Bloodstream Infection Caused by Nontoxigenic *Corynebacterium diphtheriae* in an Immunocompromised Host in the United States

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Corynebacterium species are well-known causes of catheter-related bloodstream infections. Toxigenic strains of *Corynebacterium diphtheriae* cause respiratory diphtheria. We report a bloodstream infection caused by a nontoxigenic strain of *C. diphtheriae* and discuss the epidemiology, possible sources of the infection, and the implications of rapid species identification of corynebacteria.

CASE REPORT

A 23-year-old male resident of Ohio with acute myelogenous leukemia (AML) was admitted for neutropenic fever. He had undergone induction chemotherapy and completed two cycles of high-dose cytarabine treatment since his diagnosis 2 months prior to the current illness. He had been feeling well after his last round of chemotherapy until developing sore throat and left ear pain a few days prior to admission. His medical history was significant for recurrent pharyngitis and otitis at ages 19 and 20. In both of those episodes, he responded well to therapy with azithromycin. However, throat cultures were negative for *Streptococcus pyogenes* in both instances. He had not traveled outside the United States since a visit to Quebec in 2009. He had no sick contacts. There were no known animal exposures. He and his family had received all immunization series as recommended for his age by the Advisory Committee on Immunization Practices.

On the day of admission, the patient presented with an elevated temperature of 101.2°F (38.4°C), blood pressure of 115/65, and heart rate of 130 beats/min. The tympanic membrane was visible and was not inflamed. The oropharynx was nonerythematous, although the posterior pharynx could not be seen well. There was tenderness to palpation in the left cervical area, without lymphadenopathy or soft-tissue swelling. For administration of chemotherapy, the patient had a triple-lumen Hickman catheter placed in the right internal jugular vein. The insertion site had no erythema, swelling, or drainage. The white blood cell count was 0.17 k/ μ l. Four blood cultures were drawn: one from each port of the Hickman catheter and one from his right arm. Due to allergies to penicillin and ceftazidime, administration of intravenous meropenem (500 mg every 6 h) was initiated. All four blood cultures were positive for Gram-positive, coryneform-like bacilli. A throat swab was negative for *S. pyogenes* by DNA probe (Group A *Streptococcus* Direct Test; Gen-Probe, San Diego, CA). Three more blood cultures were drawn from the Hickman catheter ports 2 days postadmission, and intravenous daptomycin (6 mg/kg of body weight daily) was added to provide therapy for treatment of a possible *C. jeikeium* infection. Due to concern for line infection, the Hickman catheter was removed 5 days posthospitalization and the catheter tip was sent for culture. The results for the second set of blood cultures and the catheter tip were negative. His sore throat improved within 4 days of initiation of meropenem therapy, and he was discharged 8 days after admission. Meropenem

therapy was discontinued, and he received intravenous daptomycin (450 mg daily) for 2 weeks as an outpatient.

Species identification, biotyping, and antibiotic susceptibility testing were performed on the organism isolated from one of the four blood cultures collected through a catheter port. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis of unextracted cells was conducted on a Microflex LT (research use only) instrument (Bruker, Billerica, MA) (1, 7). The organism was identified as *Corynebacterium diphtheriae* (score > 2.0), but the software (Biotyper software version 3.0) was unable to distinguish biotypes within the species, as was reported previously by Konrad et al. (7). The next best identifications for *Corynebacterium* species with the Biotyper software were the other toxin-producing species, *C. ulcerans* and *C. pseudotuberculosis*. However, scores of 1.397 and 1.324, respectively, for these species were unacceptably low. An identification of *Corynebacterium diphtheriae* to the *mitis/belfanti* group level was determined by biotyping performed with the API Coryne biochemical panel (bioMérieux, Marcy l'Etoile, France). Classical tube biochemicals, including glucose, maltose, nitrate, catalase, mannitol, and urea, were utilized for species confirmation. Based upon the ability of the strain to reduce nitrate, final identification of *C. diphtheriae* biotype *mitis* could be assigned (4). The organism identification was verified and the isolate was found to be nontoxigenic by the modified Elek test at the Centers for Disease Control and Prevention (CDC). Susceptibility testing was performed by broth microdilution with 5% lysed horse blood in Mueller-Hinton broth and interpreted according to CLSI guidelines (2). The isolate was susceptible to the following antibiotics: penicillin, clindamycin, erythromycin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, meropenem, imipenem, daptomycin, and vancomycin. It was resistant to ceftriaxone (MIC, 4 μ g/ml).

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Discussion. *Corynebacterium diphtheriae* bacteria are aerobic, Gram-positive rods that exhibit a “club-shaped” morphology. The species is divided into four biotypes (*gravis*, *mitis*, *belfanti*, and *intermedius*) based on colony morphology, fermentation reactions, and nitrate reduction (4, 5). Strains that harbor a lysogenic β -phage that carries an endotoxin-producing gene (*tox*⁺) cause respiratory diphtheria (9).

The Cleveland Clinic Microbiology Laboratory performs identification and susceptibility testing for coryneform organisms isolated from blood and sterile sites within 72 h of a previous positive culture. The intent of this protocol is to provide useful information to clinicians treating probable infections; however, the protocol provides limited genus information and no susceptibility data for likely contaminants. Using this procedure, 78 corynebacteria were identified in the past 11 years. The most frequently isolated species were *C. jeikeium* (17%) and *C. pseudodiphtheriticum* (27%). No species could be determined for 26% of isolates. This case was the first known isolation of *C. diphtheriae* by the Cleveland Clinic Microbiology Laboratory. As the biochemical panels utilized are able to identify *C. diphtheriae*, we believe that if *C. diphtheriae* were commonly encountered in our patient population it would have been previously observed as a bloodstream pathogen.

While commercial panels and classical biochemical methods are accurate for identification of *C. diphtheriae* and *C. ulcerans*, identification of other *Corynebacterium* species is often accurate only to the genus level with those tests. Thus, there is a need for molecular methods such as 16S rRNA or *rpoB* gene sequencing for definitive species identification (4, 5, 7). Therefore, many laboratories do not provide species results for corynebacteria. MALDI-TOF MS is gradually being incorporated as an identification tool in clinical laboratories, providing accurate identification for most species within minutes. Konrad et al. reported an analysis of 116 corynebacterial species, including 78 *C. diphtheriae* strains, that demonstrated 99% accuracy of MALDI-TOF MS compared to *rpoB* gene sequencing for identification to the species level within the corynebacteria (7). A more recent publication tested 92 clinical *Corynebacterium* species isolates, and 87% were correctly identified to the species level (1). As MALDI-TOF MS becomes more widely utilized, we believe that it is likely that more clinical laboratories will provide species data for corynebacteria, particularly when isolated repeatedly from invasive sites.

In the United States, where vaccination is widely accepted, no confirmed case of diphtheria has been reported since 2003 (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5253a3.htm> [accessed 23 January 2012]). Having isolated a nontoxigenic strain from a bloodstream infection, we were interested in determining the frequency of similar infections in the United States in order to understand the probable source and associated epidemiology. Cases of invasive nontoxigenic *C. diphtheriae* in the United States appear to be rare. Three outbreaks that included both toxigenic and nontoxigenic *C. diphtheriae* strains occurred among urban adults in Seattle, WA, from 1972 to 1982 (6). Eighty-six percent of the infections were cutaneous, and some were pharyngeal. Invasive infections were reported only for patients with toxigenic strains. A review of published literature did not yield other reports of nontoxigenic *C. diphtheriae* infections in the United States.

Isolation of nontoxigenic *C. diphtheriae* is, however, being reported with increasing frequency elsewhere, with publications from France, Poland, Switzerland, the United Kingdom, New

Zealand, Australia, Canada, and other countries (8, 10–12, 16, 17). Those articles describe invasive infections, including septicemia, endocarditis, and osteoarthritis, as well as wounds, cutaneous infections, and pharyngitis. While cardiac abnormalities have been identified in some patients with endocarditis due to nontoxigenic *C. diphtheriae*, for other invasive infections, risk factors often remain undefined (10, 15). In many of the described cases of systemic disease due to nontoxigenic *C. diphtheriae*, the patients have been intravenous drug abusers, alcohol addicts, the urban poor, or homeless (6, 8, 11, 13, 17). Given those risk factors, it appears that transmission may be facilitated by person-to-person contact in crowded, unhygienic environments (6, 8, 11). Our patient had none of the reported risk factors but was immunocompromised due to chemotherapy and had a Hickman catheter in place.

The source of our patient's infection is unclear. Possible routes of infection include extension from the skin through the catheter or invasion from the throat. The patient had no signs of skin infection, and the results of tests of follow-up blood cultures drawn through the original catheter and culture of the catheter tip were negative. However, antibiotic therapy might have prevented recovery from cultures at that time. For organisms commonly present on the skin, such as the corynebacteria, the CDC defines a central-line-associated bloodstream infection as recovery of an infecting organism from two or more blood cultures in a patient who had a central line at the time of or 48 h prior to infection. In addition, the bloodstream infection cannot be related to any other infection the patient might have (14). The presence of sore throat that improved upon antibiotic therapy casts some doubt upon the central line as the source of the bacteremia. *C. diphtheriae* is not currently considered a common cause of pharyngitis in the United States, and selective culture for this organism was not performed. As testing was negative, *S. pyogenes* was probably not the cause of his sore throat, and his symptoms were not consistent with mucositis due to chemotherapy. Given the lack of skin infection and improvement of symptoms on therapy, we hypothesize that he harbored a nontoxigenic strain of *C. diphtheriae* in his throat that lead to the bloodstream infection.

The best descriptions of nontoxigenic *C. diphtheriae* associated with pharyngitis/tonsillitis have been from the United Kingdom (3, 12). The number of *C. diphtheriae* cases reported to the Streptococcus and Diphtheria Reference Unit of the Public Health Laboratory Service rose substantially from 1986 to 1999, with 250 cases reported in 1999 alone (12). The data from the United Kingdom may represent a true increase in cases or they may be a reflection of enhanced detection due to heightened awareness and surveillance activities in the United Kingdom. Clinical data available for 238 patients with nontoxigenic strains submitted to the reference laboratory indicate that 90% had sore throats. As in our case, 62% of those patients were in the 15-to-24-year-old age group (12). The incidence of nontoxigenic *C. diphtheriae* pharyngitis in Scotland has been estimated to be 0.68 cases per 100,000 people (3). Thus, while pharyngitis associated with nontoxigenic *C. diphtheriae* occurs, it is relatively uncommon and may be limited to particular geographic regions. Susceptibility to the antibiotics used for streptococcal pharyngitis may contribute to an underestimation of the disease burden.

The experience in other countries suggests that nontoxigenic *C. diphtheriae* strains are sufficiently pathogenic to cause disease. Coupling this finding with an enhanced ability of identification to the species level by MALDI-TOF MS, we suspect that more cases

may be identified in the United States in the future. While the definitive source of the infection in our patient was not identified, this case report raises awareness of the potential for *C. diphtheriae* to cause bloodstream infection secondary to pharyngitis or skin infection.

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