RESEARCH ARTICLE

In vitro Antibiotic and Antifungal Sensitivity Pattern of Bacterial and Fungal Isolates from Otitis Externa in Dogs

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Abstract

The objective of the present study was to evaluate the *in-vitro* antibiotic and antifungal sensitivity test of the 55 bacterial and 20 fungal isolates from otitis dogs. The antibiotic sensitivity test for coagulase-positive *Staphylococcus* species revealed the highest sensitivity to ceftriaxone & tazobactam (100.00%), Coagulase-negative *Staphylococcus* species to Ceftriaxone & tazobactam and Enrofloxacin (100.00%), *Streptococcus* species to Enrofloxacin & Ofloxacin (88.88%); however, Gram-negative organisms like *Escherichia coli* and *Pseudomonas* species revealed highest (100.00%) sensitivity to enrofloxacin. *Malassezia pachydermatis* and *Candida* species revealed 100% sensitivity to Ketoconazole, Clotrimazole, and Itraconazole in AFST.

Keywords: ABST, AFST, Antimicrobial, Dogs, Otitis.

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Introduction

Otitis externa is inflammation of the epithelial lining of the external ear canal consisting of the pinna ear canal up to the level of the tympanum. The condition might be acute or chronic. The condition is more frequently found in dogs than in cats. Pendulous eared dogs are most commonly affected than erect eared dogs. There are various predisposing factors identified that might lead to otitis development, such as stenosis of the ear canal, presence of foreign bodies in the ear canal, pendulous ears, increased humidity, moisture retention (after bath), and injury. The main cause of infection includes bacteria (coagulase-positive *Staphylococcus* spp., coagulase-negative *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas aeruginosa*, *Escherichia coli*) and yeasts (*Malassezia pachidermatis*, *Candida* spp.) (Petrov *et al*., 2013).

The purpose of the present study was to determine the *In-vitro* antibiotic and *In-vitro* antifungal sensitivity test of the bacterial and fungal isolates from otitis dogs to formulate an effective therapeutic regimen in the subsequent therapeutic trials.

Materials and Methods

The present study was conducted at Veterinary Clinical Complex (VCC), College of Veterinary Science, Assam Agricultural University at Khanapara in collaboration with the Department of Veterinary Epidemiology & Preventive Medicine and Department of Animal Biotechnology, College of Veterinary Science and Central Instrument's Facility (CIF), Assam Agricultural University, Khanapara.

Collection of Material

Dogs suspected of otitis were screened based on clinical signs *viz.* shaking of the head, pawing at the ear, foul smell from the ear, lesions on the external ear, and subjected to detailed clinical examination for otitis by using indirect otoscope (by Heine Optotechnik GMBH & Co.KG). Total 110 ear swabs (55 ear swabs for bacterial and 55 ear swabs for fungal isolation) were collected from the affected ear of the suspected dog irrespective of age, breed, and sex with dry sterile cotton swabs aseptically for both antibiotic sensitivity tests (ABST) and antifungal sensitivity test (AFST).

Isolation and *In-vitro* Antibiotic Sensitivity Test

The collected sterile swabs were placed into the blood agar and kept 24 h in the incubator at 37°C for the growth and multiplication of organisms. Different single colonies

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were taken and streaked on nutrient agar and the test tube containing 5 mL of nutrient broth based on colony morphology on blood agar. Both nutrient agar and broth containing inoculated single colonies were kept 24 h in the incubator at 37°C. Changing nutrient broth consistency from clear to turbid was recorded as positive for the growth of the suspected organism.

Staphylococcus species were identified based on colony characteristics, fermentation of mannitol, catalase test, and coagulase test. Coagulase-positive *Staphylococcus* species were identified based on their positive reaction to catalase and coagulase production. Coagulase-negative *Staphylococcus* species were identified based on their catalase-positive and coagulase-negative reaction.

Identification of *Streptococcus* species was done with Gram's staining, colony characteristic, biochemically on their catalase and coagulase-negative reaction. *Escherichia coli* was identified based on their Gram-negative rods, bright pink colonies on MacConkey agar, and metallic sheen on EMB (Eosin Methylene Blue) agar. Further, the confirmatory diagnosis was done by IMViC (Indole, Methyl red, Voges Proskauer, and Citrate) biochemical test. It was found that *Escherichia coli* showed a positive reaction for Indole and Methyl Red test. *Pseudomonas* species were identified based on their specific characteristic, i.e., the organism changes color from yellow to green in nutrient agar. On Gram's staining, negative rods of *Pseudomonas* species were observed.

The antibiotic sensitivity of the isolate was done *in-vitro* by disc diffusion techniques in Muller Hilton Agar (Quinn *et al.,* 2011). A single isolate of pure culture was inoculated in 5 ml of nutrient broth and incubated for 24 h at 37°C. The MHA plates were inoculated with the pure culture grown in the nutrient broth with the help of a sterile swab. The plates were then kept uncovered for 15 min at room temperature to dry the inoculums under the biosafety cabinet. The antibiotic discs were placed using forceps over the agar plates and at least 20 mm apart from one disc. The plates were then incubated at 37°C for 24 h before reading the sensitivity. The antibiotic discs procured from Hi-Media Laboratories Pvt. Ltd., Mumbai used for antibiotic sensitivity test were: Cefpodoxime (CPD, 10 mcg), Ciprofloxacin (CIP, 5 mcg), Enrofloxacin (EX,10 mcg), Gentamicin (GEN, 10 mcg), Ofloxacin (OF, 5 mcg), Ceftriaxone and tazobactum (CTX, 30 mcg)

The result of the sensitivity pattern of isolates to various antimicrobial discs was interpreted by measuring the diameter of the zone of inhibition in millimeters as per the chart provided by the manufacturer.

Isolation and *in-vitro* Antifungal Sensitivity Test

Preparation of Inoculums

The samples were streaked over the Petri dish/plate containing Sabouraud Dextrose Agar (SDA) with chloramphenicol. The plates were sealed with parafilm and kept at room temperature for 2 weeks under observation for fungus growth. The fungal growth was taken aseptically with the sterile needle, transferred into 5 ml of nutrient broth, and incubated at 37°C for 24 h.

Inoculation of Plates

The broth culture was evenly smeared over the surface of SDA plates with the help of a sterile cotton swab. The inoculum was allowed to dry for 15 min. The antifungal discs were placed over the agar plates by maintaining 20 mm distance from one disc to another with the help of sterile forceps. The results were recorded after 72 h of incubation at 37°C by measuring the size of the zone of inhibition around each disc and compared with the standard chart (Jain, 2010). The antifungal discs used Ketoconazole (KT, 30 mcg), Clotrimazole (CC,10 mcg), Itraconazole (IT,30 mcg), Miconazole (MIC, 30 mcg). were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai.

Results and Discussion

In-vitro antibiotic sensitivity test (ABST)

In the present study (Table 1) a total of 55 numbers of ear swabs were collected for bacterial isolation from clinically suspected dogs. It was noted that coagulase-positive *Staphylococcus* species isolates (n = 22) were sensitive to ceftriaxone and tazobactum (100.00%) followed by enrofloxacin (90.90%), ofloxacin (86.36%), gentamicin (81.81%), ciprofloxacin (77.27%), and cefpodoxime (72.72%). Coagulase-negative *Staphylococcus* species (n = 8) was sensitive to ceftriaxone and tazobactum (100.00%) enrofloxacin (100.00%), ofloxacin (100.00%), followed by ciprofloxacin (87.50%), gentamicin (62.50%) and cefpodoxime (62.50%). These observations were in accordance to Kumar *et al.* (2002), Kale and Aher (2004), Lyskova *et al.* (2007), and Behera *et al.* (2016) since they also reported the highest sensitivity of *Staphylococcus* species to Enrofloxacin and Ciprofloxacin. Ceftriaxone was found to be the highest sensitive antibiotic in otitis which corroborates with the reports of Sharma *et al.* (2016).

Streptococcus species (n=9) revealed 88.88% sensitivity to enrofloxacin, ofloxacin, ceftriaxone and tazobactum followed by 77.77% to ciprofloxacin, 66.66% to Gentamicin and Cefpodoxime. Pure isolates of *Pseudomonas* species (n=2) revealed sensitivity to Enrofloxacin (100.00%) and Ciprofloxacin (50.00%) but resistance to Ofloxacin, Cefpodoxime, Gentamicin, and Ceftriaxone & tazobactum. Antibiotic sensitivity test revealed *Escherichia coli* (n=14) was 100.00% sensitive to enrofloxacin followed by ciprofloxacin (57.14%), ceftriaxone and tazobactum (28.57%), and Ofloxacin (14.28%) but resistant to Cefpodoxime and Gentamicin. These observations are in accordance with Petrov *et al.* (2013), who narrated that gram-negative bacteria were sensitive to enrofloxacin. Behera *et al.* (2016) also revealed that

Table 1: Antibiotic Sensitivity Test of Different Cultural Isolates from Otitis externa in dogs

Cultural isolate	*No. of organisms isolated*	*Antibiotic disc used*					
		Enrofloxacin (% sensitivity)	*Ciprofloxacin (% sensitivity)*	*Ofloxacin (% sensitivity)*	*Gentamicin (% sensitivity)*	*Ceftriaxone and tazobactum (% sensitivity)*	*Cefpodoxime (% sensitivity)*
Coagulase positive *Staphylococcus* species (n=22)	22	20 (90.90)	17 (77.27)	19 (86.36)	18 (81.81)	22 (100.00)	16 (72.72)
Coagulase-negative *Staphylococcus* species (n=8)	8	8 (100.00)	7 (87.50)	8 (100.00)	5 (62.50)	8 (100.00)	5 (62.50)
Streptococcus species (n = 9)	9	8 (88.88)	7 (77.77)	8 (88.88)	6 (66.66)	8 (88.88)	6 (66.66)
Escherichia coli (n = 14)	14	14 (100.00)	8 (57.14)	2 (14.28)	0 (0.00)	4 (28.57)	0 (0.00)
Pseudomonas species (n = 2)	2	2 (100.00)	1 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Table 2: Antifungal Sensitivity Test of Different Cultural Isolates from Otitis externa in dogs

Cultural isolate	*No. of organisms isolated*	*Antifungal disc used*			
		Ketoconazole (% sensitivity)	*Clotrimazole (% sensitivity)*	*Miconazole (% sensitivity)*	*Itraconazole (% sensitivity)*
Malassezia pachydermatis (n = 18)	18	18 (100.00)	18 (100.00)	17 (94.44)	18 (100.00)
Candida species (n = 2)	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)

Pseudomonas species was sensitive to ciprofloxacin, which corroborates with the present reports.

In-vitro antifungal sensitivity test (AFST)

In the present study (Table 2), an antifungal sensitivity test was done on pure isolates, and it was noted that *Malassezia pachydermatis* (n = 18) showed 100.00% sensitivity to Ketoconazole, Clotrimazole, Itraconazole followed by Miconazole (94.44%). *Candida* species (n = 2) revealed 100.00% sensitive to Ketoconazole, Clotrimazole, Itraconazole and Miconazole. These findings corroborated with Sihelska *et al*. (2019), Erika *et al*. (2007), Carlotti (1991), Kathleen *et al*. (2019), and Nakano *et al*. (2005).

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