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Culture-Negative Prosthetic Valve Endocarditis with Concomitant Septicemia Due to a Nontoxigenic *Corynebacterium diphtheriae* Biotype Gravis Isolate in a Patient with Multiple Risk Factors

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A 54-year-old female with a prosthetic mitral valve presented with a 3-day history of dizziness, subjective fever, and chills. Blood cultures were positive for a pleomorphic Gram-positive rod. Initial phenotypic testing could only support the identification of a *Corynebacterium* species. Nucleic acid sequencing (16S rRNA) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) were conclusive for *Corynebacterium diphtheriae*. Definitive phenotypic testing classified the strain as nontoxigenic *C. diphtheriae* biotype Gravis.

CASE REPORT

A 54-year-old female with a prosthetic mitral valve presented with a 3-day history of dizziness, subjective fever, and chills. In addition, she suffered from left ankle pain that was so severe that she was unable to walk. Over the next 2 days, she continued to have dizziness, fever, and chills with the addition of lethargy that kept her in bed for most of the day. On the day of admission, she was seen by her primary care physician, who referred her for hospitalization. Her physical examination was notable for an elevated heart rate of 101 beats/min and a low blood pressure of 106/44 mmHg. There was also an irregular heart rhythm present without obvious murmur or gallop. In addition, her left ankle was exquisitely tender and swollen with a joint effusion. Three sets of blood cultures were drawn peripherally from the left antecubital fossa and the right hand, and only the three aerobic bottles revealed Gram-positive rods within 24 h. An abdominal computed tomography scan showed a wedge-shaped hypodensity of the spleen suggestive of an infarct. The patient's clinical history, including fever, chills, and dizziness combined with Gram-positive bacteremia and a spleen infarct, was suggestive of infective endocarditis. She was subsequently placed on empirical antibiotics consisting of vancomycin and piperacillin-tazobactam after the positive blood culture results were obtained.

The patient's medical history included rheumatic heart disease with severe mitral valve stenosis. A 23-mm St. Jude mitral valve replacement and Maze procedure for atrial fibrillation were performed in 2009. In addition, she had peripheral vascular disease and had undergone a right femoral-popliteal artery bypass procedure. She also had a history of methamphetamine abuse that reportedly ended in 2005 and a 25-pack/year smoking history that ended in 2009 and admitted to being a current recreational marijuana user.

A transesophageal echocardiogram (TEE) was performed 2 days after the patient was admitted, and it revealed a nonmobile soft echo density along the atrial side of the prosthetic mitral valve (medial) measuring 0.7 by 1.0 cm. The appearance was most consistent with a thrombus and was not diagnostic of vegetation. A repeat TEE approximately 1 week later demonstrated an enlarging

mass on the prosthetic mitral valve in the same location as the aforementioned echo density. In addition, there was a second mobile component on one of the mechanical leaflets, as well as increased thickening along the annulus, indicating an early annular abscess. As a result, the patient underwent a repeat mitral valve replacement and was placed on penicillin, vancomycin, and gentamicin. The *C. diphtheriae* strain isolated from the blood cultures was found to be sensitive to all three antibiotics according to antimicrobial susceptibility testing (AST) (Table 1). However, because of a possible drug-related rash, the antibiotic regimen was changed to rifampin and daptomycin. Likewise, the isolate AST profile revealed susceptibility to both of these antibiotics. Susceptibility to meropenem, cefepime, and ceftriaxone was determined in house by Etest. Susceptibility to the remaining antibiotics was determined by an external reference laboratory with the Sensititre Gram-Positive MIC Plate (Trek Diagnostic Systems). Histopathologic examination of the removed mechanical mitral valve revealed fibrosis, hemosiderin, and cellular debris. However, the Gram stain was negative for bacteria and there was no growth after 5 days of tissue culture.

Gram staining of the synovial aspirate from the patient's painful, swollen left ankle revealed 1+ epithelial cells, no white blood cells, and no visible organisms. Likewise, there was no growth in culture after 5 days. However, it should be noted that the specimen was obtained after the implementation of empirical antibiotics. In addition, repeat blood cultures drawn on the same day also revealed no growth at 5 days.

The patient was discharged in stable condition, and appropriate follow-up appointments were scheduled. However, approximately 2 weeks after discharge, the patient developed a persistent

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TABLE 1 Antibiotic susceptibilities of *C. diphtheriae* biotype Gravis from blood

Antibiotic	MIC ($\mu\text{g/ml}$)	Interpretation ^a
Azithromycin	0.064	NA
Cefepime	1.0	NA
Cefotaxime	4	R
Ceftriaxone	2.0	I
Ciprofloxacin	≤ 0.50	S
Clindamycin	≤ 0.12	S
Daptomycin	≤ 0.25	S
Erythromycin	≤ 0.25	S
Gentamicin	≤ 2	S
Imipenem	0.064	S
Linezolid	≤ 0.50	S
Meropenem	2.0	S
Penicillin	0.50	S
Quinopristin-dalfopristin	≤ 0.12	S
Rifampin	≤ 0.50	S
Tetracycline	≤ 2	S
Trimethoprim-sulfamethoxazole	$\leq 0.5/9.5$	S
Vancomycin	≤ 1	S

^a NA, no interpretation according to CLSI M45-A2; R, resistant; S, sensitive; I, intermediate.

fever, despite continued intravenous antibiotic treatment. She also complained of shortness of breath. Consequently, she was subsequently readmitted to the hospital for further workup. Her physical examination was significant for an irregular heart rate and rhythm with a III/VI blowing diastolic murmur at the left sternal border. On the day of admission, a TEE revealed an immobile mechanical valve leaflet with a 1.5-cm mass that represented a thrombus or vegetation. In addition, the TEE showed severe mitral valve stenosis and aortic regurgitation. The patient had subtherapeutic anticoagulation with an international normalized ratio of 1.1. It was therefore suspected that the mitral valve findings were representative of a thrombosis. Repeat blood cultures were negative, the patient was appropriately anticoagulated, and a repeat TEE showed resolution of the thrombus, as well as recovery of valve function.

The three initial aerobic blood cultures were positive for a pleomorphic Gram-positive rod exhibiting “diphtheroid” morphology. The colony morphology on sheep blood agar revealed mucoid, gray beta-hemolysis under the colonies at 18 to 24 h of incubation (Fig. 1). The isolate was catalase positive and did not exhibit tumbling motility, which helped exclude *Erysipelothrix rhusiopathiae* and *Listeria monocytogenes* as possible etiological agents (1). Identification was obtained by 16S rRNA sequencing (MicroSeq 500 16S rDNA bacterial identification kit and RipSeq [Isentio] software) and revealed a 100% match for *Corynebacterium diphtheriae*. The 16S rRNA sequence consisted of 458 bp and matched many RipSeq (Isentio)-targeted *C. diphtheriae* sequences, including the type strain, *C. diphtheriae* ATCC 700971, NCTC 13129, biotype Gravis. 16S rRNA sequencing was not performed on the tissue associated with the mechanical mitral valve and the synovial fluid because the specimens had been discarded prior to blood culture isolate identification. The isolate was sent to the Hawaii Department of Health State Laboratories (HSL) for phenotypic confirmation and referral to the Centers for Disease Control and Prevention (CDC) for toxin testing. The HSL performed conventional testing to verify key reactions such as black

colonies with a brown halo on modified Tinsdale medium, urease negativity, and nitrate reduction positivity. Testing with API Coryne v2.0 (bioMérieux, Inc., Durham NC) provided very good identification of the organism as *C. diphtheriae* biotype Gravis with 99.7% identity and a profile of 1110326. The CDC reproduced the HSL identification of *C. diphtheriae* biotype Gravis obtained with the API Coryne system and demonstrated the strain to be nontoxigenic with the Elek immunodiffusion assay. The isolate was further identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Inc., Billerica, MA) with a high score of 2.364 (the cutoff is 2.000). The best match was with *C. diphtheriae* 1G12072319_3e MVD in the MALDI-TOF MS database. Other *C. diphtheriae* strains in the database that matched at ≥ 2.000 (cutoff value) were at 2.261 (*C. diphtheriae* subsp. *mitis* R1_36_29ISB) and 2.090 (*C. diphtheriae* DSM44123T_DSM).

C. diphtheriae is a pleomorphic, Gram-positive, club-shaped bacillus that exhibits clumping (i.e., resembles “Chinese characters”). Its colonial morphology depends on the biotype and ranges from small, gray, translucent colonies on 5% sheep blood agar to medium-size colonies that are white and opaque. Suspected *C. diphtheriae* subcultured to modified Tinsdale medium reduces tellurite and produces the characteristic black colonies with a brown “halo.” Other Tinsdale halo-positive *Corynebacterium* spp. are *C. ulcerans* and *C. pseudotuberculosis*, which are easily excluded because, unlike *C. diphtheriae*, they hydrolyze urea. Since *C. diphtheriae* was not suspected in this case and tellurite-containing medium is not commonly used in most clinical laboratories, 16S rRNA sequencing was used for bacterial identification. The use of 16S rRNA sequencing is becoming increasingly more important in the identification of esoteric or uncommon isolates in the clinical microbiology laboratory. This is especially true for the culture identification of *C. diphtheriae*. In fact, 16S rRNA sequencing has

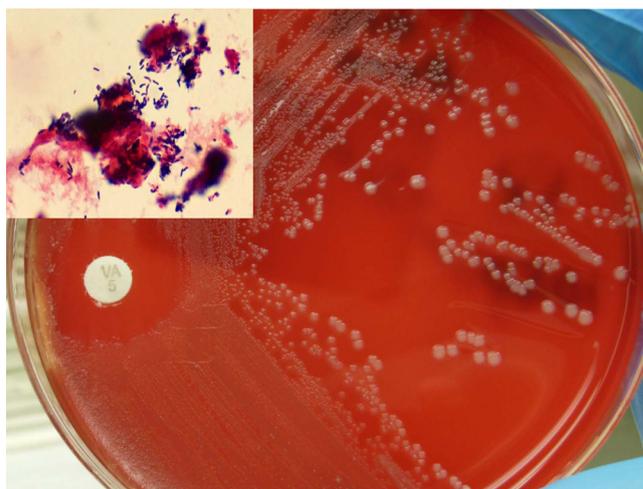


FIG 1 *C. diphtheriae* colony morphology on a sheep blood agar plate with a zone of sensitivity to vancomycin (5 $\mu\text{g/ml}$) suggestive of a Gram-positive bacillus. It should be noted that beta-hemolysis is not evident but is beneath the colonies. The inset shows direct Gram staining of a blood culture revealing the “club-shaped” microscopic morphology typical of Gram-positive bacilli ($\times 1,000$).

even been used directly on tissue in culture-negative endocarditis. In a recently reported case in the literature, 16S rRNA sequencing was used to avoid an incorrect presumptive diagnosis (2, 3).

C. diphtheriae is divided into four biotypes (Gravis, Mitis, Bel-fanti, and Intermedius) based on a variety of phenotypic characteristics. These identification methods include colony morphology, nitrate reduction, and fermentation reactions (3, 4). In addition, both toxigenic and nontoxigenic strains of *C. diphtheriae* exist based on the presence of a lysogenic β phage that harbors an endotoxin-producing gene (*tox*⁺). These toxigenic strains are required to cause clinical respiratory diphtheria (5).

The dramatic decrease in the incidence of diphtheria following the introduction of the vaccine has highlighted the emergence of invasive disease. This is evidenced by published cases of endocarditis due to nontoxigenic *C. diphtheriae*. (2, 6, 7). Some of these cases were associated with a high morbidity rate, including sepsis and death (7), even in pediatric patients (2, 6). A 2007 literature review identified 129 patients with *C. diphtheriae* endocarditis. It was found that most of these patients were adult males and one-third of the patients had an underlying valvular heart disease (8). The patient in the present case report also had underlying valvular heart disease status after mitral valve replacement. Interestingly, most of the cases of invasive disease due to nontoxigenic *C. diphtheriae* were described in European countries. We did not find any evidence in the literature to suggest that the frequency of infections due to *C. diphtheriae* in Hawaii is different from that in the rest of the United States or in other countries. A review of the literature did not reveal an increased rate of respiratory diphtheria cases or systemic infections in Hawaii. On the basis of an extensive literature and a Hawaii Department of Health record search, this is only the second case of *C. diphtheriae* septicemia with concomitant endocarditis in a prosthetic heart valve in Hawaii. According to HSL records, the first case was that of a man in 1982 who had endocarditis associated with a heart valve replacement. There are several reports suggesting that the transmission of nontoxigenic *C. diphtheriae* may be augmented by unsanitary conditions where individuals are in close proximity to one another (9–11). The patient in the present case report did not reveal any of the previously identified risk factors other than a remote history of methamphetamine use. A recent case report of *C. diphtheriae* sepsis in an immunocompromised host also suggests that a fragile immune system may facilitate invasive disease (12). Although the patient described here had a history of previous sepsis after a surgical procedure, she did not have a history of immunosuppression or any other evidence suggestive of an immunocompromised state. One possibility is that the patient was an asymptomatic carrier who was infected with the nontoxigenic strain of *C. diphtheriae* until conditions were ideal for the organism to proliferate and cause disease. Internalization of nontoxigenic *C. diphtheriae* using

cultured human respiratory epithelial cells has suggested a possible mechanism of throat colonization that could lead to asymptomatic carriage resulting in bacterial eradication failure (13).

In summary, this is a rare case of nontoxigenic *C. diphtheriae* septicemia with concomitant endocarditis in an immunocompetent host with a prosthetic mitral valve rereplacement in Hawaii.

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