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CHROMOBACTERIUM VIOLACEUM: AN UNCOMMON BACTERIUM ISOLATED FROM BLOOD CULTURE.

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ABSTRACT Chromobacterium violaceum sepsis is a rarely reported phenomenon. We report a unique case of C. violaceum sepsis in a female child. A 2-year-old girl presented to our institution with bilateral ear discharge, fever, vomiting, with poor weight gain, diminished activity and mental status. No significant Radiological findings. The infant was successfully treated with ciprofloxacin and gentamicin.

KEYWORDS : Chromobacterium violaceum, biochemical test for identification.

INTRODUCTION:

Chromobacterium violaceum a saprophytic bacterium was first discovered in water buffalo by Wooley in 1905. It is an opportunistic pathogen found in soil or water and is geographically restricted to tropical and semitropical climates and is found between latitudes 35ºN and 35ºS.[1]

It belongs to family Neisseriaceae of class Betaproteobacteria on the basis of 16s rRNA sequence analysis and by DNA-rRNA hybridization respectively.[2]

Chromobacterium violaceum is a large motile Gram negative, non-sporing bacillus, 0.6-0.9 x 1.5-4 µm, motile by means of single polar, usually, and also one to two lateral flagella.3, 4. It is facultative anaerobe. Minimum temperature for growth is 10-15ºC, maximum 40ºC; optimal growth at 30-35ºC. Optimum pH 7-8; no growth occurs below pH 5.2 Grow readily on routine culture media like nutrient media, MacConkey agar and blood agar. Colonies are moist, smooth, circular 0.5 to 1.5mm in diameter, are easily emulsified in water. Most strains produce the violet pigment 'violacein', soluble in ethanol and insoluble in water and chloroform. They are Catalase and oxidase positive.[1, 2]

Chromobacterium violaceum has been responsible for life-threatening infections with severe sepsis and metastatic abscesses particularly in children with defective neutrophil function (e.g. those with chronic granulomatous disease).[5]

Serious, and in some cases fatal, infections in humans have been reported from Argentina, Australia, Brazil, Cuba, Nigeria, Singapore, Taiwan, United States, and Vietnam. In most of these cases the route of entry was through the broken skin, following contamination with soil or water.[6]

CASE HISTORY:

A previously healthy 2-year-old girl from rural central India was transferred and admitted to our institution. One month before child was apparently all right, and then she develops bilateral ear discharge, on and off mild grade fever since 10 days, vomiting since 2 days, anaemia with poor weight gain and diminished activity and mental status.

At admission, the patient was febrile, with temperature of 38^{0}C (100.4^{0}F). Her vital sign includes a heart rate of 140 beats per minute, respiratory rate 60 breaths per minute, blood pressure of 115/60 mmHg, and her body weight was 5.6 kg.

The child was lethargic and displayed a diminished response to painful stimuli. Generalized facial oedema was noted, skin was thin and dry, hair easily pluckable, liver and spleen was not palpable. Ear discharge was purulent in nature without any specific odour.

The routine laboratory investigations showed peripheral total WBC count of 19100 cells per mm3 with 36% neutrophils, 60% lymphocytes, 3% monocytes, 1% eosinophils. A complete blood count revealed microcytic anaemia (Haemoglobin 8.3 g/dl, RBC count 3.76 million cells per mm3, PCV of 26.5%, MCV 70.4 fL, MCH 22 picogram, MCHC was 31.3g/dl, and platelets count of 96000 cells per mm3) ESR in first hour was 70mm. Malaria parasite not seen.

Blood biochemistry of patients was as shown urea 13 mg/dl, creatinine 0.68 mg/dl, sodium 136.5 mEq/L, potassium 3.84 mEq/L, uric acid 3.12 mg/dl, serum bilirubin 0.42 mg/dl (total) and 0.20 mg/dl (direct), SGOT (AST) 49 U/L, SGPT (ALT) 49 U/L, serum amylase 101.7 U/L, serum total protein 6.9 g/dl, serum albumin 3.3 g/dl, serum glucose 80 mg/dl.

Serum widal test shows a titre of 1:60, Montoux test was negative, on urine routine microscopy occasional pus cell and 1-2 epithelial cells seen per high power field. Urine culture was found negative. Analysis of blood culture and ear swab pus culture were yielded similar growth characteristics on routine culture media. Colonies on nutrient agar was circular of 0.5 to 1mm diameter in size, smooth, moist, violet in colour due to pigment production. On blood agar colonies shows hemolysis and on MacConkey agar colonies was non lactose fermenting (figure: 1 and 2).

On Gram's staining; Gram negative rods were seen under oil emersion field. They were Catalase and Oxidase positive. It was difficult to interpret the latter because the violet pigment prevented observing the result. This difficulty was overcome by incubating the agar plate anaerobically; there was no pigment formation. After a few hours under aerobic conditions, the colonies became violet again. On hanging drop they were actively motile bacilli. For further biochemical identification refer to table 1 and (figure: 3).

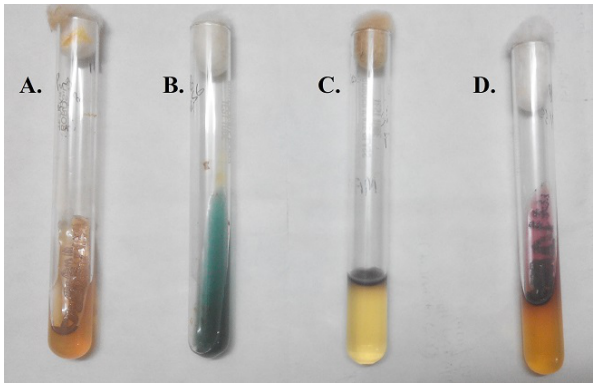
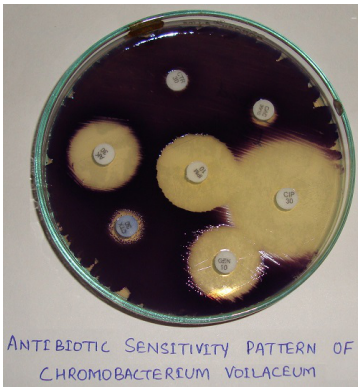


TABLE-1 Biochemical test results

Name of biochemical test	Result	Name of biochemical test	Result
Violet pigment 'violacein'	+	Urease	-
Growth on MacConkey agar	+	Citrate utilization	±
β- hemolysis	+	Acid from glucose	+
Growth in nutrient broth	Produces a violet colour ring at the surface with fragile pellicle.	Triple sugar iron	K/A
Catalase	+	H2S production	-
Oxidase	+	MR	-
Motility	+	VP	-
Indole	-	Nitrate is reduced to nitrite	+

On antibiotic susceptibility testing amikacin (30 µg), ciprofloxacin (30 µg), gentamicin (10 µg) and imipenem (10 µg) was sensitive and cefotaxime (30 µg), ceftriaxone (30 µg) and ceftazidime + clavulanic acid (30+10 µg) was resistant. (Figure: 4).



During the initial two days of hospitalization by her primary-care physician, the patient was treated with iv cefotaxime, iv amoxicillin-clavulanic acid and patient was on F75 diet 90ml/ 3hours. However, she remained febrile 3 days later; Blood culture and ear swab pus culture was done and grew a gram-negative rod bacterium after 48 hours. Her initial regimen that included iv cefotaxime, iv amoxicillin-clavulanic acid was replaced with vancomycin and metronidazole. When C. violaceum was identified, piperacillin-tazobactam and trimethoprim- sulfamethoxazole (TMP-SMX) were empirically added, replacing vancomycin and metronidazole. Once bacterial sensitivities pattern was known then ciprofloxacin and gentamicin was substituted for piperacillin-tazobactam and trimethoprim-sulfamethoxazole (TMP-SMX).

After 8 days of stay in the hospital she was afebrile, and her weight was 5.83 kg at the time of discharge.

DISCUSSION:

C. violaceum is a long gram-negative bacillus that is motile and facultatively anaerobic. Medium containing tryptophan is required to grow the organism and is the carbon source for violacein.3. When cultured; the organism produces large, smooth, convex colonies that are a violet-black colour. The organism's appellation derives from its striking purple pigment, violacein, which is soluble in alcohol but insoluble in water.[7]

The incidence of C. violaceum sepsis is low, and documented human infection is rare. Most cases have occurred during the summer, and they have been reported from such disparate locales as Australia, Southeast Asia, India, Argentina, and the south-eastern United States.8 C. violaceum has a wide distribution in soil and water in tropical and subtropical areas.[9]

Because of the rarity of C. violaceum sepsis, treatment has not been conclusively defined. C. violaceum is known to be generally resistant to cephalosporins and penicillins. Indeed, our patient received both ceftriaxone and amoxicillin-clavulanic acid without improvement of symptoms. Significant improvement came when the sensitivities were known and the patient was started on ciprofloxacin and gentamicin. This outcome is consistent with the extensive in vitro studies of Aldridge et al.[10], which found ciprofloxacin to be the most active compound against C. violaceum. Analysis of in vitro data suggests that of available antibiotics, fluoroquinolones are the most active against C. violaceum. Our patient survived a usually fatal infection after being treated with an antibiotic regimen that included ciprofloxacin. Ciprofloxacin has the most gram-negative coverage of the quinolones presently available; however, any quinolone with good coverage could be considered.[2]

These facts suggest that clinicians faced with patients critically ill from C. violaceum sepsis should consider adding a quinolone to the antibiotic regimen, even for infants.

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