

## **ISOLATION AND IDENTIFICATION OF ENTEROPATHOGENIC *AEROMONAS HYDROPHILA* FROM LAHARPUR WATER RESERVOIR, BHOPAL**

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**Abstract:** Water is essential to life, but many people do not have access to clean and safe water and many die of waterborne bacterial infections. There is increasing evidence that consumption of raw fresh produce is a major factor contributing to human gastrointestinal illness due to the potential for contamination with pathogenic microorganisms. The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination; the persistence of pathogens in water, in soil, and on crops; and the route of exposure. Laharpur water reservoir of Bhopal is built with the objective to irrigate local farms, but the water is highly polluted and it is unsuitable for irrigation. In the present study presence of enteropathogenic aeromonads was observed in the water of reservoir, it was identified as *Aeromonas hydrophila*, this water not suitable for human consumption. Antibiotic sensitivity was also monitored in the study.

**Keywords:** *Aeromonas hydrophila*, irrigation, water reservoir, polluted water, antibiotic sensitivity.

### **Introduction**

Commercial and small-scale farmers generally irrigate their produce with water from nearby rivers, streams, ponds, wells and dams most of which do not meet the required standard for irrigation (Westcot, 1997). In India number of surface water system is polluted. Water-borne diseases are any illness caused by drinking water contaminated by human or animal faeces, which contain pathogenic microorganisms. In the present study enterotoxic bacteria was isolated from the Laharpur reservoir. Laharpur reservoir is situated in the southwest of Bhopal city in the state of Madhya Pradesh, India with an objective to store water for irrigational purpose. It is receiving water from following major inlets. 1. Anna Nagar sewage fed drain, Shahpura sewage fed drain, Barkhera Pathani Nalla and Barkhera Nalla. These inlets carry untreated sewage and waste water from the southwest part of Bhopal Town (Pandey et.al, 2010). The Laharpur reservoir has both rural and urban catchments. In last few years the lake became surrounded by habitations with the growth of the city. These developments resulted in anthropogenic pressures on the lake which accelerated the

eutrophication process thereby making the water body un-fit for human consumption (Pandey et.al, 2008). The higher concentration of bacterial contamination in the water of reservoir may possibly contaminate the ground water also. Water of the reservoir is used by nearby villagers for washing their clothes. Microbiological quality of the reservoir was found to be very poor as the presence of coilform was reported in previous study (Pandey et.al, 2010). Coliform bacteria are a reliable indicator of organic pollution as they are unable to survive in clean water beyond a limited time. Use of this water for irrigation is very dangers as enterotoxic bacteria may harm the health of peoples. An enterotoxin is an exotoxin that disrupts the lining of the gastrointestinal tract, and if the enterotoxic organisms enter human body through contaminated vegetables and fruits, this may be resulting in severe epidemic. *Aeromonas* spp. are widely distributed in aquatic environments (Kingombe et.al, 2004). *Aeromonas* spp. can produce several virulence factors, including extracellular enzymes, siderophores, cytotoxic and cytotoxic enterotoxins, Shiga-like toxins, endotoxins, invasins, and adhesins (Kirov, 2001). *Aeromonas* spp. are able to grow and produce toxins in foods at refrigeration temperatures also (Kirov, et.al. 1993). The isolation of enterotoxigenic aeromonads from drinking water indicates the possible human health hazard of waterborne *Aeromonas* spp ((Saavedra et.al 2007 and Sharma et.al, 2005). Ceaser et.al 2010 and Albert et.al, 2000 isolated enterotoxic genes from children with diarrhea emphasize the importance of enterotoxins in the pathogenicity of aeromonads. Besides irrigation water of this reservoir is also used by local laundry persons; this is another risk factor as it develops skin diseases in both loundry man and the cloth owner. So the purpose of the study is to ensure that weather the water of the Laharpur reservoir is contaminated with aeromonads or not.

## **Materials and Methods**

### **Isolation of bacteria from water:**

Water samples were taken in clean sterile glassbottles, and transported from the Laharpur water reservoir to the laboratory within 30 min in ice bags and ice jackets. All collected samples were transported and prepared for immediate bacteriological test.

### **Media for isolation:**

Serially diluted water sample were spread on nutrient agar medium and plates were incubated at 37<sup>0</sup>C for 24 h. After incubation the cultures were pour plated on glutamate starch phenol red agar (GSP agar, Merck) and incubated at 37 °C again for 24-48 h.

### Characterization

On the basis of Gram staining and by biochemical test isolates were identify up to species level (Abott et.al, 2003) (Table -1).

### Test for antibiotic resistance

Antibiotic resistance was determined by using combi-disc of 12 antibiotics (Hi-media) using Mueller–Hinton agar by agar diffusion test as per Bauer-Kirby Method (1966).

### Result and Discussion

Glutamate starch penicillin (GSP) medium was used for the simultaneous isolation of aeromonads. This glutamate starch phenol-red agar contains glutamate and starch as its sole nutrients. Many accompanying microorganisms cannot metabolize these compounds (STANIER et al. 1966). Starch is degraded by *Aeromonas* with acid production causing phenol red to change the yellow, but not by *Pseudomonas*. Yellow colonies surrounded by yellow zone were picked tentatively as aeromonads. Strains were found Gram negative and rod shape. On the basis of biochemical test isolates were identified as *Aeromonas hydrophila*. All the isolates were found to be sensitive against Gentamycin (10 µg), Neomycin (5 µg), Chloramphenicol (30 µg) and Tetracyclin (30 µg). Isolates were found to be resistant towards Ampicillin (10 µg), Cephalothin (30 µg) and Penicillin (6 µg). *Aeromonas hydrophila* is associated with various diseases. *A. hydrophila*, *A. caviae*, and *A. veronii* biotype *sobria* are considered of clinical significance (Janda, 1991, Janda and Abott, 1998) and if this water is used for irrigation or washing clothes is would be very dangerous. The spectrum of infectious diseases caused by *Aeromonas* species includes gastrointestinal infections as well as extraintestinal infections such as cellulitis, wound infections, septicemia, urinary tract infections, and hepatobiliary and ear infections, among others (Janda et.al, 1995). So the presence of *Aeromonas hydrophila* in the water of reservoir is an alarming situation and it need attention.

**Table 1: Biochemical characterization of isolates**

Test	Isolates (n=37)	Test	Isolates (n=37)
oxidase	37	Glucose (Gas)	36
catalase	37	Glycerol	32

Indole	37	Arabinose	36
urease	0	Lactose	17
Simmon citrate	34	Maltose	37
MR	23	Mannose	37
VP	32	Sucrose	37
LDC	37	Esculin	37
ODC	0	gluconate	34
ADH	37	0% NaCl	37
Gelatin hydrolysis	37	3% NaCl	37
DNase	31	6% NaCl	0

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