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Fatal Wound Infection Caused by *Chromobacterium violaceum* in Ho Chi Minh City, Vietnam▽

Stephen Baker,[1,2]* James I. Campbell,[1,2] Richard Stabler,[3] Hoang V. M. Nguyen,[1,2,4] Diep S. To,[4] Dung V. Nguyen,[4] and Jeremy Farrar[1,2]

Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam[1]; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom[2]; London School of Hygiene and Tropical Medicine, London, United Kingdom[3]; and Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam[4]

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Chromobacterium violaceum **is a proteobacterium found in soil and water in tropical regions which rarely causes infection in humans. Here, we report a fatal bacteremia caused by** ***Chromobacterium violaceum*** **in Vietnam. We describe a number of clinical, microbiological, and molecular aspects associated with this bacterial infection.**

CASE REPORT

A 21-month-old human immunodeficiency virus-negative boy was admitted to the children's ward at the Hospital for Tropical Diseases (HTD), Ho Chi Minh City. The child was distressed and had a fever of 38.5°C, which peaked 3 days later at 40.8°C. The initial clinical presentation and examination suggested viral encephalitis of unknown origin. During the next 4 days, the child's condition rapidly deteriorated, and he was transferred to the Pediatric Intensive Care Unit at the HTD. A secondary examination identified a small red rash in the vicinity of his right nipple. The area had been scratched and had become inflamed, and the skin was broken; a presumptive diagnosis of sepsis of bacterial origin (*Staphylococcus aureus*) was made. By this time, the fever was slightly reduced (38°C), although he had developed respiratory distress and septic shock, characterized by a sudden drop in white-blood-cell (WBC) and platelet counts (Table 1) and cyanosis of the fingers. He was treated with high doses of intravenous oxacillin, vancomycin, and imipenem, placed on a ventilator, and monitored with intensive supportive measures.

Numerous tests were carried out upon transfer to the Pediatric Intensive Care Unit, including hematology and biochemistry lab tests, a cerebrospinal fluid investigation, a stool examination, and a blood sample test for microbiological blood culture. The blood sample was cultured in a Bactec bottle and incubated in an automated Bactec blood culture identification machine at 37°C. A positive result was recorded on the second day of incubation, and bacteria were isolated for identification. A Gram-stained film demonstrated a gram-negative bacillus. The bacteria were subcultured on blood agar and nutrient agar plates and incubated aerobically at 35°C overnight. The blood plates demonstrated numerous small colonies with a blue pigmentation, while a similar morphology was seen on the nutrient agar plates, although the colonies had a more metallic dark-violet sheen. This pigmentation is associated with *Chromobacterium violaceum* and is due to the production of a chemical called violacein (1). Identification was confirmed by using API 20NE, giving a score of 5152555 (99.9% identification, 0.72 T). The bacterium was named *C. violaceum* HTD1, and unlike the majority of previously reported cases of *C. violaceum* infection, the strain was mannitol positive (8).

C. violaceum is a gram-negative, facultative, anaerobic betaproteobacterium which can be routinely isolated from soil and water (10). It is associated in particular with slow-moving or stagnant water sources in tropical and subtropical regions. A swab from the rash on the boy's chest yielded a growth of *C. violaceum*, suggesting that this was the original entry point of the bacteria, although we were unable to confirm contact with stagnant water. These results were reported to the treating physicians 3 days after blood culture and wound sampling.

C. violaceum HTD1 was tested for antimicrobial sensitivity, and the results are presented in Table 2. The antimicrobial and MIC testing was performed on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute guidelines (3). The phenomenon of drug resistance in *C. violaceum* is well known, although the species is usually sensitive to aminoglycosides and chloramphenicol (5, 7).

TABLE 1. Hematology lab results over the course of *C. violaceum* infection[a]

Day(s) postadmission	WBC count (10^3/μl)	% Neutrophils	Hemoglobin count (g/dl)	Platelet count (10^3/μl)
1	21	47.5	12.9	320
4	19.7	86.6	10.1	240
5	1.62	61.3	10.8	159
6	8.39	81.3	10.2	86.6
8	25.9	85.9	7.83	47.9
9	22.2	83.9	7.99	52.8

[a] The normal ranges for WBC count, percent neutrophils, hemoglobin count, and platelet count are 4.3×10^3 to 10.8×10^3/μl, 45 to 74%, 14 to 18 g/dl, and 150×10^3 to 350×10^3/μl, respectively.

* Corresponding author. Mailing address: Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 190 Ben Ham Tu, Quan 5, Ho Chi Minh City, Vietnam. Phone: (84-8) 9 241 761. Fax: (84-8) 9 238 904. E-mail: sbaker@oucru.org.

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TABLE 2. Antimicrobial susceptibility results of *C. violaceum* HTD1

Antibiotic	Disc sensitivity result	MIC (μg/ml)
Etrapenem	Sensitive	Not done
Cefapime	Sensitive	6
Netacillin	Sensitive	Not done
Ciprofloxacin	Sensitive	0.006
Ofloxacin	Sensitive	0.016
Amikacin	Sensitive	16
Imipenem	Sensitive	1
Gentamicin	Sensitive	2
Ticarcillin	Intermediate	Not done
Ceftriaxone	Resistant	>256
Ceftazidime	Resistant	>256
Amoxicillin + clavulanic acid	Resistant	>256
Piperacillin/tazobactam	Resistant	24
Chloramphenicol	Sensitive	6

This particular isolate demonstrated high-level resistance to all tested cephalosporins; however, it did not exhibit typical extended-spectrum beta-lactamase activity when the combination disc method was used. This suggests a more general efflux-mediated resistance mechanism. Notably, the bacteria were sensitive to imipenem, which was one of the antimicrobials administered to the patient in the treatment cocktail.

Hematology lab results (Table 1) suggested massive bacteremia and septic shock, as the WBC count was initially $21.0 \times 10^3/\mu l$ and then dropped to $1.62 \times 10^3/\mu l$ and the platelet count dropped from 240×10^3 to $47.9 \times 10^3/\mu l$. This occurred in a short time frame (within 4 days), signifying that the sepsis was so severe that it had caused suppression of the bone marrow. The C-reactive protein result of 47.8 mg/liter (normal range, 0.0 to 10.0) was indicative of an immune response stimulated by an infectious agent. Despite the administration of an appropriate antibiotic, the patient failed to respond to treatment and died 9 days after admission.

For further characterization of the fatal bacteria, we isolated DNA from *C. violaceum* HTD1 and hybridized the DNA using an active surveillance of pathogens (ASP) oligonucleotide microarray (R. A. Stabler, L. F. Dawson, P. C. F. Oyston, R. W. Titball, J. Wade, J. Hinds, A. A. Witney, and B. W. Wren, unpublished data), thus providing data that would have potential use for future diagnosis, antimicrobial therapy, and assessing horizontally transferred genes in the strain. The ASP array included 6,110 genomic features, including resistance genes, species signature genes, and antimicrobial resistance genes from a range of bacteria. The array included 80 features from the *C. violaceum* ATCC 12472 genome sequence. *C. violaceum* HTD1 demonstrated hybridization to 78 features in total on the array, 69 of which were from the *C. violaceum* ATCC 12472 genome and 9 of which were unique to the Ho Chi Minh City strain. The nine HTD1 unique features included genes from other bacterial species that were mainly related to drug resistance, including a multidrug efflux pump from *Clostridium difficile*, a polymyxin resistance glucosyl transferase gene from *Burkholderia pseudomallei*, a bleomycin resistance gene from *Ralstonia eutropha*, and an additional multidrug resistance gene from *Caulobacter crescentus*. These data confirmed the identification of the bacteria and demonstrate continuing genetic flux and further acquisition of microbial resistance genes in *C. violaceum*, particularly with other bacteria found in similar surroundings.

In the natural environment of the organism, *C. violaceum* appears to pose little threat to humans, as infections caused by *C. violaceum* are extremely rare. The first reported human infection was in Malaysia in 1927, and until recently, only about 100 cases have been described (9, 12, 13). Although reported, infections caused by nonpigmented forms of the bacteria are less common than cases associated with the pigmented variety, although this may be due to population density within the bacterial species (14). The bacterium is exceptionally resilient and possesses the ability to survive in a range of harsh natural environments (4, 6). Therefore, *C. violaceum* is of interest in many areas of biotechnology, as it contains several biochemical pathways that could be exploited by chemical industries (11). For this reason, the genome of *C. violaceum* was sequenced and annotated to completion in 2003 (2). The genome sequence offered few clues as to the pathogenesis of the bacterium; a type III secretion system was identified but lacked some integral genes associated with invasion in other distantly related pathogenic bacteria (2). An unobvious mechanism of pathogenesis may explain the lack of human cases despite the potentially high level of exposure of many humans living in wet tropical areas. Indeed, the bacterium should possibly be described as an accidental rather than an opportunistic pathogen, as infections, like those in this report, are associated with the entry of bacteria through an open wound rather than through consumption of water from a contaminated source.

This fatal case of *C. violaceum* infection points out the need for rapid diagnosis of wounds contaminated with soil and water in subtropical and tropical areas. Prompt bacteriological isolation, identification, and susceptibility testing, especially in the young, are essential to maximize the treatment of these wounds and to prevent life-threatening sepsis. In this case, the isolation was done promptly despite the possibility of a presumptive diagnosis of *Staphylococcus aureus* sepsis. Regrettably, the patient died despite treatment with an appropriate antibiotic. The ASP array results demonstrate the utility of this system for rapid molecular identification and will play a significant role in treatment regimens and in monitoring gene acquisition in bacterial species in the future.

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