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Research Article

ASSESSMENT OF MICROBIAL POLLUTION IN CERTAIN DRINKING POND AND TAP WATER SAMPLES OF RAMANATHAPURAM DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

In the drinking pond waters of Karendal and Thiruppalaikudi, the results showed the presence of *Serratia marscescens.* But in the pond water of Thiruppalaikudi apart from the presence of *S. marscescens, Vibrio cholera* was also present. This pathogenic *Vibrio cholera* is causing the acute diarrheal disease called cholera. Cholera is a serious disease or epidemics in many developing countries. This is due to the consumption of food and water from unsafe sources. Polluted and contaminated water may create a deleterious environment for the growth and survival of *Vibrio cholerae.* Endemic cholera is a highly preventable disease, requiring only proper sanitation and safe drinking water to reduce its transmission within a community.

Keywords: *Serratia marscescens, Vibrio cholera,* Microbial contamination, Drinking pond.

INTRODUCTION

Water is considered as a vehicle for the propagation and dissemination of human associated bacteria (Faria *et al.,* 2009). Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers (WHO, 2004; Fawell and Nieuwenhuijsen, 2003).

Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption. In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes (Biyela *et al.,* 2004). These unprotected water sources can be contaminated with microbes through rainfall runoff and agricultural inputs, mixing with sewage effluents and faeces from wild life (Obi *et al.,* 2002; Sharma *et al.,* 2005), which render them unacceptable for human consumption. Faecal coliforms, *Aeromonas* and *Pseudomonas,* are used as indicators of faecal contamination in water (Webster *et al.,* 2004) and the presence of these pathogens may have severe health implications on consumers especially those that are immunocompromised (Biyela *et al.,* 2004; Dumontet *et al.,* 2000; Pavlov *et al.,* 2004).

Seasonal temperature variation could account for some of the bacterial population variation. *Aeromonas hydrophila, Shewanella putrefaciens, Corynebacterium urealyticum, Escherichia coli, Pseudomonas* sp., *Vibrio cholera and, Cellulomonas* sp. were the common species in all the bacterial populations of different seasons where the first four bacterial species are made up the most cultural assemblage. *Flavobacterium* sp., *Micrococcus* sp., *Streptococcus* sp., *Burkholderiaglumae and Pasteurella* sp. were present in some seasons of the year. *Pseudomonas fluorescens* and *Salmonella* sp. were present only in winter, where *Pasteurella pneumotropica* was found only in summer (Al-Harbia and Uddina, 2006).

Clinically EPEC (Enteropathogenic *E.* coli) presents with watery diarrhoea which can vary in its severity and duration. Indeed, in several outbreaks there has been a high mortality of up to 30% or more. The infectious dose is very high, the LD50 being about 10|8 to 10|10 organisms (Kothary and Babu, 2001).

In addition to causing outbreaks associated with contaminated drinking water, enterohaemorrhagic *E. coli* have caused outbreaks linked to recreational water contact. There have been a number of outbreaks associated with swimming pools (Friedman *et al.,* 1999; Paunio *et al.,*

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1999), a paddling pool (Brewster *et al.,* 1994; Hildebrand *et al.,* 1996) and natural lakes or other surface water (Keene *et al.,* 1994; Anon. 1996; Cransberg *et al.,* 1996; Mc Carthy *et al.,* 2001). The general assumption is that outbreaks have generally followed faecal accidents from other bathers. Swimming pool outbreaks have occurred in pools with inadequate chlorination. McCarthy and colleagues (2001) described an outbreak of haemolytic uraemic syndrome in children due to *E. coli* O121: H19, a non-O157 strain. In addition to three cases of HUS, there were eight cases of diarrhoea.

A direct epidemiological approach could be used as an alternative or adjunct to the use of index micro-organisms. Yet epidemiologic methods are generally too insensitive, miss the majority of waterborne disease transmissions (Frost *et al.,* 1996) and are clearly not preventative. Nonetheless, the ideal is to validate appropriate index organisms by way of epidemiological studies. A good example is the emerging use of an enterococci guideline for recreational water quality (WHO 1998). Often epidemiologic studies fail to show any relationship to microbial indicators, due to poor design (Fleisher, 1990, 1991) and/or due to the widely fluctuating ratio of pathogen(s) to faecal indicators and the varying virulence of the pathogens.

MATERIALS AND METHODS

Study area

Kallakulam and Karendal Oorani ponds were selected in Ramanathapuram area to analyze and monitor the extend of microbial pollution in the drinking water sources.

Kallakulam Pond: This pond is situated 24 km away from Ramanathapuram Town. This is the only drinking water source for a number of villages around Thiruppalaikudi.

Karendal Oorani: This pond is situated 8 km away from Ramanathapuram. This Oorani is the only source of drinking water for the people of many villages of that area. During rainy seasons this Oorani is receiving river water. Another drinking water samples was collected from tap water (Cauvery water)

Collection of water samples

Water samples from the drinking water sources were collected in sterilized glass bottles of 250 ml with ground glass stopper protected by Kraft paper. The water samples were transported to the laboratory immediately after collection at the earliest possible. All the samples collected were assayed within 3 hours.

Assessment of microbial population

Serial dilution agar plating method was used for the isolation and enumeration of microbial organisms present in well water sample. In serial dilution agar plate method, 1 ml of water sample to be assayed is added to 99 ml of sterile water blank and made up to a total volume of 100 ml. This dilution is named as 10^{-1}. From this 10^{-1} bacterial suspension,1 ml is taken and is added to 99 ml of sterile distilled water and is made to 10^{-2}. In this same way a serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} were made. Finally, 1 ml of aliquot of various dilutions (from 10^{-4} to 10^{-7}) are added to sterile petri dishes (triplicate for each dilution) to which are added 15 ml of sterile, cooled, molten (45^0 C) nutrient agar. After solidification the plates are incubated in an inverted position for 48 hrs to 72 hrs at 25^0 C. The number of colonies appearing on the diluted plates are counted, averaged and multiplied by the dilution factor to find out the total number of bacterial cells of the sample.

The following formula is used to count the total bacterial population.

Total number of bacterial cells counted = Number (average of 3 replicates of colonies) × Dilution factor

Membrane filter method (MFC) was followed for calculating the total population of individual strains of bacteria present among the total population of all the microorganisms.

Gram's staining: The different bacterial cultures (16 to 18 hrs.) were smeared on a clean glass slide and heat fixed. The smears were flooded with crystal violet for a minute and the stain was washed off using distilled water. The smears were flooded with Gram's stain iodine solution (fixative) for a minute and rinsed with distilled water, decolorized with acetone alcohol and rinsed out and the smears were counter stained with saffranin, air dried and examined under the oil immersion objective. Gram positive bacteria were purple or violet and Gram negative bacteria were red when observed for the respective isolates.

ONPG (o-nitro phenyl β – D - galactoside): One ONPG disc (6 mm) was placed in a sterile test tube. 0.1 ml of sterile 0.85% w/v sodium chloride solution was added (physiological saline). The colony was picked up under test with a sterile loop and emulsified in physiological saline in the tube containing the disc. It was incubated at 35-37°C. The active lactose fermenters were detected by observing the tube one hour, for up to 6 hours. The lactose fermenters were detected after incubating the tubes for upto 24 hours. MR-VP test: MRVP broth tubes were taken for bacterial culture and as control (two tubes as culture tubes and two as control). The culture was inoculated into 2 tubes and was incubated at 35^oC for 48 hours. Five drops of methyl red indicator was added into the tubes. The change in colour of methyl red test was observed. In another two tubes ten drops of VP-I reagent and 2-3 drops of VP-II reagents were added. The tubes were gently shaken and the caps were removed and waited for 15-30 minutes to complete the

reaction. Now the change in colour of the tubes was observed.

Glucose Lactose test: Three tubes containing glucose, lactose broth with 0.5% sugar along with sufficient amount of beef extract and peptone, were taken and the pH indicator phenol red for acid detection was added. Then the Durham tube was put in each tube, and the bacterial culture was inoculated and incubated at 37^{o}C for 24 hours.

Catalase test: The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid liberation of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

Coagulase test: Coagulase is an enzyme that clots blood plasma. This test is performed on Gram- positive, catalase positive species to identify the coagulase positive *S. aureus.* The culture was inoculated into a culture tube containing citrate plasma. The culture tube was incubated at room temperature for 24 hours. After incubation period the formation of clots indicated the bacterial culture as coagulase positive.

Starch hydrolysis: This test is used to differentiate bacteria based on their ability to hydrolyze starch with the enzyme a-amylase or oligo-l,6-glucosidase. It aids in the differentiation of species from the genera *Corynebacterium, Clostridium, Bacillus, Bacteroides, Fusobacterium*, and members of *Enterococcus.*

Gelatin hydrolysis: The presence of gelatinases is detected using a nutrient gelatin medium. This medium is a simple medium composed of gelatin, peptone and beef extract. When nutrient gelatin tube is stab-inoculated with a gelatinase positive organisms, the secreted gelatinases will liquefy the gelatin, resulting in the liquefaction of the medium. But the gelatinase negative organisms do not secrete enzymes and do not liquefy the medium.

Triple Sugar Ion test: Triple sugar iron agar test is used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentatively and produce hydrogen sulfide (H2S). It contains 10 parts of lactose: 10 parts of sucrose: 1 part of glucose and peptone. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H2S production respectively.

Oxidase test: Fresh growth is removed from the agar plate using a non-metallic instrument such as a sterile plastic inoculating loop or a sterile swab or wooden splint. The oxidase test strip is moistened slightly with sterile water and the growth is rubbed into the moistened paper of the strip. If the microbe has cytochrome oxidase, it will add electrons to the reagent, changing it from its colorless appearance to a deep indigo blue in a matter of 10-20 seconds.

Urease test: Using a sterile technique, each experimental organism is inoculated into its appropriately labeled tube by means of loop inoculation. Cultures were incubated 24-48 hours at 37°C.

Citrate utilization: Simmons citrate agar is inoculated lightly on the slant by touching the tip of a needle to a colony that has 18 to 24 hours old. This was incubated at 35^{o}C to 37^{o}C for 18 to 24 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium. The development of blue color was observed.

Membrane filter technique: The membrane filter (MF) technique is fully accepted and approved as a procedure for monitoring drinking water microbial quality in many countries. This method consists of filtering a water sample on a sterile filter with a 0.45-mm pore size, which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter. Many media and the incubation conditions for the MF method have been tested for optimal recovery of coliforms from water samples (Grabow and Du Preez, 1979). The most widely used medium for drinking water analysis are the m-Endo type medium in North America (APHA, 1998). Other media, such as MacConkey agar and the Teepol medium have been used in South Africa and Britain. However, comparisons among the media have shown that m-Endo agar yielded higher counts than MacConkey or Teepol agar (Grabow and Du Preez, 1979). The filters were incubated on an enriched lactose medium (m-FC) at a temperature of 44.5 °C for 24 h to enumerate FC (APHA, 1998).

RESULTS AND DISCUSSION

Total microbial population

The total population of microbes assayed in Achunthanvayal, well water was 47×10^{-2} (4,700). But the load of microbial population estimated in the drinking water ponds of Karendal and Thiruppalaikudi showed as 16×10^{-3} (16,000) and 13×10^{-3} (13,000). In the tap water (Cauvery water) the total microbial population was calculated as 12×10^{-2} (1,200/ml) and the total number of clinically important microbes such as *P. aeruginosa* was calculated as 12/100 ml and *E. coli* as 10 /s100 ml using membrane filter method (Table 1).

Total population of clinically important microbes

The total number of clinically important microbial pathogens estimated in the Karendal drinking water pond revealed the presence of *Serratia marcescens.* This bacterial population was calculated as 52/100 ml of water. But in the Thiruppalaikudi pond water, *Serratia marcescens,* and *Vibrio cholera* were identified and estimated as 32/100ml and 10/100 ml. But in the tap water the clinically important pathogenic microbes were

identified as *E. coli* and *P. aeruginosa*. The *E. coli* was estimated 10/100 ml and *P. aeruginosa* was estimated 12/100 ml (Table 2).

Biochemical assay

Biochemical characterization is very important to confirm the particular strain of bacteria present in the water sample. The particular colonies of bacterial isolates were treated with the universal biochemical tests such as, Gram's staining, Catalase, Indole, MR-VP, Citrate, ONPG, Gelatin hydrolysis, lipid hydrolysis, Urease, Oxidase and HF test. The positive and negative reaction of the microbes were studied and the results were tabulated (Table 3).

This investigation was carried over to find out the load of microbial pollution of certain drinking water samples collected from in and around Ramanathapuram district. No life without water is a common saying, as water is the essential requirement of all life supporting activities. Water can be obtained from a number of sources, among which are streams, lakes, ponds, rivers, springs, well and taps (Okohto *et al.,* 2008).

Load of total microbial pollution

The water samples collected from Thiruppalaikudi drinking water pond was having higher microbial population than the Karendal pond. The improper maintenance of drainage system and the mixing of sewage water cause the increasing number of microbial population in the Thirupallaikudi pond water than the Karendal pond water. Since the Karendal is a village area the mixing of sewage pollution is very much limited.

Assessment of clinically important bacteria in the drinking water samples revealed the presence of many pathogenic microbes in all the water samples. Most water borne diseases are related to faecal pollution. Therefore, water microbiology is largely based on the need to identify indicators of faecal pollution such as coliform and *E. coli* bacteria.

The total number of *E. coli* was calculated as 7/100 ml of water by membrane filtration method. This level of pollution is higher than the standard value prescribed by WHO. Public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic bacteria.

This investigation of Achunthanvayal pond water showed the presence of *Pseudomonas fluorescence* only. This microbe is a rod shaped, aerobic and non- lactose fermenting and gram negative bacterium. It can survive and replicate in most water reservoirs and as a result nosocomial outbreaks often lead to the investigation of water resources. This pond water is highly polluted with this strain of bacteria. It was measured as 61/100 ml of water. Because *Pseudomonads* constitute the main part of these both naturally occurring and contaminating bacteria as well as their role in opportunistic infections, the assessment of the health risk from these organisms after drinking continues to be a high interest for both of microbiologists and health workers. Therefore, as for *P. fluorescence* and *P. aeruginosa*, their occurrence in drinking water is considered as quality indicator. This investigation was carried over to find out the load of microbial pollution of certain drinking water samples

Table 1. Total microbial population estimated in the drinking water samples collected from the ponds and tap of different areas of Ramanathapuram district.

S. No.	Source of drinking water sample collected	Total population	
		Dilution factor	Total population of microbes
1	Karendhal (Pond water)	10-3 ×16	16000
2	Thirupallaikudi (Pond water)	10-3 ×13	13000
3	Tap water (Cauvery water)	10-2 ×12	1200

Table 2. Total population of clinically important pathogenic microbes estimated in different drinking water samples of pond and tap water.

S. No.	Source of drinking water sample collected	Type of pathogenic microbes	Total number of pathogenic microbes in 100 ml of water
1	Karendhal (Pond water)	*Serratia marcescens*	52
2	Thirupallaikudi (Pond water)	*Serratia marcescens*	10
		Vibrio cholera	32
3	Tape water (Cuavery water)	*Pseudomonas fluorescence*	12
		Escherichia. coli	10

Table 3. Total population of clinically important pathogenic microbes estimated in different drinking water samples of pond and tap water.

S. No.	Source of drinking water sample collected	Name of the pathogens	Biochemical tests											
			Gram's staining	Catalase	MR	VP	Coagulase	Citrate	Oxidase	Indole	Urease	Gelatin hydrolysis	Starch	Oxidase
1	Karendhal	*S. marscescens*	-ve	+	-	+	-	+	-	-	-	+	-	-
2	Thirupallakudi	*S. marscescens*	-ve	+	-	+	-	+	-	-	-	+	-	-
		V. cholerae	-ve	+	-	+	-	+	-	-	-	+	+	-
3	Tap water	*E. coli*	-ve	+	+	-	-	-	-	-	-	-	-	+
		P. aeruginosa	-ve	+	-	-	-	+	-	+	-	-	-	-

CONCLUSION

Drinking water has been suggested as an important source of human infections caused by the members of *Pseudomonas* spp. By taking into account that there is much local supplied drinking water in this area, and there is no documented and reliable information on the microbial quality of this drinking water and consequently their hygienic safety for consumptions.

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