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Pediatric bacteremia caused by *Chromobacterium haemolyticum*/*Chromobacterium aquaticum*

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ABSTRACT

We present a case of pediatric bacteremia caused by *Chromobacterium haemolyticum*, a β-hemolytic, non-pigmented, Gram-negative bacilli recovered from a blood culture and initially identified as *Chromobacterium violaceum* using phenotypic and proteomic methods. 16S rRNA sequencing of the patient isolated demonstrated a high degree of sequence homology with the type strain of *C. haemolyticum*. The patient recovered following treatment with meropenem, gentamicin, and trimethoprim/sulfamethoxazole. This case highlights the potential misidentification of *C. haemolyticum* as non-pigmented *C. violaceum* due to limitations of the currently available identification methodologies.

An 11-year-old male presented to the Emergency Department with a fever (102 °F), chest pain, chills, and a headache. This patient had a complicated history of congenital heart disease associated with double outlet right ventricle, pulmonary stenosis, and Rastelli C-type atrioventricular canal defect and has had previous cardiology surgeries involving hardware installation, most notably, a prosthetic mitral valve and an RV-PA conduit in place since 2008. The patient also had a history of *Kingella kingae* endocarditis one month prior to the current admission which had been complicated by multiple mycotic aneurysms. Three weeks prior to the current admission, the patient was admitted with febrile episodes attributed to an enteroviral infections. All blood cultures at that time were negative.

Upon presentation at this admission, the patient was clinically septic with his fever peaking at 104.4 F, tachypnea, a peripheral leukocytosis and elevated inflammatory markers (Table 1). Blood cultures were collected, and after 48–51 hours of incubation in the Bactec blood culture instrument (Becton Dickinson, Franklin Lakes, NJ), the bottle flagged as positive and Gram-negative bacilli were reported from the Gram-stained smear. Since the previous episode of endocarditis a month prior, the patient had been undergoing intravenous (IV) therapy with ceftriaxone given via peripherally inserted central venous catheter (PICC). The initial concern was that the *Kingella* bacteremia had recurred while the patient was on therapy. The blood culture was subcultured to 5% sheep blood, MacConkey and Chocolate agar plates (Becton, Dickinson, and Company, Franklin Lakes, New Jersey, USA). After 16-18 hours of incubation in 5% CO_2 at 35°C, gray (non-pigmented), β-hemolytic colonies with a 3 mm zone of hemolysis were observed on the blood agar and very small, hazy growth was observed on the MacConkey agar (Fig. 1). Identification performed by a Vitek MS MALDI-TOF (BioMérieux, Marcy-l'Étoile, France) yielded an identification of *Chromobacterium violaceum*, but the identification was listed as "unclaimed" in the VITEK MS IVD database. Additional testing was performed using the Vitek 2 system (BioMérieux, Marcy-l'Étoile, France) yielding an identification of *Chromobacterium violaceum.* Conventional biochemical testing indicated that the organism was oxidase positive but catalase and indole negative. While most reported isolates of *C. violaceum* produce a violet or purple pigment, non-pigmented strains have been reported. Furthermore, *C. violaceum* isolates are typically catalase positive. The identification of the organism was reported as *Chromobacterium violaceum*.

Two days following the initial admission, a second blood culture was collected. A phenotypically identical isolate was recovered from this culture and was also identified as *C. violaceum*. *Stenotrophomonas maltophilia* was also isolated. 16S RNA sequencing was later performed on the *Chromobacterium* isolate and the results were compared to published sequences using BLAST. This yielded 99.29% homology with a sequence from *Chromobacterium haemolyticum* and 99.13% homology with a sequence from *Chromobacterium aquaticum*.

The patient was placed on combination therapy of meropenem, gentamicin, and trimethoprim/sulfamethoxazole until antimicrobial susceptibilities were determined. His PICC line was also removed the same day the bacteremia was identified. The PICC was the likely source of the bacteremia, as peripheral cultures drawn at the same time were negative. Susceptibility testing was performed by Etest (BioMérieux, Marcy-l'Étoile, France), and the organism was reported as susceptible

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Table 1
Patient laboratory results.

	Patient value	Reference range
WBC	14.2 (H)	4.5–13.5 K/mcL
RBC	3.64 (L)	4.00–5.20 M/mcL
HGB	11.5	11.5–15.5 gm/dL
HCT	32.7 (L)	35.0–45.0%
MCV	89.8	77.0–92.0 fL
MCH	31.5	25.0–33.0 pg
MCHC	35.1	31.0–37.0 gm/dL
RDW	13.4	≤14.6%
Platelet	310	135–466 K/mcL
Procalcitonin	2.25 (H)	≤0.5 ng/mL
ESR	28	0–10 mm/hr
CRP	5.87 (H)	≤0.30 mg/dL

H = High; above reference range, L = Low; below reference range.

Table 2
Patient isolate antimicrobial results by Etest.

Antimicrobial	MIC (μg/mL)	Interpretation
Levofloxacin	0.012	S
Meropenem	4	S
Pipercillin/Tazobactam	≥256	R
Tobramycin	12	R
Ceftazidime	3	S
Amikacin	24	I
Aztreonam	3	S
Colistin	≥256	R
Cefepime	≥256	R
Trimethoprim/Sulfamathoxazole	4	S
Gentamicin	0.5	S

to levofloxacin, meropenem, trimethoprim-sulfamethoxazole, and gentamicin using guidelines published by the Clinical and Laboratory Standards Institute (Table 2) (Institute CaLS, 2014). Upon review of the susceptibility results, the patient was released from the hospital on IV ceftriaxone (continuing therapy for the previous *Kingella* infection and to address mycotic aneurysms) and oral levofloxacin. Antimicrobial therapy was discontinued at 2 months after resolution of the mycotic aneurysms. His cardiac prostheses were left in place and his infection has not recurred after 3 months of follow-up.

The genus *Chromobacterium* consists of 7 recognized species: *Chromobacterium aquaticum*, *C. haemolyticum*, *Chromobacterium piscinae*, *Chromobacterium pseudoviolaceum*, *Chromobacterium subtsugae*, *Chromobacterium vaccinia*, and *C. violaceum* (Han et al., 2008; Sivendra, 1976; Soby et al., 2013; Young et al., 2008). Of these 7 species, *C. violaceum* and *C. haemolyticum* are the only species previously reported to cause infections in humans. *C. violaceum* is a Gram-negative, facultative anaerobic bacterium that is typically found in soil and water sources in tropical and sub-tropical climates (Campbell et al., 2013; Kaufman et al., 1986). Most strains of *C. violaceum* produce a violet pigment (violacein) when grown on solid media (Fig. 2); however rare non-pigmented strains have been reported (Yang, 2011). Cases of human infections with *C. violaceum* have been reported in Vietnam, Argentina, Malaysia, Taiwan, South Korea, India, and the Southeastern portions of the United States (Baker et al., 2008; Campbell et al., 2013; Kaufman et al., 1986; Kumar, 2012; Lee et al., 1999; Moore et al., 2001; Yang, 2011; Yang and Li, 2011). Infections typically begin as skin or soft tissue infections, but may progress to septicemia with or without multiple organ abscesses (Baker et al., 2008; Kaufman et al., 1986; Moore et al., 2001; Yang, 2011). *C. violaceum* is resistant to many antimicrobials including β-lactams and colistin. However, it is generally susceptible to fluoroquinolones, tetracyclines, imipenem, and gentamicin. Infection due to *C. violaceum* is unusual, especially within the continental United States. Only 16 cases of pediatric *C. violaceum* infection have been reported in the U.S between 1971 and 2004 with 9 of those resulting in fatalities (Sirinavin et al., 2005). Of those 9 fatalities, only 4 patients received appropriate antimicrobial therapy. There have been at least 5 previous cases reported of patients infected with non-pigmented strains of *C. violaceum* (Bosch et al., 2008; Lee et al., 1999; Sorensen et al., 1985; Yang, 2011). Our findings, as well as those by Han *et al.*, indicate the possibility that previous isolates identified by conventional biochemicals (API 20E, Vitek 2, Microscan) and reported as a non-pigmented, β-hemolytic strain of *Chromobacterium violaceum* may, in fact, be *C. haemolyticum* (Han et al., 2008).

C. haemolyticum, first described by Han et al. in 2008, is a Gram-negative bacillus that does not produce a violet pigment on solid media but demonstrates strong hemolysis on sheep blood agar (Fig. 1) (Han et al., 2008). It has been recovered from lake water in a tropical region (Lima-Bittencourt et al., 2011) and in 4 clinical specimens (Han et al., 2008; Okada et al., 2013; Takenaka et al., 2015; Tanpowpong et al., 2014). It is characterized by its hemolytic activity on sheep blood agar, with large zones of hemolysis surrounding individual colonies that have reached 5 mm in diameter after 24 hours of incubation (Han et al., 2008).

C. aquaticum, described by Young, et al. in 2008, was recovered from spring water samples collected in Taiwan (Institute CaLS, 2014). Phenotypically, it is similar to *C. haemolyticum*. Both isolates produce β-hemolysis on blood agar, colonies are non-pigmented, and catalase reactions are typically negative. Both are sensitive to fluorquinolones and resistant to penicillin (Institute CaLS, 2014; Young et al., 2008). Currently, no known cases of human infection caused by *C. aquaticum* have been reported.

In this case of pediatric bacteremia, the causative agent was initially identified as *C. violaceum* and later identified as *C. haemolyticum*/*C. aquaticum*. Phenotypic testing via VITEK 2 and proteomic analysis via MALDI-TOF mass spectrometry identified this isolate as *C. violaceum*. Unlike typical isolates of *C. violaceum,* this isolate was non-pigmented, hemolytic, and catalase negative, thus further analysis was performed. 16S rRNA sequencing of this isolate yielded a sequence that had greater sequence homology with *C. haemolyticum*/*C. aquaticum*. Given the results of 16S rRNA sequencing, the lack of pigment production, and the β-hemolytic nature of this isolate, the patient isolate is likely *C. haemolyticum*/*C. aquaticum*.

In an effort to better understand the relationship between *C. violaceum*, *C. haemolyticum,* and *C. aquaticum,* further phylogenetic

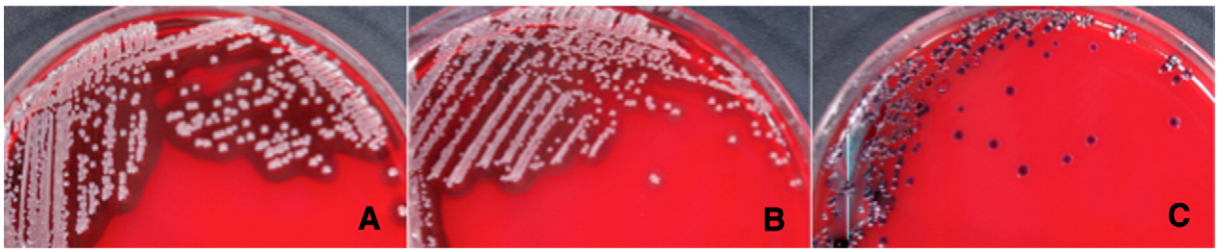

Fig. 1. Growth on 5% sheep blood agar following 24 hours of incubation at 35 °C in CO_2. (A). *C. haemolyticum* MDA0585^T. (B) Patient isolate. (C) *C. violaceum* ATCC 12472.