

Original Research Article

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Isolation and Identification of Bacterial Strains from Yamuna River at Allahabad District in Uttar Pradesh, India

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ABSTRACT

Keywords

River Yamuna, Water pollution, *Salmonella* spp, *Vibrio* spp, Isolation, Identification

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The aim of this work was to detect the presence of bacterial species in Yamuna river water from six different sites namely (S₁) Mahewa Ghat, (S₂) Gau Ghat, (S₃) Old Naini Bridge, (S₄) New Naini Bridge, (S₅) Arail Ghat, (S₆) Saraswati Ghat. Total 36 samples of water were used for isolation purpose. *Salmonella* were detected in 5 samples with highest ranking (13.9%). *Salmonella* spp. isolates belonged to the species *S. typhi* (two isolates), *S. enterica*, *S. choleraesuis*, *S. gallinarum* while *Vibrio* were detected in 4 samples (11.2%). *Vibrio* spp. isolates belonged to the following species *V. cholera* (two isolates), *V. parahaemolyticus*, *V. navarrensis*. The presence of bacteria species indicates the pollution status, due to the sewage, human activities and industrial activities etc, the quality of river water has deteriorated which affects human as well as aquatic life.

Introduction

Yamuna is one of the most polluted rivers in India, especially around Delhi, because of the large amount of the wastewater discharge. According to the Centre for Science and Environment, approximately 75 to 80 percent of the river's pollution is the result of raw sewage, industrial runoff and the garbage thrown into the river and it totals over 3 billion litres of waste per day (Mishra, 2010 and Martínez *et al.*, 2009). Today water resources have become the most exploited natural system since man strode the earth.

According to united nation report, freshwater is gradually becoming a matter of concern with nearly 900 million people affected by diarrhoea each year and an equal number suffering from disease caused by various worm. Unclean water ranks at top of the world population problem (Goel and Grad, 2008). The industrial pollutants associated with organic matter, inorganic dissolved solids and other unwanted chemicals cause serious problems in the water quality (Radha *et al.*, 2007). Water quality can also be evaluated through chemical and physical parameters

including heavy metals, trace metals, total suspended solid and turbidity. These trace elements present in virtually potable water, some of which play a role in metabolism. Major ions in drinking water are correlated with palatable mineralization that affects the quality of drinking water (Delpla *et al.*, 2009). Water of good quality is of basic importance to human physiology and man's continued existence depends very much on its availability (Lamikanra *et al.*, 1999; Ottaviani *et al.*, 2005). Pathogens such as *Salmonella* spp, *Vibrio cholera* and *E.coli* that are shed into water body through faecal contamination perpetuate many diseases (Faparusi *et al.*, 2011). These cause typhoid fever and dysentery. Other agents of water borne diseases are protozoan that cause diarrhoea; *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli* and *Cryptococcus parvum* (Kelly *et al.*, 1997). *Salmonella* is a ubiquitous enteric pathogen with a worldwide distribution that comprises a large number of serovars characterized by different host specificity and distribution (Pond, 2005) just like other enteric bacteria, *Salmonella* is spread by the fecal-oral route of contamination. This microorganism can enter the aquatic environment directly with feces of infected humans or animals or indirectly, *e.g.*, via sewage discharge or agricultural land run off (Wray *et al.*, 2000; Lightfoot *et al.*, 2004; Dolejska *et al.*, 2009). *Salmonellae* are frequently found in environmental samples. They (10^3 - 10^4) are usually present in large numbers in raw sewage CFU/L) and can still be present in wastewater effluent after advanced secondary treatment including coagulation, filtration and disinfection (Maier *et al.*, 2000; Wéry *et al.*, 2008). Temporal and spatial variation of *Salmonella* frequencies have been commonly observed in surface water (Lemarchand and Lebaron, 2003; Bonadonna *et al.*, 2006; Meinersmann *et al.*, 2008; Till *et al.*, 2008; Byappanahalli *et al.*, 2009; Haley *et al.*, 2009; Wilkes *et al.*, 2009;

Jokinen *et al.*, 2010). It is possible that variations in the occurrence of *Salmonella* in ambient water may be governed, in part, by environmental parameters such as temperature, water chemistry, and solar radiation that influence survival and transport of the microorganism (Polo *et al.*, 1999; Baudart *et al.*, 2000; Schets *et al.*, 2008; Wilkes *et al.*, 2009). *Vibrios* are primarily aquatic bacteria. Species distribution depends on sodium concentration and water temperature (Farmer and Hickam-Brenner, 2003). *Vibrios* are autochthonous to the oceanic, estuarine, and freshwater ecosystem (Kaneko and Colwell, 1973). They are found in sediments (Vezzulli *et al.*, 2009) and are known to produce bio-films on surfaces. They either swim freely in the water column, or adhere to associated with other organisms. Moreover, ample numbers of *Vibrio* species have developed adaptive features that enable them to predominantly thrive in salty and even river environments (Hood and Winter, 1997; Mccarter, 1999; Lipp *et al.*, 2002; Thompson *et al.*, 2004; Grau *et al.*, 2005; Pruzzo *et al.*, 2005). Several investigations have also shown the prevalence of *Vibrio* species in surface water throughout the world, and their prevalence in the environment is influenced by season and location (Johnson *et al.*, 2012). They are usually linked to eruptions of *Vibrio* infections as a result of consuming undercooked seafood and water contaminated with sewage (Lee *et al.*, 2002). *Vibrio* species have been reported to be capable of surviving in many different environmental conditions due to the development of a spectrum of adaptive responses to nutrient deficit, variations in salinity and temperature, and a resistance to predation by heterotrophic protists and bacteriophage (Colwell, 2009). Numerous studies that have investigated the distribution of *Vibrio* species suggest that pathogenic subpopulations of the genus *Vibrio* are potential reservoirs for disease epidemics (Lutz *et al.*, 2014). Previous studies have

established that it is almost impossible to understand the effect of single physico-chemical parameters on *Vibrio* species since all parameters are interdependent and the influence of the environmental conditions varies from one species to another. The incidence and the rate distribution of *Vibrio* species have been linked to a vast array of environmental factors, most notably organic matter, salinity, temperature, and the association with aquatic animals depending on the pathogen and its habitat, and the geographic location (Cavallo and Stabili, 2002; Janelidze *et al.*, 2011; Jaiani *et al.*, 2013; Arunagiri *et al.*, 2013). Developing countries are extremely affected because of their paucity of resources, infrastructure and disaster awareness systems (Sur, 2000). The aim of this work was to detect the presence of *Salmonella spp.* and *Vibrio spp.* from Yamuna river water.

Materials and Methods

Study area

The study area was divided into six sampling sites (Table 1). River water samples were collected from six sampling sites from Allahabad district as (S₁) Mahewa Ghat, (S₂) Gau Ghat, (S₃) Old Naini Bridge, (S₄) New Naini Bridge, (S₅) Arail Ghat, (S₆) Saraswati Ghat.

Collection of water sample

The Yamuna river water samples were collected during April to September 2016 from the Mahewa Ghat to Saraswati Ghat for the assessment of physico-chemical factors. River water Samples were collected (3 Times) of every month in BOD bottles and sterile one litter politeness bottles. The closed bottles were immersed in the river at the depth of 0.5 to 0.7 m and the stopper was opened in bottom of river and was closed again to the river water. After sample collection, the bottles were kept in ice box and transferred

immediately to the laboratory for further analyses (Sivamanikandan and Ahmed John, 2015). Total 36 water samples were used for *Salmonella spp.* and *Vibrio spp.* isolation and identification purpose.

Isolation of *Salmonella*

1ml of water sample was inoculated in 9 ml of buffered peptone water and incubated at 37°C for 18 hrs for pre-enrichment. After enrichment, a loopful of inoculums was then streaked on xylose lysine desoxycholate (XLD) agar and incubated at 37°C for 24 h. The presumptive *Salmonella* colonies appearing slightly transparent red halo with a black centre surrounded by a pink-red zone on XLD agar and were sub-cultured and for each sample, a representative pure colony was selected and stored on sterile nutrient agar, colonies were confirmed by gram staining and biochemical characterization.

Isolation of *Vibrio*

1ml of water sample was inoculated in 9 ml of alkaline peptone water and incubated at 37°C for 8 h for pre-enrichment. After enrichment, a loopful of inoculums was then streaked on thiosulphate citrate bile salt sucrose (TCBS) agar and incubated at 37°C for 24 h. Yellow smooth and slightly flattened colonies with opaque centers and translucent peripheries appearing after 24h of incubation were presumably considered as *Vibrio* species and were sub-cultured and for each sample, a representative pure colony was selected and stored on sterile nutrient agar, colonies were confirmed by gram staining and biochemical characterization.

Morphological analysis

The pure culture obtained was then observed for colony and other morphological characteristics, the culture was gram stained.

Biochemical analysis

Gram Staining, Motility, Urease, ONPG, Catalase, Oxidase, Indole production, Voges Proskauer, Nitrate Reduction, Citrate Utilization, String (for *Vibrio*), H₂S, Methyl Red test was used for both colonies of *Salmonella spp* and *Vibrio spp* as described Bergey's Manual of Systematic Bacteriology.

Results and Discussion

Out of total 36 water samples analyzed. Origins and numbers of samples in each of them are presented in the Table 1. *Salmonella* were detected in 5 samples (13.9%) and *Vibrio* were detected in 4 samples (11.2%). The site with highest incidence was the Gau Ghat, where the *Salmonella* organism was found in 3 samples, followed by Arail Ghat, and Mahewa Ghat with one strain each one. The site with highest incidence was the, Mahewa Ghat where the *Vibrio* organism was found in 2 samples, followed by Arail Ghat, and Gau Ghat with one strain each one. Both the organisms were found motile and strictly aerobic.

Results for *Salmonella spp.* motility, catalase, nitrate MR, H₂S reaction are found positive and similar results have been reported by (Nesaet *al.*, 2011; Khan *et al.*, 2007), the negative results of Voges-Proskauer and Indole, urease, ONPG, oxidase, citrate. The

carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose. Similar results have been reported by Meinersmann *et al.*, (2008).

Results for *Vibrio spp.* motility, string, citrate, indole, oxidase, ONPG, nitrate reaction are found positive the negative results of MR, H₂S and urease. The carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose. Similar results have been reported by Kaper *et al.*, (1980). Results for *Vibrio spp.* motility, string, citrate, indole, oxidase, ONPG, nitrate reactions are found positive the negative results of MR, H₂S and urease.

The carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose. Similar results have been reported by (Kaper *et al.*, 1980) (Table 2–5).

Table.1 Geographical location of water sampling sites

| Site | Latitude | Longitude |
|------------------|---------------|---------------|
| MahewaGhat | 25° 25' 2" N | 81° 50' 18" E |
| GauGhat | 25° 25' 27" N | 81° 50' 45" E |
| Old Naini Bridge | 25° 25' 37" N | 81° 50' 59" E |
| New NainiBridge | 25° 25' 25" N | 81° 51' 41" E |
| ArailGhat | 25° 25' 18" N | 81° 52' 54" E |
| SaraswatiGhat | 25° 25' 50" N | 81° 52' 6" E |

Table.2 Origins and numbers of samples

| Site of sampling | Type of site | Number of samples |
|------------------|--------------|-------------------|
| Mahewa Ghat | River | 6 |
| Gau Ghat | River | 6 |
| Old Naini Bridge | River | 6 |
| New Naini Bridge | River | 6 |
| Arail Ghat | River | 6 |
| Saraswati Ghat | River | 6 |
| Total | | 36 |

Table.3 Occurrence % of *Salmonella* spp and *Vibrio* spp from Yamuna river

| No. of Water samples | No. of <i>Salmonella</i> spp | No. of <i>Vibrio</i> spp |
|----------------------|------------------------------|--------------------------|
| 36 | 5 (13.9%) | 4 (11.2%) |

Table.4 Cultural and Morphological analysis for *Salmonella typhi* and *vibrio cholera*

| | Characteristics | XLD Agar | TCBS Agar |
|-------------------------------|-----------------|---------------------|-----------------------|
| Cultural Characteristics | Colour | Red with Black dots | Yellow, blue to black |
| | Margin | Regular | Regular |
| | Elevation | Convex | Umbonate |
| | Opacity | Opaque | Opaque |
| | Pigmentation | Red and Black | Yellow |
| | Size (in mm) | 0.5 – 0.7 | 1-2 |
| | Texture | Butyrus | Butyrus |
| Morphological characteristics | Staining | Gram's | Gram's |
| | Reaction | -ve | -ve |
| | Shape | Rod | Curved(comma) |
| | Size (in µm) | 0.4-0.5 | 0.3-0.4 |

Table.5 Sugar fermentation test for *Salmonella typhi* and *Vibrio cholera*

| S. No | Carbohydrates | <i>S.typhi</i> | | <i>V.cholerae</i> | |
|-------|---------------|-----------------|-----------------|-------------------|-----------------|
| | | Acid | Gas | Acid | Gas |
| 1 | Arabinose | -ve | -ve | + ^{ve} | + ^{ve} |
| 2 | Lactose | -ve | - ^{ve} | - ^{ve} | - ^{ve} |
| 3 | Maltose | + ^{ve} | + ^{ve} | + ^{ve} | + ^{ve} |
| 4 | Mannitol | + ^{ve} | + ^{ve} | - ^{ve} | - ^{ve} |
| 5 | Sucrose | -ve | - ^{ve} | - ^{ve} | - ^{ve} |
| 6 | Xylose | + ^{ve} | - ^{ve} | - ^{ve} | - ^{ve} |
| 7 | Glucose | + ^{ve} | + ^{ve} | + ^{ve} | + ^{ve} |

Table.6 Biochemical analysis of *Salmonella typhi* and *Vibrio cholerae*

| Biochemical Characteristics | Name of the test | <i>Salmonella typhi</i> | <i>Vibrio cholerae</i> |
|-----------------------------|---------------------|-------------------------|------------------------|
| | Gram Staining | -ve | -ve |
| | Motility | + ve | +ve |
| | Urease | -ve | -ve |
| | ONPG | -ve | +ve |
| | Catalase | +ve | -ve |
| | Oxidase | - ve | +ve |
| | Indole production | - ve | +ve |
| | Voges-Proskauer | -ve | -ve |
| | Nitrate reduction | + ve | +ve |
| | Citrate utilization | -ve | -ve |
| | String | - | +ve |
| | H ₂ S | +ve | -ve |
| | Methyl Red | +ve | -ve |

The biochemical properties were estimated using Bergey's Manual of Systematic Bacteriology. Results for *Vibriospp.* motility, string, citrate, indole, oxidase, ONPG, nitrate reaction are found positive the negative results of MR, H₂S and urease. The carbohydrate profile of the bacteria under investigation depicts that it could utilize glucose, maltose and mannitol with weak production of acid and little or no gas production; but could not utilize xylose, lactose, sucrose and arabinose. Similar results have been reported by (kaper *et al.*, 1980). The biochemical properties were estimated using Bergey's Manual of Systematic Bacteriology (Table 6).

The isolation of *Salmonella spp.* and *Vibrio spp.* from water sample means that the direct consumption of such water without treatment

May be very risky. This study reveals that contamination of Yamuna water is by humans and other animal sources like bathing, farming and washing.

In conclusion, present study confirmed the presence of *Escherichia coli*, *Salmonella spp.*, and *Vibrio spp.* as water-borne pathogens in Yamuna water of Allahabad district in Uttar Pradesh, India. It also showed the important public health problem in of Allahabad district in Uttar Pradesh, India. The result of this study revealed that the water sources in these communities are moderate polluted and is not safe for use by the communities for drinking or other domestic needs without prior treatment. There is need to monitor regularly and mitigate the effects of community behaviour on surface waters.

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