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Isolation and Identification of *Corynebacterium* Species from cases of skin and ear infection of dogs

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Abstract

The present study was carried out to investigate role of *Corynebacterium* bacteria in dogs suffering from skin infection and ear infection. Total 100 samples comprising 70 samples of skin and 30 samples of ear collected from dogs showed skin lesions and otitis externa, respectively. *Corynebacterium* spp. was isolated and identified on the basis of cultural characteristics, staining and at molecular level by PCR. The involvement of *Corynebacterium* spp. was observed as 7.14% and 6.66% in skin samples and ear samples, respectively. *In vitro* antibiotic susceptibility testing of bacterial isolates showed higher sensitivity against amikacin (85.71%), cefpodoxime (85.71%), enrofloxacin (85.71%) and ceftriaxone (71.42%), while higher resistance was recorded against amoxycylav (100%), clindamycin (71.42%) and erythromycin (71.42%).

Keywords: *Corynebacterium*, otitis, PCR, skin infection

Introduction

Skin infection also known as bacterial pyoderma and otitis externa are most commonly encountered problems in small animal practices (Chitra *et al.*, 2015 [5]). Pyoderma is the most frequent bacterial disease observed in dogs and considered as frustrating problem in pet animals which has public health significance also. Bacteria like *Staphylococcus*, *micrococcus* and *Corynebacterium* are normally found on the dog's skin (Cerasela, 2013 [4]). The skin infection in dogs requires much attention due to its complex etiological agents, treatment cost and management aspects. Therefore the identification of main etiological agent is main step for successful therapeutic treatment.

Occurrence of otitis externa in dogs is mainly associated with normal flora of ear canal that includes various bacteria viz., *Staphylococcus*, *Pseudomonas* as well as yeast like *Malassezia pachydermatis*. From this, *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Streptococcus canis* are most commonly reported organisms in canine otitis externa infection (Aalbaek *et al.*, 2010) [1]. *Corynebacterium* is considered as normal bacterial flora of the skin in dogs (Angus, 2004) [2]. Involvement of this bacterium has been reported occasionally from cases of canine otitis (Graham-Mize and Rosser, 2004) [7].

The prevalence of antimicrobial resistant bacteria has been increasing over the years and it is highly associated with the overuse and misuse of antibiotics in human and veterinary medicine, agriculture and industry. Furthermore, multidrug-resistant bacteria have been increasingly reported as a cause of infections, which makes this issue a major concern in clinical practice worldwide (Syed, 2019; Christaki *et al.*, 2020) [11, 6].

Bacteria in the genus *Corynebacterium* are Gram-positive rods, facultative anaerobes, and catalase positive and oxidase negative. The rods appear straight to slightly curved with tapered ends, some of which may exhibit a clubbed shape (Boynosky and Stokking, 2015) [3].

Though *Corynebacterium* is a commensal bacteria found on skin as well as ear canal of dogs, very little information is available regarding involvement of this bacteria in both pyoderma and ear infection cases of dogs. Therefore present study was planned to determine involvement of *Corynebacterium* from cases of dogs suffering from pyoderma and otitis externa infection.

Materials and Methods

Sample Collection

In present study 70 skin swabs from dogs showed various pyoderma lesions like pustule, vesicle, nodules and 30 ear swabs from dog suffering from otitis externa were aseptically collected from Veterinary Clinical Complex, Veterinary College, Navsari and transported to the Microbiology Department.

Cultural isolation and identification of *Corynebacterium* spp

For primary isolation of bacterial agents, all the samples were streaked on 5 % sheep blood agar and incubated aerobically at 37 °C for 18- 24 hrs. Colonial morphology characters and gram staining technique was employed for preliminary identification of bacterial agents.

Molecular detection of *Corynebacterium* spp

For molecular confirmation of *Corynebacterium*, *rpoB* gene specific primer previously described by Torres *et al.*, 2015 [12] were used. Briefly, DNA extraction from bacterial colony was carried out by manual heating and chilling method as per Chitra *et al.*, 2015 [5]. For PCR, reaction mixture was prepared in 25 µl quantity containing 3.0 µl template DNA, 12.5 µl of 2x PCR master mix, 1.0 µl of forward (*rpoB*-F-CGTATGAACATCGGCCAGGT) and 1.0 µl of reverse primer (*rpoB*-R- TCCATTTCCGCCGAAGCGCTG) and 7.5 µl sterile nuclease-free water. Cycling condition was set as initial denaturation at 95 °C for 10 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 45 s with a final extension step at 72 °C for 10 min. PCR product was visualized after electrophoresis using 1.5% agarose gel and with UV Transilluminator (SynGene, UK).

Antibiotic susceptibility testing of *Corynebacterium* spp

In vitro antibiotic-susceptibility profile of *Corynebacterium* isolates was performed using the disc diffusion method as per standard protocol. Amikacin (30 µg), Amoxyclav (10 µg), Cefpodoxime (10 µg), Clindamycin (2 µg), ceftriaxone (30 µg), erythromycin (15 µg), and Enrofloxacin(10µg) antibiotics were applied on Mueller-Hinton agar plate as per standard method and plate was aerobically incubated at 37 °C for 18-24 hrs. Next day, zone of inhibition around antibiotic disc was measured and interpreted as susceptible, intermediate or resistant.

Results

Out of 70 Skin swab samples 5 (7.14%) and out of 30 ear swab, 2(6.66%) samples showed pale yellow colonies on blood agar (Figure 1). Microscopically, Gram's staining of colonies revealed typical Chinese letter appearance (Figure 2) which is presumptively considered as a *Corynebacterium* spp. All the isolates were found positive for catalase while negative for oxidase test.

In PCR, all 7 *Corynebacterium* isolates were amplified at 445 bp for *rpoB* gene and molecularly confirmed as a *Corynebacterium* spp. (Figure 3).

In - vitro antibiogram of all the *Corynebacterium* spp. isolated is recorded as per Table 1. Higher sensitivity was observed against against amikacin (85.71%), cefpodoxime (85.71%), enrofloxacin (85.71%) and ceftriaxone (71.42%), while higher resistance was recorded against amoxyclav (100%), clindamycin (71.42%) and erythromycin (71.42%).

Table 1: Antibiotic sensitivity pattern of *Corynebacterium* spp. (N=7)

Antibiotic	Sensitive	Resistant
Amikacin (30 µg)	6(85.71%)	1(14.28%)
Amoxyclav (10 µg)	0(0%)	7(100%)
Cefpodoxime (10 µg)	6(85.71%)	1(14.28%)
Clindamycin (2 µg)	2(28.57%)	5(71.42%)
Ceftriaxone (30 µg)	5(71.42%)	2(28.57%)
Erythromycin (15 µg)	2(28.57%)	5(71.42%)
Enrofloxacin (10µg)	6(85.71%)	1(14.28%)

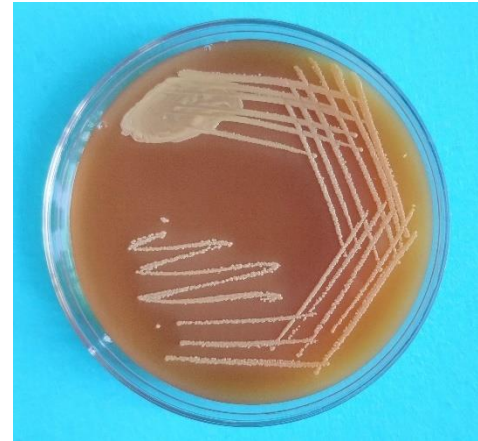


Fig 1: *Corynebacterium* spp. on blood agar

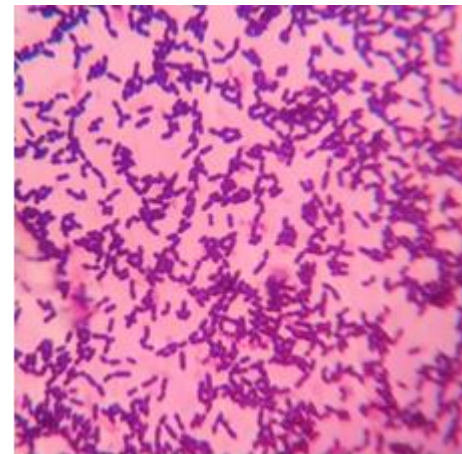


Fig 2: Chinese letter appearance under Microscope

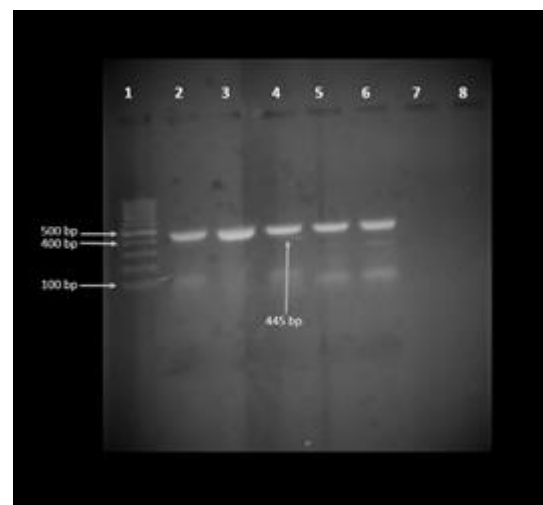


Fig 3: *rpoB* gene specific PCR of *Corynebacterium* spp.

Discussion

Multiple studies have investigated the role of *Corynebacterium* spp. in canine otitis externa, little information has been reported about the involvement/function of these organisms in canine dermatitis (Henneveld *et al.*, 2012; Aalbaek *et al.*, 2010; Boynosky & Stokking, 2015) [8, 1, 3].

In the present study overall involvement of *Corynebacterium* in canine dermatitis and otitis externa was found as 7.0 % (7 out of 100). Involvement of in skin infection was reported as 7.14 % and in ear infection as 6.66 %. Henneveld *et al.* 2012 [8] found 16% involvement of this bacteria for otitis externa in dogs. Many others have reported various coryneform bacteria in samples from canine otitis externa (Graham-Mize and Rosser, 2004; Zdovc, 2004; Aalbaek *et al.*, 2010) [7, 13, 11].

All the isolates of *Corynebacterium* showed 100 % resistance for beta lactam antimicrobials in this study. These results were in agreement with those from earlier reports of antibiogram pattern of *Corynebacterium* spp. in which resistance to beta lactam antimicrobials was common (Henneveld *et al.*, 2012; Soriano *et al.*, 1995) [8, 9].

The antibiogram study for the *Corynebacterium* isolates for this study as compared with previous study showed contrast finding for some antibiotics. In the present study sensitivity for erythromycin was found as 28.57 %, in contrast to this Boynosky & Stokking, 2015 [3] reported sensitivity for erythromycin as 74 %.

As Coryneform bacteria are regarded as part of the normal flora of the skin in dogs (Angus, 2004 [2]) and many have reported role of specific species like *C. auriscanis* in dermatitis (Boynosky & Stokking, 2015) [3] and otitis externa infection (Aalbaek *et al.*, 2010) [11], Further species level identification of *Corynebacterium* is necessary to investigate role of major species involvement in canine skin and ear infection.

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