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ISOLATION AND MOLECULAR DETECTION OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* FROM A CASE OF UMBILICAL ABSCESS IN A KID

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Abstract: The present study involves the isolation and molecular detection of *Corynebacterium pseudotuberculosis* (*C pseudotuberculosis*) from cutaneous abscesses in a female crossbred kid aged one and a half month. The animal was presented to the clinics with an abscess near the umbilicus. Pus samples were collected aseptically and brought to the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy for bacteriological culture and identification. On culturing on 7 % Blood Agar (BA), Gram positive bacilli could be isolated. On antibiotic sensitivity, the organisms were found to be sensitive to Tetracycline and Ceftriaxone and resistant to Amoxicillin-Clavulanic acid, Enrofloxacin and Cotrimoxazole. Cultural, morphological and biochemical characteristics of the organisms were done to confirm the organism as *C pseudotuberculosis*. The colonies were subjected to polymerase chain reaction targeting *rho* gene of *C pseudotuberculosis.* Positive amplicons of 209 bp confirmed the isolate as *C pseudotuberculosis*.

Keywords: *Corynebacterium pseudotuberculosis*, kid, Polymerase chain reaction, caseous lymphadenitis, cutaneous abscess, Kerala.

INTRODUCTION

Caseous lymphadenitis is a chronic contagious disease affecting sheep and goats caused by the bacterium *Corynebacterium pseudotuberculosis* (*C pseudotuberculosis*). The disease is characterized by abscessation in or near peripheral or internal organs and lymph nodes. In goats, external form of the disease is more commonly seen. Economic losses include death, condemnation of infected carcasses, hide and wool loss, loss of sales for breeding animals, and premature culling of affected animals from the herd or flock. Once established in a farm, infection is maintained by contamination of the environment with active draining lesions.

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Animals with the internal form of the disease contaminate the environment through nasal discharge or coughing.

C pseudotuberculosis is a Gram-positive, facultative, intracellular bacillus (Brown and Olander, 1987). Two biotypes have been identified based on the ability of the bacteria to reduce nitrate. Nitrate-negative group infects sheep and goats, and a nitrate-positive group infects horses. The most common site of entry is the skin. Once the bacteria have entered the body, they move to the lymph nodes via the regional draining lymphatic system. Internally, the bacteria establish infection not only in the lymph nodes but also in the viscera. The incubation period varies from one to three months, culminating in development of encapsulated abscesses. *C pseudotuberculosis* is hardy in the environment and can survive on fomites such as bedding and wood for two months and in soil for eight months. The present study deals with the isolation and identification of *C pseudotuberculosis* from a case of cutaneous abscess in a kid.

MATERIALS AND METHODS

A female crossbred kid aged one and a half month was brought to the University Veterinary Hospital, Mannuthy with abscess near the umbilicus. Pus sample was collected aseptically on puncture from the abscess and processed in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Kerala. The sample was inoculated to 7 per cent Blood Agar (BA) and incubated at 37°C for 48 hours. Gram positive bacilli obtained were inoculated to Cystine tellurite blood agar and incubated at 37°C for 24 hours. The colonies were subjected to catalase, oxidase, indole, methyl red, Voges Proskauer, nitrate reduction, esculin hydrolysis, urease and fermentation of sucrose, maltose, and glucose (Quinn *et al*., 1994). Antibiogram of the isolate was done by disc diffusion method (Cockerill *et al*., 2013).

The DNA of the isolate was extracted by phenol chloroform method (Sambrrok and Russel, 2001) and subjected to PCR using primers designed targeting the *rho* gene of *C pseudotuberculosis*.

A 12.5 µl reaction mixture was set up for the single PCR reaction consisting of

10X PCR master mix	6.25µL
Forward Primer	1µL
Reverse Primer	1µL
Template DNA	3µL
Nuclease Free Water	1.25µL

The PCR tubes were placed in a thermal cycler (Eppendorf) and reaction was run as per the following protocol:

Initial denaturation	95°C for 60 sec	
Denaturation	95°C for 45 sec	30 cycles
Annealing	60°C for 60 sec	
Elongation	72°C for 60 sec	
Final extension	72°C for 7 min	

Identification of the PCR product was done in a submerged agarose gel electrophoresis system using one per cent agarose stained with ethidium bromide, and Tris Borate EDTA buffer was used as the matrix at a voltage of 50V. The gel was visualised under a UV transilluminator and results were documented on gel documentation system (Biorad).

RESULTS AND DISCUSSION

The colonies on blood agar were small, grey and dry with a narrow zone of haemolysis. On Cystine tellurite BA, the colonies were black, dry and opaque (Figure 1). On Gram's staining, the colonies from BA and Cystine tellurite BA, revealed Gram positive bacilli exhibiting palisade arrangements as per Baird and Fontaine (2007) (Fig. 2).

Figure 1. Black, dry opaque colonies on Cystine tellurite BA

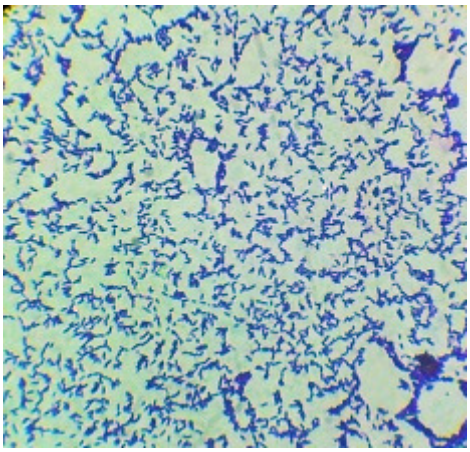

Figure 2. Gram staining of *C. pseudotuberculosis*

The organisms were positive for catalase and negative for oxidase. It produced a small zone of haemolysis on BA. On IMViC test, the organisms were positive for methyl red and fermentation tests were positive for sucrose, maltose and glucose. The organisms were positive for urease and negative for nitrate reduction and esculin hydrolysis. Thus, from the cultural characters and biochemical tests, the organism was identified as *C. pseudotuberculosis*. Similar results were obtained by Dorella *et al*. (2006).

On antibiogram, the organisms were found to be sensitive to Tetracycline and Ceftriaxone and resistant to Amoxicillin-Clavulanic acid, Enrofloxacin and Cotrimoxazole. The most active agents were reported to be penicillins, macrolides, tetracyclines, cephalosporins, lincomycin, chloramphenicol, and rifampicin. Most isolates were resistant to aminoglycosides, nitrofurans, polymyxins, nalidixic acid, and cycloheximide (Judson and Songer, 1991). This shows the variation in resistance of the organism over years.

The organism was confirmed to be *C pseudotuberculosis* by *rho* gene specific PCR which revealed the presence of 209bp (Figure 3) amplicon.

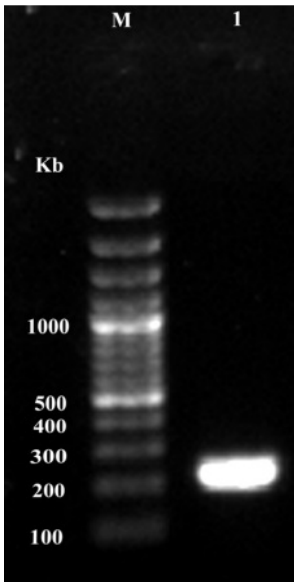

Figure 3. Amplification of *Rho* gene by Polymerase Chain Reaction

Lane M: Molecular weight marker (100 bp)

Lane 1: *C pseudotuberculosis* positive sample (209 bp)

Caseous lymphadenitis in sheep and goat results in severe economic losses to farmers resulting in loss of fertility, gradual emaciation and condemnation of carcass at abattoirs (Conner *et al.*, 2000). Even though it commonly affects small ruminants, it also affects bovines, equines, pigs, deers, camels and humans, showing its zoonotic relevance. Hence, apart from economic loss, its zoonotic potential also puts the consumers at risk from

contaminated meat and milk (Bastos *et al*., 2012). This makes detection of clinical and subclinical cases in susceptible species important. According to Ilhan (2013), PCR assay proves to be a sensitive and rapid method for the detection of *C. pseudotuberculosis* in lymph node samples from naturally infected animals. Reports from other parts of Kerala (Mohan et al., 2007) also implicated the need for control of the disease through awareness, proper management and improved vaccines.

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