

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

R. Manickam¹, T. Lurthu Reetha² and B. Puvarajan³

¹Asst.Prof., ²Prof. & Head, ³Assoc. Prof.

Department of Veterinary Microbiology, Veterinary College and
Research Institute, Orathanadu, Tamil Nadu, India

E-mail: manickam75vet@gmail.com (¹Corresponding Author)

Abstract: 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 36 bacterial samples were evaluated for susceptibility towards 10 antimicrobial agents. The results of the antimicrobial susceptibility tests show that the bacterial isolates were susceptible to enrofloxacin, gentamicin, cephalothin, chloramphenicol and neomycin. Resistance was most common to penicillin G, oxacillin and ampicillin. Among the coagulase-negative staphylococci isolates, *S.intermedius* was susceptible to enrofloxacin (90.90%), gentamicin (100%), cephalothin (90.90%) and neomycin (81.81%). Coagulase positive *S. aureus* (CPS) isolates presented more or less the same susceptibility pattern.

The current treatment of otitis externa usually implies the topical application of antibiotics (enrofloxacin, gentamicin, chloramphenicol, polymixin B and neomycin) in combinations with anti inflammatory drugs and ear cleaning solutions. Systemic therapy can be also employed, mostly when the inflammation 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. Hence the study was undertaken to assess the antibiotic resistance pattern of the bacterial isolates and for the selection of appropriate antibiotics.

Keywords: Antibiotic resistance, *Staphylococcus spp.*, otitis externa, dog.

Introduction

Otitis externa, the most common disease of the canine ear canal, is well known for the frustrating difficulty in electing a suitable treatment, frequently been associated 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]. There are many studies describing the antimicrobial susceptibility profiles of canine otitic pathogens, most of them pointing out different antibiotic resistance patterns, but all suggesting the emergence of the antimicrobial resistance for isolated bacteria ^[2]. The increase in the isolation of multidrug-resistant pathogens

belonging to the *Staphylococcus* genera is of growing concern in both human and veterinary medicine^[3].

Materials and methods

Specimens:

A total of 42 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). The isolation of staphylococci was performed using swabs taken from the external auditory meatus of each dog.

Isolation of staphylococci:

The collected swabs are pre-enriched in nutrient broth (HiMedia Pvt. Ltd.) at 37°C for 18-24 hours. The culture was then inoculated onto blood agar (Blood Agar Base, HiMedia Pvt. Ltd), MSA (Mannitol Salt agar, HiMedia Pvt. Ltd.) and the selective agar, Baird Parker Agar (BPA) (HiMedia Pvt. Ltd.). The agar plates are incubated in aerobic conditions at 37°C for 24-48 hours, depending on the rate of growth of the bacteria.

Initial bacteriological characterization was performed by evaluating the morphology of the colonies and the presence and type of hemolysis on blood agar. Characteristic appearance of jet black colonies surrounded by a white halo on Baird Parker Agar (BPA) and golden yellow coloured colonies on Mannitol Salt agar are considered to be presumptive *S. aureus*. The pure cultures are streaked on Nutrient agar (HiMedia Pvt. Ltd.) and incubated for 24 hours at 37°C and were further characterized by biochemical tests.

Identification of Staphylococcus species:

The *Staphylococcus* species (n=36) are identified based on the colony characteristics, Gram stain, catalase and coagulase activity and also using the API ID Staph System (bioMerieux).

The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes.

Biochemical Identification

Biochemical tests were performed to confirm *S. aureus* using Catalase test, Coagulase test, DNase test, Acetoin production, Oxidase test and D-mannitol fermentation, free coagulase test, tests for bound coagulase (clumping factor), catalase test using the commercially available media and reagents procured from HiMedia Pvt. Ltd.

Susceptibility to antimicrobials:

Susceptibility to antimicrobials was assessed by Bauer - Kirby disk diffusion method^[4]. The antibiograms included 10 antibiotic agents: P - Penicillin G (10UI), AMP - ampicillin (10mg), OX - oxacillin (5mg), CEP - cephalotin (30mg), CHL - chloramphenicol (30mg), ERY - erythromycin (15mg), GEN - gentamicin (10mg), STR - trimethoprim-sulfamethoxazole (25mg), N - neomycin (30mg), and EX - enrofloxacin (10mg).

Results and discussions

A total of 36 staphylococcal strains were isolated and identified from ear canals of dogs with otitis externa. Of these, 9 were coagulase - positive (CPS) (25.00%) and 7 were coagulase-negative (CNS) (19.44%). 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 (n=36)

Species	Coagulase	Isolates number	%
<i>Staph. intermedius</i>	positive	11	30.55
<i>Staph. aureus</i> (CPS)	positive	9	25.00
<i>Staph. aureus</i> (CNS)	negative	7	19.44
<i>Staph. hyicus</i>	positive	1	2.77
<i>Staph. saprophyticus</i>	negative	1	2.77
<i>Staph. haemolyticus</i>	negative	1	2.77
<i>Staph. epidermitis</i>	negative	6	16.66
	Total	36	99.96

Coagulase-positive species were most commonly found and the most frequently isolated *Staphylococcus* species were *Staph. intermedius* (30.55%) and Coagulase positive *Staph. aureus* (25.00%). Other species such as coagulase negative staphylococci (19.44%), *Staph. epidermitis* (16.66%), *Staph. hyicus* (2.77%), *Staph. saprophyticus* (2.77%) and *Staph. haemolyticus* (2.77%) were also identified.

These results pointed out *S. intermedius* as the major coagulase-positive staphylococci (CPS) associated with chronic otitis externa such as described by some authors^[5]. *Staphylococcus intermedius*, as well as other staphylococci are 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^[6], for CNS, such as *Staph. saprophyticus*, *Staph. haemolyticus*,

Staph.epidermitis, a definitive role (pathogens, opportunistic or part of the normal microflora) has not been established yet.

The results of the antimicrobial susceptibility tests for coagulase-positive and coagulase negative staphylococci are given in Table 2. Bacterial isolates were susceptible to enrofloxacin, gentamicin, cephalothin, chloramphenicol and neomycin. Resistance was most common to penicillin G, oxacillin and ampicillin. Among the coagulase-negative staphylococci isolates, *S.intermedius* was susceptible to enrofloxacin (90.90%), gentamicin (100%), cephalothin (90.90%) and neomycin (81.81%). *S. aureus* (CPS) isolates presented more or less the same susceptibility pattern.

Table 2: Antimicrobial susceptibility tests for coagulase-positive and coagulase negative staphylococci (n=36)

Microorganisms (No.)	Antimicrobials									
	P	AMP	OX	CE	CH	ERY	GEN	STR	N	EX
				P	L					
<i>S. intermedius</i> (11)	4	7	4	10	8	8	11	7	9	10
<i>S.aureus</i> (CPS) - 9	3	4	3	8	8	4	8	5	7	9
<i>S.aureus</i> (CNS) - 7	2	3	2	6	6	4	6	5	5	7
<i>S. hyicus</i> (1)	1	0	1	1	1	0	1	1	1	1
<i>S.saprophyticus</i> (1)	0	1	1	1	1	1	1	1	0	1
<i>S.haemolyticus</i> (1)	0	0	1	1	1	1	1	1	0	1
<i>S.epidermitis</i> (6)	3	4	4	5	5	4	6	4	6	6
Susceptibility rate	36.1	52.7	44.4	88.8	83.3	61.1	94.4	66.6	77.7	97.2

DISCUSSION

Inflammatory disease of the external ear is commonly encountered in dogs. Despite advances in therapeutic approach, refractory cases of the otitis externa remain common. In view of this fact, the identification of microorganisms involved in this pathology and the determination of their antimicrobial susceptibility rates are important tools in canine practice. Results from 36 staphylococcal isolates showed that *S.intermedius* as the major coagulase-positive staphylococci (CPS) associated with chronic otitis externa such as described by some authors [7].

Despite CPS are considered to be the main bacterial agents in canine otitis [8], in the present study, CNS were the most common bacteria (19.44%) isolated among them. CNS has a

questionable pathogenicity, rarely considered in canine otitis ^[9]. However they are considered as potential pathogens since they were isolated in canine deep pyoderma ^[10] and are the most frequent reported as bloodstream pathogens in hospitals ^[11] or reservoirs and transmitters of antibiotic resistance genes in the hospital environment ^[12]. There are some reports on the presence of CNS in other animal species causing otitis and they have been isolated from skin surface of clinically normal cats ^[13]. Similar results were described for identification of staphylococci from clinical lesions, using another simplified identification systems ^[14].

In canine practice, the topical application of antibiotics in otitis externa is especially effective, because with this procedure it is possible to reach higher antibiotic concentrations. In the present study, antibiotics as enrofloxacin, gentamicin, chloramphenicol, cephalothin and neomycin are indicated for treatment of clinical cases as demonstrated by antimicrobial susceptibility tests and similar suggestions were given by others ^[15]. Different antibiotic susceptibility patterns presented by *Staphylococcus* species from otitis of dogs was also recorded ^[16]. This fact supports the idea that the increasing resistance of staphylococci to different antibiotics are given by its indiscriminate use in clinical practice causing the appearance of bacterial resistance.

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. These findings suggest that CNS plays an important role in the pathogenicity of canine chronic otitis externa. The test scheme has a value for identification of staphylococcal species and should be performed, following by antimicrobial susceptibility tests.

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