CHARACTERIZATION OF *Staphylococcus aureus* ISOLATED FROM DOGS WITH CORNEAL DISEASES

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Abstract

Corneal swabs were brought to the Department of Veterinary Microbiology from Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Mannuthy for cultural isolation and identification. By biochemical characterization, the most predominant organism was identified as Staphylococcus aureus. The isolate was subjected to molecular confirmation by Polymerase Chain Reaction.

Keywords: *Corneal swabs, Staphylococcus aureus, PCR*

Dogs are considered as the best companion animal. In case of corneal disease conditions, the organisms that are thought to be of ocular commensals may become the pathogens. The corneal pathologic conditions are usually regarded as secondary rather than primary, so the resolution is best achieved by identification and treatment of primary cause. The present investigation was, therefore, planned for the isolation and identification of predominant bacterial pathogens associated with corneal diseases in dogs.

Materials and Methods

A total of 25 corneal swabs were collected from Teaching Veterinary Clinical complex, College of Veterinary and Animal Sciences, Mannuthy. Sterile swabs were used for the collection of samples. Samples were cultured onto Brain Heart Infusion Agar (BHIA), blood agar (BA) and incubated at 37 ºC under aerobic condition. After incubation, the colonies obtained from both the medium were subjected to Gram's stain and were subcultured on BHIA. The pure culture colonies were streaked in potassium tellurite blood agar and mannitol salt agar and incubated overnight at 37 ºC. The isolates were subjected to characterization by cultural, morphological and biochemical tests as per Quinn *et al*. (1994).

Polymerase Chain Reaction (PCR) was carried out using genus specific 16S rRNA gene primers (Forward primer – TAACGGCTTACCAAGGCAAC and Reverse primer - TAATTCCGGATAACGCTTGC) with amplicon size of 303 bp, for confirmation of the isolates. For *Staphylococcus,* PCR mixture was formulated (Table 1) and reaction was performed with the given protocol (Table 2).

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Table 1. Composition of PCR mixture for the detection of *Staphylococcus*

Component	Quantity (µL)
10 X PCR Taq buffer (with $MgCl_2$)	2.50
dNTP (2.5 mM each)	1.00
Taq polymerase (3U/µL)	0.33
DNA template	5.00
Primer: F (10 pmol/µL)	0.50
Primer: R (10 pmol/µL)	0.50
Nuclease free water	15.17
Total	25.00

Table 2. PCR protocol for the detection of Staphylococcus

Steps	Temperature	Time	Cycles
Initial denaturation	94ºC	4 min	1
Denaturation	94ºC	45 sec	30
Annealing	57ºC	45 sec	
Extension	72ºC	50 sec	
Post extension	72ºC	10 min	1

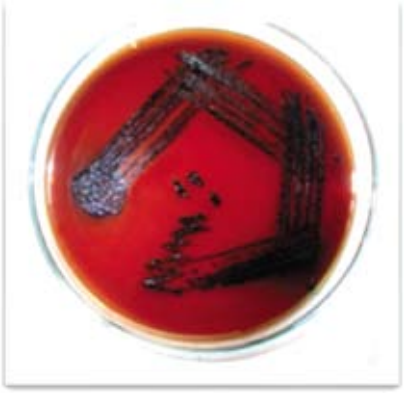

Fig. 1. *Staphylococcus aureus colonies* on potassium tellurite blood agar *colonies* (black colonies)

Fig. 2. *Staphylococcus aureus* on mannitol salt agar (yellow coloured colonies)

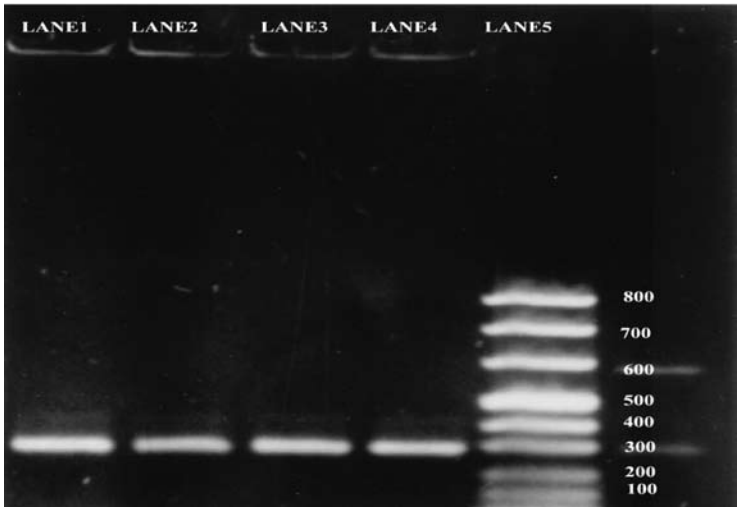

Fig. 3 PCR analysis of *Staphylococcus* genus, Lane(1-4) – *Staphylococcus* isolates, Lane 5 – 100 bp ladder

Results and Discussion

Out of 25 swabs, 12 isolates were Gram positive cocci. The colonies obtained were large, smooth and yellow coloured on BHIA. Small, smooth, round, opaque and hemolytic colonies were observed on blood agar. On Gram's staining, Gram positive cocci arranged in clusters or as bunches of grapes were observed. Black coloured colonies were documented on potassium tellurite blood agar (Fig.1). The isolates produced yellow coloured colonies on mannitol salt agar (Fig.2). The biotyping results of the isolates were depicted in (Table 3) which was in accordance with Quinn *et al*. (1994).

Based on the result of these tests, the isolates were identified as *Staphylococcus aureus* (*S*. *aureus*). Kundirkiene *et al*. (2006) reported that *S. aureus* was the predominant microbial agent isolated from dogs with ophthalmic infections. Several researches reported the isolation of *S. aureus* from dogs with corneal diseases such as microbial keratitis and deep corneal ulcers (Moriyama and Lima 2008, Al-Mujani *et al*., 2009 and Vongsakul *et al*., 2009).

The *S. aureus* isolates were subjected to PCR amplification by targeting the 16S rRNA gene genus specific primer pairs which generated a product of approximately 303 bp size (Fig. 3). Bizot *et al*. (2004) reported that PCR was the method of choice for the rapid and reliable identification of *Staphylococcus* spp.

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References

Al-Mujaini, A., Kharusi, N.A., Thakral, A. and Wali, K.U. 2009. Bacterial keratitis: Perspective on epidemiology, clinico-pathogenisis, diagnosis and treatment. *SQU +Med. J*. **9**: 184-195.

Bizot, S.C.M., Talon, R. and Leroy, S. 2004. Development of a multiplex PCR for the identification of *Staphylococcus*

Table 3. Biotyping of the *Staphylococcus aureus* isolates

Test	Result
Gram's staining and morphology	Gram positive cocci, grape like clusters
Catalase	Positive
Oxidase	Negative
Indole	Negative
Methyl red	Positive
Vogesproskauer	Positive
Citrate	Positive
Urease	Positive
Coagulase	Positive
OF test	Fermentative
Cellobiose	Negative
Fructose	Positive
Lactose	Positive
Maltose	Positive
Mannitol	Positive
Mannose	Positive
Raffinose	Negative
Sucrose	Positive
Trehalose	Positive
Xylose	Negative

genus and four Staphylococcus species isolated from food. *J. Appl. Microbiol*. **97**: 1087-1094.

Kudirkiene, E; Zilinscas, H. and Siugzdaite, J. 2006. Microbial flora of the dog eyes. *Veterinarija ir zootechnika* **34** (56) : 18-21.

Moriyama, A.S. and Lima, A.L.H. 2008. Contact lens associated microbial keratitis. *Arq. Bras. Oftalmol.* **71**: 31-36.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. 1994. *Clinical veterinary microbiology*. Wolf publishing, Spain, pp. 209-236.

Vongsakul, S., Tuntivanich, P., Sirivaidyapong, S. and Kalpravidh, M. 2009. Canine amniotic membrane transplantation for ocular surface reconstruction of created deep corneal ulcers in dogs. *Thai J. Vet. Med.* **39** (2): 135-144. ■