

A Case Of Non-Toxigenic *Corynebacterium Diphtheriae* Endocarditis In A Pediatric Patient With Tetralogy Of Fallot

Sarumathi Dhandapani¹, Ramit Kundu¹, Abhishek Singh², Sriram Krishnamurthy², Madhileti Sravani², Mahalakshmi Kumaresan¹, Apurba S Sastry¹

¹Department of Microbiology, JIPMER, Puducherry

²Department of Pediatrics, JIPMER, Puducherry

Corresponding author: Dr. Apurba S Sastry, Additional Professor of Microbiology

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I. INTRODUCTION

One of the frequent complications of cyanotic heart disease in paediatric age group is infective endocarditis (IE) [1,2,3]. The most commonly encountered organisms in IE are Streptococci or Staphylococci [4,5]. *Corynebacterium diphtheriae*, the causative agent of diphtheria is a rare cause of IE [6]. It is found to be an uncommon etiology associated with mycotic aneurysm, septic arthritis, splenic abscess, and osteomyelitis. Isolation of such uncommon organisms is made feasible with the help of automated systems in diagnostic microbiology laboratory which also reduces turnaround time of patients reports. Though many sporadic cases of *C.diphtheriae* endocarditis have been reported, recommended guidelines for treating this infection is unavailable. We report a case of *C.diphtheriae* endocarditis in a paediatric patient with congenital cyanotic heart disease.

II. CASE PRESENTATION

A 7-year-old girl, follow up case of congenital cyanotic heart disease was referred from a peripheral hospital for evaluation of febrile illness. The patient had a history of progressive breathlessness and history of chest pain on and off since two years of age. The patient was evaluated only at the age of 6 years when echocardiography demonstrated Tetralogy of Fallot (TOF) with subaortic ventricular septal defect, pulmonary atresia, over-riding of aorta and right ventricular hypertrophy. The patient did not undergo any surgical or medical management for these conditions. The patient also had bilateral moderate sensorineural hearing loss from birth with facial dysmorphism and overriding of the fourth toes of the bilateral foot. At present, the patient presented to the paediatric casualty of our hospital with history of episodes of mild fever for past 4 days along with chest pain and breathlessness. The patient didnot have cyanotic spells and syncope in the past. The patient was appropriately vaccinated for age and there was no history of similar illness in the family. The patient had history of dental extraction 2 weeks ago. On examination of oral cavity, there was no membrane over tonsils and fauces. The cardiovascular system examination revealed grade 4 pansystolic murmur in the mitral area, radiating to the tricuspid area, in axilla and at back, heard best with the diaphragm of stethoscope and increases with expiration. An early soft diastolic murmur, decrescendo murmur, of grade 2 heard in aortic area. Systolic thrill present in mitral and tricuspid area and grade 2 parasternal heave with epigastric pulsation was also present.

One set of blood culture comprising of two bottles was taken from left dorsum of hand following sterile precautions, inoculated in BacT/Alert and sent to microbiology laboratory. Second blood culture set comprising of one bottle which was collected from right hand dorsum was repeated next day. All the blood culture bottles were received and immediately loaded into continuous blood culture monitoring system, BacT/Alert Virtuo system (BioMérieux, France) instrument. WBC levels were $24.22 \times 10^3/\text{mm}^3$. PT-INR and APTT levels were prolonged and D-dimer levels were elevated (5.716 $\mu\text{g}/\text{ml}$) indicating disseminated intravascular coagulation. All the biomarkers of sepsis are elevated as follows— procalcitonin level –120.35 ng/ml, C- Reactive Protein – 1.2 mg/dl, Ferritin – 793.2 ng/ml.

Echocardiography was performed which suggested vegetations on mitral and tricuspid valve. The patient was diagnosed with infective endocarditis and was started on intravenous ceftriaxone and vancomycin. The first blood culture set flagged positive with a time-to-positivity of 13 hours and 11 hours. The flagged bottles were processed as per blood culture laboratory protocol. Gram staining was performed from a drop of blood which shows gram-positive rods with clubbed ends arranged parallelly or in an L-shaped pattern (**Figure 1**). Subculture

was done onto 5% sheep blood agar and MacConkey agar and the plates were incubated at 37 °C. After overnight incubation, sheep blood agar showed white colonies with smooth surface with rounded edges. Gram staining from the colonies grown on SBA showed short gram-positive bacilli in cuneiform arrangement with club-shaped swelling on one or both ends. Albert staining performed from the culture growth showed green-colored bacilli with bluish black metachromatic granules at polar ends which are arranged in a cuneiform pattern (**Figure 2**). The organism was identified to be *C. diphtheriae* using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF MS) (VITEK MS version 3.0) with a confidence interval of 99%. The second blood culture bottle which was collected from different site also grown *C. diphtheriae*. The organism identification was also confirmed by conventional method such as urease test and Hiss serum sugars. Urea was not hydrolyzed and fermentation test performed on Hiss serum sugar media revealed that the organism fermented glucose, maltose, and starch accompanied by the presence of acid without gas production. It produced jet black-colored colonies on potassium tellurite blood agar (**Figure 3**). In-house conventional PCR was performed for the presence of *tox* gene (*tox A* and *tox B*) and *dtxR* gene from the culture growth. The result of PCR showed that the isolate was negative for *tox* genes but positive for *dtxR* gene (**Figure 4**). This confirms that the isolate is a non-toxigenic strain of *C. diphtheriae*. Antimicrobial susceptibility testing was performed with penicillin(10µg), erythromycin(15µg) and clindamycin(2µg) disks in 5% sheep blood agar plate and results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and all these three antibiotics were found to be susceptible.

The child's antibiotic regimen was changed to intravenous crystalline penicillin 4.1 lakh units and subsequent three sets of blood culture which was repeated turned out to be sterile. Next generation sequencing revealed homozygous pathogenic mutation in exon2 of GJB2 gene (C. 71G>A) confirming deafness 1A, the cause for sensorineural hearing loss of the child. After a week of admission, the patient developed cyanotic spells and sudden cardiac arrest during which patient was revived with cardiopulmonary resuscitation. She developed multiple organ dysfunction and acute kidney injury and further acute respiratory distress syndrome which worsened the condition requiring ventilator support. Clinical condition was further complicated by pulmonary edema, pneumothorax and massive pulmonary haemorrhage which ultimately resulted in death of the patient in spite of aggressive management.

III. DISCUSSION

Corynebacterium diphtheriae is the causative agent of classic diphtheria – a toxin mediated acute infectious disease affecting the upper respiratory tract and occasionally the skin [7,8]. The toxin produced by *C. diphtheriae* also affects other parts of the body including the heart and nervous system, causing paralysis and cardiac failure. The immunization for *C. diphtheriae*, based on toxoid vaccine, has been the base for reducing the morbidity and mortality in cases of diphtheria, especially in children. In many countries where vaccination against *C.diphtheriae* is stringently followed, the spectrum of illness caused by the organism demonstrated a change. Non-toxigenic strains of *C. diphtheriae* (NTCD) are potential emerging pathogens as they are also capable of causing severe disease. Vaccine does not prevent infections by non-toxigenic strains. The virulence factors such as surface K antigens, cord factors, and the enzymes neuraminidase appear to determine the invasiveness of non-toxigenic *C.diphtheriae*[9]. Systemic illness due to nontoxigenic strains of *C.diphtheriae* has been increasingly observed recently in many parts of the world. The most important risk factor for bloodstream infection due to *C. diphtheriae* is the skin and oral colonization [10]. Non-toxigenic *C. diphtheriae* endocarditis has been related to the presence of valve prosthesis, use of injectable drugs and underlying cardiac disease.

The most common bacterial species of IE are *S. aureus*, followed by viridans group streptococci and enterococci. These organisms along with platelets and fibrin attach to previously formed lesions in the heart valves and form vegetations. These cause valvular dysfunction and can manifest as heart failure. Subsequently under the impact of turbulent blood flow, pressure and vegetation instability, a rupture may occur in vegetations thus forming emboli. Those in turn may cause septic emboli and ischemic areas in various organ systems. Modified Duke criteria are being used to diagnose IE where positive blood culture and positive echocardiogram findings are included as major criteria.

Hotlthouse DJ et al reported two cases of *C.diphtheriae* endocarditis [11]. Both of these cases required valve replacement and they showed complications such as cerebral embolic events and pseudoaneurysm formation in lower limb arteries. Muttaiyah et al., while describing 10 cases of *C. diphtheriae* endocarditis, showed a good prognosis with less complication and no mortality in all these 10 cases [12]. In our patient, the patient could be the oral carrier of non-toxigenic *Corynebacterium diphtheriae* which might have gained access into the blood stream following dental extraction. The pre-existing unresolved TOF with IE and massive pulmonary haemorrhage may have resulted in mortality in this patient.

IV. CONCLUSION

We described a rare case of *C. diphtheriae* endocarditis in patient with tetralogy of fallot. For the successful treatment of this IE, early diagnosis is important and diagnostic microbiology laboratory should be equipped with automated blood culture system for adequate recovery of organism and automated identification system for initiating antimicrobial therapy at right time.

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Figures:

Figure 1 - Gram stain from blood culture bottle shows gram-positive bacilli arranged parallelly or in an L-shaped pattern (100 X magnification):

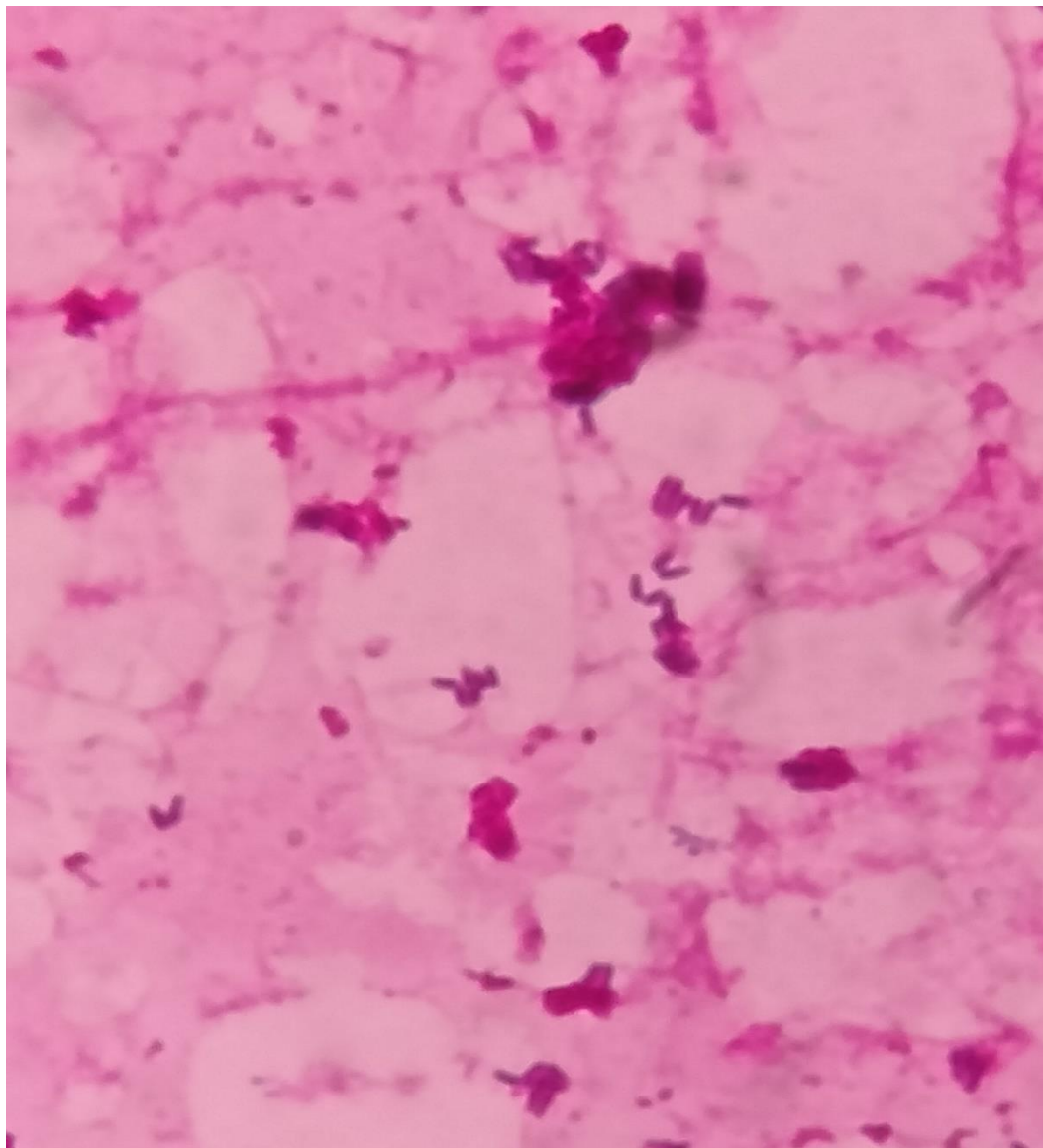


Figure 2 - Albert stain showing green-colored bacilli with bluish-black metachromatic granules at polar ends (100 X magnification):



Figure 3 - Growth of *Corynebacterium diphtheriae* on commercial 5% sheep blood agar (left) and 0.04% potassium tellurite blood agar (right):



Figure 4 - Detection of *tox* (*toxA* and *toxB*) genes and *dtxR* gene in *Corynebacterium diphtheriae* by conventional PCR:

