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RESEARCH **P**APER

Identification of vibrio species from various water samples of Bareilly, using multiplex PCR

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The purpose of this study was to investigate the prevalence of Human pathogenic vibrio species in water bodies of Bareilly, U.P. using the traditional culture and multiplex PCR methods. Vibrio species are very much prevalent in water bodies of tropical region. The study was conducted on 25 various water samples collected from rivers and ponds of Bareilly, U.P. For this, species specific primers were designed targeting the toxgene of the five pathogenic species. Confirmation was done using multiplex PCR technique for rapid detection of the five selected pathogenic species including *V. cholera, V. alginolyticus, V. parahaemolyticus, V. mimicus* and *V. vulnificus.* Out of the five targeted species three were present *i.e. V. cholera, V. alginolyticus, V. parahaemolyticus.* Water is essential to life, but many people do not have access to clean and safe drinking water and many die of waterborne bacterial infections. In this review a general characterization of the most important bacterial diseases transmitted through water like cholera is present.

Key words : TCBS, Water samples, *Vibrio cholerae*

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INTRODUCTION

In particular, waterborne infections like typhoid fever, cholera, dysentery and traveler's diarrhea, caused by different types of bacterial pathogens pose a major public health hazard (Hunter, 1997) especially in developing countries. The spectrum of waterborne infections is also expanding, and many infectious diseases once believed to be conquered are on the rise (Marshall *et al.,* 1997). An outbreak of cholera began in Haiti's Artibonite Department in October 2010 and rapidly spread across all 10 Haitian departments and Port-au-Prince. Initial investigations indicated that drinking untreated water was the principal risk factor for infection (O'Connor *et al.,* 2011) in order to protect public health, regular monitoring of waterborne pathogens is required. However, the deficiency of precise and cost-effective diagnostic methods is a major obstacle in the prevention and control of infections and outbreaks transmitted by waterborne pathogens. Vibrio, a diverse genus of aquatic bacteria, currently includes 72 species, 12 of which occur in human clinical samples. Members of the vibrio genus are gram negative, halophillic bacteria indigenous to coastal marine systems (Thompson *et al.*, 2003). While these common bacteria persist as a natural component of the marine microbial flora, a small per centage of environmental isolates carry the genetic determinants for human pathogenesis (Nishibuchi and Kaper, 1995; Chakraborty *et al.*, 2000 and Rivera *et al.*, 2001). The aim of this study is to screen different water bodies (rivers, estuarine, lakes and ponds) for

the presence of vibrio species and determine their presence by multiplex PCR method.

Analysis of water samples :

Water samples were collected from various, rivers, and ponds of Bareilly. Selective agar media *i.e.* TCBS was selected for the growth of colonies having vibrio species. 200 ml of fresh water sample was taken and was spread onto TCBS agar plates and was left for incubation upto 48 hours, for the development of the colonies. After obtaining green and yellow colonies on TCBS plates, single colonies were taken and streaked onto Tryptone Soya Agar plates for obtaining discrete colonies. Thereafter, the broth was prepared for obtaining pure culture of the colony which would help in DNA isolation.

Isolation of genomic DNA :

Isolation of genomic DNA was performed using the Phenol chloroform method. Extraction of bacterial DNA was done by broth. 1 ml broth was taken in an eppendorf tube and centrifuged at 10000 rpm for 10 mins. There after supernatant was discarded and the pellet obtained, was dissolved in 900 ml T.E. buffer.10 µl of 10 per cent SDS was added and tubes were kept in waterbath for cell lysis. After 1-2 hours tubes were again centrifuged and the supernatant was collected. Phenol chloroform Isoamyl mix was added in the ratio 25:24:1 and the samples were again centrifuged. Double the volume of ice cold isopropanol was added into this upper layer of DNA. Centrifugation was done at 10000 rpm for 10 min. Supernatant was discarded and the pellet obtained was dissolved in 50 µl of TE buffer and stored at 4°.

PCR amplification and gel electrophoresis :

After performing gel electrophoresis of the isolated DNA on 0.8 per cent gel, we performed PCR on the isolated samples using species specific primers. The PCR mix was made for a total of 20ml. PCR was performed on a thermocycler and the total number of cycles set was 30. Further the PCR products were run on agarose gel electrophoresis for checking amplification. The amplified products were visualized after electrophoresis at 50 V for 45 minutes on a 1.2 per cent agarose gel by ethidium bromide staining.

Isolation of vibrio species :

The yellow and green colonies were grown, when water sample was spread onto the TCBS media and kept in incubation for 48 hrs. The yellow colonies indicate to be sucrose positive. The green colonies were sucrose negative (Fig. 1 and 2).

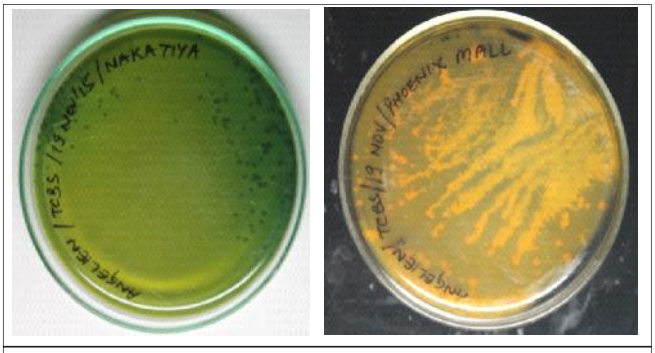

Fig. 1 and 2 : Isolated green and yellow colonies on TCBS selective media

Isolation of genomic DNA from isolated vibrio species:

After isolation of vibrio species on TCBS media from different water samples, the colonies were inoculated on TSA broth for isolation of genomic DNA from vibrio species. The Phenol Chloroform Isoamyl method was used for isolation of DNA. The precipitated DNA was reconstituted using 1xTE Buffer. Quality and quantity of DNA extracts were also verified on 1.2 per cent agarose gels stained with ethidium bromide.

Amplification by multiplex PCR :

Amplification of the samples was performed by multiplex PCR method and out of the five targeted species, three of them were present in the samples *i.e. Vibrio cholera, Vibrio alginolyticus* and *Vibrio parahaemolyticus* and were used for identification. The amplified products were run in agarose gel electrophoresis for visualization of the amplified products

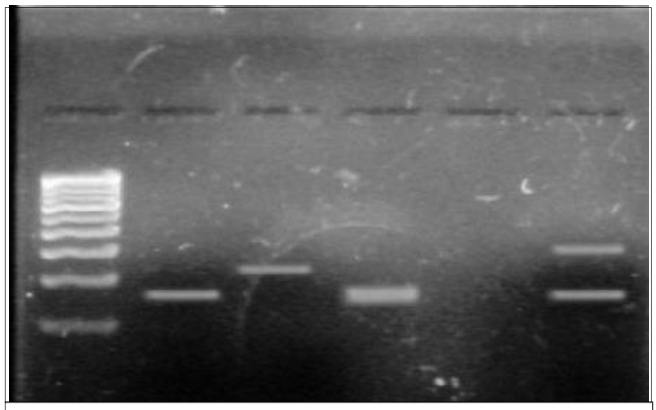

Fig. 3: Visualization of the amplified DNA

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