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RESEARCH ARTICLE

Isolation and Biochemical identification of *Yersinia species* of Narmada River from Khalghat, M.P.

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Abstract

A study was conducted with the objectives to isolate and identify *Yersinia species* in the water of the Narmada River. Samples were collected from Khalghat one of the major station of the River having different sociobiological activities around it. The confluences of Narmada form the major outlets of domestic sewage and into the water. The presence of large numbers of Gram negative Yersinia within the water is a matter of concern. The Bacteria which has been identified are the indicators of pollution in the water. Presence of this bacteria may cause many infectious disease to human health such as plague, diarrhea. Its presence basically shows the contamination of water by human feces. The present finding indicates the urgency of detailed investigations on the aspects, especially with reference to pathogenic organisms. The paper discusses this aspect in detail.

Introduction

Bacterial diversity and number ranges from a few too many millions in a millimeter of water depending upon the source and the level of contamination. River is very rich in bacteria because of the large quantity of dissolved organic matter present in water. Bacterial population is one of the major problems with respect to fresh water pollution. *Yersinia* is a genus of bacteria in the family *Enterobacteriaceae*. *Yersinia* are Gram-negative rod shaped bacteria, a few micrometers long and fractions of a micrometer in diameter, and are facultative anaerobes (Ryan et al, 2004). Some members of *Yersinia* are pathogenic in humans; in particular, *Y. pestis* is the causative agent of the plague. Rodents are the natural reservoirs of *Yersinia*; less frequently other mammals serve as the host. With the exception of *Yersinia pestis*, *Yersinia* species are ubiquitous organisms. They are distributed globally in a wide range of environments. Typically, isolates are recovered from soil samples, surface water, and human and canine feces (Brenner et al, 1988). *Yersinia* species are potentially pathogenic. Three species produce disease in humans. *Yersinia pestis* is the causative agent of the plague, while *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* cause diarrhea, mesenteric lymphadenitis, and septicemia. All other *Yersinia* species are opportunistic pathogens, meaning they cause infection only in immune compromised individuals (Brenner et al, 1988).

The Narmada is a river in central India and the fifth largest river in the Indian subcontinent. It forms the traditional boundary between North India and South India and flows westwards over a length of 1,312 km (815.2 mi) before draining through the Gulf of Cambey (Khambat) into the Arabian Sea, 30 km (18.6 mi) west of Bharuch city of Gujarat. It is one of only three major rivers in peninsular India that runs from east to west (largest west flowing river) along with the Tapti River and the Mahi River. It flows through the states of Madhya Pradesh (1,077 km (669.2 mi)), Maharashtra, (74 km (46.0 mi)) – (35 km (21.7 mi)) border between Madhya Pradesh and Maharashtra and (39 km (24.2 mi) border between Madhya Pradesh and Gujarat and in Gujarat (161 km (100.0 mi)). The Narmada basin, hemmed between Vindya and Satpuda ranges, extends over an area of 98,796 km2 (38,145.3 sq mi) and lies between east longitudes 72 degrees 32' to 81 degrees 45' and north latitudes 21 degrees 20' to 23 degrees 45' lying on the northern extremity of the Deccan Plateau. The basin covers large areas in the states of Madhya Pradesh

(86%), Gujarat (14%) and a comparatively smaller area (2%) in Maharashtra. In the river course of 1,312 km (815.2 mi) explained above, there are 41 tributaries, out of which 22 are from the Satpuda range and the rest on the right bank are from the Vindhya range.

The present paper deals with the identification pathogenic bacteria called *Yersinia spp* of the river water collected from Khalghat one of the major confluent point of the River in Madhya Pradesh, India.

MATERIALS AND METHODS

The water sample was collected in the year 2010 to 2012 to isolate and identify *Yersinia* Species according to APHA (2002).

Sampling Station

Khalghat

Khalghat is a town and a municipality in Dhar district in the state of Madhya Pradesh. It has an average elevation of 150 metres (495 feet). It is located on the banks of Narmada River and National Highway 3 Agra-Indore-Dhule-Mumbai. Its latitude 21 º06'N and longitude 75 º27'E.

Morphological and Biochemical Identification

In the lab, the experiment was done by isolating "unknown" bacterial samples from the River water for biochemical identification. There are many methods for identifying bacteria. Traditionally an observational and biochemical approach has been used. Bacteria are categorized as "Gram Positive" or "Gram Negative" according to whether or not they are stained by a chemical dye, a common biochemical technique.

Yersinia is a Gram negative bacterium, so further biochemical experiments were carried on Gram negative bacteria isolated from the water. Bacteria were identified from isolated colonies through morphology and staining reactions, culture characteristics and biochemical reactions..

1.Culture method for detection and identification of *Yersinia* Species.

1.1 Enrichment

Cold enrichment for 2 weeks in low selective phosphate-buffered saline broth supplemented with 1% mannitol and 0.15% bile salts (Niskanen et al., 2002).

1.2 Plating Media

Different media such as MacConkey agar media, Bile esculin agar media, Christensen's urea agar media were used for morphological identification of *Yersinia* after inoculation followed by incubation for 24 to 72 hours.

2. Biochemical identification of *Yersinia* Species.

For Biochemical identification following tests was performed:

IMViC: A battery of biochemical tests known as IMViC was used in the clinical lab to distinguish between enteric microorganisms. The acronym IMViC stands for indole, methyl red, Voges Proskauer and citrate. The "i" in the acronym is added for pronunciation purposes.

Tryptone broth/Indole test ("I"): Used to demonstrate the ability of a bacterium to produce the enzyme tryptophanase. This enzyme acts on the amino acid to produce "indole".

Methyl Red ("M") – an indicator of low pH (red below pH of 4.4) – used to show the mixed acid fermentation ability of bacteria.

VP -Voges-Proskauer Test ("Vi") – used to show bacterial production of acetoin, also known as 2,3-butanediol.

Simmons citrate slant ("C") – Simmons citrate agar tests for the ability of a gram-negative organism to import citrate for use as the sole carbon and energy source. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from uninoculated slant.

U -Urea broth: demonstrates the ability of a bacterium to produce the enzyme urease, capable of hydrolyzing urea. Phenol red indicator is added (fuchsia above pH 8.4) to show rise in pH due to accumulation of ammonia.

RESULT AND DISCUSSION

Identification of *Yersinia spp* by Morphological characterization of bacterial colony grown in different media:

1. Enrichment Media:

As a psychrotrophic organism, Yersinia is able to multiply at 4ºC and enrichment at this temperature for 2-4 weeks has been used. The growth rate of competitive bacteria at 4°C is slowed sufficiently to enable Yersinia to multiply to numbers necessary for isolation of plating media.

2. 2. MacConkey Agar Media:

- Lactose negative colonies
- flat, colorless, or pale pink
- 1-2 mm diameter

The Above characters give presumptive positive test for *Yersinia*.

3. Bile Esculin Agar media:

- Colourless colonies

Other *Enterobacteriaceae* gives black colour in this media).

4. Christensen's Agar media:

- Pink colour colonies which shows urease positive bacterial colonies of *Yersinia.*

Table 01: Identification of *Yersinia spp* by Biochemical characterization

S.NO	NAME OF THE TEST	RESULT
1	Indole	-
2	Methyl Red	-
3	Voges-Proskauer	-
4	Citrate Utilization	-
5	Urease Production	+
6	H_2S Production	-

CONCLUSION

The present study indicates extremely high levels of bacterial populations in the Narmada River indicating adverse effects of pollution and deterioration of the environment through the organic and inorganic pollutant that enter the water. The presence of *Yersinia* bacteria in the water is a matter of concern. The present findings indicate the urgency of detailed investigations on this aspect, especially with reference to the pathogenic organisms in this region.

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