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Original article

MICROBIOLOGICAL AND ANTIBACTERIAL RESISTANCE PROFILE IN CANINE OTITIS EXTERNA – A COMPARATIVE ANALYSIS

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Summary

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The aim of the present study was to compare the prevalence of microbial agents involved in canine otitis externa and their sensitivity to antibacterial drugs in two periods: 2007–2011 and 2013–2017. For 2013–2017, coagulase-positive staphylococci were the dominating bacterial species (186 isolates), followed by *Pseudomonas aeruginosa* (82 strains). The rate of isolated yeasts (mainly *Malassezia pachydermatis)* was substantially high (152 isolates). Compared to the earlier period (2007–2011) a tendency to more frequent occurrence of co-infections was noted – 61.7% and more than 80% of co-infections involved yeasts. Antibacterial resistance patterns showed a clear trend to increased resistance of coagulase-positive staphylococci and β-haemolytic streptococci to amoxicillin/clavulanic acid (42% and 50% respectively) and gentamicin (29%, 40%). Increased resistance of *P. aeruginosa* was established to gentamicin (16%) and amikacin (18%). The prevalence of pseudomonads resistant to enrofloxacin was lower (27%).

Key words: antimicrobial susceptibility, dog, microbial profile, otitis externa

INTRODUCTION

Otitis externa is acute or chronic inflammation of the ear auricle, horizontal and vertical ear canal and the outer tympanic membrane wall (Smith, 2015). The disease could be either primary (foreign bodies, ectoparasites etc.) or secondary following complications from ear canal stenosis, drooping ears, excessive hair in the ear canal, water in the ear canal, obstructions, atopic dermatitis, allergic skin reactions to food, metabolic diseases, abnormal keratinisation, autoimmune diseases, injury during manipulations etc. Otitis externa is particularly common in canine pets with affection rates of 5–20% (Angus, 2004; Greene, 2006; Lyskova *et al.,* 2007; Terziev & Urumova, 2018).

The normal microflora of the ear canal involves mainly *Staphylococcus* spp. (coagulase-positive and coagulase-negative), β-haemolytic *Streptococcus* spp., *Bacillus* spp. (August, 1988, Lyskova *et al.,* 2007). Also, the presence of *Malassezia pachydermatis (M. pachydermatis), Microsporum canis* and *Otodectes cynoti* was reported in ears without inflammation (Bornand, 1992; Hariharan *et al.,* 2006; Aalbæk *et al.,* 2010; Malayeri *et al.,* 2010).

The commonest microbial pathogens associated to otitis externa are members of genera *Staphylococcus*, *Streptococcus* spp., *Corynebacterium* spp.; *Pseudomonas* aeruginosa (*P. aeruginosa*), *Proteus mirabilis* (*P. mirabilis*), *Escherichia coli (E. coli*) and *Klebsiella pneumoniae* (Gotthelf, 2004). Principal staphylococcal species reported to be involved in the etiology of the condition are coagulase-positive *S. pseudintermedius* or former *S. intermedius* (Devriese *et al.*, 2009), *S. aureus, S. schleiferi* subs. *coagulans* and coagulase-negative *S. epidermidis*, *S. schleiferi* subs. *schleiferi*, *S. simulans* and *S. saprophyticus* (Lilenbaum *et al.*, 2000; Hoekstra *et al.,* 2002; Nagase *et al.,* 2002; May *et al.,* 2005). *M. pachydermatis* is also a common finding. Yeasts are widespread in animals having undergone continuous treatment with antibiotics. It should be stated that bacteria and yeasts are not primary pathogens of otitis externa but opportunistic species that replicated under favourable conditions created by another primary cause (Rosser, 2004; Miller *et al.,* 2013).

The treatment of otitis externa in dogs still remains a great concern. Local antimicrobial therapy aimed at eradication of bacterial or yeast infections is commonly applied in small animal veterinary practices (Angus, 2004). The identification of the primary cause for ear inflammation is however essential for the success of the therapy (Jacobson, 2002). In most cases, antibacterial therapy is prescribed without identification of the microbial pathogen and its sensitivity to antimicrobial drugs. This approach is often inefficient when microorganisms are resistant to applied chemotherapeutics resulting in recurrence of otitis when the primary cause is not eliminated or due to resistant strains selection.

The aim of the present study was to investigate the changes in the prevalence of microbial agents of canine otitis externa and their sensitivity to antibacterial drugs in 2013–2017 in comparison to the period 2007–2011 (Petrov *et al.,* 2013).

MATERIALS AND METHODS

The study was carried out between January 2013 and December 2017 in the microbiology lab of the Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Animals and samples

A total of 185 dogs with otitis externa were sampled. One hundred and twenty-four of dogs were with bilateral otitis, so a total of 248 samples were collected from both ears. The number of samples from dogs with unilateral otitis was 61. All 309 swab samples with secretion from the external ear canal were sent from private veterinary clinics for bacteriological examination and antibacterial sensitivity testing. They were stored in Amies transport medium under refrigeration conditions and transported to the lab by logistic companies.

The disease history in most cases comprised chronic course, empirical treat-

ment without satisfactory outcome and frequent recurrencies.

Microbiological examination

Samples were parallelly cultured onto blood agar (base) (Merck, Germany) with 5% sheep blood and McConkey agar (Merck, Germany). Cultures were incubated aerobically for 24–48 h at 37 ºC. For the mycological examination, the same samples were inoculated for 2–7 days on Sabouraud 4% dextrose agar (Merck, Germany), supplemented with 0.4 g/L chloramphenicol (>99.0% HPLC, Fluka, China) and 0.5 g/L actidione (cycloheximide, >93.0% HPLC, Fluka, China), at 37ºC and aerobic conditions. Staphylococcal isolates were identified as described in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) and Manual of Clinical Microbiology (Murray *et al.,* 2003), on the basis of colony appearance, Gram staining, production of pigments, presence of haemolysis and biochemical behaviour (catalase, oxidase, coagulase production).

The sensitivity of bacterial isolates to antimicrobial drugs was tested by the disk diffusion methods and interpretation of results – by the Bauer-Kirby scoring system (Bauer *et al.,* 1966), according to the Clinical and Laboratory Standards Institute (2013). The following disks loaded with antimicrobial substances were used: amoxicillin/clavulanic acid (20/10 µg), cefquinome (30 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), enrofloxacin (5 µg), marbofloxacin (5 µg), chloramphenicol (30 µg), lincomycin/spectinomycin (9/100 µg) and polymyxin B (10 µg).

Statistical analysis

The determination of 95% confidence limits was performed with the help of statistical software GraphPad InStat v. 3.00 (GraphPad Software Inc., La Jolla, CA).

RESULTS

Negative microbiological finding was detected in 35 out of tested 309 swab samples. From the 274 samples with microbial growth, 505 strains were isolated (Table 1).

Monoinfections were established in 105 (38.3%) of the 274 positive samples. Most commonly, the isolate was a coagu-

Table 1. Number and frequnecy of isolation of canine otitis externa pathogens for the period 2013–2017.

Microbial pathogens	Number	Isolation rate (%)	95% confidence limits
Staphylococci (coagulase positive)	186	36.83	33.1÷40.8
M. pachydermatis	152	30.01	26.1÷33.4
P. aeruginosa	82	16.24	13.4÷19.2
P. mirabilis	18	3.56	2.5÷5.6
E. coli	16	3.17	2.4÷5.8
Staphylococci (coagulase negative)	15	2.97	2.1÷5.1
Streptococci (β-haemolytic)	15	2.97	2.1÷5.1
Candida spp.	12	2.38	1.3÷2.7
Bacillus spp.	5	0.99	0.3÷1.9
Corynebacterium spp.	4	0.79	0.2÷1.6
Total	505	100.00	

Table 2. Antibiotic resistance (%) of Gram+ bacterial agents isolated from dogs with otitis externa (2013–2017)

Antibiotics	Staphylococci (coagulase positive)	Confidence limits	Staphylococci (coagulase negative)	Confidence limits	Streptococci (β-haemolytic)	Confidence limits
Amoxicillin/clavulanic acid	42%	33.4÷50.8	20%	2.3÷48.8	50%	20.9÷79.0
Cefquinome	29%	21.3÷37.2	22%	3.1÷51.2	22%	2.5÷52.9
Gentamicin	29%	21.4÷37.1	27%	5.5÷57.1	40%	13.3÷70.3
Tobramycin	28%	17.4÷40.9	25%	0.1÷72.0	33%	0.2÷85.3
Amikacin	20%	12.7÷28.4	38%	9.7÷71.8	33%	7.9÷65.0
Enrofloxacin	39%	30.5÷47.8	33%	7.9÷65.0	80%	51.1÷98.6
Marbofloxacin	28%	16.1÷41.7	NA	–	NA	–
Chloramphenicol	39%	29.5÷50.8	71%	34.8÷96.1	40%	5.9÷81.3
Lincomycin/Spectinomycin	50%	38.5÷56.5	50%	20.9÷79.0	30%	7.1÷60.3
Polymyxin B	48%	36.1÷60.0	67%	28.0÷95.6	NA	-

NA – not assayed.

Table 3. Antibiotic resistance (%) of Gram– bacterial agents isolated from dogs with otitis externa (2013–2017)

Antibiotics	*P. aeruginosa*	Confidence limits	*P. mirabilis*	Confidence limits	*E. coli*	Confidence limits
Amoxicillin/clavulanic acid	90%	79.2÷97.1	75%	48.9÷93.8	25%	3.0÷58.5
Cefquinome	55%	40.7÷68.8	42%	16.7÷69.7	38%	9.7÷71.8
Gentamicin	15%	6.6÷25.9	27%	8.3÷51.4	38%	9.7÷71.8
Tobramycin	26%	14.5÷39.5	NA	–	0%	–
Amikacin	18%	8.3÷30.4	33%	10.4÷60.8	0%	–
Enrofloxacin	27%	15.5÷40.3	7%	0÷23.3	38%	9.7÷71.8
Marbofloxacin	35%	14.3÷59.2	NA	–	NA	–
Chloramphenicol	92%	31.1÷99.1	88%	58.3÷99.9	43%	11.3÷78.3
Lincomycin/Spectinomycin	93%	83.3÷98.6	85%	61.4÷98.4	29%	4.8÷62.9
Polymyxin B	50%	25.7÷74.2	57%	21.7÷89.6	0%	–

NA – not assayed.

Table 4. Antibiotic resistance (%) of Gram+ bacterial agents isolated from dogs with otitis externa in 2007–2011 and 2013–2017

	Staphylococci (coagulase positive)		Streptococci (β-haemolytic)	
	2007–2011*	2013–2017	2007–2011*	2013–2017
Amoxicillin/clavulanic acid	5%	42%	5%	50%
Gentamicin	20%	29%	9%	40%
Tobramycin	26%	28%	23%	33%
Amikacin	15%	20%	21%	33%
Enrofloxacin	32%	39%	100%	80%
Chloramphenicol	40%	39%	0%	40%
Lincomycin/Spectinomycin	59%	50%	40%	30%
Polymyxin B	66%	48%	100%	NA

* from Petrov *et al.* (2013); NA – not assayed.

Table 5. Antibiotic resistance (%) of G- bacterial agents isolated from dogs with otitis externa in two periods – 2007-2011 and 2013-20

	P. aeruginosa		*E. coli*		*P. mirabilis*	
	2007–2011*	2013–2017	2007–2011*	2013–2017	2007–2011*	2013–201
Amoxicillin/clavulanic acid	100%	90%	32%	25%	30%	75%
Gentamicin	2%	15%	18%	38%	28%	27%
Tobramycin	20%	26%	5%	0%	0%	NA
Amikacin	0%	18%	0%	0%	0%	33%
Enrofloxacin	38%	27%	14%	38%	22%	7%
Chloramphenicol	100%	92%	0%	43%	74%	88%
Lincomycin/Spectinomycin	40%	93%	0%	29%	55%	85%
Polymyxin B	0%	50%	0%	0%	74%	57%

* from Petrov *et al.* (2013); NA – not assayed.

lase-positive *Staphylococcus* and *P. aeruginosa* (39 and 31 cases, respectively), followed by *M. pachydermatis* (23 cases). Monoinfections induced by coagulase-negative staphylococci, *P. mirabilis, Corynebacterium* spp. and β-haemolytic streptococci were rare (in 5, 3, 3, 1 cases respectively). Co-infections were far more commonly seen (169 cases or 61.7%), with main combinations between coagulase-positive staphylococci and *M. pachydermatis* (67 cases); coagulase-positive staphylococci and *P. aeruginosa* (11 cases); *M. pachydermatis* and *P. aeruginosa* (10 cases). In 44 samples, three microbial species were simultaneously isolated, also with predominance of associations between coagulase-positive staphylococci, *M. pachydermatis* and *P. aeruginosa* (15 cases) and coagulase-positive staphylococci, *M. pachydermatis* and β-haemolytic *Streptococcus* spp. (10 cases). From another ten samples, 4 microbial species were isolated at a time.

In 104 dogs, microbial findings were the same in left and right ears, so a total of 334 isolates including 92 yeasts strains were analysed. The antimicrobial sensitivity of 242 bacterial isolates was tested (Tables 2 and 3). Antibiotic resistance was a common finding. A very high resistance to amoxicillin with β-lactamase inhibitor was exhibited by coagulase-positive staphylococci. These bacteria were often resistant also to lincomycin/spectinomycin, polymyxin B, chloramphenicol and fluoroquinolones. Staphylococci isolated from canine otitis externa were most sensitive to aminoglycosides and cefquinome.

As expected, the greatest resistance to antimicrobial drugs was demonstrated by pseudomonads, in particular against amoxicillin/clavulanic acid, lincomycin/spectinomycin and chloramphenicol. *P. aeruginosa* isolates had a relatively well preserved sensitivity to gentamicin and amikacin, although very resistant to enrofloxacin and marbofloxacin.

DISCUSSION

The comparison of data from 2013–2017 and those from the previous period 2007–2011 (Petrov *et al.,* 2013) showed that staphylococci, *M. pachydermatis* and *P. aeruginosa* were the predominating microbial species isolated from dogs with otitis externa. In 2013–2017, coagulase-positive staphylococci were involved in more than 70% of cases. Marked increase of samples with yeast isolates (*M. pachydermatis* and *Candida* spp.) – over 65% and *P. aeruginosa* – 33% was noted as compared to the previous period with prevalence rates of 40% and 17%, respectively. This increase was probably due to the higher number of clinical samples from patients with chronic otitis, history of unsuccessful treatment or recurrent disease. Similar high prevalence of *M. pachydermatis* in dogs was reported by Crespo *et al.* (2002) in Spain, Nardoni *et al.* (2014) in Italy and Bardshiri *et al.* (2014) in Iran. Schick *et al.* (2007) have isolated *P. aeruginosa* in 53% of chronic canine otitis externa in line with our study.

The presence of *M. pachydermatis* monoinfection in 23 cases provided evidence for failure of antibiotic treatment prescribed without identification of the involved microbial agent. This is a commensal species on animal and human skin proliferating in the presence of favourable factors e.g. ear canal stenosis, excessive hairs, increased humidity and prolonged antibiotic treatment (Nardoni *et al.,* 2014). In comparison to our previous research however (Petrov *et al.,* 2013) the number of samples from which only yeasts were

isolated has decreased. This could be probably attributed to the higher number of chronic otitis patients with polymicrobial associations. In this study, co-infections with participation of yeasts were present in almost 80% (140 out of 169 samples with more than one isolate), vs 50% (66 samples from 132 co-infections) in the previous study.

The analysis of sensitivity tests of bacterial isolates demonstrated high resistance of Gram+ bacteria to classical antimicrobial drugs: β-lactams and lincospectin. A special attention should be paid on the substantial increase of Gram+ bacteria resistant to the combination amoxicillin/clavulanic acid. Our data for sensitivity to amoxicillin/clavulanic acid are comparable to those of De Martino *et al.* (2016) and Saputra *et al.* (2017), but significantly higher that rates reported by Robaj *et al.* (2015); Metiner *et al.* (2015); Dziva *et al.* (2015) and Terziev & Urumova (2018). The established increase of resistance of staphylococcal isolates to aminoglycosides, although not as pronounced, exceeded several times the rates from other studies. For instance Dziva *et al.* (2015) reported 17% resistance to gentamicin, De Martino *et al.* (2016) – 11%, and Metiner *et al.* (2015) – only 2.5%. Only 1.1% of staphylococcal strains isolated from dogs were not sensitive to amikacin in the research of Saputra *et al.* (2017). Similar to our resistance rates in staphylococci were reported by Robaj *et al.* (2015): more than 30%.

The comparison of resistance of canine otitis externa bacterial isolates in the present study and the previous one (Petrov *et al.,* 2013) showed that the resistance of Gram+ bacteria (coagulase positive staphylococci and β-haemolytic streptococci) to amoxicillin/clavulanic acid has increased 8–10 times: from 5% to 42% and 50%. Resistance rates to the other tested antibiotics varied at a lesser extent, whereas the sensitivity of coagulase-positive staphylococci to polymyxin B was even increased from 34% to 52%. A similar tendency: reduction of resistant isolates from 59% to 50% was observed with respect to the combination lincomycin/spectinomycin (Table 4).

The established higher resistance of streptococci to almost all tested antibiotics, in particular to amoxicillin/clavulanic acid, gentamicin and chloramphenicol as in line with the results of De Martino *et al.* (2016). It should be noted that streptococci are rarely isolated from canine otitis patients. Its occurrence rather in co-infections than in monoinfections suggest unsatisfactory outcome of antibiotic treatment undertaken without preliminary identification of the pathogen and its antimicrobial resistance profile.

With respect to Gram- bacteria, a more detailed analysis could be made for *P. aeruginosa* isolates because the number of *E. coli* and *P. mirabilis* strains was low. Expectedly, the percentage of pseudomonads resistant to chloramphenicol, amoxicillin/clavulanic acid and lincomycin/spectinomycin was very high. The detected resistant rates among *Pseudomonas* isolates in this study were higher than those reported by other researchers (Robaj *et al.,* 2015; De Martino *et al.,* 2016), but similar to those reported by Sutkevičiūtė (2015) in Latvia.

The clear trend to increase in *Pseudomonas* strains resistant to the usual antimicrobial drugs used in clinical setting – aminoglycosides, mainly gentamicin (Table 5) is of special concern for practicing veterinarians. On the other hand, a certain decline in the percentage of strains resistance to other used group of antimicrobials – fluoroquinolones (enrofloxacin in

particular). The observed resistance in about one-third of *P. aeruginosa* isolates to enrofloxacin and marbofloxacin suggested a wrong approach to the therapy of *Pseudomonas* otitis in dogs resulting in selection of resistant strains. This pattern is probably similar in other countries as seen from reports about lower sensitivity of pseudomonads to enrofloxacin: 52% (Martin *et al.,* 2000), 56.5% (De Martino *et al.,* 2016), only 35% (Sutkevičiūtė, 2015).

A similar tendency was found out with respect to resistant *P. mirabilis* strains. This microbial species was outlined with the highest resistance to chloramphenicol, lincomycin/spectinomycin and amoxicillin/clavulanic acid. *Proteus* isolates were from chronic co-infections, mainly associated with *P. aeruginosa* and *M. pachydermatis*. Therefore, the resistance to these antimicrobial drugs had not a considerable effect on therapy efficacy as pseudomonads were also very resistant to them. The finding that almost one-third *P. mirabilis* strains were resistant to gentamicin and amikacin, particularly associations with pseudomonads for which these antibiotics are strategic, affirms the opinion on the wrong treatment approach to chronic canine otitis.

Among *E. coli* isolates, the resistance to gentamicin and enrofloxacin was almost twice increased. This fact suggests that the prescription of these antibacterial drugs without need is still a current practice and colibacteria are bacteria that evolve resistance very easily. This is not only a national problem. A study conducted in Australia has shown that 40% of veterinarians use fluoroquinolones empirically for therapy of canine otitis externa, and when cytology detected Gram-negative bacterial rods, their proportion increased to 61% (Hardefeldt *et al.,* 2017). This results in rapid evolution of antimicrobial resistant in bacterial strains, especially after prolonged treatments (Penna *et al.,* 2010).

Similar results were found out with regard to resistance rates to chloramphenicol and lincomycin/spectinomycin – in the previous study of ours all *E. coli* isolates were sensitive to them while in the recent study, 30–40% of isolates were already resistant.

The yet preserved 100% sensitivity of *E. coli* isolates to amikacin, tobramycin and polymyxin B requires attentive use of these strategic antimicrobial drugs in the future.

CONCLUSION

The comparative analysis of prevalence of isolates from canine otitis demonstrated increased occurrence of commensal species *M. pachydermatis* and *P. aeruginosa*, along with increased resistance of bacteria to commonest antibacterial drugs. Therefore, the emphasis should be placed on the accurate etiological diagnosis and sensitivity tests to chemotherapeutics. This approach would allow for scientifically justified well-targeted antibiotic therapy.

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