Aeromonas caviae Septicemia in Immunocompetent Gastrointestinal Carriers

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Aeromonas caviae strains have been isolated from blood and stool cultures of three immunocompetent patients, residents of Northern India, who presented with community acquired septicemia without any recent history of diarrhea. Cell culture infectivity test performed on Hep-2 cells have shown substantial degree of invasiveness in the isolated strains. This case unleashed a possibility of asymptomatic gastrointestinal carriage of such strains of A. caviae in a very large population of India, as several areas of India have very high rates of Aeromonas induced acute diarrhea/gastroenteritis (up to 13%). It needs to be appraised further in India as well as other countries having high rates of Aeromonas induced acute diarrhea/gastroenteritis.

Key-Words: Aeromonas caviae, septicemia, gastrointestinal carriage.

Aeromonas species are Gram-negative, motile, facultative anaerobic, rod shaped, oxidase positive bacteria of the family Aeromonadaceae [1]. Aeromonas species are environmental bacteria which are widely distributed in aquatic environment (both fresh and saltwater), soil and agricultural products [1]. Aeromonads cause acute diarrheal disease of short duration or chronic loose stools in children, the elderly, or the immunocompromised, and they have been implicated as a cause of travelers’ diarrhea.

The significance of Aeromonas species as causative agent of human diarrhea has been well established [2]. Incidence of diarrheal disease caused by Aeromonas species is higher in developing countries than to developed countries [3] and extra-intestinal infections caused by these organisms are being recognized with increasing frequency [4]. These extra-intestinal infections include occasional primary infections in normal hosts (like cystitis and wound infections) as well as severe infections (like septicemia, peritonitis, endocarditis, osteomyelitis, meningitis, necrotizing fasciitis etc.) in immunocompromised patients [1]. There are presently 17 species in the genera Aeromonas. At least 10 of these have been identified in human diseases, but important species are Aeromonas hydrophila (A. hydrophila), A. caviae and A. veronii biovar sobria [5]. Most invasive infections are caused by A. hydrophila in patients with compromised immune systems, usually in association with malignancies or liver cirrhosis [6].

Here we are reporting three cases of septicemia with asymptomatic gastrointestinal carriage of A. caviae (having substantial degree of invasiveness), in immunocompetent patients.

Case Report

We have isolated three strains of A. caviae from the blood of patients (residents of Lucknow, U.P., India) with community acquired bloodstream infections in the year 2008 (since Jan 2008 to June 2008) from patients getting admitted to Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, a tertiary care hospital based in the largest state of north India. All the patients were apparently healthy before the onset of symptoms of septicemia and had no recent history of diarrhea, previous hospitalization, peritonitis, endocarditis, osteomyelitis, meningitis or necrotizing fasciitis.

Stool samples (3 samples, 24 hours apart) from each patient were also collected. Stool samples were cultured directly on MacConkey agar, Ampicillin sheep blood agar, and Xylose deoxycholate citrate agar. Simultaneous enrichment was also done in Gram-negative broth and alkaline peptone water, incubated overnight at 37ºC and then sub cultured on the above mentioned media. Stool samples from two of the patients showed heavy growth of the A. caviae (as confirmed by the standard biochemical tests) on the primary isolation medium [7]. While from one patient’s stool it was grown only after enrichment.

Antimicrobial susceptibility testing was performed using Mueller-Hinton agar (Oxoid, UK) by the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations [8]. A. caviae isolates of each patient from both sources (blood and stool) showed similar antibiotic susceptibilities. All the six isolates were susceptible to Gentamicin, Amikacin and Imipenem; Resistant to Amoxycillin, Cotrimoxazole, Cephalothin and Cefazolin; 4 isolates were sensitive to Cefotaxime (2 isolates from one patients being resistant), while only 2 isolates (from one patient) were sensitive to Ciprofloxacin.

To test invasiveness of the strains Hep-2 cells were grown in minimal Eagle’s medium (MEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine and 20 mM HEPES (Hi Media). These cells were grown at 37ºC in a humidified atmosphere under 5% CO₂. Twenty hours prior to the infection, approximately 4 x 10⁵ cells were seeded per well of 6-well tissue culture plates (BD Falcon, U.S.).

Bacteria were grown in BHI broth at 28ºC for 12 hours and added to the cell monolayer at a multiplicity of infection (MOI) of 50:1. To determine the invasiveness of bacteria, at 3 hours of infection, Hep-2 cells were washed thrice with Phosphate Buffer Saline (PBS) and incubated further for 90 min in MEM containing 100 µL/mL of gentamicin. The numbers of intracellular bacteria were determined by lysing the cells in

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Figure 1. *Aeromonas caviae* cell invasion to epithelial cells. The cells were incubated with bacteria for 3 hours, then washed and further incubated with MEM/Gentamicin for 90 minutes. Thereafter, washed, fixed and stained with Giemsa. Slides were examined by light microscopy with 10x objective lens.

PBS containing 0.1% digitonin and plating on trypticase soy agar (TSA). Cell invasion tests were found positive in all the isolate, 0.069% to 0.098% (mean being 0.073%) of the inoculated bacteria have invaded the Hep-2 cells. This cell-line invasion test was performed in Department of Biochemistry, Lucknow University, Lucknow, UP, India.

Slow intravenous infusion of Amikacin in a dose of 7.5 mg/kg body weight was given to the each patient every 12 hours for a period of 10 days and the patients were treated successfully. Follow-up cultures of blood and stool taken on 7th and 10th day were negative for *A. caviae*. Patients were asymptomatic after the antibiotic course. HIV ELISA was performed and was found negative in all the three patients.

Discussion

*Aeromonas* spp. cause cellulitis or wound infections following traumatic injury in an aqueous environment. They also cause various infections associated with hospitalization such as rare urinary tract infections, surgical wound infections, meningitis, peritonitis, endocarditis, or other serious infections [1,7]. Major predisposing conditions for *Aeromonas* infections include cirrhosis or other hepatic disease, hematologic malignancies and hepatobiliary diseases [9]. *Aeromonas* bacteremia is a rare disease usually seen in patients with multiple medical problems and in immunocompromised hosts, especially those with malignant or hepatobiliary diseases [6]. Sepsis may also occur as a result of contamination of wounds from fresh water or soil sources [1] or with the use of medicinal leeches [1]. The most common species involved in *Aeromonas* septicemia is *A. hydrophila* (just as in case of diarrhea) [6]. Although, from China bacteremia caused by *A. caviae* has been reported among patients with underlying illnesses like liver cirrhosis, malignancy and hepatobiliary diseases [9]; no case of *A. caviae* septicemia is reported from India.

We have found cases of septicemia with asymptomatic gastrointestinal carriage of *A. caviae* in immunocompetent patients having no history of diarrhea or previous hospitalization. These strains of *A. caviae* having considerable degree of invasiveness might have invaded the gastrointestinal tract and gained access to the bloodstream (just as in case of *Salmonella typhi*, *Yersinia enterocolitica*, *Shigella dysenteriae* etc).

Asymptomatic gastrointestinal carriage of invasive *A. caviae* strains is an unusual finding and is of epidemiological importance as several areas of India have very high rates of *Aeromonas* induced acute diarrhea/gastroenteritis (up to 13%) [3], which may lead to asymptomatic gastro-intestinal carriage later on. Finding of these cases unleash a possibility of asymptomatic gastrointestinal carriage of such invasive strains of *A. caviae* in a very large population of India, which needs to be evaluated further in India as well as other countries having high rates of *Aeromonas* induced acute diarrhea/gastroenteritis.

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