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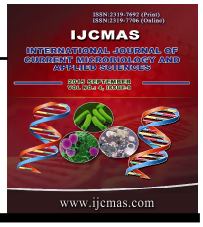

Original Research Article

Isolation and Identification of *Vibrio sps* from Laharpur Water Reservoir, Bhopal, India

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Keywords

Vibrio sps, Irrigation, Water reservoir, Polluted water

A B S T R A C T

Environmental waters if contaminated with sewage waste are an important reservoir for *pathogenic microorganism*. The occurrence of pathogenic microorganism was previously observed in Laharpur water reservoir. 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. 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. In the present study, presence of enteropathogenic *Vibrio sps* was observed in the water of reservoir, it was identified as *Vibrio sps* and due to presence of it this water not suitable for human consumption.

Introduction

Vibrio cholera has long been known to be responsible for the life threatening secretory diarrhea termed as Asiatic cholera or epidemic cholera (Ryan and Ray, 2004; Faruque and Nair, 2008). *Vibrio cholerae* is a "comma" shaped Gram-negative bacteria with a single, polar flagellum for movement (Heidelberg *et al.*, 2000). Although cholera is primarily known as a water-borne disease in the endemic regions including Bangladesh, contamination of foods can also be an imperative mode for cholera transmission (Glass and Black, 1992). Many studies have reported that foods including vegetables, fruits, seafoods, dairy products, poultry and meat products and others can become contaminated with *Vibrio* spp. through improper handling, undercooking, washing with unhygienic water and by the use of untreated night soil (Feachem, 1981; Huq *et al.*, 1983; Rabbani and reenough, 1999). Due to poverty and poor sanitation, cholera is highly prevalent in developing countries where many outbreaks has been implicated to the consumption of faecally-contaminated foods such as rice, millet gruel, and vegetables (Rabbani and Greenough, 1999). 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. Enterotoxic *Aeromonas hydrophila* and coliforms was previously reported in Laharpur water reservoir (Tripathi and Choudhary, 2014; Pandey *et al.*, 2010) make this water unsuitable for irrigation purpose. Presence of aeromonads and coliform bacteria arise a question for the possibility of *Vibrio* sps in the reservoir. So the purpose of the study is to ensure that weather the water of the Laharpur reservoir is contaminated with *Vibrio* sps or not.

Materials and Methods

Isolation of bacteria from water

Water samples were taken in clean sterile glass bottles, 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

Water sample was filtered by 0.45 µm membrane (Millipore) filter. Membranes filter were placed in alkaline peptone water (pH 8.6 ± 0.2) and incubated for 24 hours at 37^0C. After proper incubation one ml culture was pour plated on TCBS agar (Thiosulfate citrate bile salts sucrose agar) (Kobayashi *et al.*, 1963) and incubated at 37°C again for 24–48 h.

Characterization

On the basis of Gram staining and by biochemical test isolates were identified up to species level (Table 1).

Results and Discussion

Bacteria present in water sample enriched in alkaline peptone water for 24 hours. Peptones provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance and encourages the growth of *Vibrio cholerae*. It is claimed that raising the medium's pH leads the medium's alkalinity to inhibit most of the unwanted flora background, leaving the viability of the *Vibrio* species intact. Culture from alkaline peptone water was inoculated in TCBC agar medium. It is highly selective for the isolation of *Vibrio spp* and *V. parahaemolyticus*. TCBS has a very high pH (8.5-9.5) which suppresses growth of intestinal flora other than *Vibrio* spp (Koneman, 2006). The medium consists of plant and animal proteins, a mixture of bile salts, one percent sodium chloride, sodium thiosulfate, ferric citrate, sucrose, and yeast extract. The bile salts inhibit growth of gram-positive microorganisms; one percent sodium chloride is incorporated into the medium to provide optimum growth and metabolic activity of halophilic *Vibrio* spp.; sodium thiosulfate provides a source of sulfur and also acts in combination with ferric citrate to detect the production of hydrogen sulfide; sucrose serves as the fermentable carbohydrate that, with the help of bromothymol blue and thymol blue indicators, allows for the differentiation of

those *Vibrio* spp. which utilize sucrose. Yellow colonies on TCBS agar was presumed as *Vibrio spp* and subjected to biochemical characterization and after this 22 isolated were identified as *Vibrio spp.*

Cholera is a disease of great public health importance. Water has been recognized as the primary vehicle of cholera transmission. Thus, to interrupt the transmission cycle, effective public health measures should be undertaken to prevent sewage contamination of irrigation water supplies, as well as to establish sanitary disposal and sewage treatment systems. So the presence of as *Vibrio spp* in the water of reservoir is an alarming situation and it needs attention and whether these isolates are pathogenic or not would be the base of next investigation. To reduce the risk of water-borne transmission of cholera, it is recommended that reservoir water should be treated against pathogenic bacteria and then use for irrigation; farmer should also take safety measures for preventing water-borne transmission of cholera (Colwell, 1996).

Table.1 Biochemical characterization of isolates

Test	Isolates (n=22)	Test	Isolates (n=22)
Colonies in TCBS	yellow	Acid from:	
oxidase	22	Sucrose	14
catalase	22	mannitol	19
Indole	22	Arabinose	0
urease	0	Lactose	0
Simmon citrate	17	Maltose	22
VP	9	DNase	9
LDC	22	0% NaCl	22
ODC	22	1% NaCl	22
ADH	7	3% NaCl	8
Gelatin hydrolysis	22	6% NaCl	0

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