



Isolation, Characterisation and AntibioGram of *Actinomyces viscosus* from a Dog

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Abstract

Actinomyces viscosus is a gram-positive, non-acid-fast, facultative, catalase-positive, filamentous, or diphtheroidal bacteria. It is the main pathogenic species of *Actinomyces* found in the dogs and humans, commonly causing localized granulomatous abscess of the skin and subcutis. The present study deals with the occurrence of actinomycosis in a three-year-old Labrador dog. The dog was presented with the history of suppurative oral and cutaneous lesions. Pus from the lesion was cultured and the isolated pure culture of the organism was identified as *Actinomyces viscosus* based on cultural and biochemical characteristics, which distinguishes it from other morphologically similar organisms. The organism was found to be sensitive to most of the broad spectrum antibiotics incorporated in antibiotic sensitivity test. On mice inoculation test, the organism did not produce death of the mice.

Keywords: Actinomycosis, *Actinomyces viscosus*, dog

Introduction

Actinomycosis is a chronic suppurative and granulomatous bacterial infection caused by *Actinomyces* species in animals and man. (Quinn *et al.*, 1994). *Actinomyces viscosus* is a gram-positive, catalase-positive, non-acid-fast, filamentous or diphtheroidal bacteria and is the main pathogenic species found in the dogs. (Pelle *et al.*, 2000). They are aerobic or microaerophilic commensal organisms found in the oral cavity of animals and humans.

It is associated with periodontal disease, granulomatous abscess of skin and subcutis in dogs. Deep form of actinomycosis can also occur characterised by pyothorax with granulomatous lesions of the thoracic tissues and accumulation of pleural and pericardial fluid. Cutaneous actinomycosis is the most common manifestation in dogs and *Actinomyces viscosus* is the most frequent isolate. (Kirpensteijn and Fingland, 1992). This study planned a detailed investigation on *Actinomyces viscosus* infection in a dog.

Materials and Methods

Case History

A three year old Labrador dog was presented to the Veterinary Hospital, Kokkalai, Thrissur with the history of suppurative oral and cutaneous lesions. Pus from the lesions was collected aseptically using sterile swabs. Inoculation was done on blood agar plates containing 5% defibrinated bovine blood in brain heart infusion agar (BHIA) and brain heart infusion broth (BHIB). The plates were incubated aerobically and microaerobically at 37°C. Colonies were observed after 7-10 days of incubation. Gram staining of the colonies was done to examine the morphology of the organisms. The organisms were further inoculated in Sabaroud's dextrose agar (SDA) to determine growth.

Biochemical Tests: Tests for catalase, indole, nitrate reduction, methyl red reduction, gelatin hydrolysis, urease, citrate and various sugar fermentation were done as per Barrow and Feltham (1993) for the identification of the isolated organism.

Animal Pathogenicity Test: Experimental inoculation of the organism was done on mice to determine the pathogenicity as recommended by Georg *et al.* (1972).

Antibiotic Sensitivity Test: AntibioGram using commonly used antibiotics such as amoxycillin, enrofloxacin, gentamicin, cefotaxime, streptomycin, oxytetracycline and chloramphenicol was conducted by standard disc diffusion method as per Bauer *et al.* (1966).

Results and Discussion

The organism was identified as *Actinomyces viscosus* by its Gram's staining reaction, cellular morphology, and biochemical characteristics. Cultures from the lesions grew equally well under both aerobic and microaerophilic conditions when incubated at 37°C for 48 hours. Colonies were small, white to grey, rough and dry, 0.5-1 mm in diameter. Gram's staining of the colonies revealed gram-positive, branching, pleomorphic diphtheroid forms and were capable of producing knobs or clubbed swellings as

described by Davenport *et al.*, (1975). In brain heart infusion broth, the organisms grew diffusely and produced a moderate to heavy sediment. They failed to grow on SDA.

For identification and discrimination of the actinomycetes species, biochemical tests such as catalase, urease, sugar fermentation etc. were done. A key biochemical test for identification of *A. viscosus* is positive catalase test. (Daie *et al.*, 2009). Since urease and catalase were positive but mannitol fermentation was negative, the isolate was identified as *Actinomyces viscosus*. The results of the biochemical and fermentation tests are shown in Table.

Table 1 . Biochemical reactions of *Actinomyces viscosus*

Determination	Results
Catalase	Positive
Urease	Positive
Indole	Negative
Nitrate reduction	Positive
Methyl red	Positive
Gelatin hydrolysis	Negative
Voges-Proskauer	Negative
Lactose	Positive
Sucrose	Positive
Mannitol	Negative
Raffinose	Positive
Salicin	Positive
Sorbitol	Negative
Trehalose	Positive
Xylose	Negative

On mice inoculation test, the organism did not produce death of the animal. This finding was in accordance of Merchant and Packer (2002) who reported lower virulence of *A. viscosus* on animal inoculation test. The organism was found to be sensitive to most of the broad spectrum antibiotics, namely amoxycillin, enrofloxacin, gentamicin and cefotaxime incorporated in antibiotic sensitivity test. The animal was successfully treated with amoxicillin at recommended dosage and showed considerable improvement.

So far, skin actinomycosis due to *A. viscosus* has been reported from an Arabian 12 year old stallion in the USA. (Spect *et al.*, 1991). *A. viscosus* was isolated from the inflammatory tissues by Bestetti *et al.* (1997) from a three-year-old female domestic cat, with suppurative granulomatous lesion of the tail and sacral area penetrated into epidural space, causing paraplegia. Similar clinical lesions and pictures were reported by Pelle *et al.* (2000). The morphology, cultural examination and biochemical characterisation of the organism identified was in accordance with Gerencser and Slack (1969) and Daie *et al.* (2009). The key biochemical test for the identification of *A. viscosus* is positive catalase reaction. They are sensitive to many broad spectrum antibiotics. Effective therapy often requires high doses of antimicrobials, administered long term.

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