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Review

# Chromobacterium violaceum infection in China: Three case reports and literature reviews

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*Chromobacterium violaceum*, Gram-negative *Bacillus*, is a common inhabitant of soil and stagnant water found in tropical and subtropical regions of the world. It is a rare cause of severe, often fatal, human disease. In the report, 3 cases of patients infected with *C. violaceum* were described in Anhui Province, China. Routine and bacteriological investigations were carried out to establish the aetiological diagnosis. Moreover, the patients were treated with appropriate antimicrobial agents and auxiliary therapy. To our knowledge, a total of 42 cases have been reported previously from Chinese mainland in the recent 20 years, with a review of the literatures.

Key words: Chromobacterium violaceum, infection, China.

### INTRODUCTION

Chromobacterium violaceum commonly inhabits soil and stagnant water in tropical and sub-tropical regions which usually produces a violet pigment known a violacein, solute in ethanol and insoluble in water and chloroform. However, infections caused by nonpigmented strains are described rarely. The bacterium, motile, oxides-positive rod-shaped, facultatively anaerobic, fermentative, and Gram-negative, is classified into the opportunistic pathogen (Groves et al., 1969; Kaufman et al., 1986). It is only Chromobacterium species that are pathogenic to humans. Despite its ubiquitous distribution, humans infected with C. violaceum are rare. As the clinical manifestations of infection are not representative and the organisms are not sensitive to the antimicrobial agents, the pathogens result in systemic and severe disease with a high fatality rate. The bacteria may be responsible either singly or in combination for wound or soft tissue infection. Their main clinical features rapidly progress to sepsis with multiple organ abscesses, including the lungs, liver, and spleen.

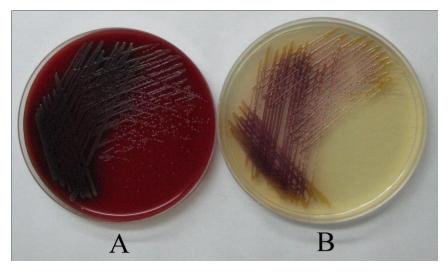
Thus, the infection often results in severe, systemic diseases with high rate (60%) (Díaz Pérez et al., 2007; Groves et al., 1969). *C. violaceum* was firstly identified in 1881 and its pathogenic potential was first described by Woolley (1905), which was isolated from a fatal infection in buffalo in Philippines. The first human case infected with *C. violaceum* was found in Malaysia by Lessler in 1927 (Sneath et al., 1953). Since then, there have been more than 150 human cases reported in India, Srilanka, Southeast Asia, Taiwan, Hongkong, Argentina, Australia, Brazil, and the southeastern region of the United States (Chang et al., 2007; Kim et al., 2005; Miller et al., 1988; Ray et al., 2004; Wen and Chen, 2000). Three cases in Anhui Province with the pigmented strains of *C. violaceum* were present in the report.

### CASE REPORTS

### Case 1

In June 2005, a 76-year- old farmer man who lived in Chaohu, Anhui Province was admitted to the Burn Department of a local hospital, because he sustained flame burns in the upper part of his body, in a farmland.

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**Figure 1.** *Chromobacterium violaceum* colonies on (A) sheep blood agar and (B) Mueller-Hinton agar.

Unfortunately, a serious wound contaminated by lake water in a rural area was detected at this time. Then, the conditions of the patient worsened quickly and he was transferred to the First Affiliated Hospital of Anhui Medical University. He denied any other disease except for hypertensive disease and chronic cardiac dysfunction. Physical examination revealed the following vital signs: temperature 38.8°C, heart rate 84 beats/min, respiratory rate 23 breaths/min, blood pressure was not examined due to noncompliance. The patient had burned more than 40% of the surface areas which were mainly located in both upper extremities and chest (I degree 12%, deep II degree 28%). Laboratory investigation upon admission showed: leukocyte count 13.0×10<sup>9</sup>/L, with 83.3% neutrophils, erythrocyte count 5.7×10<sup>12</sup>/L, hemoglobin 105 g/L, platelet count 169×10<sup>9</sup>/L; total protein 39.7 g/L, albumin 21.5 g/L, A/G 1.18, total bilirubin 15.43 mol/L, ALT 17 U/L, AST 27 U/L, urea nitrogen 5.12 mmol/L, creatinine 63 mol/L, blood sodium 130 mmol/L, potassium 2.88 mmol/L, blood sugar 7.76 mmol/L.

A Gram-negative bacillus formed smooth and violaceous colonies on sheep blood agar and Mueller-Hinton agar was cultured from purulent discharge of putrid skin on admission (Figure 1). No growth of organisms had been detected from blood culture. No evidence of abnormality was found in chest X-ray. The bacillus was identified as C. violaceum by API 20 NE and Vitek system. The clinical strain was susceptible to imipenem, levofloxacin and ciprofloxacin, intermediate to amikacin, gentamicin and tobramycin, but resistant to aztreonam, cefepime, cefotaxim, ceftazidime, cefatrizine, piperacillin, and trimethoprim-sulfamethoxazole. During hospitalization, the patient was suspected to infection with Gram-negative organism and empirically treated with intravenous penicillin (2 g, every 4 h) for 3 days. When

identification and susceptibility reports were available on the fourth day, the therapeutic schedule was changed into intravenous levofloxacin (0.4 g, one time per day). The wound was debrided twice per day. On eighth day after hospitalization, the patient's symptoms disappeared. There was no fever, wound gradually healed, and liver function recovered. Serial blood culture was again obtained, but no growth of organisms had been detected.

### Case 2

A 42-year-old-male patient who lived in Angin, Anhui Province had multiple traumas on exposed parts of his body due to a tractor accident while plowing on December 6, 2007. He was first admitted to ICU of the local hospital for management of shock and then transferred to ICU, the First Affiliated Hospital of Anhui Medical University. He had hemopneumothorax and respiratory failure, rib and pelvis fracture, pancreas hematoma and back contusion. On admission, physical examination revealed the following vital signs: temperature 38.6°C, respiratory rate 26 breaths/min, HR138 beats/min, BP 130/80 mmHg, and oxygen saturation 94%. The laboratory investigation showed: hemogram (leukocyte count 19.89×10<sup>9</sup>/L, with 78.6% neutrophils, erythrocyte count 2.6×10<sup>12</sup>/L, hemoglobin 17 g/L, platelet count  $59 \times 10^9$ /L), liver and kidney function (urea nitrogen 11.33 mmol/L, creatinine 180 mol/L; total protein 44.7 g/L, albumen 21.5 g/L, A/G 0.93, total bilirubin 16.2 mol/L, ALT 11 U/L, AST 22 U/L), routine urinalysis (occult blood 2+, protein +). Blood sodium, potassium, calcium levels were within normal limits. Chest X-ray showed mixed infiltrations in bilateral lungs, bilateral pleural effusion, and the fracture of left seventh

rib. Ultrasonography of abdomen confirmed seroperitoneum. The ascites cell count showed: WBC  $3000 \times 10^6$ /L (monocaryon 75%, polynucleation 25%), RBC 200 × 10^6/L; however, biochemical data were within normal limits.

The patient empirically treated with intravenous cefoperazone (1 g, every 8 h) and ciprofloxacin (0.75 g, one time per day) in addition to symptomatic treatment. On the fourth day, ascetic culture was inoculated and identified as C. violaceum, with antibiograms showing sensibility to imipenem, amikacin, gentamicin, and tobramvcin. resistance to aztreonam, cefepime. cefotaxim, ceftazidime, cefatrizine, piperacillin, ticarcillin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The successful treatment with antimicrobial regimen initiated intravenous imipenem (1.0 g, every 8 h) for 7 days according to the sensitivity of the isolated organisms. Seven days after admission, the patient's symptoms disappeared. There was no fever, cough and expectoration relieved, liver function parameters were normalized, renal function recovered, routine urinalysis was normal, and he accepted oral food well. Ultrasound examinations showed disappearance of ascites. Chest Xray showed disappearance of chest pleural effusion. Abscesses in liver and kidney were not showed by the computed tomographic scan of the abdomen. Serial blood cultures were again obtained, however, no growth of organisms had occurred. The patient recovered 2 months later, and then was discharged.

# Case 3

An 81-year old man who lived in Hefei, Anhui Province was admitted to the department of nephrology on October 20, 2008, presenting with a history of hypertension, coronary heart disease, installation of pacemaker, and chronic renal failure for over 2 years. Through examination, patients were found with hypodynamia, odvnuria, and edema for a month. The patient slipped and fell on land near a pond when he went for a walk in the park 5 days ago. Physical examination revealed the following vital signs: temperature 39.2°C, respiratory rate 19 breaths/min, heart rate 93 beats/min, BP 110/80 mmHg. Blood analysis on admission showed an abnormal hemogram (leucocyte count 14.52×10<sup>9</sup>/L, with 81.3% neutrophils, erythrocyte count 4.2×10<sup>12</sup>/L, hemoglobin 96 g/L, platelet count 57×10<sup>9</sup>/L) and an aggravating renal impairment (urea nitrogen 44.27 mmol/L, creatinine 1037 mol/L, occult blood 3+, protein 2+). Blood sodium, potassium, calcium levels were within normal limits. The parameters of liver function revealed total protein 51.7 g/L, albumin 31.5 g/L, total bilirubin 21.7 mol/L, ALT 21 U/L, AST 27 U/L. The patient's empirical treatment with antimicrobial agents received intravenous cefotaxime (2 g, every 8 h). On the fifth day, bacillus was inoculated from the

midstream urine and subsequently identified as C. violaceum. Blood cultures did not grow C. violaceum. The strain was resistant to aztreonam, cefotaxime, piperacillin, or ticarcillin, intermediate to cefotaxim or ceftazidime, and susceptible to amikacin, tobramycin, cefepime, imipenem, or trimethoprim- sulfamethoxazole. Hemodialysis was given to the patient lasting for 3 days and antimicrobial agents was switched to intravenous imipenem (0.5 g, every 8 h) in the light of results of susceptibility tests. On the tenth day after hospitalization, the patient's symptoms disappeared. There was no fever, routine urinalysis and liver function recovered. Another blood and midstream urine culture were again obtained, but no growth of organisms had occurred. The patient felt better then and was discharged from the hospital.

# DISCUSSION

More than 150 cases of patients infected with C. violaceum have been reported worldwide (de Sigueira et al., 2005; Wen and Chen, 2000). Since the first case of human infection was described in 1987 in Zhejiang Province of China, 45 cases have been found in Chinese mainland in the recent 20 years. Among literatures, 30 cases with partly clinic materials were documented in different periods (Chen and Dong, 1990; Gu et al., 1987; Wu et al., 1988; Xu et al., 1993; Zhang et al., 1990; Zhou and Yang, 2006; Zhu et al., 1991) (Table 1). Of 42 patients of previous reports and 3 at present, most cases infected with C. violaceum occurred in the Southern coastal areas of the Chinese mainland (Figure 2). Moreover, C. violaceum is frequently confined to tropic and southern subtropic zones and the central subtropical zone (Figure 2). 3 cases of human infection with C. violaceum have been reported in Hong Kong (1 discharged and 2 died) (Teoh et al., 2006). In 1968, a 5year old child who died of sepsis was the first case infected with C. violaceum in Taiwan (Wu et al., 1986). According to 7 cases reported from Taiwan, 3 successfully recovered and 4 unfortunately died (Wu et al., 1986).

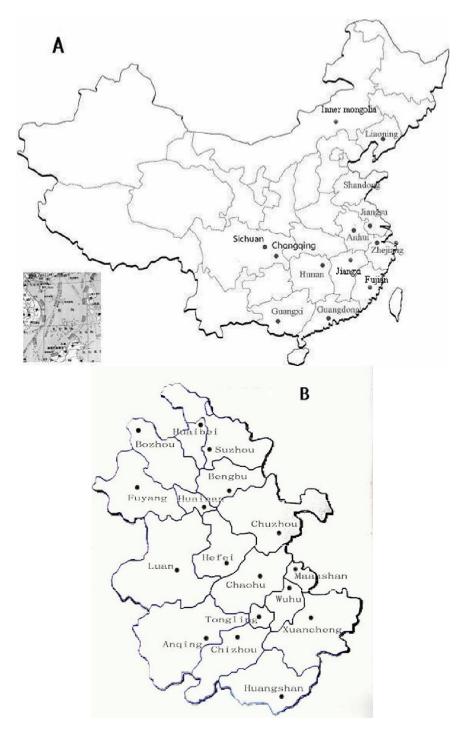
From the age characteristics, the young people had high incidence compared with the teenagers and middleaged people. Only 9 cases infected with *C. violaceum* ranging from 16 to 45 years have been reported in the literature, while 11 cases were teenagers (from 0 to 15 years) and 10 middle-aged people (older than 45 years). The youngest patient was a 13-day-old infant with respiratory infection associated with left eye infected with *C. violaceum*. The oldest patient, who was an 81-year-old man with the history of kidney failure appeared urinary tract infection by *C. violaceum* and was discharged without adopting any antimicrobial therapy. *C. violaceum* is an opportunistic pathogen. Nevertheless, the incidence of exposure to infection in teenagers and the elderly is

No.	Sex	Age	Year	Province	Clinical presentation	Specimen	Outcome	Reference NO.
1	F	2.5	1992	Anhui	Septicemia	Blood	Died	Xu and Xu (1996)
2	Μ	20	1992	Zhejiang	Skin wound	Wound pus	Recovered	Lei et al. (1994)
3	Μ	51	1994	Jiangxi	Hepatapostema	Puncturefluid of abscess	Unknown	Liu (1995)
4	F	13	1994	Zhejiang	Skin wound, septicemia	Blood	Died	Jiang (1997)
5	F	40	1995	Zhejiang	Septicemia	Blood	Died	Jiang (1997)
6	Μ	49	1995	Zhejiang	Skin ulcer	pus	Recovered	Wu et al. (1998)
7	Μ	30	1997	Inner Mongolia	Finger injury	Wound pus	Unknown	Li (1997)
8	Μ	8	1997	Zhejiang	Septicemia	Blood	Died	Wu (1999)
9	Μ	73	1998	Liaoning	Laryngocarcinoma	Sputum	Died	Zhang (1998)
10	Μ	4	1999	Shandong	Left ear suppuration	Ear secretions	Recovered	Wang and Mi (1999)
11	F	24	2000	Chongqing	Puerperal infection	Vaginal secretions	Recovered	Xie et al. (2001)
12	F	26 days	2000	Shandong	Sepsis of newborn	Blood	Recovered	Wei et al. (2001)
13	Μ	7	2001	Guangdong	Septicemia	Pus, blood	Recovered	Fu et al. (2002)
14	Μ	9	2001	Hunan	Septicemia	Heart blood	Died	Zhou and Guo (2004)
15	Μ	76	2001	Anhui	Extensive burn	Wound pus	Died	Present study
16	Μ	25	2002	Guangxi	comminuted patella fracture	Wound pus	Recovered	Yao (2003)
17	Μ	50	2003	Jiangsu	Septicemia, MODS	Blood	Died	Yang et al. (2005)
18	Μ	65	2005	Sichuan	Septicemia	Blood	Recovered	Xia et al. (2007)
19	F	55	2005	Shandong	Hepatapostema, septicemia	Blood	Unknown	Wang and Zhong (2005)
20	F	1	2006	Zhejiang	Pneumonia, septicemia, MODS	Sputum, Blood	Died	Hua et al. (2006)
21	F	13 days	2006	Fujian	Left eye conjunctivitis, neonatal respiratory infection	Ocular secretion	Recovered	Hu et al. (2009)
22	F	9	2006	Zhejiang	Left ear suppuration, hepatapostema, septicemia	Pus, blood	Recovered	Tong et al. (2007)
23	Μ	42	2007	Anhui	Multiple trauma	Peritoneal drainage fluid	Recovered	Present study
24	Μ	81	2008	Anhui	Renal insufficiency	Midstream urine	Unknown	Present study
25	Μ	5	unknown	Guangdong	Multiple abscesses	Pus	Recovered	Ge (1995)
26	Μ	49	unknown	Zhejiang	Skin and soft tissue infections	Pus, blood	Recovered	Zhu and Zhu (1996)
27	М	59	unknown	Zhejiang	Multiple liver abscess	Blood	Died	Huang et al. 2006
28	Μ	26	unknown	Zhejiang	suppurative lung infection, septicemia	Blood	Died	Huang et al., 2006
29	Μ	38	unknown	Zhejiang	Right arm abscess, infectious shock	Pus, blood	Unknown	Huang et al., 2006
30	М	45	unknown	Guangxi	Cholangitis, septicemia	Blood	Died	Wei, 2007

Table 1. Clinical manifestation of 30 cases caused by C. violaceum in Chinese mainland.

Clinical manifestations of the other 15 cases did not show integrity, so they were not described.

higher than in young people which may be due to immunocompromise, leading to the dissemination of infection. Thus, one of the important factors was immunocompromised, caused by many underlying diseases, old age and serious injuries, which could involve the pathogenesis of case 2 and case 3, respectively. From the point of view of gender difference, the majority of patients infected with *C. violaceum* were male (21 cases),



**Figure 2.** The geographic distribution of 12 provinces and 1 municipality in Chinese mainland (A), the map of Chinese mainland shows 12 provinces and 1 municipality, in which Chromobacterium violaceum isolates were reported (From 1998 to 2009, Zhejiang (17 cases), Guangdong (9 cases), Anhui (4 cases), Shandong (3 cases), Hunan (3 cases), Guangxi (2 cases), Sichuan (1 case), Chongqing (1 case), Jiangsu (1 csae), Jiangxi (1 case), Liaoning (1 case) Inner mongolia (1 case), Fujian (1 case) were reported in Chinese mainland; Liaoning, Shandong, Jiangsu, Zhejiang, Fujian, Guangdong, Guangxi are adjacent to the oceans in Chinese mainland) . (B), the map shows the 17 cities in Anhui Province (Anhui Province locates in eastern China with the population of 66.757 million and covers the area of 139,600 square kilometers, the Yangtse River and Huaihe River traverse the province boundary; with Huaihe River as the separatrix, the partly moist monsoon climate of warm temperate zone belongs to the north Anhui, the moist monsoon climate of subtropical zone belongs to the south Anhui).

which accounted for 70.0%. C. immunocompromise, leading to the dissemination of infection. Thus, one of the important factors wasimmunocompromised, caused by A few patients, only 13 cases, recovered after many underlying diseases, old age and serious injuries, hospitalization. 9 patients were dead of septicemia which could involve the pathogenesis of case 2 and case caused by C. violaceum (Table 1). Thus, poor prognosis 3. respectively. From the point of view of gender and high mortality were described in patients infected difference, the majority of patients infected with C. with C. violaceum, if the disease was quickly developed violaceum were male (21 cases), which accounted for into a fatal systemic infection (Sneath et al., 1953). 70.0%. C. violaceum is often found in the soil and However, symptoms were not obvious and typical, when environmental water. the initial stage of infection although the human infectivity Moreover, men usually took up the impaired occupation of C. violaceum is low. According to some related (cultivating and fishing) and easily injured or literatures, the mortality rate of local infection with C. contaminated skin lesions, which increased the incidence violaceum was more than 65%, and of septicemia was of exposure to pathogen. In the report, 3 patients infected higher than 80% (Chang et al., 2007; Miller et al., 1988; with C. violaceum were male; moreover, the history of Jin et al., 2003). As effective antimicrobial treatment in exposure to soil and stagnant water on account of time, 3 cases described in the report did not progress to cutaneous injury caused by various factors was described severe bacteremia and sepsis, which was important to in 3 cases. Most infection caused by C. violaceum the treatment of C. violaceum infection. occurred in summer, especially from July to September. Diagnosis requires a high index of suspicion and is In Chinese mainland, the definite time of C. violaceum made on the basis of isolation of the organism from infection was detected in 20 cases as following (1 case in specimen cultures. The organism is usually sensitive to January, 2 cases in June, 2 cases in July, 7 cases in carbapenems and fluoroquinolones, but resistant to August, 3 cases in September, 2 cases in October, 2 penicillins, aminoglycosides, and cephalosporins. Thus, cases in November, 1 case in December). The reason of the rational use of carbapenems and fluoroquinolones increasing incidence might attribute to the suitable plays an important role in the pathogenic treatment. Howtemperature for the growth of C. violaceum which was ever, it should be mentioned that there were discrepancy more than 30°C. The patients usually wear thin clothes of sensitivity and efficiency to aminoglycosides and when the weather got hot. C. violaceum usually made cephalosporins in different regions. incursions into the bodies via broken skin. In the meantime, it is the importance of early diagnosis However, it was cold in December, which was not and empirical treatment, timely with antimicrobial agents suitable for bacterial growth. In the report, the patient in in order to avoid progression to sepsis and multiple organ case 2 probably was low immunity owing to sever failure. The patient should receive not only antimicrobial injuries. Thus, opportunistic infection was caused by C. treatment but also symptomatic and supportive treatment violaceum from contaminated wound. The main clinical in time for the benefit of infection control, which could symptoms included fever (22 cases), the increase of evidently improve the prognosis (Rahal et al., 1998). In leukocyte (15 cases), the increase of neutrophils (14 the report, Cases 1, 2, and 3 have been rapid recovery cases), focal or multiple abscesses (5 cases), and with accurate identification and susceptibility test of the regional lymphadenectasis (4 cases), etc. If patients were pathogen in time, as well as effectively supportive care, showing hypoimmunity with some predisposing factors, a apart from appropriate antimicrobial therapy. localized infection would be rapidly developed into the In sum, the most severe complication of infection with systemic infection, which could lead to the progression to C. violaceum was sepsis, infectious shock, and multiple sepsis and multiple organ failure involving the liver, organ failure. Distinctive features of infection were low kidney, and spleen (Díaz Pérez et al., 2007; Jin et al., morbidity and high mortality. Clinical manifestations of C. 2003). violaceum infection often initially show minor localized 13 cases were related to skin ulceration caused by abscess. Then, local lesions rapidly developed into trauma or insect bite and had the contact history of soil sepsis, multiple organ failure, even death owning to their and water; however, some infection routes were still immunocompromise or irrational use of antimicrobial unclear. Systemic infection was even possibly due to the agents. intrusion of swallowing sewerage including pathogens As the opportunistic pathogen, it was rare that human (Kim et al., 2005; Miller et al., 1988; Xu and Li, 2004). infections were caused by C. violaceum. However, micro- Thus, we suspected that ingestion of seafood which was biological researchers and physicians should pay more freeze-drying processed and stored, might have been the attention to the increasing incidence in recent years (Ge, source of infection in some cases because of more and 1995). The history of exposure to stagnant water and soil more convenience to transportation. Some patients could not be ignored, which was important to early denied ever going into the sea or other direct exposure to diagnosis of infection. Empirical use of antimicrobial agents in time, the adjustment of therapeutic schedule timely on the basis of susceptibility tests, and intensive nursing also played important role in disease control and improving the prognosis (Ray et al., 2004).

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