ANTIBIOTIC RESISTANCE LEVEL IN *STAPHYLOCOCCUS SPP*. STRAINS ISOLATED FROM DOGS WITH OTITIS EXTERNA

MIHAELA NICULAE, MARINA SPÎNU, CARMEN DANA ŞANDRU, F. BRUDAŞCĂ, D. CADAR, A. UNGVARI, I. SCURTU, P. BOLFĂ, C.I. MATEŞ, M. TĂUȚAN

University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5, Manastur Street, 400372, Cluj-Napoca, Romania
E-mail: niculaemihaela@hotmail.com

Summary

The purpose of this study was to determine the antibiotic resistance pattern of *Staphylococcus spp.* strains isolated from dogs with otitis externa. A total of 82 bacterial samples were evaluated for susceptibility towards 16 antimicrobial agents. The results indicated a high level of antimicrobial resistance for the tested isolated, most frequently impliying antimicrobials like penicillin, erythromycin, gentamicin, kanamycin, streptomycin, lincomycin, sulphamethoprim, cephalotin, polimixin B and amoxycillin. This study highlights the multidrug resistance of canine *Staphylococcus spp.* and emphasizes the need for susceptibility testing before electing the antimicrobial therapy.

Key words: antibiotic resistance, *Staphylococcus spp*., otitis externa, dog

Otitis externa (OE), the most common disease of the canine ear canal, is well known for the frustrating difficulty in electing a suitable treatment, frecquently been asociated with a chronic or recurrent course despite all therapeutic approaches. This aspect can be the direct consequence of the complex multifactorial aetiology and also of the emergence of resistance towards antimicrobial agents among the microorganisms isolated, case of *Staphylococcus spp.* and *Pseudomonas aeruginosa* (1, 6, 7, 9, 12). There are many studies describing the antimicrobial susceptibility profiles of canine otic pathogens, most of them pointing out different antibiotic resistance patterns, but all suggesting the emergence of the antimicrobial resistance for isolated bacteria (2, 4, 5, 10, 11).

The increase in the isolation of multidrug-resistant pathogens belonging to the *Staphylococcus* genera is of growing concern in both human and veterinary medicine (e.g., oxacillin-resistant *S. intermedius* and methicillin-resistant *Staphylococcus aureus*) (3, 8, 13).

The purposes of this retrospective study were to determine the antibiotic resistance pattern in *Staphylococcus spp.* strains isolated from dogs with otitis externa.

Materials and methods

Collection and Processing of Samples: a total of 116 ear exudates samples were aseptically collected from the external auditory canal of canine

patients showing clinical signs of otitis externa (erythema, otorrhoea, pain, itch, lesions of the ear tegument).

Identification of Isolates: The *Staphylococcus* species (n=82) were identified based on the colony characteristics, Gram stain, catalase and coagulase activity and also using the API ID Staph System (bioMerieux).

Susceptibility to antimicrobials was assessed by Bauer – Kirsby disk diffusion method. The antibiograms included 16 antibiotic agents: penicillin G (10UI), gentamicin (10µg), oxacillin (1µg), tetracycline (30µg), trimethoprim-sulphamethoxazole (25µg), ampicillin (10µg), enrofloxacin (5µg), ciprofloxacin (5µg), norfloxacin (10µg), chloramphenicol (30µg), polymyxin B (300UI), amoxicillin-clavulanic acid (30µg), cefalexin (30µg), erythromycin (15UI), neomycin (30µg), and cephalotin (30µg).

Results and discussions

A total of 82 staphylococcal strains were isolated and identified from ear canals of dogs with otitis externa. Of these, 52 were coagulase-positive (CPS) (63.41%) and 30 were coagulase-negative (CNS). Also, based on the API ID Staph System, the following species were identified (table 1):

Table 1

Different *Staphylococcus spp.* isolated from dogs with otitis externa

Species	Coagulase	Isolates number	%
Staph. intermedius	positive	27	32.92
Staph. aureus	positive	21	25.60
Staph. hyicus	positive	1	1.21
Staph. schleiferi	positive	3	3.65
Staph. saprophyticus	negative	1	1.21
Staph. haemolyticus	negative	1	1.21
Staph. sciuri	negative	2	2.43
Staph. epidermitis	negative	15	18.3
Staph. auricularis	negative	11	13.42
Total		82	100

Coagulase-positive species were most commonly found and the most frequently isolated *Staphylococcus* species were *Staph. intermedius* (32.92%) and *Staph. aureus* (25.60%). Other species, such as *Staph. hyicus* and *Staph. schleiferi* were also identified. Regarding the coagulase-negative staphylococci, the microbiological assays pointed out several species: *Staph. saprophyticus*, *Staph. haemolyticus*, *Staph. sciuri*, *Staph. epidermitis*, *Staph. auricularis.*

These results pointed out *S. intermedius* as the major coagulase-positive staphylococci (CPS) associated with chronic otitis externa such as described by some authors (1, 5, 13). *Staphylococcus intermedius*, as well as other staphylococci, is habitant of the normal bacterial flora of the dog and can be isolated from healthy dogs, especially from the anal region. *S. intermedius* is also an important skin pathogen in dogs. While CPS are considered to be the main bacterial agents in canine otitis (4, 9, 12), for CNS, such as *Staph. saprophyticus*, *Staph. haemolyticus*, *Staph. sciuri*, *Staph. epidermitis*, *Staph. auricularis*, a definitive role (pathogens, opportunistic or part of the normal microflora) has not been established yet.

The results of the antimicrobial susceptibility, obtained using the Bauer – Kirsby disk diffusion method, are presented for the coagulase-positive strains (CPS) in figure 1.

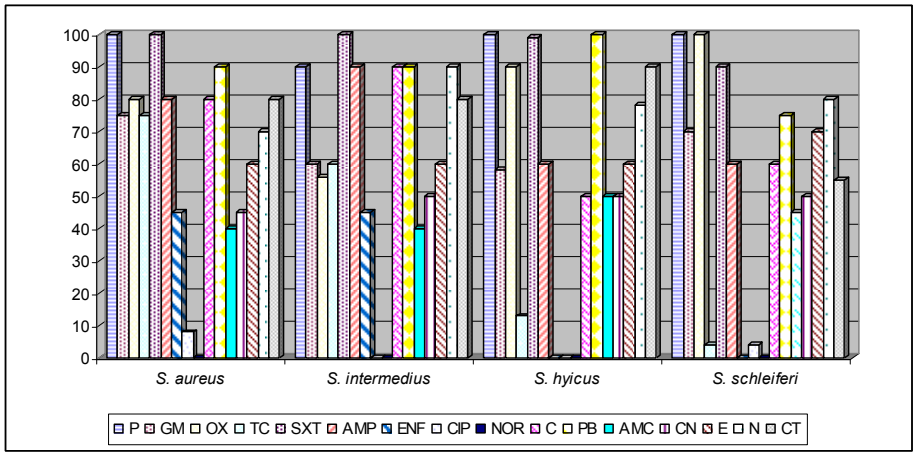

Fig.1. Antibiotic resistance level in *Staphylococcus spp*. strains isolated from dogs with otitis externa (penicillin G (P), gentamicin (GM), oxacillin (OX), tetracycline (TC), trimethoprim-sulphamethoxazole (SXT), ampicillin (AMP), enrofloxacin (ENF), ciprofloxacin (CIP), norfloxacin (NOR), chloramphenicol (C), polymyxin B (PB), amoxicillin-clavulanic acid (AMC), cefalexin (CN), erythromycin (E), neomycin (N) and cephalotin (CT))

Resistance to antibiotics was manifested by all the tested stains isolated from dogs with otitis externa and it can be considered high as all the tested strains were resistant to at least three antibiotics. Sensitivity of the isolated bacteria interested a relatively reduced number of antimicrobials, case of: cefalexin (%), enrofloxacin, norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid. Different levels of resistance were observed also for polymyxin B, neomicin, penicillin G,

ampicillin, tetracycline, oxacillin, neomycin, erythromycin, chloramphenicol, trimethoprim-sulphamethoxazole, cephalotin and cefalexin.

S. intermedius and *S. aureus* isolates presented a very similar susceptibility pattern.

These results confirmed some aspects presented in previous reports, but we found different resistance profile. Lilenbaum et al. (10) reported that 90.9% of the *Staphylococcus* strains isolated from canine otitis externa expressed resistance to at least one drug. The most active antimicrobial agents against these strains were rifampin and oxacillin. Fernandez et al. (4) showed that the most effective antibiotics were tobramycin (100% susceptible), marbofloxacin (91.3%) and ceftazidime (91.3%). Ticarcillin and gentamicin, commonly used for the treatment of otitis externa, were also active (susceptibility of strains was 86 and 65.2% respectively). Lower susceptibility was found using enrofloxacin (52.1%) probably due to its indiscriminate use.

Development of antibiotic resistance in bacteria represents a problem of great concern for both human and veterinary medicine. From this point of view, it is important to monitor the level of antimicrobial susceptibility.

In canine practice, despite advances in therapeutic approach, refractory cases of the otitis externa remain common. The current treatment of otitis externa usually implies the topical application of antibiotics (enrofloxacin, gentamicin, chloramphenicol, polimixin B and neomycin) in combinations with anti inflammatory drugs and ear cleaning solutions. Systemic therapy can be also employed, mostly when the inflamation becomes chronic or recurrent. Many practitioners treat otitis externa on the basis of their clinical experience, usually without laboratory tests. However, if data on sensitivity are continuously up gradated, they can serve as a basis for empirical therapy.

Conclusions

In conclusion, our results highlight the high level of the antibiotic resistance displayed by the canine *Staphylococcus* strains isolated from the clinical cases of otitis externa and once more emphasize the need for bacterial culture with species identification and susceptibility testing of swab specimens from the ear canal in order to choose appropriate antimicrobial agents.

References

1. **Cole, L.K., Kwochka, W.K., Kowalski, J.J., Hillier, A.**, Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media, J. Am. Vet. Med. Assoc., 1998, 212, 4, 534-538.

2. **Colombini, S., Merchant, S.R., Hosgood, G.**, Microbial flora and antimicrobial susceptibility patterns from dogs with otitis media. Vet. Derm., 2000, 11, 235-239.
3. **Duquette, R. A., Nuttall, T. J.,** Methicillin-resistant Staphylococcus aureus in dogs and cats: an emerging problem? Journal of Small Animal Practice, 2006, 45, 12, 591-597.
4. **Fernandez, G., Barboza, G., Villalobos, A., Parra, O., Finol, G., Ramirez, R. A.,** Isolation and identification of microorganisms present in 53 dogs suffering otitis externa, Revista Cientifica, 2006, 16, 1, 23-30.
5. **Ganiere, J.P., Medaille, C., Etore, F.,** In vitro antimicrobial activity of orbifloxacin against Staphylococcus intermedius isolates from canine skin and ear infections, Res Vet Sci, 2004, 77, 1, 67-71.
6. **Graham-Mize, C.A**., **Rosser, E.J. Jr.,** Comparison of microbial isolates and susceptibility patterns from the external ear canal of dogs with otitis externa, J Am Anim Hosp Assoc., 2004, 40, 2, 102-8.
7. **Guedeja-Marrom, S.S., Blanco, J.L., Ruperez, C., Garcia, M.E.,** Susceptibility of bacterial isolates from chronic canine otitis externa to twenty antibiotics, Vet.Med.B., 1998, 48, 507-512.
8. **Hanselman, B.A**., **Kruth, S**., **Weese, J.S**., Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital, Vet Microbiol., 2008, 126, 1-3, 277-81.
9. **Hariharan, H., Coles, M., Poole, D., Lund, L., Page, R.,** Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa, Can Vet J., 2006, 47, 3, 253–255.
10. **Lilenbaum, W., Veras, M., Blum, E., Souza, G.N.**, Antimicrobial susceptibility of staphylococci isolated from otitis externa in dogs, Letters in Applied Microbiology, 2001, 31, 1, 42-45.
11. **Lis, Christina de Oliveira, Lopes Leite, C.A., Raimunda Sâmia, N. Brilhante, Cibele Barreto, Carvalho, M.,** Etiology of canine otitis media and antimicrobial susceptibility of coagulase-positive Staphylococci in Fortaleza city, Brazil, Braz. J. Microbiol., 2006, 37, 2.
12. **May, Elizabeth R., Hnilica, K.A., Linda, Frank, A., Jones, R.D, Bemis, D.A**., Isolation of Staphylococcus schleiferi from healthy dogs and dogs with otitis, pyoderma, or both, Journal of the American Veterinary Medical Association, 2005, 227, 6, 928-931.
13. **Vanni, M., Tognetti, R., Pretti, C., Crema, F., Soldani, G., Meucci, V., Intorre L.,** Antimicrobial susceptibility of Staphylococcus intermedius and Staphylococcus schleiferi isolated from dogs, Research in Veterinary Science, Article in Press.