Cronobacter sakazakii in Adults - A Rare Case Report.

Shah P¹, Mundhada S², Madhu G³, Rai M⁴, Waghmare P⁵, Shaikh NK⁶. Dr. Vaishampayan Memorial Government Medical College, Solapur(Maharashtra).

Abstract- A case report of 60 yrs old male presented with fever, neck stiffness and vomiting. CSF was sent for bacteriological examination. Motile gram negative bacilli with yellow pigmented colonies on blood agar were further subjected for biochemical reaction and Antibiotic suspeceptibility testing. It was suspected to be Enterobacter species and yellow pigmented colonies on blood agar raised doubt of Enterobacter sakazakii. The isolated was sent to BJMC Pune where it was confirmed to be Cronobacter sakazakii (previously known as Enterobacter sakazakii) by Phoenix 100 ID/AST system(Becton Dickinson Co., Sparks, MD). Keywords: Cronobacter sakazakii, Enterobacter sakazakii, Pott''s spine, Yellow colonies.

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-sporeforming bacteria of the family *Enterobacteriaceae*. *Enterobacter sakazakii* is now known as *Cronobacter sakazakii* (Farmer et al,1980). *Cronobacter sakazakii* is an emerging opportunistic pathogen which can cause life threatening meningitis, necrotising enterocolitis, and sepsis mostly in premature and full term infants especially fed on powdered milk. However adult infection is also known.^[1,2]

I. Case Report

A 60 year old patient was admitted in orthopaedic ward with history of fever, neck stiffness, convulsions, vomiting since 15 days, also tingling sensation and numbness was present bilaterally. There were radicular symptoms. On examination, the patient had stooping forward gait. There was decreased sensation in left thigh. His muscle power was grade 4. Kerning's sign and Brudzinski sign were also positive. Due to signs and symptoms of meningitis, CSF was collected aseptically and was sent for microbiological examination.

Patient had past history of tuberculosis 1 year back and was on anti-tubercular treatment and was suspected for tubercular meningitis and so CSF was sent for AFB also.

Microscopy- Wet mount revealed 4-5 polymorphonuclear lymphocytes/hpf along with motile bacilli. Gram stain revealed plenty pus cells and Gram negative bacilli.

Culture was done on Blood agar and MacConkeys agar and was incubated overnight at 37°C.

Next day on blood agar 2-3 mm, yellow pigmented, circular, raised, entire margin, smooth surface, non-haemolytic colonies were present which were catalase positive and oxidase negative.



Figure 1: Yellow pigmented colonies on C. sakazakii on Blood agar. ^[3,4]



(figure 2):Yellow pigmented colonies of *C. sakazakii* compared with white colonies of *Enterobacter aerogens* ATCC 13048.

MacConkeys agar showed lactose fermenting, smooth, circular, raised, entire margin colonies, 3-4 mm colonies. After examination of colonies, they were inoculated for biochemical reactions and AST. The blood sample of the patient was also cultured. It also revealed similar colonies. However, the AFB examination of the patient was negative(both microscopy and culture).

Biochemical reactions-Indole -negative, Methyl red-negative, VP-positive, Citrate-positive, Urease-negative, TSI-Acid/acid with abundant gas. Nitrate was reduced to nitrite and Ortho dinitrophenyl beta galactoside(ONPG) was hydrolysed.



figure 3: Moeller's decarboxylase media

Ornithine decarboxylase was positive.(figure 3)

Arginine dihydrolase test was also positive. .(figure 3)

Antibiotic susceptibility testing done by Kirby Beaur disk diffusion method- It was sensitive to amikacin, gentamicin, imipenam, ceftazidime, cefotaxime, aztreonam, ciprofloxacin, cotrimoxazole and piperacillin + tazobactam and resistant to ampicillin.(according to CLSI 2014).

Yellow pigmented colonies on Blood agar and Acid/acid with abundant gas on TSI raised doubt of *C.sakazakii*^[3,4] and so sample was sent to BJMC, Pune from where it was confirmed as *C.sakazakii* by Phoenix 100 *ID/AST* system(Becton Dickinson Co.,Sparks,MD).

Antibiotic susceptibility pattern by Phoenix 100-It was sensitive to amikacin, gentamicin, imipenam, cefuroxime, ceftazidime, cefotaxime, aztreonam, ciprofloxacin, tetracycline, cotrimoxazole and piperacillin + tazobactam and resistant to ampicillin and piperacillin.

Colonies were further grown on-

- 1) Tryptone soy agar, which revealed yellow pigmented colonies which differentiates from white colonies of *Enterobacter aerogens* ATCC^[1,5]
- ²⁾ Violet Red Bile Glucose agar-pinkish purple colonies with purple halo around it.^[1,5]

Other tests-DNA was not hydrolysed(25°C), Esculin showed hydrolysis, Gelatin was not hydrolysed(22°C).^[4]



Figure 4-Comparision of yellow pigmented colonies of *C.sakazakii* on tryptone soy agar with creamish white colonies of *Enterobacter aerogens* ATCC.



Figure 5-Pinkish purple colonies of *C.sakazakii* on Violet red bile glucose agar.

Pathological examination-

Haemoglobin-11.4g/dl, RBCs-4.74million/cmm,WBC-15600/cmm,Neutrophils-55% Lymphocytes-43%, Monocytes-1%, Eosinophils -1%, basophils-0%.Platelet-481000/cmm.

Biochemical parameters of Blood-

Protein-5.8g/dl, albumin-2.8g/dl, globulin-3.0g/l, SGOT-29IU/L, SGPT-32IU/L, sodium-135meq/L, potassium-4.3meq/L, creatnine-1.5mg/l, BSL-72, BUL-56, MCHC-28.64g/dl

Parameters of CSF-Protein-262mg/dl,Glucose-35mg/dl,WBC-689 cells/micro litre. There was neutrophilic predominance.

The patient was already on AKT and for treatment of meningitis piperacillin + tazobactam (PipTaz) and cefotaxime was added to it.

II. Discussion

C.sakazakii infection is seen mostly in powdered fed infants. However cases have been reported in elderly and immunocompromised.^[6]In a Food Safety Authority Ireland report (FSAI, 2007) it is reported that at least 111 cases have been reported in infants and children of whom 26 were fatal. Lai et al in 2001, and it is not usually life threatening in adults.^[7] All six species in the genus *Cronobacter* should be considered to be pathogenic-WHO article 2008.Patients who are the most susceptible to acquiring *Enterobacter* infections are those who stay in hospital (especially in the intensive care unit) for prolonged periods.^[8] *Enterobacter* infections are observed most frequently in neonates and elderly individuals .^[2] Iversen & Forsythe (2003) and Drudy et al. (2006a) confirmed that *E. sakazakii* infections usually affected adults with fatal underlying diseases.^[9] There is no information on *E. Sakazakii* cases from developing countries. ^[10]As *C.sakazakii* infection mainly spreads by foodborne route, it is unknown in our case how infection is obtained. But in all reported cases it is common amongst immunocompromised this is seen in our study also as the patient had tuberculosis. *C.sakazakii* is sensitive to most of the antibiotics. However, some studies have found resistance to ampicillin.^[11,12] this finding correlates with our study also. The patient recovered completely after treatment , and his CSF and blood samples were repeated after few days and second time both were found to be negative.

This indicates patient was successfully treated. To best of our knowledge, this is the first reported case of *C.sakazakii* in adults from CSF. As, most of the cases are found in infants, the elderly cases may be ignored.^[11]

III. Conclusion

C. sakazakii can cause adult infection especially in immune-compromised patient and must be looked for in any unusual presentation in such patients.

Acknowledgement

Special thanks Dr. Renu Bharadwaj Professor and Head of Microbiology Department BJMC, Pune for identification of this isolate and antibiotic susceptibility pattern by Phoenix 100 *ID/AST system(Becton Dickinson Co.,Sparks,MD)*..

References

- [1]. Biering, G., Karlsson, S., Clark, N.C., Jonsdottir, K.E., Ludvigsson, P., and Steingrimsson, O. 1989. Three cases of neonatal meningitis caused by Enterobacter sakazakii in powdered milk. J Clin Microbiol. 27(9):2054-6.
- [2]. Nazarowec-White & Farber et al, 1997a;. Enterobacter sakazakii: a review. International Journal of Food Microbiology, 37: 103-113.
- [3]. BUCHANAN, J.G. (co-ed) & GIBBONS, N.E. (co-ed). 1974. Bergey's Manual of Determinative Bacteriology 8th edition. Baltimore, Md.: Waverly press in Farmer et al 1980.
- [4]. Winn WC, Allen SD, Janda WM, Koneman EW, Precop GW, Schreckenberger PC. Koneman's color atlas and textbook of diagnostic microbiology. Chap. 7. 6th ed. New York: Lippincott; 2006. p. 303-91.
- [5]. Food and Agriculture Organization. 1994 Codex Alimentarius: code of hygienic practice for foods for infants and children. CAC/RCP 21-1979. Food and Agriculture Organization of the United Nations, Rome, Italy.
- [6]. Friedemann, M. "Enterobacter sakazakii in food and beverages (other than infant formula and milk powder)". International Journal of Food Microbiology. 2007. Volume 116 (1). p. 1-10.
- [7]. LAI, K.K. 2001. Enterobacter sakazakii among neonates, infants, children and adults: case reports and a review of the literature. Medicine, 80: 113-122.
- [8]. SANDERS, W.E. & SANDERS, C.C. 1997. Enterobacter spp: pathogen poised to flourish at the turn of the century. Clinical Microbiology Reviews, 10(2); Apr.: 220-241.
- IVERSEN, C. & FORSYTHE, S. 2003. Risk profile of Enterobacter sakazakii an emergent pathogen associated with infant milk formula. Trends in Food Science and Technology, 14: 443-454.
- [10]. FAO/WHO. 2004. Enterobacter sakazakii and other microorganisms in powdered infant formula: Meeting report, MRA 6. Available from: http://www.who.int/ 99 foodsafety/publication/micro/enterobacter_sakazakii/en [Accessed 27 April 2007].
- [11]. Lucas Mofokeng. ISOLATION AND CHARACTERIZATION OF ENTEROBACTER SAKAZAKII STRAINS FROM POWDERED INFANT FORMULAE AND OTHER FOOD SOURCES IN SOUTH AFRICA. Department of biotechnology and food technology. Tshwane University of Technology 2009.
- [12]. Md. Fakruddin Md. Mizanur Rahaman, Monzur Morshed Ahmed, Md. Mahfuzul Hoque.International Journal of Biomedical And Advance Research. ISSN: 2229-3809 (Online)Journal DOI:10.7439/ijbar CODEN:IJBABN.Review Article-Cronobacter sakazakii (Enterobacter sakazakii): An Emerging Food borne Pathogen.