Usefulness of 16S rDNA sequencing for the diagnosis of infective endocarditis caused by *Corynebacterium diphtheriae*

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We report a rare case of infective endocarditis caused by *Corynebacterium diphtheriae* in an 8-year-old boy, 2 years after a right ventricular outflow tract reconstruction with a bovine Contegra valved conduit. The patient recovered well after an RV–PA conduit enblock explantation and replacement with an aortic homograft with antibiotic treatment. All bacteriological cultures of excised tissue and blood were negative. The aetiological agent was identified as *C. diphtheriae* subsp. *gravis* by 16s rDNA sequencing.

**Introduction**

Infective endocarditis (IE) is a severe disease and identification of the aetiologic agent is necessary to facilitate rapid and effective therapy. Conventional methods used in most clinical laboratories to diagnose IE include detection of micro-organisms in blood cultures, excised cardiac valves or vegetations. These methods often fail because of previous antimicrobial therapy or the involvement of fastidious, slow-growing or non-cultivable micro-organisms. To overcome this, culture independent molecular techniques based on the amplification and direct sequencing of ribosomal sequences have been developed (Breitkopf et al., 2005).

We describe the clinical usefulness of broad range 16S rDNA amplification in the identification of *Corynebacterium diphtheriae* from an explanted Contegra bovine jugular vein conduit.

**Case report**

An 8-year-old male born to non-consanguineous parents was referred to our hospital on 30 December 2010 with a clinical diagnosis of infective endocarditis (IE) and right ventricular outflow tract obstruction. He had a history of congenital heart disease and in November 2008, underwent intra-cardiac repair with a valved right ventricle–pulmonary artery (RV–PA) 18 mm Contegra conduit for Tetralogy of Fallot in a city hospital. The patient was asymptomatic until November 2010, when he developed intermittent fever with facial oedema and severe anaemia. He was treated for suspected endocarditis in a local hospital with penicillin, amikacin and piperacillin–tazobactam. Since he had symptoms of persistent infection and fever continued in spite of medical management, he was referred to our hospital.

On physical examination, the child appeared thin, pale and generally unwell and was febrile. Blood pressure was 100/60 mmHg, heart rate was 118 min⁻¹ and auscultation revealed an ejection systolic murmur of grade 4/6. He had mild pallor and oedema but no cyanosis or icterus. His parents reported that he had been given all the childhood immunizations as scheduled. Chest X-ray showed leovocardia, cardiomegaly with enlarged right atrium and normal lung vascularity.

Laboratory studies revealed an erythrocyte sedimentation rate of 50 mm h⁻¹; haemoglobin of 9.6 g dl⁻¹; total white blood cell count of 9.8 x 10⁹ l⁻¹, with a differential count of 85% neutrophils, 12% lymphocytes and 0.3% eosinophils; and C-reactive protein of 51.30 mg l⁻¹. Kidney and liver function tests were normal. Two sets of blood cultures, which were processed using the Bact Alert 3D system (bioMérieux), showed no growth.

**Abbreviations:**
IE, infective endocarditis; RV–PA, right ventricle–pulmonary artery.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the *Corynebacterium diphtheriae* isolate obtained in this study is JQ396647.
Transthoracic echocardiography revealed a large echogenic mass (vegetation) measuring 1.72 cm² in the proximal right ventricular outflow tract below the level of the conduit valve, causing severe obstruction with a peak gradient of 76 mmHg.

The patient underwent RV–PA conduit enblock explantation and replacement with a 21 mm aortic homograft on 12 January 2011. The large vegetation, attached to the pulmonary end of the RV–PA conduit valve, was excised and sent for microbiological investigation (Fig. 1). A Gram-stained smear of the vegetation revealed irregularly stained Gram-positive cocci in pairs and chains, resembling disintegrating cocci as well as pleomorphic Gram-positive coccobacillary forms (Fig. 2). A potassium hydroxide wet mount showed no fungal elements. Both blood and excised valve vegetation cultures did not yield any growth.

Based on the Gram stain findings a presumptive diagnosis of streptococcal endocarditis was made.

The patient was treated with vancomycin and amikacin. The post-operative period was uneventful and he was discharged on the 6th post-operative day after advice to continue intravenous vancomycin 250 mg three times a day along with amikacin 250 mg once daily for 3 weeks.

A sample of heart valve was stored at −20 °C and DNA from the tissue was extracted by using an in-house prepared tissue digestion buffer (50 mM Tris/HCl, pH 8; 100 mM EDTA; 100 mM NaCl; 1 % SDS; and 10 mg proteinase K ml⁻¹). DNA was amplified using the broad-range 16S rRNA gene primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′). The PCR mixture consisted of 10 × PCR buffer (15 mM MgCl₂), dNTP mix (10 mM each dNTP), primers (2 μmol each), Taq DNA polymerase (1 unit), and PCR-grade water to a final volume of 50 μl. The PCR amplification was carried out with initial denaturation at 95 °C for 3 min followed by 37 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min with final extension at 72 °C for 7 min. The amplicons (expected size 1500 bp) were resolved in 0.8 % agarose gel by electrophoresis for ~20 min at 100 V and analysed using a gel documentation system (Bio-Rad).

Identification was performed by 16S rDNA sequencing (Weisburg et al., 1991). The sequences generated (GenBank accession no. JQ396647) were compared to those available in the NCBI database and results of the BLAST (http://www.ncbi.nlm.nih.gov/Blast.cgi) search showed 99 % similarity to C. diphtheriae subsp. gravis.

Infective endocarditis caused by C. diphtheriae is uncommon. There has been a resurgence of infections caused by C. diphtheriae in many parts of the world and non-toxigenic strains have been increasingly reported as a cause of invasive disease, including endocarditis, in the recent past (Menon et al., 2010; Mishra et al., 2005; Reacher et al., 2000; Holthouse et al., 1998).

The source of infection in our patient is unknown. The Contegra bovine jugular vein conduit is used for the reconstruction of congenitally abnormal RV–PA tracts. Endocarditis of the Contegra conduit has been shown to be very rare (Bajraktari et al., 2009).

The Gram stain morphology of the organism in the excised tissue gave the impression that the causative agent may be of the genus Streptococcus. It is possible that the antibiotics used to treat his infection could have altered the microscopic morphology of C. diphtheriae and lead to the misidentification of the organism as a species of Streptococcus.

The patient recovered well and survived in spite of the large vegetation owing to surgical intervention and subsequent prolonged antibiotic treatment with vancomycin and amikacin.

Hence, broad-range 16S rDNA PCR, which is a simple molecular test, would be a useful adjunct to the existing battery of tests used to diagnose IE and is useful in the identification of culture-negative endocarditis.
References


