

## Agricultural Injury–Associated *Chromobacterium violaceum* Infection in a Bangladeshi Farmer

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**Abstract.** *Chromobacterium violaceum* is an emerging environmental pathogen that causes life-threatening infection in humans and animals. In October 2017, a Bangladeshi farmer was hospitalized with high-grade fever due to an agricultural injury–related wound infection. Bacteriological and 16S rRNA gene investigation detected *C. violaceum* in the wound discharge. The patient recovered successfully after a combination treatment with meropenem and ciprofloxacin, followed by prolonged medication to avoid recurrence. We strongly propose to incorporate *C. violaceum* in the differential diagnosis of wound and skin infections occurring in tropical and subtropical regions, especially when the injury was exposed to soil or sluggish water.

### INTRODUCTION

*Chromobacterium violaceum* is a  $\beta$ -proteobacterium that belongs to the Neisseriaceae family. It is a saprophytic bacterium found in tropical and subtropical regions of the world.<sup>1</sup> In 1927, Lessler recorded the first human infection case from Malaysia.<sup>2</sup> Since then, world over, 154 clinical cases were reported; among them, 23 cases were documented from the Southeast Asian countries, including India, Thailand, Sri Lanka, and Nepal.<sup>3</sup> This bacterium rarely causes human infection; if infected, it causes severe systemic disease with a high fatality rate (~60%).<sup>4</sup> *Chromobacterium violaceum* infection outcome may range from cellulitis and skin abscesses to septic shock and systemic abscesses including vital organs, such as lungs, liver, and spleen.<sup>5,6</sup> Herein, we describe a case of agricultural injury–related wound infection caused by *C. violaceum*. To our knowledge, this is the first successfully treated *C. violaceum* infection case from Bangladesh.

### CASE PRESENTATION

A 40-year-old male farmer from Gazipur district of Dhaka Division in Bangladesh was admitted to the local hospital on October 3, 2017. He was suffering from high fever, chills, pain in the lower abdomen, insufficient urination, and excessive sweating. He had a laceration (4 cm in length, 1 cm diameter, and 1.5 cm deep) on the ankle of his left leg. The patient recollected the precise details of an incident that happened 15 days back where he met with a tractor accident during work in a paddy field, and the wound immediately was exposed to stagnant muddy water. He took immediate treatment from the village physician, medicated with oral cefixime (400 mg) twice a day along with paracetamol (500 mg) for 1 week. However, the infection did not respond to the treatment and persisted with continuous pus discharges. On hospital admission, the patient's general condition was found to be satisfactory. However, he was febrile with 102°F body temperature, and his pulse, respiratory rate, and blood pressure were detected to be 115 pulses/minute, 21 breaths/minute, and 145/95 mmHg, respectively. The patient's leukocyte count was 15,000 cells/

mm<sup>3</sup>, with 84% neutrophils, 25% lymphocytes, 2% eosinophils, and 2% monocytes. Blood hemoglobin was 9.6 g/dL, the erythrocyte sedimentation rate was 49 mm/hour, aspartate aminotransferase was 45 U/L, and alanine aminotransferase was 49 U/L. Moreover, absolute platelet counts, random blood glucose, blood urea, electrolytes, and other blood cell parameters remained normal. Abdominal ultrasonography inspection revealed a regular abdomen. After physical examination, surgical debridement of the wound was performed, and intravenous meropenem (500 mg/8 hours) was started immediately.

The aseptically collected pus specimen was subjected to bacteriological investigation. We cultured the specimen on MacConkey agar and 5% sheep blood agar plates as described previously.<sup>5</sup> After overnight incubation, significant growth of distinguishable violet-pigmented colonies were detected which produced  $\beta$ -hemolysis reaction on a blood agar plate (Figure 1). Gram staining of the culture revealed Gram-negative coccobacilli. The pathogen was identified as *C. violaceum* through traditional biochemical analysis and

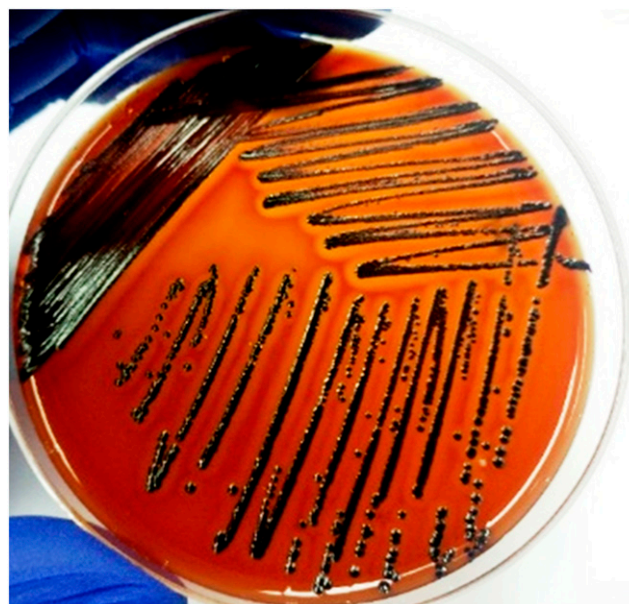


FIGURE 1. *Chromobacterium violaceum* RDN09 produces violet-pigmented colonies with  $\beta$ -hemolysis reaction on a blood agar plate. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

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TABLE 1  
Phenotypic characteristics of *Chromobacterium violaceum* RDN09, isolated from infected wound

Bacteriological tests performed	Test results
Gram stain	Gram-negative bacilli
Biochemical reaction	
Catalase test	Positive
Oxidase test	Positive
Kligler iron agar	
a. Acid production in slant	Negative
b. Acid production in butt	Positive
c. Hydrogen sulfide production (H <sub>2</sub> S)	Negative
d. Gas production	Negative
e. Alkali production in slant	Positive
Motility indole urea test	
a. Motility	Positive
b. Indole production	Negative
c. Urea hydrolysis	Negative
Simmons citrate reaction test	Positive
Nitrate reduction	Positive
Gelatin liquefaction	Positive
Esculin	Negative
Lysine decarboxylase	Negative
Ornithine decarboxylase	Negative
Arginine dihydrolase	Positive
Sugar fermentation	
a. Glucose	Positive
b. Lactose	Negative
c. Sucrose	Negative
d. Maltose	Negative
e. Mannose	Negative
f. Arabinose	Negative
g. Salicin	Negative
h. Mannitol	Negative
i. Inositol	Negative
Culture media type	
a. Chocolate agar	Dark black color colony
b. Blood agar	Dark violet colony with β hemolysis
c. MacConkey	Violet-pigmented colony
d. Mueller–Hinton	Violet-pigmented colony
e. Gelatin agar plate	Violet-pigmented colony
Salt (NaCl) tolerance (%)	
0	Positive
1	Positive
2	Positive
3	Negative
4	Negative
5	Negative
6	Negative
8	Negative
Growth temperature	26–42°C (aerobic) 35°C in 5% CO <sub>2</sub>
VITEK 2 (bio-number)	0067000140541210
API 20 NE (number)	5150555

reconfirmed with both the API 20 NE test and VITEK 2 compact system using GN card (BioMérieux, Marcy-l'Étoile, France); both these identification systems were able to detect *C. violaceum* with 99% probability (Table 1). We further subjected the bacterium for molecular identification and extracted the genomic DNA from a pure culture using a QIAamp DNA Mini Kit (Qiagen, Dusseldorf, Germany), as per the manufacturer's instructions. We then performed amplification and sequencing of the partial 16S rRNA gene as described previously,<sup>7</sup> and the sequence was submitted to GenBank (accession number MH061318). Furthermore, we constructed the phylogenetic tree to show genetic relationships between *C. violaceum*-RDN09 isolate and other *Chromobacterium* species using sequences from the GenBank database. The neighbor-joining method and the pair-wise deletion of the

gap were used to construct the phylogenetic tree; the evolutionary distance was calculated by the p-distance implementation in MEGA 6.<sup>8</sup> The phylogeny analysis indicated high bootstrap score between the studied strain and the sequences of *C. violaceum* ATCC 12472, whereas other *Chromobacterium* species demonstrated low bootstrap scores (Figure 2). Antibiotic susceptibility was tested by the disk diffusion method using 21 different antibiotics. Results were interpreted referring Clinical & Laboratory Standards Institute guideline for non-lactose fermenting Gram-negative bacteria.<sup>9</sup> The isolate *C. violaceum* RDN09 was found to be resistant to ampicillin, Augmentin, cefixime, colistin, cefotaxime, polymyxin B, ceftriaxone, and ceftazidime. However, the isolate was susceptible to the following 13 antimicrobial agents: amikacin, azithromycin, ciprofloxacin, cotrimoxazole,

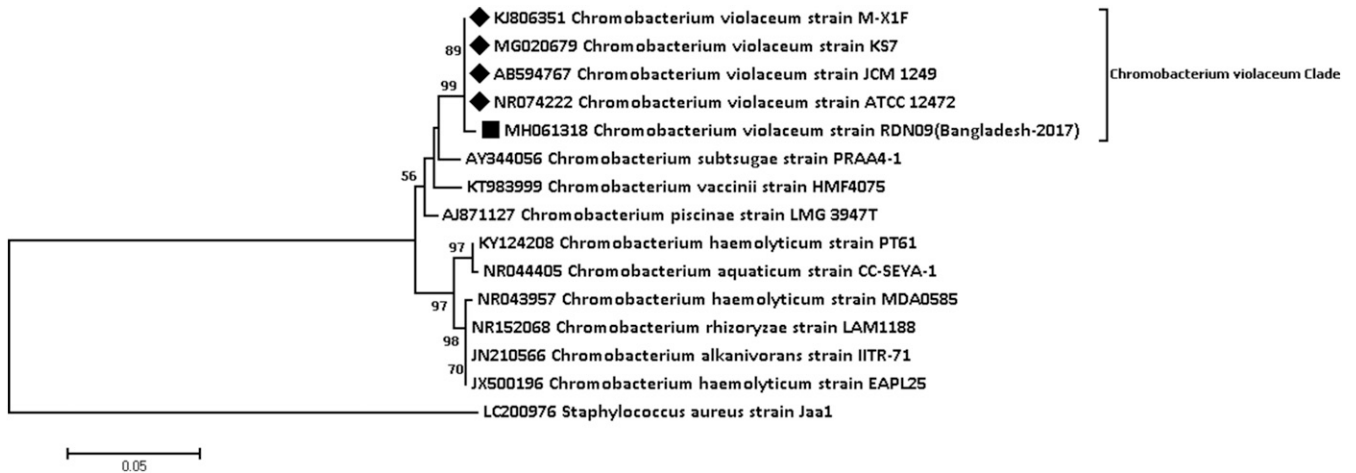


FIGURE 2. Phylogenetic tree based on 16S rRNA partial sequences, illustrating the homology between the 16S rRNA gene sequence of *Chromobacterium violaceum* RDN09 (black box) and other reported 16S rRNA gene sequences of *C. violaceum* retrieved from the National Center for Biotechnology Information gene bank (black diamond). Mega 6 software package was used to construct the phylogenetic tree following the neighbor-joining method with 1,000 bootstraps. Percentage bootstrap support is indicated by values at each node (values < 50 are omitted). As an out-group, we used *Staphylococcus aureus* strain Jaa1.

ertapenem, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, netilmicin, piperacillin plus tobramycin, and tobramycin. After the culture identification and antimicrobial susceptibility results, the clinician changed the course of therapy to a combination of meropenem (500 mg/8 hours) and ciprofloxacin (500 mg/12 hours) for 14 days. During hospital discharge, the patient was afebrile and was advised to continue oral ciprofloxacin (500 mg/12 hours) for 2 months to avoid the recurrence of infection. After 2 months, the wound healed completely and demonstrated a negative blood culture.

## DISCUSSION

*Chromobacterium violaceum* is the only species of its genus that is associated with human infections presenting high morbidity and mortality rates. It escapes the human defense mechanism because of the elevated levels of superoxide dismutase and catalase activity.<sup>10</sup> In the recent decade, the worldwide incidence of *C. violaceum* infections has increased dramatically, particularly, in Southeast Asia because of the recent global climate dynamics.<sup>11</sup> The subject of this case was infected when the average temperature in Dhaka was around 31°C<sup>12</sup>; such a weather condition supports *C. violaceum* growth in the environment.

As the patient reported a recent incident of an agricultural injury, after which he developed the infection. Perhaps, the farmland stagnant water could be the source of *C. violaceum*, and the injury acted as a portal of entry for infection.<sup>13</sup> Previous case studies suggest several predisposing factors for *C. violaceum* infection such as neutrophil dysfunction, immunocompromised state, chronic granulomatous disease, and glucose-6-phosphate dehydrogenase deficiency.<sup>11</sup> However, the subject in our case was unaffected by such factors. He was healthy with no history of immunocompromised illnesses, and this could also be a reason for his complete recovery without any complication. Diagnosis of *C. violaceum* infection is completely dependent on the bacteriological culture followed by biochemical characterization.<sup>3</sup> We found that the conventional biochemical identification process is time-consuming

and requires expertise in result interpretation, whereas the API 20 NE and VITEK 2 systems have reduced turnaround time compared with the traditional biochemical methods.

We also evaluated the utility of partial 16S rRNA gene sequencing for precise molecular detection of a clinical pathogen that cannot be easily identified by phenotypic methods.<sup>14</sup> Analysis of partial 16S rRNA gene sequence of the strain showed 99% homology with the *C. violaceum* ATCC 12472 type strain. All *C. violaceum* strains analyzed shared the same clade in the phylogenetic tree (Figure 2). We suggest that the 16S rRNA gene sequencing technique described in this study can be used alternatively to phenotypic methods for detecting *C. violaceum*, in case advanced bacteriological identification systems such as API 20NE and VITEK 2 are unavailable. Antibiotic susceptibility trends on *C. violaceum* remain sparse because of fewer reports from clinical specimens.<sup>15</sup> However, as per the available reports, the bacterium is susceptible to aminoglycosides, chloramphenicol, fluoroquinolone, and carbapenems and extremely resistant to penicillin and broad-spectrum cephalosporins.<sup>16</sup> In this case, the combination of meropenem and ciprofloxacin regimen showed the best treatment outcome when used together with oral ciprofloxacin as prolonged maintenance therapy.<sup>15,17</sup> *Chromobacterium violaceum* may become a significant human pathogen because of the ongoing global climate dynamics. This work is expected to sensitize clinicians from tropical and subtropical areas on rapid diagnosis and appropriate antimicrobial treatment for the remission of this life-threatening infection. Therefore, we suggest incorporating *C. violaceum* in the differential diagnosis of wound and skin infections, especially when the injury comes in contact with soil and/or sluggish water.

Received April 18, 2020. Accepted for publication May 18, 2020.

Published online June 22, 2020.

Acknowledgments: This research study was funded by core donors who provide unrestricted support to icddr, b for its operations and research. Current donors providing unrestricted support include the governments of Bangladesh, Canada, Sweden, and the United Kingdom. We gratefully thank our core donors for their support and commitment to icddr, b's research efforts. We would like to thank the

study participant for sharing the case details. We are thankful to Niyaz Ahmed for his critical review of the manuscript. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

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