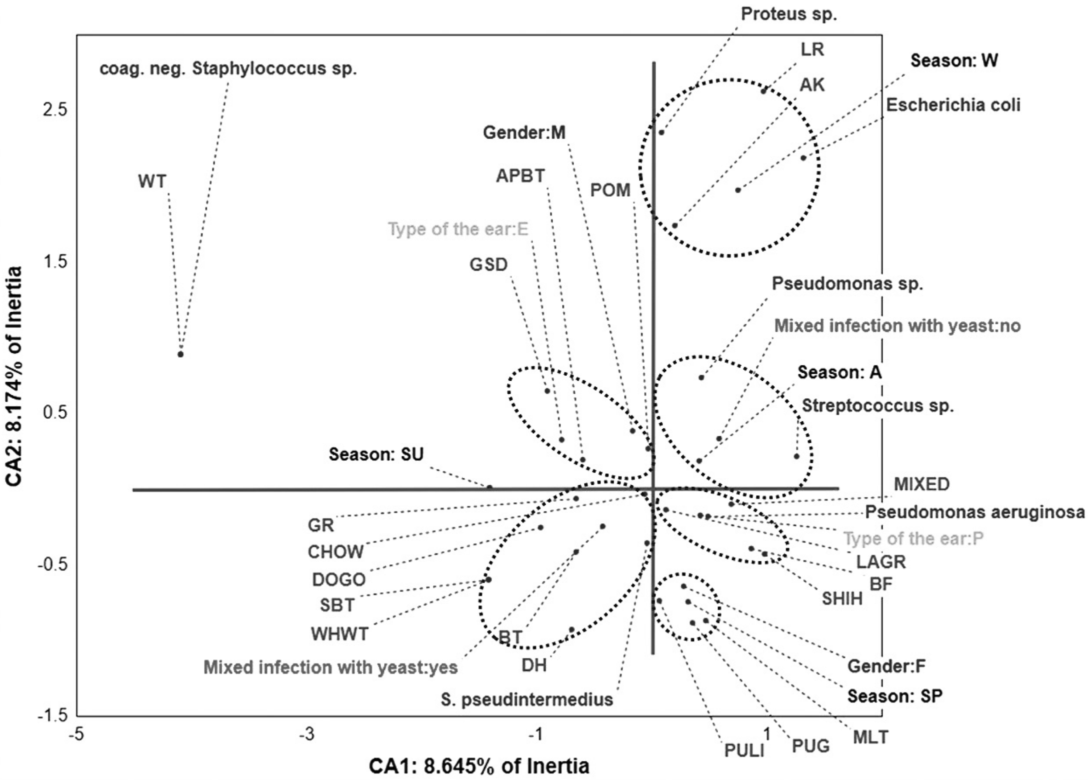

Fig. 2. Multivariate correspondent analysis - the position of the evaluated variables in the space defined by the first two correspondent axes.

After *S. pseudintermedius*, just like in other researches, the most often isolated bacterial pathogens are *P. aeruginosa* (7.55%) and *Proteus* spp. (5.66%) (Bornand 1992, Kumar et al. 2002). The approximate results for these two bacteria have got Forster et al. (2018) and Bornard (1992) in a study conducted in Australia. Moreover, similar results (6.8%) regarding *Proteus* spp. were reported by Bugden (2012), but in this study, contrary to our results, *P. aeruginosa* was the most frequently isolated microorganism with incidence of 35.5%. Furthermore, other researches claim that these two agents are isolated from dogs with chronic infection of otitis externa, or in dogs that did not respond well to previous antibiotic therapy (Martín Barrasa et al. 2000, Fernández et al. 2006, Petrov et al. 2013).

In the current study the greatest resistance was recorded in case of *S. pseudintermedius* against penicillin and amoxicillin. Also, high resistance to clindamycin, lincomycin and azithromycin has also been reported. Similar results of *S. psudintermedius* resistance to penicillin in dogs was reported by other authors (Lyskova et al. 2007, Zamankhan Malayeri et al. 2010, Bourély et al. 2019). On the other hand, the most effective antibiotics against *S. pseudintermedius* were cefoxitin, amoxicillin + clavulanic acid, ceftriaxone and enrofloxacin. Moreover, all these antibiotics, except cefoxitin, which was not tested, also showed good sensitivity in the research conducted by Zamankhan Malayeri et al (2010). Furthermore, De Martino et al. (2016) got comparable results, with the fact that in their research the percentage of resistance to amoxicillin + clavulanic acid was higher (35.5%, versus 10 and 17.65% in ours research). The selection of an appropriate antibiotic for initial therapy of uncomplicated canine otitis externa can be aided by historical knowledge of the most common bacterial isolates in a geographical area and their antibiotic susceptibility patterns (Bugden 2012) since there are variations in regional isolates and their susceptibility to different antimicrobials (Wong et al. 2015). So, local and recent data are crucial for choosing appropriate treatment considering that the differences in microbial prevalence and susceptibility across different geographical areas and over time can vary (Scarborough et al. 2020). This could be the reason why it is necessary that every country implements their own efficacy supportive, well established measures, such as representative monitoring of AMR and consumption records on national level guided by AMS.

Furthermore, as expected, our research results have shown that G- bacteria strains showed higher degree of resistance compared to G+ isolated bacteria. Besides, in the study of Zamankhan Malayeri et al. (2010) G- bacteria manifested full resistance against erythromycin and penicillin, whereas in our research that were tetracycline and vancomycin. Moreover, the percentage of resistance to penicillin was very high in our research also, as well as to amoxicillin, doxycycline, azithromycin, ampicillin and nitrofurantoin. In addition, aminoglycosides and fluoroquinolones, as a second choice, are recommended in the topical therapy of G- bacteria caused otitis externa (Greene 1998, Prescott et al. 2000, Bugden 2012). In the current study gentamicin, neomycin, enrofloxacin and ciprofloxacin, antibiotics that belong to these two drug groups, were the most efficient against these bacteria. Bacterial resistance to each of these antibiotics was only 11%. Other drugs from this group were less, but sufficiently effective. In the study of Lyskova et al. (2007) all G- rods were susceptible to ampicillin, ciprofloxacin, enrofloxacin, gentamicin, chloramphenicol, clindamycin and tetracycline, which partially coincides with ours. Furthermore, good sensitivity to gentamicin and enrofloxacin was also reported in the study of Hariharan et al. (2006). In addition, neomycin was found to be the best drug against *Pseudomonas* spp. Moreover, *P. aeruginosa* is known as an emerging pathogen because of the increase of AMR (Hosseini et al. 2012, Bassetti et al. 2018, Langendonk et al. 2021). Although it was isolated in a small percentage in our study, it showed the highest level of resistance, on 12 of 24 tested antibiotics. Multidrug resistant of *P. aeruginosa* was also present in the study performed in Italy (De Martino et al. 2016) including neomycin with 65.2% of resistance. These results suggest urgent need for development and implementation of guidelines for prudent use of antibiotics in veterinary medicine as an important tool of risk management to reduce the consumption of antibiotics and the consecutive development of AMR. Furthermore, preventive strategies such as regular vaccination, deworming, appropriate nutrition and probiotics (Morley et al. 2005, Sharma et al. 2018, Patel et al. 2020) are also important as part of antimicrobial stewardship.

Antibiotics used in veterinary practice are identical or very close to those used in human medicine and may cause cross-resistance to antimicrobial drugs (Ungemach et al. 2006). Moreover, special attention should be focused on the critically important antimicrobials (CIA) for human medicine, since their use in veterinary field represents another concern, especially in small animal practice (EMA/CVMP 2015). The World Health Organization (WHO) has classificated 3rd to 5th generation cephalosporins, polymyxins, quinolones, glycopeptides, and macrolides as those of prime concern currently (WHO/AGISAR, 2019). Since our research has shown high resistance to vancomycin and colistin, continual educations of current veterinarians have to be addressed on this issue to combat AMR with focus on CIA.

In addition, our research included the relation between dog breed, type of ears (pendulous or erect), gender, age, type of infection and season of the year in regard to otitis externa bacteria. As to the relationship between the bacterial otitis externa and dog breeds, the most frequent breed types were Maltese and Mixed breed, followed by Bichon Frise, Labrador Retriever, German Shepherd Dog and Pug. All these breeds, except Mixed Breed and German Shepherd Dog have pendulous ear type that is predisposing factor and increase the risk of these breeds for otitis externa (Murphy 2001, Scott et al. 2001, Rosser 2004, Saridomichelakis et al. 2007). It was also shown that in relation to the total number of bacterial otitis, a larger incidence is characteristic for dogs with ears down orientated.

Moreover, gender was recognized as a weak but still predisposing factor for otitis externa in previous study in which male dogs had 1.21 more chances than females to get this disease (O'Neill et al. 2021). These results are in line with our study where males were more susceptible as well, with the exception that it took into account only bacteria as the cause. Interestingly, Kumar et al. (2014) got almost same results as in our study in which the incidence of male cases were 61.11% and females 38.88%. This research that was conducted in India also reported that in relation to age, bacterial otitis externa appeared most often in dogs of three years or above three years, then dogs of one to three and under one year of age. Moreover, the largest number of the affected dogs in our study belonged to the 2 to 5 year old group, followed by older than 10 years and dogs up to one year of age. Furthermore, the obtained results indicate that bacterial otitis externa is more common with older dogs, but also could affect younger dogs in some degree. It is similar with other results where almost all dogs greater than five years old had cocci and rods at significantly higher levels, which authors explained by chronic changes and the possibility that milder otitis had not been diagnosed when these dogs were younger (Zur et al. 2011).

On the other hand, the age of animals did not statistically affect the presence of yeast coinfection ($U=168$, $z=0.74$, $p=0.46$). Also, our study shows that there was not significant difference in incidence of mixed infection with yeast compared to independent bacterial ones. However, it has been proven that *S. pseudintermedius* infections are usually accompanied by yeast in many different breeds such as Golden Retriever, Chow chow, Dogo Argentino, Staffordshire Bull Terrier, West Highland White Terrier, Boston Terrier and Dachshund breeds. Furthermore, season of the year with the highest number of diagnosed cases were spring and autumn, while in the winter the smallest number of otitis cases was reported. However, totally opposite results from ours were reported in the study conducted in Poland by Święcicka et al. (2015) where bacteria otitis externa incidence was the highest during the winter months (18.8%), lower in the summer (11.3%); followed by autumn and spring, both somewhat above 5%. These results point out that the difference between the seasons may indicate that the season of the year also played a role in the incidence rate. The connection between the occurrence of inflammations in the auditory meatus and climate change, such as air humidity is determined by other authors (Staroniewicz et al. 1995, Ziółkowska and Nowakiewicz 2004).

Conclusion

In addition to corticosteroids and antifungals, antibiotics play an important role in the treatment of canine otitis externa. The results of the antimicrobial sensitivity testing in our study showed that the isolated bacteria, especially G- bacteria, were the most sensitive to aminoglycosides and fluoroquinolones. These are good news, considering that the pharmaceutical formulations available in Serbia for topical treatment of otitis externa are mostly based on antibiotics from these groups that are usually primarily prescribed for ear infection in dogs. On the other hand, our research has shown high resistance to some critically important antimicrobials for human medicine (vancomycin and colistin), so continual educations of current veterinarians have to be addressed on this issue to combat AMR. Furthermore, it is important that antibiotic susceptibility testing are regularly performed in order to determine the appropriate antibiotic therapy. In addition, implementation of other antimicrobial stewardship principles, including surveillance and monitoring programs, represent essential tools in reducing antimicrobial resistance.

With increasing AMR, further research is required to develop antibiotic alternatives against bacteria, such as phytotherapy.

Acknowledgements

This work was supported by the Ministry of Science and Technological Development, Republic of Serbia [Grant Number 451-03-47/2023-01/ 200117].

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