

## Case Study

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# Isolation of *Aeromonas salmonicida* from Human Blood Sample: A Case Report

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## ABSTRACT

### Keywords

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*Aeromonas salmonicida* belonging to the genus *Aeromonas* is a common pathogen that causes furunculosis and septicemia in variety of fishes. It infects cold blooded vertebrates living at low temperature mainly salmonid fish hence named salmonicida. *Aeromonas salmonicida* is considered to be a fish pathogen, non-pathogenic for humans as it cannot grow at 37°C. However, In our laboratory culture plates, blood culture bottle and broths were incubated twice at 37°C and each time same type of colonies were isolated which were identified as *A. salmonicida* by Vitek 2 compact automated system. By far one case has been reported regarding its isolation from human biological sample. Here we present the second case report of *A. salmonicida* isolated from the human blood.

## Introduction

*Aeromonas salmonicida* belonging to the genus *Aeromonas* is a common pathogen that causes furunculosis and septicemia in variety of fishes. It infects cold blooded vertebrates living at low temperature mainly salmonid fish hence named salmonicida. *Aeromonas salmonicida* is considered to be a fish pathogen, non-pathogenic for humans as it cannot grow at 37°C.

By our best knowledge, till date Tewari *et al.*, study has been reported first case of

*Aeromonas salmonicida* from human blood (Tewari *et al.*, 2014).

## Case Report

A 12-year-old Muslim female child, visited medicine Out Patient Department (OPD), at AIIMS, Jodhpur in December 2015 with complaints of off and on low grade fever, pain abdomen associated with weakness and malaise for last 20 days. There was no history of chills and rigor, sore throat, diarrhea, urinary discomfort, any other

localized infection, or chronic illness suggestive of immune compromised state except history of RHD for which she is taking medicines regularly. There is history of fish ingestion from local pond, few days before symptoms arise. All the routine investigations were found to be normal except leucocytes which were slightly raised.

Blood sample was sent to our Department of Microbiology, Dr. S. N. Medical College, Jodhpur lab for culture and sensitivity. After overnight incubation, turbidity and haemolysis were reported in blood culture bottle. Blood culture broth was sub cultured on blood agar plate and MacConkey agar plate and incubated overnight. Next day, pinpoint yellowish colonies, friable, not easily emulsifiable and non-hemolytic and having entire margin grew on blood agar plate. (figure-1) No growth was observed on MacConkey Agar. On Gram's staining, gram negative bacilli with no specific arrangement were seen. (figure-2) The organism was nonmotile, catalase positive and oxidase positive. Triple sugar iron (TSI) was K/K, hydrogen sulphide production negative, indole negative, urease negative and citrate utilization test negative. (Baron J and Finglod S., 1996) As identification could not be clearly made by these biochemical test hence blood agar plate was re-incubated. Next day, colour of colonies turned golden yellow where as size of colonies became larger.

As identification of organism could not be done by conventional biochemical reactions second sample was collected on her second visit. Sample was processed by BacT alert (BioMeriux, New York) and sub cultured on Blood and MacConkey agar and incubated overnight at 37°C. Next day colonies with same morphology (as of first sample) were isolated. For second sample identification

was done and confirmed by Vitek 2 compact system. To our surprise report showed isolation of *Aeromonas salmonicida*.

Antibiotic sensitivity was determined by Kirby Bauer's disk diffusion method as per CLSI guidelines. The isolate was found to be sensitive to drugs (disks of Himedia) Imipenem (10 mcg), Ceftazidime (30 mcg), Ceftriaxone (30 mcg), Levofloxacin (5 mcg), and Amikacin (30 mcg) while resistant to Ampicillin (10 mcg) & Tetracycline (30 mcg).

### **Results and Discussion**

Member of the genus *Aeromonas* have been recognized since 1891, when Sanerelli, first reported them in frog and produced septicemia and other diseases upon re-inoculation into cold and warm blooded animals. Through the use of improved techniques for isolation and identification from biological specimens their importance in human has recently become better appreciated. Much advancement has occurred regarding their taxonomy, disease spectrum and pathogenicity over many past years. (J.M. Janda, S. Duffey, 1988)

*Aeromonas salmonicida* belonging to the genus *Aeromonas*, was first discovered in a Bavarian brown trout hatchery by Emmerich and Weibel in 1894. (Amos, k., 2013) It is facultative anaerobe, Gram negative, non motile bacterium which readily ferments and oxidizes glucose and give catalase and oxidase test positive. (Kim *et al.*, 2011) It is considered as primary pathogen in variety of fishes (Kim *et al.*, 2011; Ishiguro *et al.*, 1981) and not in humans as they cannot grow at 37°C. Optimal temperature required for its growth has been reported as 22-25°C. (Austin, 1986) In 90% of the strains, its virulence was found to be lost if cultured at 30°C and above. (Ishiguro *et al.*, 1981)

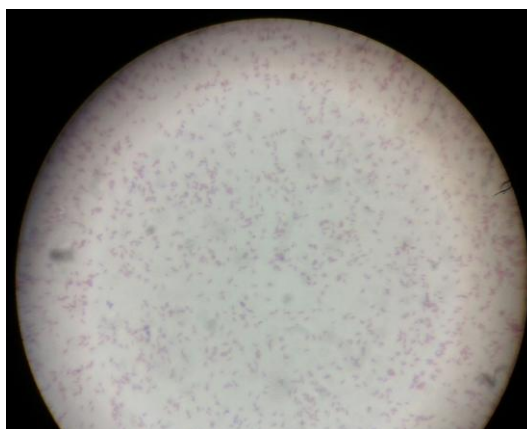
Previously other *Aeromonas* species like *A. hydrophila*, *A. caviae*, *A. veronii* etc. were also considered as pathogen in cold blooded animals only, including fish, amphibians and reptiles but gradually recognized as opportunistic pathogen for human. However these organisms have increasingly been identified as a primary pathogen for humans in normal individual as well as in immune-compromised patient mainly in

gastrointestinal infections and septicemias. (Kao *et al.*, 2003) Major sources described for *Aeromonas* species in gastrointestinal infection are environment-water-animal complex and ingestion of contaminated foods whereas sources reported for extra-intestinal infection are either direct soil or water contact or ingestion of contaminated food followed by bacteremic dissemination from gastro intestinal tract. (Abott, 1998)

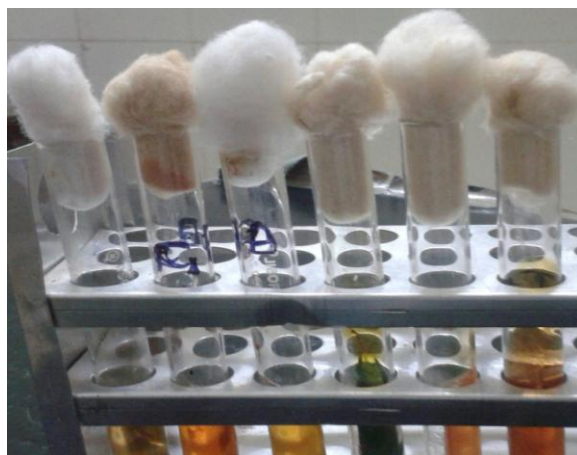
**Figure.1** *Aeromonas salmonicida* colony on Blood Agar



**Figure.2** *Aeromonas salmonicida* seen on Gram's Stain



**Figure.3** Biochemical Reaction Pattern of Isolate



**Figure.4** Antimicrobial Susceptibility Test of Isolate



Compared with other pathogenic *Aeromonas* *A. salmonicida* can also be found in environment, diseased fish and water and may be transmitted by all these sources. Also it has pathogenic factor S– layer which mediates tissue adherence as in other *Aeromonas* like *A. hydrophila*, *A. veronii* so it may also act as pathogen in humans like other species. (Ishiguro *et al.*, 1981; Igbiosa *et al.*, 2012) Although previously documented that growth is unlikely at 37°C and so in humans (Ishiguro *et al.*, 1981) which is contradictory to our report where blood samples were cultured by two different methods (conventional and BacT alert) and sub-cultured plates were incubated at 37°C and for both samples (first and

second) same colonies were isolated which suggests that either *A. salmonicida* has undergone some changes and can grow at 37°C or it has been misidentified by Vitek 2 system.

We have limited resources and facilities to work on molecular basis in our setup hence this Vitek 2 report could not be confirmed further but we can throw the light on this organism and make the scientists to rethink and work more on this organism to find any change undergone by this organism in pathogenicity, virulence, disease spectrum and antibiotic resistance.

Our isolate was found sensitive to drugs

(disks of Himedia) Imipenem (10 mcg), Ceftazidime (30 mcg), Ceftriaxone (30 mcg), Levofloxacin (5 mcg), and Amikacin (30 mcg) while resistant to Ampicillin (10 mcg) & Tetracycline (30 mcg). Isolate found in Tewari *et al.*, study was sensitive to all drugs (by Kirby Bauer's method) Recently antibiotic resistant *A. salmonicida* strains have been recognized as a serious concern owing to their potential health risk to humans and animals (Amos, 2013). Has this problem been evolved by humans only who are devising techniques and facilities to cross all the boundaries and getting interacted with all types of living and non living things?

To conclude, society is already overburdened with resistant organism and if nonpathogenic organisms will change their host preference, virulence and sensitivity, it will be difficult for clinicians and scientists to tackle this problem.

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