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Prevalence of vibrio cholerae species in water bodies of Bareilly, Uttar Pradesh

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Abstract
Vibrio cholerae, the etiologic agent of cholera, is autochthonous to various aquatic environments, but despite intensive efforts its ecology remains an enigma. The facultative human pathogen *Vibrio cholerae* can be isolated from estuarine and aquatic environments. *V. cholerae* is well recognized and extensively studied as the causative agent of the human intestinal disease cholera. In former centuries cholera was a permanent threat even to the highly developed populations of Europe, North America, and the northern part of Asia. Today, cholera still remains a burden mainly for underdeveloped countries, which cannot afford to establish or to maintain necessary hygienic and medical facilities. Especially in these environments, cholera is responsible for significant mortality and economic damage. During the last three decades, intensive research has been undertaken to unravel the virulence properties and to study the epidemiology of this significant human pathogen. More recently, researchers have been elucidating the environmental lifestyle of *V. cholera*. Better understanding of V. cholerae ecology can help reduce the times that human beings come into contact with this pathogen and thus minimize the health risk it poses. The purpose of this study was to identify the presence of *Vibrio* species in the water samples collected from different locations of Bareilly, Uttar Pradesh. Water samples were collected from different locations. *Vibrio* species were identified by using TCBS agar medium.

Keywords: *Vibrio*, isolation, TCBS, water samples

Introduction
Water is a vital natural resource because of its basic role to life, quality of life, the environment, food production, hygiene, industry, and power generation (Meays *et. al*., 2004) [7]. The scarcity of water does not only threaten food security, but also the production of energy and environmental integrity. This often results in water usage conflicts between different communities, and water contamination when humans and animals share the same source of water (Kusiluka *et. al*., 2005) [6].

With the rapid increase in world population and increased urbanisation, there is a massive strain on the existing water supply and sanitation facilities (UNDPI, 2005) [9]. In the developing world, poor access to safe water and inadequate sanitation continues to be a danger to human health (WHO, 2004) [8].

Cholera, a life threatening diarrhoeal disease, still kills thousands annually and remains one of the few bacterial diseases known for its pandemicity. Of more than 200 O-antigen serogroups so far identified among *V. cholerae* isolates, only two serogroups, O1 and O139, are known to cause epidemics and pandemics (Sack *et al.*, 2004). Non-O1/non-O139 strains have not been found to be involved in epidemic cholera, but they are associated with non- Ol/non-O139 *V. cholerae* gastroenteritis. Although rare, non-Ol/ non-O139 *V. cholerae* gastroenteritis can cause septicaemic infections and even prove lethal. The predominant symptoms associated with this illness are diarrhoea, abdominal cramps, and fever, together with vomiting and nausea and the appearance of blood and mucus in the infected individual's stools (Cheasty *et. al*., 1999) [2]. *V. cholera* O1, O139 and non-O1/O139 comprises a single taxonomic species and their environmental habitats are likely to reveal great similarities (Levin, 1996) [3].

Vibrio spp. are gram negative aquatic bacteria that are widely distributed in sea and brackish water environments, both as free-living organisms and bound to a variety of substrates, including suspended mineral particulates, plants and the exoskeleton of zooplankton (Hervio *et. al*., 2002 and Jores *et. al*., 2003). [11, 12] Only a few species, including *Vibrio cholerae, V. parahaemolyticus, V. mimicus, V. vulnificus,* and *V. fluvialis* have been linked to human food-borne infections. The diarrhoeal disease, cholera, which has caused epidemics and pandemics, continues to be a global threat to public health and is caused by infection with

V. cholera (Faruque *et. al*., 2003) [13]. Every year millions of cholera episodes occur throughout the world especially in developing countries and thousands of cases are reported to be fatal (Gaffga *et. al*., 2007) [14].

Molecular biological DNA-based diagnostic methods, especially polymerase chain reaction (PCR), have been studied and developed for accurate and rapid identification of Vibrio spp. These methods provide advantages that complement standard microbiological culture-based methods (Kaysner *et. al*., 1998 and Jones *et. al*., 2012) [4, 5].

Material and methods

Collection of water samples: Water samples were collected from different water bodies and various ponds of Bareilly.

Isolation and Identification of Vibrios: Thiosulfate–citrate–bile-salts–sucrose agar (TCBS) is a highly selective differential medium that is most commonly used for the isolation of *V. cholerae*; its selective ingredients suppress the growth of most of the interfering organisms such as coliforms, pseudomonads, aeromonads, and other Gram-positive bacteria (Kobayashi *et al*., 1963) [10]. The advantage of TCBS is its sucrose–bromothymol blue diagnostic system, which distinguishes the yellow sucrose-positive colonies of *V. cholerae* from other colonies. 200 ug of each water sample was spread onto TCBS, (Hi-Media, India) plates (Pinto *et. al*., 2011) [15]. All the plates were incubated at 37ºC for 24 h. Sucrose positive colonies showing yellow and green slightly flattened with opaque centre and translucent peripheries from each plate were subcultured on trypticase soy agar (TSA). These sub-cultured peripheries were cultured in Tryptone soy broth (TSB) for obtaining pure cultures.

DNA Isolation and PCR Assay

PCR amplification of the target DNA was carried out by multiplex PCR assay, in a Thermal Cycler (Genei); with 200 µL PCR tubes with a reaction mixture volume of 20 µL. Cells were grown overnight at 37°C on Luria Bertani broth (LB broth) and various steps included centrifugation, followed by suspension in 10% SDS and after isolating, dissolving the DNA sample in T.E buffer. The amplification consisted of Initial denaturation at 94°C for 2 minutes, followed by 30 cycles consisting of 94°C for 1 minute, 62°C for 1 minute and 72°C for 1 minute and a final extension at 72°C for 10 minutes. PCR products thus obtained, were electrophoresed through 1.2% (wt/vol) agarose gel with ethidium bromide (final concentration 0.5 µg/mL), to resolve the amplified products and visualized.

Results and Discussion

Various samples were collected from different locations of Bareilly which are listed in the Table below

Table 1: Collection of water samples from various locations

Sr.	Coding	Location Name	Region
1.	KL	Keshlata (near hospital campus, Delapeer road)	Bareilly
2.	4CL	4 civil lines (in front of NBA academy)	Bareilly
3.	PN	Phoenix mall (near Jingle bells school)	Bareilly
4.	SSN	Suresh sharma nagar (suresh sharma nagar chauraha)	Bareilly
5.	JN	Jagriti nagar (BDA Colony road)	Bareilly
6.	SC	Soncity (near chaurasi ghanta mandir)	Bareilly

Isolation of *Vibrio* spp. from water sample on TCBS media (samples showing both green and yellow colonies)

Green colonies and yellow colonies of *Vibrio* organism were observed. On TCBS, yellow colonies were presumptively identified as *V. alginolyticus*, *V. cholerae*, and *V. mimicus*. Green or blue-green colonies were assumed to be *V. parahaemolyticus*, and *V. vulnificus*.

Fig 1: Spreading on TCBS medium showing green and yellow coloured colonies of vibrios species**.**

Table 2: Differentitation of samples based on colour of the colony obtained

Sr. no.	Coding	Location name	Yellow colony	Green colony
1.	KL	Keshlata	✓	✓
2.	4CL	4 Civil lines	✓	✓
3.	PN	Phoenix mall	✓	✓
4.	SSN	Suresh sharma nagar	✓	✓
5.	JN	Jagriti nagar	✓	✓
6.	SC	Soncity	✓	✓

Sub culturing on TSA and obtaining pure culture from TSB

Sucrose positive colonies showing yellow and green slightly flattened with opaque centre and translucent peripheries from each plate were sub cultured either on trypticase soy agar (TSA). These sub cultured peripheries were cultured in Tryptone soy broth (TSB) for obtaining pure cultures.

Fig 2: Streaking done on TSA media plate to obtain colonies of the specific species,

Fig 3: Broth cultivation of sample inoculated and stored at 37° C for 48 hours for incubation

Primer specific detection of the above obtained colonies

Table 3: Primer specific identification

S. No.	Coding	Location name	Vibrio spp present
1.	JN	Jagriti nagar	*Vc*
2.	SC	Soncity	*Vc*
3.	KL	Keshlata	V_A
4.	SSN	Suresh Sharma Nagar	*Vv*

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