RESEARCH ARTICLE

Isolation, Characterization, Antibiogram and Molecular Detection of Antibiotic Resistance Genes from Bacteria Isolated from Otitis Externa in Dogs

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Abstract

Otitis externa is one of the most common diseases of the canines, defined as an acute or chronic inflammation of the external ear canal. In the present study, 124 samples were collected, out of which 94 samples were from 75 dogs suffering from otitis externa and 30 from 15 healthy dogs from the regions of Anand and Vadodara districts of Gujarat. Total 129 bacteria comprised of *Staphylococcus* spp. (58.40%), *Pseudomonas* spp. (10.62%), *Proteus* spp. (10.62%), *Streptococcus* spp. (7.08%), *Corynebacterium* spp. (6.19%), *E. coli* (4.42%), *Klebsiella* spp. (1.77%) and *Enterobacter* spp. (0.88%) were isolated from otitic and healthy ears of dogs based upon colonial, microscopic, and biochemical characteristics. The bacterial isolates showed least resistance towards tobramycin (1.51%) followed by enrofloxacin (5.52%), ofloxacin (11.98%), amikacin (15.20), gentamicin (17.07), and amoxycillin clavulanic acid (17.53%), while they showed the highest resistance towards ampicillin (63.90%). The multidrug resistance was detected in 29.46% (34/129) of the total bacterial isolates. Beta-lactam resistance genes were detected in *E. coli, Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas* spp.; quinolone resistance genes were detected in *E. coli,* and aminoglycoside resistance genes were detected in *E. coli, Proteus* spp. and *Staphylococcus* spp.

Keywords: Antibiotic resistance genes, Antibiotic susceptibility testing (ABST), Bacterial isolates, Dogs, Otitis externa.

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Introduction

Otitis externa is one among the foremost common diseases of the canines. It is defined as an acute or chronic inflammation of the external ear canal with its prevalence between 5% to 20% (Fernandez *et al*., 2006; Kumar *et al*., 2010; Boda *et al*., 2011). The predisposing causes of otitis externa are anatomical ear canal stenosis, pendulous ears, haired concave side of ears, increased humidity, moisture retention, washing, injury during manipulations, foreign bodies, prolonged antibiotic treatment, obstructive diseases (neoplasms), parasites, hypersensitivities, autoimmune diseases, and keratinization disorders. The bacteria or yeasts associated with cases of the otitis externa are only opportunists and are not the primary pathogens (Scott *et al.,* 2001, Rosser, 2004). The most often isolated bacteria include *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Escherichia coli*, *Klebsiella pneumoniae,* and *Enterobacter* spp. (De Martino *et al.,* 2016; Petrov *et al.,* 2019). Treatment of otitis externa usually involves antibiotic therapy. But day by day, due to overuse or misuse of antibiotics, there is an emergence of antibiotic resistance (AMR). Thus, detection of antibiotic resistance genes (ARGs) amongst the bacterial isolates has become a must. The antibiotic resistance genes cause multiple drug resistance in bacteria. The present study aimed to determine the prevalence of otitis externa in dogs, isolate and identify bacterial agents from infected and healthy ears of dogs, determine *in vitro* antibiotic susceptibility pattern of isolates, and detect ARGs in the bacterial isolates using polymerase chain reaction (PCR).

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Materials and Methods

Collection of Ear Swabs from Otitic and Healthy Dogs

The study was performed from April 2019 to March 2020. Before sample collection, the ears of dogs were appropriately cleaned with betadine. Total 124 samples were collected aseptically using sterile ear swabs. Out of which 94 samples

were from 75 dogs suffering from otitis externa and 30 from healthy ones, from both genders, from different breeds, and from 5 months to 15 years old, all with clinical signs specific for otitis externa. Dogs were presented with unilateral as well as bilateral otitis externa.

Microbiological Examination

The identification of bacterial isolates was made as per the method described by Markey *et al*. (2013). For bacteriological culture examination, ear swab samples were processed in aseptic conditions and streaked using the sterile platinum loop on 5% sheep blood agar (BA) for primary bacterial isolation, and the plates were incubated at 37°C for 24-48 h. After the incubation, the plates were examined for bacterial growth, and the morphological characteristics of bacterial colonies were noted. The bacteria were identified using Grams staining, production of pigments, presence of hemolysis, primary biochemical test (catalase, KOH, and oxidase tests), sugar fermentation test, and Indole, Methyl-red, Voges-Proskauer, and Citrate test (IMViC).

In-Vitro Antibiogram Pattern of Bacterial Isolates

The *in vitro* antibiotic susceptibility tests (ABST) of the isolates were conducted as per the method of Bauer (1996), and interpretation of results was made according to the Clinical and Laboratory Standards Institute (2019). The antibiotics used were ampicillin (10 mcg), amoxicillin-clavulanic acid (30 mcg), gentamicin (10 mcg), amikacin (30 mcg), tobramycin (10 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), enrofloxacin (10 mcg), moxifloxacin (5 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg) and norfloxacin (10 mcg). Additionally, for *Staphylococcus* spp. Isolates antibiotic drugs methicillin (5 mcg) and oxacillin (1 mcg) were used.

PCR

The template DNA was extracted by boiling method from total of 129 bacterial isolates obtained from otitic and healthy ears of dogs. It was subjected to the detection of various ARGs, *viz.,* quinolone resistance genes [*qnrA, qnrB, qnrS* (Robicsek *et al.,* 2006)], aminoglycoside resistance genes [*aac(3)-1V, aadAl* (Van *et al.,* 2008)] and beta-lactam resistance genes [$bla_{OXA,}$ $bla_{SHV,}$ and bla_{TEM} (Shehata *et al.,* 2016). and bla_{CMY} (Van *et al.,* 2008)]. The extracted DNA of *Staphylococcus* spp. isolates were subjected to PCR for detection of one ARG, *viz.,* resistance to methicillin (*mecA)* (Louie *et al.,* 2002). The primers used PCR conditions, and the expected size of PCR product for ARGs was as per the protocols described by respective workers. Agarose gel electrophoresis was carried out to confirm the targeted amplification where PCR products from each tube were electrophoresed along with 100 bp DNA molecular weight marker (Thermo Fisher) on 2.0 % agarose (low EEO, SeaKem, USA) gels containing 0.5 µg/ml ethidium bromide (Sigma-Aldrich, USA) at 80 V in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by a gel documentation system (Genetix Biotech Pvt. Ltd., Delhi).

Results and Discussion

Among 75 dogs suffering from otitis externa and 15 healthy dogs screened from the regions of Anand and Vadodara districts of Gujarat, the breed wise prevalence percentage of otitis was highest in Labrador (36.00%) followed by Non-descriptive (25.33%), German Shepherd (16.00%), Pomeranian (6.67%), Cocker Spaniel (5.33%), Rottweiler (4.00%), Daschund (2.67%), Beagle, Doberman and Saint Bernard (1.33%). The age-wise prevalence was highest in 0-4 years age group (53.33%) followed by 5-10 years (40.00%) and 11-15 years (6.66%). The sex-wise prevalence was higher in males (54.67%) than in females (45.33%). The prevalence of otitis was 38.67% in the left ear and 36.00% in the right ear. The bilateral prevalence was 25.33%. Subapriya *et al.* (2015) and Parmar *et al*. (2020) also reported more or less very similar prevalence of otitis externa in dogs concerning the breed, age, and sex.

In the present study, out of 94 ear swabs collected from otitic ears, 86 (91.49%) ear swabs were found positive for bacteria. Based upon morphological, cultural, and biochemical characters, a total of 113 bacteria isolated from otitic ears were *Staphylococcus* spp. (58.40%, 66/113), *Pseudomonas* spp. (10.62%, 12/113), *Proteus* spp. (10.62%, 12/113), *Streptococcus* spp. (7.08%, 8/113), *Corynebacterium* spp. (6.19%, 7/113), *E. coli* (4.42%, 5/113), *Klebsiella* spp. (1.77%, 2/113) and *Enterobacter* spp. (0.88%, 1/113). For 30 ear swabs collected from healthy ears, 12 (40.00%) ear swab samples were found positive for bacterial isolation. Bacteria isolated were *Staphylococcus* spp. (87.50%, 14/16) and *Proteus* spp. (12.5%, 2/16). Similarly, from the dogs with otitis externa, Malayeri *et al.* (2010) isolated *Staphylococcus* spp. (83.70%), *Pseudomonas* spp. (10.87%), *Proteus* spp. (3.26%) and *E. coli* (1.09%); Demirbilek and Yilmaz (2019) isolated *Staphylococcus* spp.(18.8%), *Pseudomonas* spp. (13.02%), *Proteus* spp. (9.4%), *Corynebacterium* spp. (5.48%), *Streptococcus* spp. (5%), *E. coli* (2.9%) and *Enterobacter* spp. (0.96%) and Petrov *et al.* (2019) isolated *Staphylococcus* spp. (39.38%), *P. aeruginosa* (16.24%), *Proteus* spp. (3.56%), *E. coli* (3.17%), *Streptococcus* spp. (2.97%) and *Corynebacterium* spp. (0.79%). In dogs with healthy ears, reported bacteria were *Staphylococcus* spp. (58.8%), *Streptococcus canis* (29.9%), *Proteus* spp. (14.4%) and *E. coli* (10.3%) (Lyskova *et al.,* 2007).

In our study, out of a total of 80 staphylococci isolates (66 from otitic and 14 from healthy ears), 6.25% (5/80) showed yellow pigmentation, while 93.75% (75/80) showed white pigmentation on nutrient agar. On the 5% SBA, 10.00, 82.50, and 7.50% isolates showed complete, partial, and no β-hemolysis. Sugar fermentation test revealed 18.75% (15/80) isolates of *Staphylococcus* spp. with acid production from mannitol, maltose, mannose, and trehalose, whereas negative for xylose fermentation were identified as *Staphylococcus*

aureus. Moreover, 81.25% (65/80) isolates of *Staphylococcus* spp. revealed acid production from maltose, mannose, and trehalose, whereas negative for mannitol and xylose were identified as *Staphylococcus pseudintermedius*. Among 8 streptococci isolates, 25% isolates produced β-hemolysis, and 75% isolates produced no hemolysis on 5% SBA. *Pseudomonas* spp. produced diffusible pigmentation on nutrient agar, *viz.,* 58.33% (7/12) isolates produced pyoverdin (greenish-yellow), 33.33% (4/12) isolates produced pyocyanin (blue-green), and 8.33% (1/12) isolate produced pyomelanin (brownish-black) pigments and were positive for oxidase test. *Proteus* spp. produced swarming growth on nutrient agar. *E. coli* isolates showed IMViC pattern "++--" and *Klebsiella* spp. and *Enterobacter* spp. showed IMViC pattern "--++."

Overall, the most effective antibiotic found was tobramycin (98.49%) followed by enrofloxacin (90.67%), ofloxacin, amikacin, amoxycillin clavulanic acid, gentamicin (81.00 to 87.00%) norfloxacin, moxifloxacin, cefotaxime, ciprofloxacin, ceftriaxone (70.00 to 75.00%) and ampicillin (63.90%) having the highest resistance. (Table 1). In an earlier study, bacterial isolates showed 80.00% susceptibility towards amoxycillin clavulanic acid, enrofloxacin, and ofloxacin (Lyskova *et al.,* 2007). Demirbilek and Yilmaz (2019) showed higher susceptibility towards amikacin (77.00%), gentamicin, and tobramycin (72.00% each) but contradictory to the present study, high resistance towards amoxycillin clavulanic acid (45.00%) and enrofloxacin (47%). Petrov *et al.* (2019) recorded higher susceptibility towards amikacin, tobramycin, and gentamicin (79.20 to 70.20%), while in contradiction to current research, higher resistance towards amoxycillin clavulanic acid (56.40%) and enrofloxacin (38.20%) was reported. However, Malayeri *et al.* (2010) and Bugden (2013) too reported higher resistance towards amoxycillin clavulanic acid (55.03%) and enrofloxacin (29.00%), respectively.

Table 1: Overall antibiotic susceptibility and resistance of bacterial isolates (n = 129)

Sr. No.	*Antibiotics*	*Susceptibility (%)*	*Intermediate (%)*	*Resistance (%)*
1.	Ampicillin	36.09	-	63.90
2.	Amoxycillin clavulanic acid	82.44	-	17.53
3.	Ceftriaxone	69.42	2.24	28.34
4.	Cefotaxime	70.77	5.63	22.98
5.	Gentamicin	81.44	1.51	17.07
6.	Amikacin	84.64	0.16	15.20
7.	Tobramycin	98.49	-	1.51
8.	Enrofloxacin	90.67	3.80	5.52
9.	Moxifloxacin	73.47	0.63	25.90
10.	Ofloxacin	87.55	0.49	11.98
11.	Ciprofloxacin	70.61	5.63	23.77
12.	Norfloxacin	75.52	0.47	24.01

The multidrug resistance (MDR) was detected in 29.46% (34/129) of the total bacterial isolates. In the present study, 1.55% of isolates showed resistance to two drugs, 7.75% to three drugs, 6.20% to four drugs, 5.23% to five drugs, 1.55% to six drugs, 3.88% to seven drugs, 1.55% to nine drugs, 0.78% to ten drugs and 0.78% to twelve drugs (Table 2). MDR was detected in *Staphylococcus* spp., *Corynebacterium* spp., *E. coli, Enterobacter* spp. and *Pseudomonas* spp., but not in *Streptococcus* spp., *Klebsiella* spp. and *Proteus* spp.

In the present study, out of 80 isolates of *Staphylococcus* spp. 11.25% (9/80) of the isolates showed resistance to methicillin and oxacillin via ABST, but via PCR *mecA* couldn't be detected in any of those isolates. Ozturk *et al.* (2010) also phenotypically (ABST) isolated 9.25% of the *Staphylococcus* isolates methicillin resistant, but weren't able to detect *mecA* gene. Similarly, Findik *et al.* (2009) isolated 80 methicillin resistant *Staphylococcus* spp. via ABST, but only 3.75% (3) isolates were detected positive for the presence of *mecA* gene. Yoon *et al.* (2010), Dziva *et al.* (2015) and Njoroge *et al.* (2016) detected *mecA* in 17.60, 8.69, and 13.00 % of oxacillin resistant strains, respectively.

Beta-lactam resistance gene, bla_{SHV} was detected in 60.00% (3/5) isolates of *Escherichia coli*, 100% isolates of *Klebsiella* spp. (2/2), and *Enterobacter* spp. (1/1), and 75.00% (8/12) isolates of *Pseudomonas* spp. (Fig. 1); bla_{OXA} was detected in 40.00% (2/5) isolates of *Escherichia coli* and

Table 2: Detection of Multidrug Resistance Isolates

Sr. No.	*Isolates resistant to*	*Overall isolates*	*Isolates*				
			Staphylococcus spp.	*Corynebacterium spp.*	*E. coli*	*Enterobacter spp.*	*Pseudomonas spp.*
1.	Two drugs	1.55% (2/129)	-	-	-	-	16.67% (2/12)
2.	Three drugs	7.75% (10/129)	6.25% (5/80)	-	-	-	41.67% (5/12)
3.	Four drugs	6.20% (8/129)	5% (4/80)	% 42.85(3/7)	20% (1/5)	-	-
4.	Five drugs	5.23% (7/129)	5% (4/80)	% 14.29(1/7)	20% (1/5)	-	8.33% (1/12)
5.	Six drugs	1.55% (2/129)	1.25% (1/80)	-	-	-	8.33% (1/12)
6.	Seven drugs	3.88% (5/129)	1.25% (1/80)	14.29% (1/7)	20% (1/5)	% (1/1)	8.33% (1/12)
7.	Nine drugs	1.55% (2/129)	2.5% (2/80)	-	-	-	-
8.	Ten drugs	0.78% (1/129)	1.25% (1/80)	-	-	-	-
9.	Twelve drugs	0.78% (1/129)		-	-	-	8.33% (1/12)

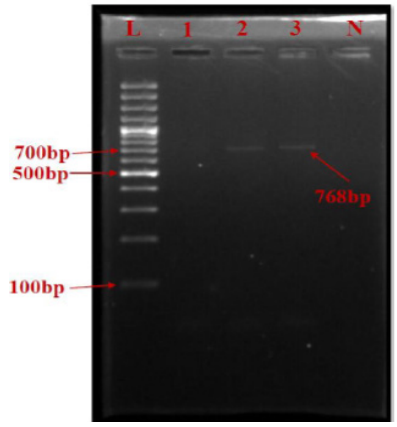

Fig. 1: Agarose gel showing amplified product for bla_{SHV}(768bp)for bacterial isolates
L- 100-1500bp N-Negative, 1,2- bacterial isolates

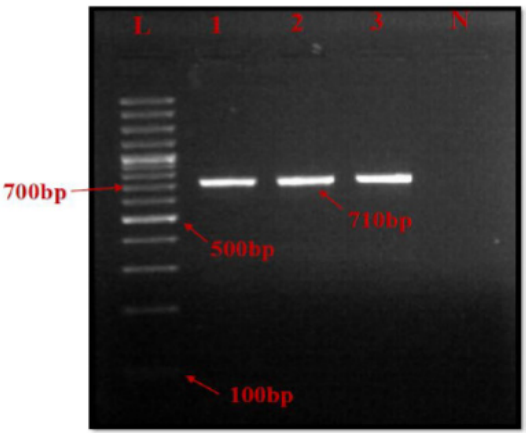

Fig. 2: Agarose gel showing amplified product for bla_{OXA} (710bp) for bacterial isolates
L- 100-1500bp N-Negative, 1-3- bacterial isolates

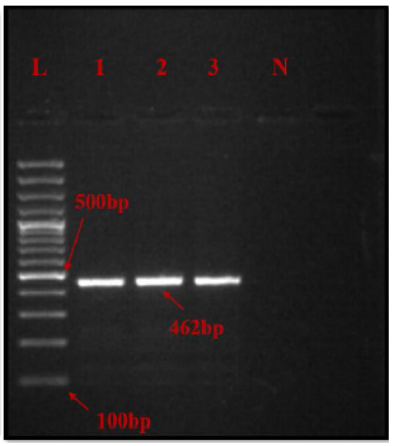

Fig. 3: Agarose gel showing amplified product for bla_{CMY} (462bp) for bacterial isolates
L- 100-1500bp N-Negative, 1-3- bacterial isolates

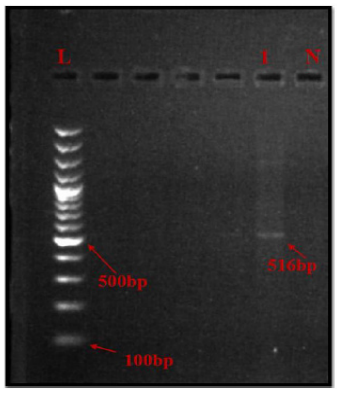

Fig. 4: Agarose gel showing amplified product for *qnrA* (516bp) for bacterial isolates
L- 100-1500bp N-Negative, 1- bacterial isolate

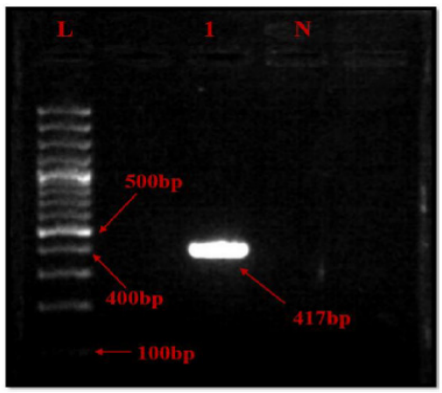

Fig. 5: Agarose gel showing amplified product for *qnrS* (417bp) for bacterial isolates
L- 100-1500bp N-Negative, 1- bacterial isolate

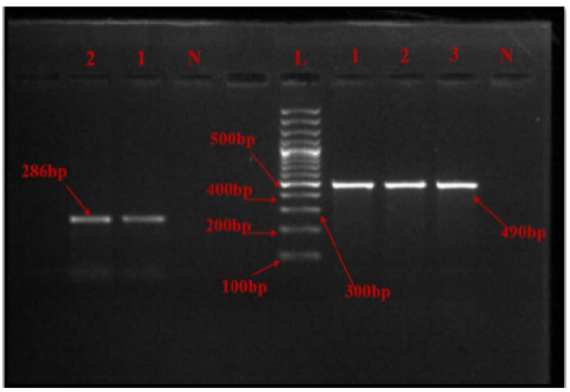

Fig. 6: Agarose gel showing amplified product for *aac(3) -IV* (286bp) and *aadA1* (490bp) for bacterial isolates
L- 100-1500bp N-Negative,
1-3- bacterial isolates

8.33% (1/12) isolates of *Pseudomonas* spp. (Fig. 2) and bla_{CMY} was detected in 80.00% (4/5) isolates of *Escherichia coli* and 50.00% (1/2) isolates of *Klebsiella* spp. (Fig. 3). However, bla_{TEM} couldn't be detected in any of the bacterial isolates. Similarly, Chikwendu *et al.* (2011) identified bla_{SHV} in 42.90% isolates of *Pseudomonas* spp.; Lina *et al.* (2014) identified bla_{OXA} in 47.50% isolates of *E. coli*; Ojdana *et al.*(2014) identified bla_{SHV} in 100.00% isolates of *Klebsiella* spp.; Nematolahi *et al.* (2016) identified bla_{SHV} in 11.11% isolates of *Enterobacter* spp.; Shehata *et al.* (2016) identified bla_{SHV} and bla_{OXA} in 60.00% and 80.00% isolates of *E. coli,* respectively; Alam *et al.*(2018) identified bla_{SHV} and bla_{OXA} in 52.40% and 90.50% isolates of *Pseudomonas* spp. respectively, and Rizi *et al.* (2020) identified bla_{CMY} in total of 16.7% isolates of *E. coli* and *Klebsiella* spp. Further, Chikwendu *et al.* (2011) identified bla_{TEM} in 42.90% isolates of *Pseudomonas* spp.; Lina *et al.* (2014) identified bla_{TEM} in 82.50% isolates of *E. coli*; Ojdana *et al.* (2014) identified bla_{TEM} in 16.67%, 100.00%, and 91.67% isolates of *E. coli, Klebsiella* spp. and *Proteus mirabilis,* respectively.

Quinolone resistance gene, *qnrA,* and *qnrS* were detected in 20% (1/5) isolates each of *E. coli* (Fig. 4, 5). But *qnrB* couldn't be detected in any of the bacterial isolates. Similar findings were reported by Diwan *et al.*(2012), who identified *qnrA* in 3.33% isolates of *E. coli* and Lina *et al.* (2014), who identified *qnrS* in 12.50% isolates of *E. coli*., while Diwan *et al.*(2012) identified *qnrB* in 6.67% isolates of *E. coli.*

Aminoglycoside resistance genes, *aadA1* was detected in 60.00% (3/5) isolates of *E. coli,* and *aac (3)-1V* was detected in 80.00% (4/5) isolates of *E. coli*, 21.43% (3/14) isolates of *Proteus* spp. and 2.5% (2/80) isolates of *Staphylococcus* spp. (Fig. 6). Similar findings were reported by Torkan *et al.* (2016), who identified *aadA1* in 35.7% isolates and *aac (3)-1V* in 28.6% isolates of *E. coli.*

Conclusions

In the present study, the most predominant bacteria were *Staphylococcus* spp. (51.60%) followed by *Pseudomonas* spp. (9.37%), *Proteus* spp. (9.37%), *Streptococcus* spp. (6.25%),

Corynebacterium spp. (5.47%), *E. coli* (3.91%), *Klebsiella* spp. (1.56%) and *Enterobacter* spp. (0.78%). From healthy dogs, the predominant bacteria isolated were *Staphylococcus* spp. (63.64%) and *Proteus* spp. (9.09%). Antibiotic susceptibility test revealed that the isolates were most sensitive to antibiotics tobramycin (98.49%), enrofloxacin (90.67%), and ofloxacin (87.55%), while most resistant to ampicillin (63.90%). The bacterial isolates showed multidrug resistance in 29.46% (34/129) for two to nine antibiotics. The ARGs were detected in most of the Gram-negative bacteria.

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References

Alam, A.N., Sarvari. J., Motamedifar. M., Khoshkharam, H., Yousefi, M., Moniri, R., & Bazargani. A. (2018). The occurrence of bla_{TEM}, bla_{SHV} and bla_{OXA} genotypes in Extended-Spectrum β-Lactamase (ESBL)-producing *Pseudomonas aeruginosa* strains in Southwest of Iran. *Gene Reports, 13,* 19-23.

Bauer, A.W. (1996). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology, 45,* 149-158.

Boda, C., Liège, P., & Rème, C.A. (2011). Evaluation of owner compliance with topical treatment of acute otitis externa in dogs: A comparative study of two auricular formulations. *International Journal of Applied Research in Veterinary Medicine, 9(2),* 157.

Bugden, D.L. (2013). Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Australian Veterinary Journal, 91(1-2),* 43-46.

Chikwendu, C.I., Ibe, S.N., & Okpokwasili, G.C. (2011). Detection of bla_{SHV} and bla_{TEM} beta-lactamase genes in multi-resistant *Pseudomonas* isolates from environmental sources. *African Journal of Microbiology Research, 5(15),* 2067-2074.

De Martino, L., Nocera, F.P., Mallardo, K., Nizza, S., Masturzo, E., Fiorito, F., & Catalanotti, P. (2016). An update on microbiological causes of canine otitis externa in Campania region, Italy. *Asian Pacific Journal of Tropical Biomedicine, 6(5),* 384- 389.

Demirbilek, S.K., & Yilmaz, O. (2019). Identification and antibiotic susceptibility of microbial agents of otitis externa in dogs. *Medycyna Weterynaryjna-Veterinary Medicine-Science and Practice, 75(2),*107-110.

Diwan, V., Chandran, S.P., Tamhankar, A.J., StålsbyLundborg, C., & Macaden, R. (2012). Identification of extended-spectrum β-lactamase and quinolone resistance genes in *Escherichia coli* isolated from hospital wastewater from central India. *Journal of Antibiotic Chemotherapy, 67(4),* 857-859.

Dziva, F., Wint, C., Auguste, T., Heeraman, C., Dacon, C., Yu, P., & Koma, L.M. (2015). First identification of methicillin-resistant *Staphylococcus pseudintermedius* strains among coagulase-positive staphylococci isolated from dogs with otitis externa in Trinidad, West Indies. *Infection Ecology and Epidemiology, 5(1),* 29170.

Fernández, G., Barboza, G., Villalobos, A., Parra, O., Finol. G., & Ramírez, R. (2006). Isolation and identification of microorganisms present in 53 dogs suffering otitis externa. *Revista Cientifica, 16(1),* 23-30.

Findik A, Akan N, Onuk EE, Çakiroğlu DandCiftci A.(2009). Methicillin resistance profile and molecular typing of *Staphylococcus aureus* strains isolated from noses of the healthy dogs. *KafkasUniv Vet FakDerg ,15,* 925-930.

Kumar, K.S., Selvaraj, P., Vairamuthu, S., Shammi, M., & Kathiseran, D. (2010). Antibiogram pattern of microbes isolated from otitis externa of dogs. *Tamil Nadu: Tamilnadu Journal Veterinary and Animal Sciences, 6(3),* 145-147.

Lina T T, Khajanchi B K, Azmi I J, Islam M A, Mahmood B, Akter M ... andCravioto A.(2014). Phenotypic and molecular characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* in Bangladesh. *PloS one, 9(10),* e108735.

Louie, L., Goodfellow, J., Mathieu, P., Glatt, A., Louie, M., & Simor, A.E. (2002). Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *Journal of Clinical Microbiology, 40(8),* 2786-2790.

Lyskova, P., Vydrzalova, M., & Mazurova, J. (2007). Identification and antibiotic susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. *Journal of Veterinary Medicine Series A, 54(10),* 559-563.

Malayeri, H.Z., Jamshidi, S., & Salehi, T.Z. (2010). Identification and antibiotic susceptibility patterns of bacteria causing otitis externa in dogs. *Veterinary Research Communications, 34(5),* 435-444.

Markey B, Leonard F, Archambault M, Cullinane AandMaguire D. (2013). Clinical veterinary microbiology e-book. Elsevier Health Sciences, Edn. 2nd , pp: 9-47.

Nematolahi, S., Mosadegh, A., Mardaneh, J., & Poorabbas, B. (2016). Identification of ESBL-producing and blaSHV gene Harboring *Enterobacter* spp. Isolated from bloodstream infections of hospitalized patients during 10 Years in South of Iran (Shiraz). *Iranian South Medical Journal, 19(4),* 536-548.

Njoroge, C., Mande, J.D., Mitema, S.E., Kitaa, J.M.A. (2016). Phenotypic and molecular characterization of methicillin resistant *Staphylococcus aureus* from surgical patients and normal dogs. *Doctoral dissertation,* University of Nairobi, Nairobi.

Ojdana, D., Sacha, P., Wieczorek, P., Czaban, S., Michalska, A., Jaworowska, J., & Tryniszewska, E. (2014). The Occurrence of bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes in extended-spectrum β-lactamase-positive strains of *Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis* in Poland. *International Journal of Antibiotics.https://doi.org/10.1155/2014/935842.*

Ozturk, D., Avki, S., Turutoglu, H., Yigitarslan, K., & Sagnak, S. (2010). Methicillin resistance among coagulase-positive staphylococci isolated from dogs with otitis externa, skin wounds and pyoderma.*KafkasU¨niversitesiVeterinerFaku¨ltesiDergisi,16(4),* 651-656.

Parmar, J.J., Neha Rao, Shah, A.I., Sadhu, D.B., Bhanderi, B.B., & Patel, D.M. (2020). Clinical studies on ear infections, microbiological evaluation and therapeutic management in canines. *Interantional Journal of Current Microbiology and Applied Science, 9(01),*1496-1501.

Petrov, V., Zhelev, G., Marutsov, P., Koev, K., Georgieva, S., Toneva, I., & Urumova, V. (2019). Microbiological and antibacterial resistance profile in canine otitis externa-A comparative analysis. *Bulgarian Journal of Veterinary Medicine, 22(4), https://doi.org/10.15547/ bjvm.2151.*

Rizi, K.S., Mosavat, A., Youssefi, M., Jamehdar, S.A., Ghazvini, K., Safdari, H., & Farsiani, H. (2020). High prevalence of bla_{CMY}AmpC Beta-Lactamase in ESBL co-producing *Escherichia coli* and *Klebsiella* spp. clinical isolates in Northeast of Iran. *Journal of Global Antibiotic Resistance,https://doi.org/10.1016/j.jgar.2020.03.011*

Robicsek, A., Jacoby, G.A., & Hooper, D.C. (2006). The worldwide emergence of plasmid-mediated quinolone resistance. *The Lancet Infectious Diseases,* 6(10), 629-640.

Rosser, E.J. (2004). Causes of otitis externa. *Veterinary Clinics: Small Animal Practice, 34(2),* 459-468.

Scott, D.W., Miller, W.H., & Griffin, C.E. (2001). *Muller and Kirk's Small Animal Dermatology*. diseases of eyelids, claws, anal sacs, and ears. 6th edition, Philadelphia: W.B. Saunders, 1203-1232.

Shehata, M.E., Gamal, M., Amira, H.M., & Hesham, M.S. (2016). Molecular and phenotypic characterization of some antibiotic resistance genes in *Escherichia coli* isolated from human and Broiler Chickens. *International Journal of Current Microbiology and Applied Sciences, 5,* 953-965.

Subapriya, S., Senthil, N.R., Vairamuthu, S., Nagarajan, B., Kavitha, S., & George, R.S. (2015). A study on microbial profile and trend in antibiotic susceptibility of canine otitis. *International Journal of Livestock Research, 5(2),* 43-48.

Torkan, S., Bahadoranian, M.A., Khamesipour, F., & Anyanwu, M.U. (2016). Detection of virulence and antibiotic resistance genes in *Escherichia coli* isolates from diarrhoiec dogs in Iran. *Archivos de MedicinaVeterinaria, 48(2),* 181-190.

Van, T.T.H., Chin, J., Chapman, T., Tran, L.T., & Coloe, P.J. (2008). Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *International Journal of Food Microbiology, 124(3),* 217-223.

Wayne, P. A. (2019). Clinical and laboratory standards institute; (2019). *Performance standards for antimicrobial susceptibility testing. CLSI document M100-S17.*

Yoon, J.W., Lee, K.J., Lee, S.Y., Chae, M.J., Park, J.K., Yoo, J.H., & Park, H.M. (2010). Antibiotic resistance profiles of *Staphylococcus pseudintermedius* isolates from canine patients in Korea. *Journal of Microbiology Biotechnology, 20(12),* 1764-8.