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The Brief Case: Recurrent *Chromobacterium violaceum* Bloodstream Infection in a Glucose-6-Phosphate Dehydrogenase (G6PD)-Deficient Patient with a Severe Neutrophil Defect

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CASE

A 37-year-old male with a medical history of glucose-6-phosphate dehydrogenase (G6PD) deficiency (Beaumont variant) presented at the emergency department (ED) with fatigue, malaise, generalized joint pains, and chills. On exam, he was febrile (40.2°C) and tachycardic (143 beats/min) while normotensive (124/75 mm Hg) and oxygenating well on ambient air (99% SpO_2). His blood chemistries were remarkable, with elevated lactic acid at 3.27 mmol/liter (range, 0.5 to 2.20 mmol/liter) and procalcitonin at 0.35 ng/ml (range, <0.05 ng/ml). Additional lab findings showed a normal white blood cell (WBC) count (7,192 cells/μl; range, 4,000 to 11,000/μl), with 80% neutrophils, and signs of acute hemolytic anemia, with high reticulocytes at >17.97% (range, 0.59 to 2.24%), low hemoglobin (Hgb; 9.9 mg/dl; range, 12.2 to 16.4 mg/dl), hyperbilirubinemia (unconjugated bilirubin, 3 mg/dl; range, 0.1 to 1.1 mg/dl), and high ferritin level (3,240 ng/ml; range, 18 to 464 ng/ml). He was given a dose of 1 g meropenem intravenously (i.v.) and admitted for further management of suspected septicemia.

The urine and sputum cultures collected at the ED were negative, but aerobic blood cultures became positive after 18 h of incubation. The initial Gram stain showed Gram-negative rods (Fig. 1A); however, the Verigene Gram-negative blood culture nucleic acid test (BC-GN; Luminex Co., Austin, TX) did not identify any organisms. Blood bottle subcultures grew purple colonies of Gram-negative rods on both 5% sheep blood (SBA) and MacConkey (MAC) agars (Fig. 1B and C). Identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS; bioMérieux, Inc., Hazelwood, MO) revealed *Chromobacterium violaceum*, which was confirmed by 16S rRNA sequencing (GenBank accession number MH790126) with 99.93% (1,470/1,471 nucleotides) identity to *C. violaceum* ATCC 12472^T. Antimicrobial susceptibility testing was performed using a Sensititre Gram-negative nonfermenters MIC plate (Thermo Fisher Scientific, Waltham, MA) and an Etest (bioMérieux Inc., Hazelwood, MO) for meropenem. The MIC values were reported with no interpretation (Table 1). He was given 14 days of high dose meropenem (2 g every 8 h [q8h] i.v.) and doxycycline (100 mg twice a day oral [BID]). Due to a continuous decline in his Hgb and platelets, he was given 1 unit of packed red blood cells and platelet transfusion on hospital day (HD) 2. Subsequent blood cultures drawn 48 and 96 h postbacteremia were negative. On HD 12, he was discharged with doxycycline maintenance therapy. At

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.00314-19 in this issue.

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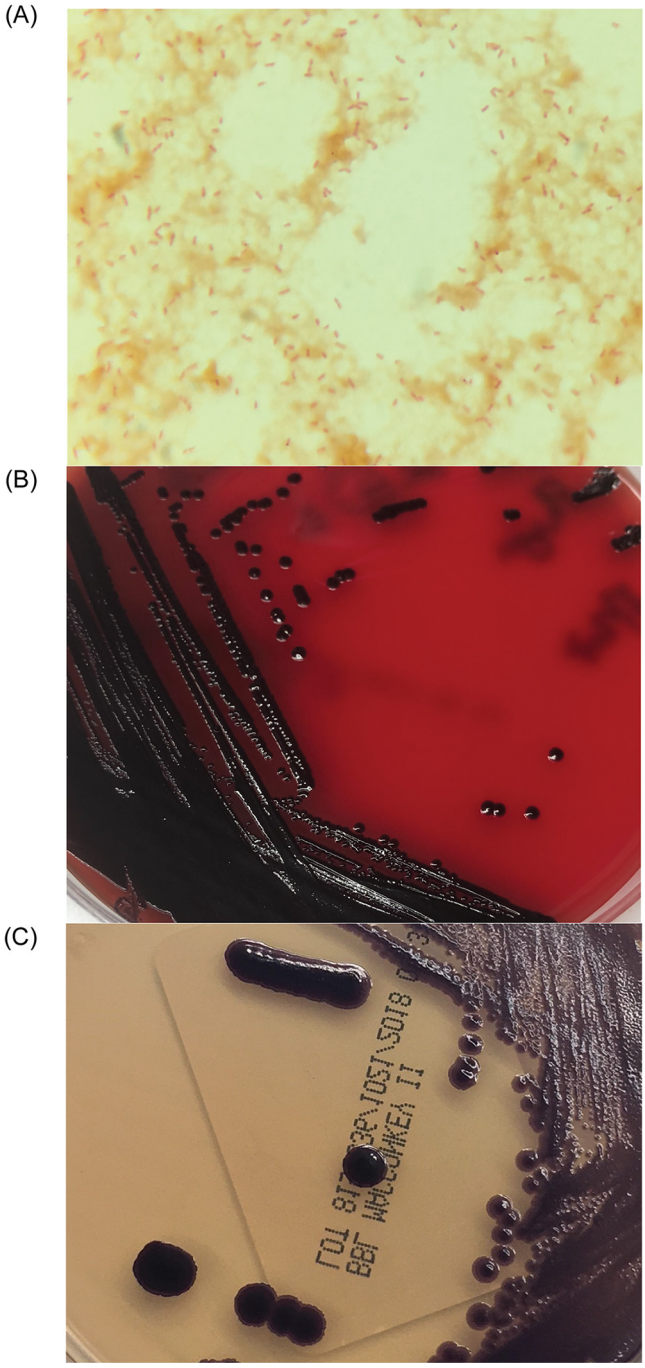

FIG 1 (A) Gram stain of *Chromobacterium violaceum* from positive aerobic blood culture bottle showing Gram-negative bacilli (×1,000). (B and C) Purple-colored colonies of *C. violaceum* isolated from the overnight subcultures (5% sheep blood agar [B] and MacConkey agar [C]) of positive blood culture bottle.

follow-up visits at 2 and 4 months, examination revealed no signs of infection, and inflammatory markers were within normal ranges.

Interestingly, the patient had had a previous episode of *C. violaceum* bacteremia 10 months earlier secondary to a leg abscess from which the same organism was also grown. The wound developed after wading in the floodwaters of a hurricane in Texas. The isolate from his positive blood cultures was identified at another hospital; susceptibility testing was not performed. He was transferred to our hospital, and *C. violaceum* from his wound culture was identified in our laboratory. The susceptibility profile of the

TABLE 1 MIC values for *Chromobacterium violaceum* isolated from the wound and positive blood cultures

Antibacterial drug	MIC (μg/ml) from:	
	Wound culture during previous admission	Positive blood culture during current admission
Piperacillin	8	32
Ampicillin-sulbactam	>16/8	>16/8
Piperacillin-tazobactam	≤8/4	16/4
Ticarcillin-clavulanic acid	>128/2	>128/2
Ceftazidime	16	>16
Ceftriaxone	32	>32
Cefotaxime	>32	>32
Cefepime	4	8
Aztreonam	4	8
Imipenem	≤1	
Meropenem		0.19[a]
Amikacin	16	8
Gentamicin	4	4
Tobramycin	4	2
Tetracycline	≤1	≤1
Levofloxacin	≤0.12	≤0.12
Ciprofloxacin	≤0.25	≤0.25
Trimethoprim-sulfamethoxazole	≤0.5/9.5	≤0.5/9.5
Chloramphenicol	4	4

[a]Performed by Etest (bioMérieux Inc., Hazelwood, MO).

organism from the wound was similar to that of the organism isolated from the blood during the current admission (Table 1). He was given meropenem (2 g i.v.) and levofloxacin (100 mg BID) and discharged with a 6-week course of levofloxacin, which was discontinued 1 day early due to the development of arthralgias. His computed tomography (CT) scan showed hepatomegaly, splenic microinfarcts, hepatic lesions, and pulmonary nodules, all of which greatly improved at 6- and 9-week follow-up visits. Of note, his abscess was completely healed when he was admitted for the current episode, and he denied repeat exposure to stagnant water.

DISCUSSION

C. violaceum is a facultative anaerobic Gram-negative bacillus that is ubiquitously found in environments, such as soil and stagnant water, in tropical and subtropical environments worldwide (1, 2). It is catalase positive and is typically recognized for production of a violet pigment called violacein (3). As it gives variable reactions for oxidase and indole assays and its pigmentation can affect interpretation, other diagnostic measures should be taken for absolute identification (3). Colonies of *C. violaceum* grow well on 5% SBA and MAC and produce an almond-like smell (1). Although human infections by *C. violaceum* are uncommon, it has emerged as an opportunistic environmental pathogen and is associated with severe morbidity and mortality (1, 2), with a historic mortality rate as high as 65.6% (1). Certain immunodeficiencies, including chronic granulomatous disease (CGD), G6PD deficiency, and diabetes, appear to be important predisposing conditions for *C. violaceum* infection (1, 2, 4, 5).

C. violaceum infection usually originates from a localized contaminated wound (6). Several case reports have demonstrated *C. violaceum* infection in children with a history of exposure to contaminated soil and water, or soldiers with battle wounds (1, 6). The organism can rapidly disseminate through the bloodstream and cause multiorgan abscesses and fatal sepsis (1, 6). Relapses and recurrent infection are common and have been reported (1).

The ability of *C. violaceum* to secrete an extracellular protein collagenase and the possession of flagella (7, 8) would likely explain the rapid dissemination of *C. violaceum* from a localized wound into multiorgan abscesses. Fulminant septicemia caused by *C. violaceum* can be attributed to the production of hemolysin and other cytolytic toxins

(8). Interestingly, the violacein pigment produced by *C. violaceum* has been a major interest in biotechnological applications due to the antimicrobial potency of this pigment (7). Although violacein has certain cytotoxic activity, it is not clear whether it is a major virulence factor for *C. violaceum* pathogenicity, since nonpigmented strains display similar pathogenicity to the pigmented ones (9).

The organism can lose pigmentation upon subsequent cultures from the parental strain or exposure to stressful environments, such as freeze-thaw cycles in laboratories (3). While pigmented colonies are easily identifiable, nonpigmented isolates can be mistaken for *Aeromonas* or *Vibrio* species (3). As *C. violaceum* can be β-hemolytic like most *Aeromonas* species, these organisms can be differentiated by biochemical characteristics, such as positive acid production from maltose and mannitol by *Aeromonas* species (3, 10). Currently, diagnostic laboratories can identify *C. violaceum* by automated biochemical systems (Vitek, MicroScan, and Phoenix), MALDI-TOF MS systems, or 16S rRNA sequencing (11). Of note, both pigmented and nonpigmented colonies of *C. violaceum* were recovered from the frozen isolates of the wound culture of our patient and were confirmed by 16S rRNA sequencing, with 99.87% (1,492/1,494 nucleotides) and 99.93% (1,471/1,472 nucleotides) identity to *C. violaceum* ATCC 12472^T, respectively (GenBank accession numbers MG938492 and MG938493). Only pigmented colonies were observed with the bloodstream isolate during the current episode.

There are currently no recommended guidelines for interpreting antimicrobial susceptibility testing data for *C. violaceum*, most likely due to its rare occurrences in clinical settings. *C. violaceum* displays resistance to most penicillins and cephalosporins and to some β-lactam/β-lactamase inhibitor combinations (e.g., amoxicillin-clavulanate) (1, 12). It also shows various levels of resistance to other classes (1) and natural resistance to polymyxins (e.g., colistin) (13). Although β-lactam resistance appears to be increasing over time, most isolates show sensitivity to meropenem, imipenem, and piperacillin-tazobactam (1, 12). Ciprofloxacin is reported to be the most effective antibiotic among 25 antibiotics of various classes tested *in vitro* (1). Other antibiotics with adequate activity against *C. violaceum* are trimethoprim-sulfamethoxazole, tetracyclines, aminoglycosides, and chloramphenicol (1). Proposed mechanisms for antibiotic resistance include β-lactamase production and lipid A modification against β-lactams and polymyxins, respectively (12, 13).

One of the primary mechanisms of host immune responses in combating *C. violaceum* infection is by antimicrobial activities of neutrophils via expression of superoxide dismutase enzyme and the release of reactive oxygen species (ROS) (14). G6PD is essential in maintaining redox equilibrium of cellular NADPH that results in the production of ROS, including hydrogen peroxide (H_2O_2), for killing pathogens (14). Microorganisms also produce H_2O_2 from their metabolic processes, which the host cells can utilize to fight against infection. Catalase-positive organisms can neutralize endogenous H_2O_2, unlike catalase-negative microbes (14). Therefore, individuals with neutrophil deficiency or functional defects in intracellular bactericidal activity, such as CGD, G6PD deficiency, or diabetes, are especially prone to infection by catalase-positive organisms like *C. violaceum* (1, 2, 14). Based on the World Health Organization classification of G6PD deficiency, our patient displayed class I severe G6PD deficiency ($<$10% activity). Importantly, the nitroblue tetrazolium (NBT) and bacterial killing assays, which test for neutrophil superoxide production and intracellular killing of *Staphylococcus aureus*, respectively, conducted on the blood samples of our patient demonstrated a severe defect in bactericidal activity (5). His neutrophil G6PD activity was only 3.51% of normal (5). Besides the exposure of floodwaters to the leg wound, G6PD deficiency (Beaumont variant) with severe neutrophil defect was one of the most significant risk factors that predisposed our patient to *C. violaceum* infection. Interestingly, the twin brother of our patient also had G6PD deficiency and died at age 3 from septic infection with *C. violaceum* after playing in the mud (5). Therefore, patients with G6PD, CGD, or similar intracellular bactericidal defects should promptly seek medical treatment due to the risk of developing severe infections from the environment.

A case series in 2011 reported that reinfection with *C. violaceum* occurred in 7 out

of 106 cases within a median of 135 days (1). Interestingly, asymptomatic colonization with *C. violaceum* in gastrointestinal and respiratory sites has been documented (4). The reported duration of antibiotics in successfully treated patients varies and is likely affected by host factors, source control, and the tissue penetration of selected agents. Patients without CGD typically received 6 weeks to 3 months of therapy (1). Still, significantly delayed relapses have been described (1, 2). Our patient appears to have relapsed at 10 months without any clinical risk factors for reinfection—no recent wounds nor re-exposure to standing water. It is likely that he suffered a relapse due to incomplete clearance of the previous infection caused by ineffective neutrophil killing. Possible sources of relapse may originate from the previous microabscesses in the lungs, liver, and spleen. In this situation, he was placed on lifelong suppressive therapy.

In summary, this case highlights a severe form of G6PD deficiency as a prominent risk factor in recurrent *C. violaceum* infection. Since *C. violaceum* displays several virulence factors, and the recovery of this organism from clinical specimens often indicates severe infection, the isolate should be reported, susceptibility testing performed, and an infectious disease team consulted for proper antibiotic selection. Because of the severity and increasing incidence of *C. violaceum* infection in clinical settings, further studies focusing on the establishment of standardized antimicrobial guidelines may be necessary in order to optimize treatment options.

Data availability. Sequences were deposited in GenBank under accession numbers MH790126, MG938492, and MG938493.

SELF-ASSESSMENT QUESTIONS

1. What antibiotic would be ineffective in treating *Chromobacterium violaceum* infection?
    a. Imipenem
    b. Colistin
    c. Ciprofloxacin
    d. Trimethoprim-sulfamethoxazole
2. Which condition is not shown to be a predisposition for *Chromobacterium violaceum* infection?
    a. Chronic granulomatous disease
    b. Diabetes
    c. Factor VIII deficiency
    d. Glucose-6-phosphate dehydrogenase deficiency
3. What activity is associated with *Chromobacterium violaceum* infection?
    a. Inhaling cigarette smoke
    b. Eating raw oysters
    c. Drinking unpasteurized milk
    d. Swimming in a creek

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