

Original Research Article

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Isolation and Identification of *Corynebacterium jeikeium* from Synovial Fluid of a Joint Ill Affected Calf

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ABSTRACT

Corynebacterium sp. is a Gram-positive, non-motile and facultatively anaerobic bacilli present in the environment as ubiquitous organism. A 45 day-old Holstein Friesian calf from University Livestock Farm (ULF), Kerala Veterinary and Animal Sciences University, Mannuthy was reported with joint ill in both of its forelimbs. Using aseptic procedures, synovial fluid was collected from the right knee joint and inoculated on to blood agar and incubated at 37⁰ C for 24 to 48 hrs to isolate and identify the bacterial aetiology involved. Pin-point gray colonies with hazy haemolysis could be observed. On Gram's staining, Gram-positive bacilli with palisade arrangement could be observed. On sub culturing on to Potassium tellurite blood agar, black coloured colonies suggestive of *Corynebacterium* sp. was observed. Biochemical tests were performed both by conventional method and using Vitek system and the organism was identified as *Corynebacterium jeikeium*. On antibiotic sensitivity test (ABST), the organism was found to be highly sensitive to gentamicin, and highly resistant to ampicillin. For molecular confirmation, *rpo* B gene specific polymerase chain reaction (PCR) was performed and an expected 452 bp amplicons was obtained. Hence, the present case reports the isolation and characterisation of *Corynebacterium jeikeium* from a case of joint ill in a calf using conventional and molecular methods.

Keywords

Calf, *Corynebacterium jeikeium*, joint ill, polymerase chain reaction, *rpo* B gene

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Introduction

Joint ill, a suppurative polyarthritis condition is one of the major causes of calf mortality in the age group of one week to one month (Bagga *et al.*, 2009). As explained by Abdulla *et al.*, (2015), the severe inflammation of the affected joints as a sequel of infection from

umbilicus and its associated structures led to critical lameness in young farm animals. Commonly, the joint ill is observed in neonatal period due to unhygienic environmental conditions of the calving shed and improper umbilical cord asepsis (Radostitis *et al.*, 2007). Some of the implied causes of joint ill in calves include

Trueperella pyogenes, *Fusobacterium necrophorum*, *Staphylococcus* spp., etc. Other causes of synovitis and arthritis in cattle include *Mycoplasma bovis*, *Brucella abortus*, etc.

Corynebacterium spp. are ubiquitous organisms present in the environment, which are Gram-positive bacilli, non-motile and facultative anaerobic in nature. It is a common habitant in the mucous membrane and skin of the animals (Markey *et al.*, 2013). The morphology has been described as a typical Chinese letter like arrangement (Indranil, 2013). Most commonly *C. bovis* isolates were observed to cause various pathological conditions like mastitis, polyarthritis etc. *C. jeikeium* was rarely isolated from bovine and has been reported to cause mastitis in cattle (Watt *et al.*, 2000). So far, no published reports have found on the fact that it could cause arthritis in cattle. The present study reports the identification of *C. jeikeium* from a polyarthritis case.

Materials and Methods

Collection of Synovial Fluid

A 45 day-old Holstein Friesian calf (Fig. 1) from University Livestock Farm (ULF), Kerala Veterinary and Animal Sciences University, Mannuthy was reported to be suffering from joint ill in both of its forelimbs. Using aseptic procedures, the synovial fluid was collected from the right knee joint.

Isolation and Identification of the Bacterial Agent

The sample was inoculated on to 5 to 10 % blood agar (BA) and incubated at 37^o C both aerobically and micro aerobically for 24 to 48 hrs. Selective isolation was done by sub-culturing onto Potassium tellurite blood agar and incubated at 37°C for 24 to 48 hours.

Isolates were presumptively identified based on colony morphology, Gram's staining and biochemical tests and further confirmed by genus specific PCR. The biochemical tests include catalase, oxidase, IMViC (Indole, Methyl red, Voges- Proskauer and Citrate), urease and sugar fermentation tests. Vitek system was used for the confirmation of at species level.

Molecular Confirmation by PCR

The DNA was extracted by boiling and snap chilling method (Suresh *et al.*, 2018). Molecular confirmation at genus level of the isolate was done by PCR using the primers targeting *rpo B* gene (Khamis *et al.*, 2004). PCR assay was optimized with 12.5 µl reaction mixture containing 3 µl of DNA template, 6.25 µl of 2 X master mix (Taq Green Master Mix, Emerald), 1 µl each of forward and reverse primers (10 pmol/µl) (Table- 1) and the rest of the volume is made by adding nuclease free water. The cycling conditions were as follows: initial denaturation at 95°C for 2 min; 35 cycles of 94°C for 30 sec, 51.4°C for 30 sec and 72°C for 2 min and a final elongation step at 72°C for 10 min. The PCR products were subjected to gel electrophoresis using 1.5% agarose with ethidium bromide as fluorescent dye and visualized using gel documentation unit (Biorad, USA).

Antibiotic Sensitivity Test (ABST)

The procedure of ABST was followed as per the guidelines established by Clinical and Laboratory Standards Institute (CLSI, 2015) with minor modification. The sensitivity of the isolate was tested in Muller Hinton agar against 15 selected antibiotics disc of Hi-Media, Mumbai (Table-2). The antibiotics were selected based on the interest of the large animal practitioners to treat joint ill infected calves in studied farms. Measurement of the

growth inhibition zone permitted the classification of each isolates as sensitive, intermediate and resistant according to data provided by Oxoid Ltd., Basingstoke, Hampshire, England and CLSI (2015).

Results and Discussion

Small, greyish white, hazy haemolytic colonies were observed on blood agar (Fig. 2). On Gram's staining, the isolate revealed Gram- positive bacilli with Chinese letter arrangement which was in agreement with Indranil, (2013). The black colony appearance on potassium tellurite agar (Fig. 3) and the other biochemical characters matched with the findings observed by Barrow and Feltham (1993) which supported for the confirmation of *Corynebacterium jeikeium*.

The isolates could ferment sugars like glucose and maltose but showed negative results for lactose, salicin, mannitol, sucrose, trehalose and xylose (Fig. 4). Based on the primary and

secondary identification, the organism was identified as *Corynebacterium jeikeium*. Species confirmation was done using Vitek system.

The PCR was standardised for the identification of *Corynebacterium* spp. The amplicon size by targeting the gene *rpo B* obtained was 434 bp (Fig. 5) which was supported by the research done by Khamis *et al.*, (2004). *Corynebacterium jeikeium* was isolated from the present study but Goodarzi *et al.*, (2015) could isolate 20 per cent *Corynebacterium bovis* from the polyarthritic samples *Corynebacterium* spp. was observed as a common habitant of the skin and mucous membrane of animals by Markey *et al.*, 2013. Watt *et al.*, (2000) could isolate *Corynebacterium jeikeium* from mastitis affected cattle. Hence it could be concluded that the organism might caused joint ill in the present study as an outcome of cross contamination from the mastitis affected cattle.

Table.1 Details of the Primers

Primer	Sequence	Amplicon size
<i>Rpo F</i>	5'-CGWATGAACATYGGBCAGGT-3'	434 bp
<i>Rpo R</i>	5'TCCATYTCRCCRAARCGCTG- -3'	

Table.2 List of antibiotic discs and their concentration

Sl. No.	Name of antibiotic discs	Concentration
1	Gentamicin (G)	30 µg
2	Ceftriaxone (CTR)	30 µg
3	Tetracycline(T)	30 µg
4	Penicillin (P)	10 Units
5	Cefalexin (CN)	30 µg
6	Amoxyclav (AMC)	30 µg
7	Enrofloxacin (EX)	10 µg
8	Amoxycillin (AMX)	30 µg
9	Streptomycin (S)	25 µg
10	Amikacin (AK)	30 µg
11	Ceftriaxone/ Sulbactam (CIS)	30 µg
12	Ceftriaxone/ Tazobactam (CIT)	30 µg
13	Cefuroxime (CXM)	30 µg
14	Cefotaxime (CTX)	30 µg
15	Ampicillin	10µg

Fig.1 Calf showing inflammation on both the knee joint



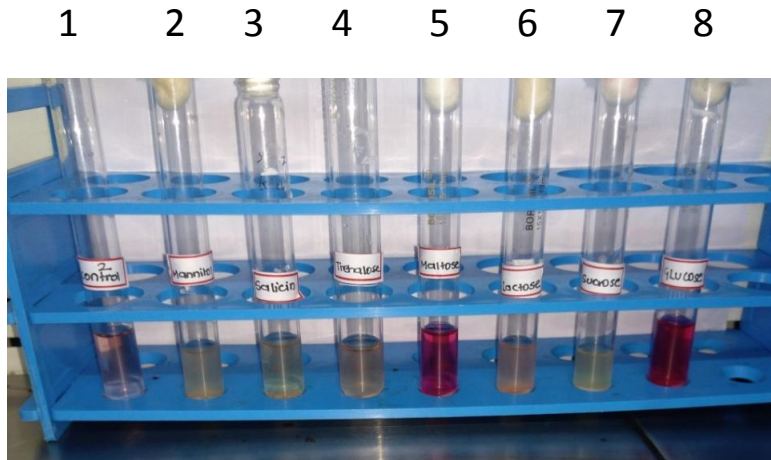
Fig.2 Greyish white colonies with hazy haemolysis on BA



Fig.3 Black coloured colonies on Potassium tellurite agar



Fig.4 Sugar fermentation tests of *Corynebacterium jeikeium*



1) Control; 2) Mannitol; 3) Salicin; 4) Trehalose
5) Maltose; 6) Lactose; 7) Sucrose; 8) Glucose

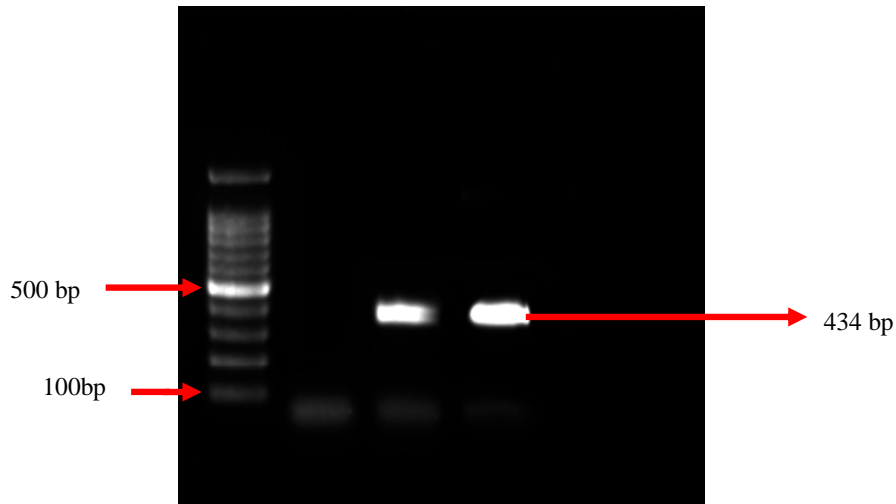


Fig. 5 PCR amplification of *rpoB* gene of *Corynebacterium* spp.
Lane 1- 100 bp Ladder
Lane 2, 5 and 6- Negative isolates
Lane 3 - Positive control
Lane 4- Positive isolate

The results of ABST revealed the organism was sensitive to gentamicin, tetracycline, enrofloxacin, amikacin and streptomycin; intermediate sensitive to penicillin-G, ceftriaxone/ tazobactam, cefuroxime and cefotaxime and resistant towards ampicillin, ceftriaxone, cefalexin, amoxycylav and amoxycillin.

Corynebacterium jeikeium is rarely isolated from bovine but reports have found on that isolates could cause mastitis in cattle but rare or almost nil reports have found on the fact that it could cause arthritis in cattle. The isolation of *Corynebacterium jeikeium* from a case of joint ill in a calf using conventional and molecular methods is highlighting the

importance of maintaining calving shed hygiene, naval asepsis and timely treatment of infected calves.

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