Case Report

Chromobacterium violaceum – an unusual pathogen. Perspectives to ponder!!

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ABSTRACT

Human infection due to *Chromobacterium violaceum* is rare, even though the bacteria are ubiquitous in distribution. Without appropriate antimicrobial therapy, the consequences can be rapidly fatal if septicemia sets in. Both pigmented and nonpigmented varieties are equally virulent. Mortality rates are quite high despite treatment. The history of trauma and simultaneous exposure to water and soil should alert clinician about this entity. Fluoroquinolones and aminoglycosides show good sensitivity, whereas penicillin and early cephalosporins are poor therapeutic options. Treatment for an extended period beyond clinical cure is indispensable to prevent relapse. Combination of prompt diagnosis, optimal antimicrobial therapy, and adequate therapeutic duration for *C. violaceum* infection is the key for successful therapy. We present here two cases of *C. violaceum* infections with interesting presentations with a brief review of literature.

Key words: *Chromobacterium violaceum; Pigment; Relapse; Septicemia; Urinary tract infection*

Usual pathogens cause a vast array of infections that we come across every day. Rarely, some unusual microbes may be isolated found ubiquitously in our environment. They could be pathogens or contaminants in certain precarious clinical conditions. One among the many of such sort, *Chromobacterium violaceum* (*C. violaceum*) naturally resides in soil and stagnant water of tropical and subtropical regions as saprophytes [1,2]. Although it was first identified in 1881, its pathogenic potential was illustrated only in 1905 by PG Woolley from a fatal case of a buffalo in Philippines. Human case of infection caused by this pathogen was first established by JE Lessler from Malaysia in 1927 [2]. Since then less than 180 cases have been documented in literature till late 2017 in Asia, Africa, South America, Australia, and southeastern United States [3,4]. Most strains of *C. violaceum* synthesize a non-diffusible violet pigment called violacein. This pigment is ethanol and acetone-soluble and insoluble in water, chloroform, ether, and benzene [5].

Infection by non-pigmented strains is equally fatal. The predominant portal of entry is through broken skin exposed to the organism through trauma, exposure to water and soil or both [2,3]. Though rarely implicated in human infections, infections caused by this bacterium can be fatal. Rapid progression to sepsis with metastatic abscesses and multidrug resistance are striking features of *C. violaceum* infections. Mortality rates are substantially high (~55–60%) despite treatment [3]. We report here a case series of *C. violaceum* infections with varied presentations from Port Blair, Andaman & Nicobar Islands. These are the first such reports from these remote islands.

Case 1

A 22-year-old male presented to the emergency room of the hospital with complaints of high persistent fever, severe headache, vomiting, severe pain and a small pustular lesion on the dorsal aspect of his right foot. He

had a toxic appearance with marked prostration. On admission, he was febrile with a temperature of 102˚F, continuous in character, pulse rate of 106 beats/min, blood pressure of 130/86 mmHg, respiratory rate of 28/min, oxygen saturation of 92%. Examination showed scleral icterus, tender hepatomegaly and a suppurative skin lesion, which had started ulcerating. Three weeks before presentation, the patient had sustained an injury on the dorsal aspect of his right foot while working in a shrimp factory. He did not seek any medical help at that time, but gradually developed fever over last 5 days and a pustule within the last 2 days. On close observation, the site around the lesion was swollen, tense, erythematous with a collection of pus, peeling of skin, sloughing off, presence of sinuses, and discharge of pus material.

He was provisionally diagnosed with osteomyelitis of first metatarsal and started on Inj. ceftriaxone 2 gm/24 h, Inj. vancomycin 1 gm/8 h and oral metronidazole 400 mg bd. Hematological and blood chemistry results are represented in Tables 1 and 2. A pus sample drawn from the site was obtained, gram staining done and inoculated for culture on nutrient agar, blood agar and Mac Conkey agar and incubated overnight at 37°C. The next day numerous rounds, convex, butyrous colonies with a violet non diffusible pigment were obtained on all three media. Biochemically, it was identified as *Chromobacterium violacuem* (Table 3).

Table 1: Results of blood tests for Case 1

Blood tests	Results	Reference/level
Haemoglobin (g/dl)	8.4	[11–14]
Red cell count (cell/mm3)	3.3 million	4.0–5.5 million
Platelets (cell/mm3)	275,000	150,000–400,000
White blood cell (cell/mm3)	14,500	4000–11,000
Neutrophils (%)	82	13–35
MCV (fl)	82	78 ± 6
MCH (pg)	30	27 ± 2
MCHC (g/dl)	37	34 ± 2
ESR (mm/hr)	120	10–15
C-reactive protein (mg/dl)	21	<0.5

MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular HAemoglobin Concentration, ESR- Erythrocyte Sedimentation rate

Antibiotic susceptibility testing was conducted by Kirby Bauer's disc diffusion method in Mueller-Hinton agar according to CLSI guidelines for non-Enterobacteriaceae [6]. The isolate was sensitive for amikacin, gentamicin, ciprofloxacin, meropenem, cefoperazone-sulbactam, cotrimoxazole, chloramphenicol and tetracycline. Ceftazidime and ceftriaxone were resistant for the isolate. Two days post admission with non-resolution of fever and ulceration, a second sample was sent for microbiological evaluation. Blood and urine samples for culture were unremarkable. On receipt of antimicrobial susceptibility report for the first sample, therapy was changed to Inj. cefoperazone-sulbactam 2 gm/24 h and inj. metronidazole 15 mg/kg/6 h.

Table 2: Blood chemistry results on admission (Case 1)

Blood tests	Results	Reference/level
Random blood sugar (mg/dl)	90	70–110
Blood urea (mg/dl)	27	15–45
Serum creatinine (mg/dl)	0.7	0.6–1.1
Sodium (mEq/l)	139	135–146
Potassium (mEq/l)	4.2	3.5–5.2
SGOT (AST) (IU/l)	38	<40
SGPT (ALT) (IU/l)	43	<35
ALP (IU/l)	185	90–460

Abbreviations: ALT - alanine aminotransferase; ALP - alkaline phosphatase; AST - aspartate aminotransferase; SGOT - serum glutamic oxaloacetic transaminase; SGPT - serum glutamate pyruvate transaminase

The identification and antimicrobial sensitivities of 2nd sample were similar to the 1st sample. However, after 4 more days, a 3rd pus sample was sent after deterioration of his clinical condition with appearance of multiple discharging sinuses in right lower limb and the ulcer burrowing deep inside tissues with extensive sloughing. The patient was subjected to debridement of ulcer, necrotic material and sloughed off tissues were removed and the wound was washed with beta dine, hydrogen peroxide and normal saline (Fig. 1). *C. violaceum* was again isolated and identified, however cefoperazone-sulbactam turned resistant by now. A second blood sample sent for culture was also positive for *C. violaceum* infection. With the imminent danger of sepsis-induced mortality, therapy was re-evaluated and the patient put on Inj ciprofloxacin 400 mg/8h and Inj. gentamicin 1.5 mg/8hy + Inj. clindamycin 600 mg/6 h. The patient responded very quickly to this

regimen. Fever disappeared and the ulcer healed within next 10 days. He was discharged with oral ciprofloxacin 500 mg BD for next 2 months. There was no evidence of recurrence after 6 months of evaluation.

Table 3: Biochemical reactions of the isolate in Case 1

Biochemical tests	Results
Gram stain	Gram negative bacilli
Catalase test	Positive
Oxidase test	Positive
Triple Sugar Iron agar	Glucose fermenter, Lactose & Sucrose non fermenter, No Gas & Hydrogen sulphide production
Motility	Positive
Indole	Negative
Urea hydrolysis test	Negative
Citrate utilization test	Positive
Methyl red test & Voges-Proskauer test	Negative
Hugh Leifson Oxidation Fermentation test	Fermentative
Lysine decarboxylase	Negative
Ornithine decarboxylase	Negative
Arginine dihydrolase	Positive
Pigment	Violet (violacein)
Esculin hydrolysis	Negative
O-Nitro Phenol Galacto-pyranoside hydrolysis	Negative

Case 2

A 15-year-old male presented to the outpatient department of the hospital with complaints of burning micturition, pain abdomen, weakness, high grade continuous fever and chills, intermittent dysuria for 5 days. There was no history of any other concurrent illness. Abdominal examination revealed suprapubic tenderness. The patient was diagnosed with urinary tract infection and started on Inj. Cefotaxime 1 gm/day. He divulged that a week ago, he had gone for catching fish with nets in a stagnant pond near his locality.

A routine urine wet mount showed >30 pus cells/ high power field with abundant bacteria and occasional granular casts. Lab investigations showed raised leucocyte count (>16,800 cells/mm3) with predominant neutrophilia (>85%) in an otherwise unremarkable routine hematological examination.

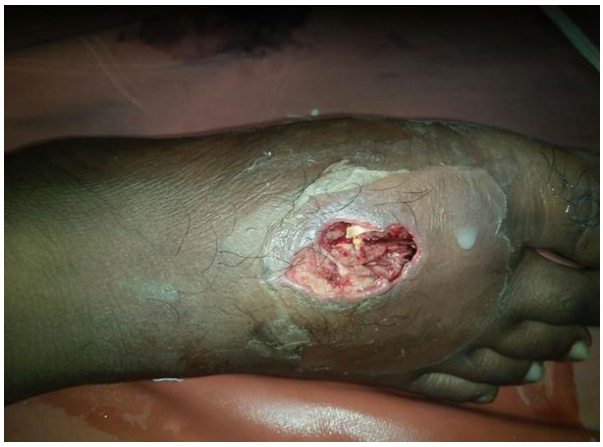

Figure 1: Deep ulcerating lesion on dorsal aspect of right foot (case 1)

A clean catch mid-stream urine sample was obtained, inoculated on CLED medium and incubated at 37°C overnight. Dark violet pigmented round, convex, regular colories were observed in the culture plate the next day (Fig. 2). On identification, the gram negative motile bacilli were catalase positive, oxidase positive, fermented glucose with acid production but without gas. The bacteria utilized citrate, reduced nitrate, and were a lactose/sucrose non fermenter, showed fermentative character on Hugh Leifson medium, indole negative, urea hydrolysis negative, arginine dihydrolase positive and lysine, ornithine decarboxylase negative. The reactions were consistent with the identification of *C. violaceum*.

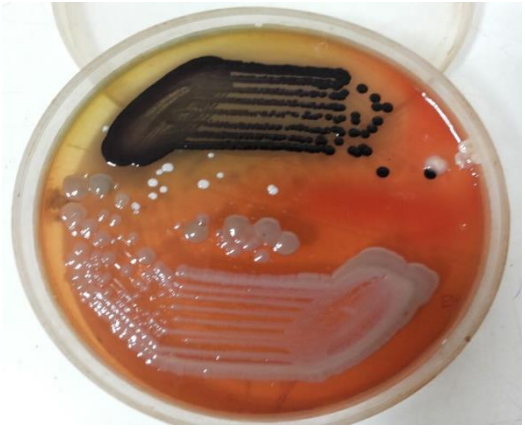

Figure 2: Deep violet coloured colony in a blood agar plate (top half)

Figure 3 - Violet non diffusible pigment of *Chromobacterium violaceum* in a sensitivity plate

Antimicrobial sensitivity testing was performed by Kirby Bauer's disc diffusion method (Fig. 3)**.** The isolate was sensitive to imipenem, cotrimoxazole, nitrofurantoin, ciprofloxacin, tetracycline, gentamicin, chloramphenicol and resistant to amoxiclav, cefepime, cefotaxime. A second sample sent on the alternate day was also identified as the same organism with the same sensitivity.

The initial empiric therapy was revised and the patient put on Inj. Ciprofloxacin 400 mg bd followed 2 days later with oralCiprofloxacin 500 mg bd for 5 days. Blood samples sent for culture, on the pretext that such infections develop septicemia quite rapidly, were negative on two occasions. The patient recovered well within the next week. A 3rd urine culture examination 1 week after discharge did not find any bacteria present. He was continued on a regimen of cotrimoxazole over the next 2 months to prevent relapse. Follow-up history was uneventful for next 6 months.

DISCUSSION

C. violaceum is a facultative anaerobe, motile gram negative bacillus producing a distinctive violet pigment. In spite of their ubiquitous distribution human infections are quite rare. Contrary to the widely regarded notion of them being nonpathogenic organisms, infections caused by them have the potential to trigger serious life threatening clinical conditions. *C. violaceum* infection is acquired mostly through the exposure of broken skin coming in contact with contaminated soil and water [7]. Severe infections after swimming in stagnant water, recreational theme parks or stagnant muddy water as well as post-surgical cases have also been reported. *C. violaceum* infection frequently presents with localized cutaneous infection followed by sepsis and multiple abscesses in the lungs, spleen, or liver with high mortality [3]. Orbital and periorbital cellulitis, osteomyelitis, urinary tract infection, pneumonia and meningitis are less frequently reported [8].

The virulent strains of this bacterium have increased the levels of superoxide dismutase and catalase that protects this organism from phagocytic attack in humans [9]. Pigment production is not a predictor of virulence in the organism. Mortality rates are close to ~55%. Important risk factors for mortality include young age, neutrophil dysfunction, chronic granulomatous disease, presence of shorter clinical course and inappropriate antimicrobial therapy [3]. Septicaemia if set in, can be very fatal unless promptly treated [2]. In the first case described here, inappropriate antimicrobial therapy was responsible for septicaemia setting in. However, a prompt change in targeted therapy helped save the patient.

Diagnosis of the condition requires microbiological confirmation by culture. The organism grows mostly on ordinary media with overnight incubation at 37°C. The presence of the spectacular deep violet pigment (violacein) is another clue for the identification of these bacteria. However, 9–11% of strains are nonpigmented and difficult to differentiate from organism like *Aeromonas spp.*, *Pseudomonas spp.*,*Vibrio spp.*, *Flavobacterium spp*. because of almost similar biochemical properties [7].

The organism is uniformly sensitive to fluoroquinolones, aminoglycosides, carbapenems, chloramphenicol, tetracycline, cotrimoxazole [10]. Genes associated with intrinsic resistance to penicillin, ampicillin and early cephalosporins have been described [11]. Alridge et al. found ciprofloxacin to be the most effective drug to treat *C. violaceum* infections [12]. A combination of a fluroquinolone with a amino glycoside/carbapenem has been found to be most successful therapy [3,12].

However, it is noteworthy that several patients report relapse or recurrence of infections after successful completion of therapy for clinical cure [13]. It is possible for occult microabscess or hidden focus to persist in internal organs despite adequate clinical cure. Hence, it is prudent to treat patients for a longer duration and follow up patients closely to prevent relapse [3].

CONCLUSION

Though human infections with *C. violaceum* are rare, the consequences developed due to this infection can be highly fatal. Clinicians should consider *C. violaceum* infection for patients with a history of trauma and concomitant exposure of contaminated water. A high index of suspicion, prompt diagnosis, aggressive, targeted antimicrobial therapy and prolonged therapeutic duration is recommended for successful management *C. violaceum* infection.

REFERENCES

1. Lima-Bittencourt CI, Astolfi-Filho S, Chartone-Souza E, Santos FR, Nascimento AM. Analysis of Chromobacterium sp. natural isolates from different Brazilian ecosystems. BMC Microbiol 2007; 7:58.
2. Ray P, Sharma J, Marak RS, Singhi S, Taneja N, Garg RK, et al. *Chromobacterium violaceum* septicaemia from north India. Indian J Med Res. 2004; 120:523–526.
3. Yang CH, Li YH. *Chromobacterium violaceum* infection: a clinical review of an important but neglected infection. J Chin Med Assoc. 2011; 74:435–441.
4. Batista JH, da Silva Neto JF. *Chromobacterium violaceum* pathogenicity: updates and insights from genome sequencing of novel chromobacterium species. Front Microbiol. 2017; 8:2213.
5. Janda WM, Mutters R. Pasteurella, Mannheimia, Actinobacillus, Eikenella, Kingella, Capnocytophaga, and other miscellaneous gram-negative rods. In Borriello SP, Murray PR, Funke G.(eds.) Topley and Wilson's Microbiology and Microbial Infections, Bacteriology Vol 2. 10th ed. London: Hodder Arnold ASM Press; 2005: 881–923.
6. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 27th ed, 2017. CLSI supplement M100, Wayne PA, USA. Available at: http://www.facm.ucl.ac.be/intranet/CLSI/CLSI-2017-M100-S27.pdf.
7. Tiwari S, Pattanaik S, Beriha SS. Nonpigmented strain of *Chromobacterium violaceum* causing neonatal septicemia: A rare case report. Indian J Pathol Microbiol. 2017; 60:427–429.
8. Moore CC, Lane JE, Stephens JL. Successful treatment of an infant with *Chromobacterium violaceum* sepsis. Clin Infect Dis. 2001; 32:E107–110.
9. Miller DP, Blevins WT, Steele DB, Stowers MD. A comparative study of virulent and avirulent strains of *Chromobacterium violaceum*. Can J Microbiol. 1988; 34:249–255.
10. Parajuli NP, Bhetwal A, Ghimire S, Maharjan A, Shakya S, Satyal D et al. Bacteremia caused by a rare pathogen – Chromobacteriumviolaceum: a case report from Nepal. International J Gen Med. 2016; 9: 441–446.
11. Fantinatti-Garboggini F, Almeida Rd, Portillo Vdo A, Barbosa TA, Trevilato PB, Neto CE, et al. Drug resistance in *Chromobacterium violaceum*. Genet Mol Res 2004; 3:134–147.
12. Aldridge KE, Valaninis GT, Saners CV. Comparison of the in vitro activity of ciprofloxacin and 24 other antimicrobial agents against clinical strains of *Chromobacterium violaceum*. Diagn Microbiol Infect Dis 1988;10: 31–39.
13. Yang CH. Non pigmented *Chromobacterium violaceum* bacteremic cellulitis after fish bite. J Microbiol Immunol Infect 2011;44:401–405.

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