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Research Article

ASSESSMENT OF THE LOAD OF MICROBIAL POLLUTION IN CERTAIN DRINKING WELL WATER SAMPLES COLLECTED FROM THE VARIOUS PLACES OF RAMANATHAPURAM DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The drinking well water sources of Pattinamkathan, Mandapam Camp and Achunthanvayal area of Ramanathapuram District were collected to assess the microbial pollution. This investigation reveals that the drinking well water of Mandapam Camp is polluted with many clinically important pathogenic microbes such as *Escherichia coli, Salmonella typhi, Streptococcus, Shigella, Klebsiella pneumonia* and *Enterococcus aerogenes*. This well water is highly polluted with the residues of sewage water through percolation of drainage water into the ground water.

Keywords: Microbial pollution, Well water, Sewage residues, Percolation.

INTRODUCTION

Water is a vital natural resource which is essential for our survival. Water is also used as a reaction medium, a solvent, a scrubbing medium and a heat transfer agent. As an important source of life for man, plants and other forms, it cannot be replaced. No known organism can live without it. Our drinking water today is far from being pure, contains bacteria, viruses, inorganic minerals (making the water hard) and a chemical cocktail that is unsuitable (if not deadly) for human consumption. The particular group in this regard is the coliforms which are considered as a warning signal and the water is subject to potentially dangerous pollution even with single coliform bacterium. Even the water used for washing and animal drinking purpose should not contain more than 50 coliform bacteria (Central Pollution Control Board, 2002).

Physico-chemical parameters were studied an important criterion for evaluating the suitability of water for irrigation and drinking purposes. The water samples were analyzed for Bacteriological Screening, temperature, pH, electrical conductivity, alkalinity, salinity, phosphate, hardness, dissolved oxygen and biological oxygen demand. The zooplankton population shows positive significant correlation with higher value of physico-chemical parameters like, temperature, alkalinity, phosphate, hardness and biological oxygen demand, whereas negatively correlated with rainfall and salinity. Bacterial isolates were isolated from all studied ponds and they were identified as *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aerugionosa, Enterobacter aerogenes*, *Bacillus* sp., *Proteus* sp., *Klebsiella pneumonia, Flavobacterium* sp., *Acinetobacter* sp. (Kumar *et al.*, 2011).

Urban and suburban areas are dominated by impervious cover. During storms, rainwater flows across these impervious surfaces, by mobilizing contaminants. The pollutants carried in runoff originate from a variety of urban and suburban nonpoint sources. Contaminants commonly found in storm water runoff include fecal and pathogenic microbes (United States Environmental Protection Agency, 2009).

The presence of pathogenic bacteria such as *E. coli, K. pneumoniae, S. typhi, Shigella dysenteriae, S. aureus,* Group D *Streptococcus, Vibrio cholerae* and *V. parahaemolytics* indicated that the water is not potable (Shittu *et al.*, 2008).

The recent history of waterborne outbreaks in the UK and US has highlighted the role of pathogens that are less susceptible to chlorination than most bacterial pathogens. In particular, many outbreaks of cryptosporidiosis occur in water supplies that have not failed coliform testing (Hunter, 1997). Several prospective studies have found a small but statistically significant increase in gastrointestinal illness in populations who drink water that apparently complies with the coliform standard (Payment *et al.*, 1991).

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Microbiological examination of water samples is usually undertaken to ensure that the water is safe to drink or bathe in. Many potential pathogens could be associated with water; it is thus impractical to screen samples for all possible pathogens. Instead, various indicator organisms have been used as surrogate markers of risk. Most waterborne disease is related to faecal pollution of water sources, therefore water microbiology is largely based on the need to identify indicators of faecal pollution such as coliforms and *E. coli*, but the use of enterococci and *Clostridium perfringens* is increasing (Hunter, 1997). Methods routinely used in water quality testing laboratories include; membrane filtration (MF) (Eckner 1998); and defined substrate technology (DST) systems (Mc Feters *et al.,* 1993).

In this juncture it is very important to protect and conserve the surface and ground water from microbial contamination for the human consumption. Hence it is the duty of the environmentalists to involve their research approaches towards the evaluation of drinking water resources for the betterment of human society. In order to preserve the aesthetic values of natural drinking water resources, conservation and management are very important. The successful microbial pollution abatement depends not only on treatment and control but also on efficient monitoring of drinking water resources. For this, research approaches are very important for providing potable and safe drinking water to the humanity. The aim of the present study was to analyze and monitor the microbial pollution of drinking well water.

MATERIALS AND METHODS

Study area

The study was conducted in drinking well water sources of Pattinamkathan, Mandapam Camp and Achunthanvayal area of Ramanathapuram District.

Pattinamkathan: This drinking water source from the well of Pattinamkathan area was collected for the assay to assess the load of microbial pollution.

Mandapam: This drinking water sample was collected from the well situated in Mandapam Camp which is 25 kms away from Ramanathapuram.

Achunthanvayal: Another drinking water source from the well of Achunthanvayal area was also collected for the assay to assess the load of microbial pollution.

Collection of water samples

Water samples from the drinking water sources were collected (as per the procedure given in the text book-Chakraborthy) 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.

Determination of total microbial population

Serial dilution agar plating method was used for the isolation and enumeration of microbial organisms present in well water sample (Dubey *et al.,* 2014). 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 upto 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 (Duby *et al.,* 2012).

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 elaboration 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 (H_2S). 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 H_2S 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 DISCUSSIONS

The assay of total microbial population in Pattinamkathan well water showed as 12×10^{-4} (12,0000). But in the well water collected from Mandapam Camp showed a higher population of microbes than the other drinking water samples collected from various parts of Ramanathapuram (Table 1).

The disinfection treatment of drinking water has important role in reducing the water borne epidemics. The contamination of clinically important microbes (such as *E. coli, Klebsiella, Salmonella, Shigella, Pseudomonas*) and the inadequate disinfection treatment of drinking waters result in the provision of unsafe drinking water to the people which can pose a great threat and risk of water borne epidemics by clinically important bacterial pathogens to the population consuming it. Such negligence may result in a catastrophic disaster.

In this investigation the total number of clinically important microbes, present in the Pattinamkathan well water showed *E. coli* bacteria as 7/100 ml of water sample and *S. auerus* as 28/100 ml. But in the well water collected from Mandapam Camp revealed the presence of many pathogenic bacteria. The assay of this drinking water showed the presence *of E. coli, E. aerogens, S. typhi, K.*

pneumonia, *Shigella* and *Streptococcus.* The total population of these above pathogens was calculated as 52/100 ml, 65/100 ml, 1/100 ml, 19/100 ml, and 12/100 ml of water respectively. In the Achunthenvayal well water the pathogenic microbe was identified as *P. fluorescence*. The number of *P. fluorescence* was calculated as 61/100 ml (Table 2).

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, Coagulase, Citrate, Starch hydrolysis, ONPG, Gelatin hydrolysis. TSI, Urease, Oxidase. The positive and negative reaction of the microbes was studied and the results were tabulated (Table 3).

Drinking water samples were collected from the wells of Pattinamkathan and Mandapam Camp. The load of total microbial population showed higher in Mandapam well water than the Pattinamkathan. But in the well water of Pattinamkathan the load of microbial population is lesser than the Mandapam well water. The increasing population of microbes in Mandapam well water may be due to the mixing and percolation of sewage water and also the water released from fish residues. The well water of Achunthanvayal showed a lesser load of microbial pollution than these two well waters. Here the mixing of sewage pollution is lesser than the above two areas.

In this investigation, the well water of Pattinamkathan also showed the presence of *Staphylococcus aureus.* The total number of these pathogenic microbes were 27/100 ml of water. This result shows that the well water is highly polluted with this bacterial population.

Staphylococcus aureus was enumerated by membrane filtration method. The total number of *S. aureus* was calculated as 28/100ml in the well water of pattinamkathan. The colonies of *S. aureus* were inoculated into coagulase plasma and the coagulase reaction was interpreted according to the method of Sperber and Tatini. Coagulase positive bacteria were further identified as *S. aureus* by oxidase test, catalse test, gram staining and morphology. The ability of the bacteria to ferment glucose and mannitol anaerobically was determined by using the media and procedures recommended by the subcommittee on taxonomy of Staphylococci and Micrococci (1965).

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

S. No.	Source of drinking water sample collected	Total population	
		Dilution factor	Total population of microbes
1	Pattinamkathan (Well water)	10^{-4} ×12	120000
2	Mandapam Camp (Well water)	10^{-5} ×2	200000
3	Achunthanvayal (Well water)	10^{-2} ×47	4700

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

S.No.	Source of drinking water sample collected	Type of pathogenic microbes	Total number of pathogenic microbes in 100 ml of water
1	Pattinamkathan (Well water)	*Escherichia coli*	7
		Staphylococcus aureus	28
2	Mandapam Camp (Well water)	*Escherichia. coli*	52
		Salmonella typhi	1
		Streptococcus aureus	12
		Shigella dysenteriae	19
		Enterococcus aerogenes	65
		Klebsiella pneumoniae	1
3	Atchunthanvayal (Well water)	*Pseudomonas fluorescence*	61

Table 3. Biochemical characterization of clinically important pathogenic microbes isolated from different drinking water samples of well water.

	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	TSI	Urease	ONPG
1	Pattinamkathan	*E. coli*	+ve	+	+	-	-	-	+	-	-	+	-	+	-	-	+
		S. aureus	-ve	+	-	+	+	+	-	+	-	-	+	-	+	-	-
2	Mandapam Camp	*E.coli*	-ve	+	+	-	-	-	+	-	-	+	-	+	-	-	+
		E. aerogenes	+ve	+	-	-	-	-	-	-	+	-	-	-	-	+	-
		K. pneumonia	-ve	+	-	+	-	+	-	-	+	+	-	-	-	+	+
		S. typhi	-ve	+	+	-	-	-	-	-	-	+	-	-	-	-	+
		Shigella	-ve	+	+	-	-	-	-	-	-	+	-	-	-	-	+
		Streptococcus	+ve	+	+	-	-	-	-	-	-	+	+	-	-	-	+
3	Atchunthanvayal	*P. fluorescence*	-ve	+	+	-	-	+	+	+	-	-s	-	+	+	-	-

Note: (+), Positive. (-), Negative.

The presence of *Staphylococcus aureus* in the well water showed the well water is highly contaminated. This is due to improper placement of wells, lack of sanitary seals proximity of grazing of animals to the wells and lack of knowledge of the significance of contaminated water were all found to be factors contributing to the poor quality water supply. This investigation also demonstrated the importance of education for the consumers who use individual supplies for their drinking water. It is also suggested that more frequent monitoring was helpful in detecting contaminated water supplies which otherwise would have gone unsuspected particularly during and after rainfall periods. Consumers need to know that testing water upon installation of a well is an inadequate measure of potability of a water supply.

In the well water of Mandapam camp numerous pathogenic microbes were identified. Among the clinically important bacteria, *Enterobacter aerogenes* were higher in number. Next comes *E. coli*. The same well water was also polluted with *Salmonella typhi*, *Klebsiella pneumonia*, *Shigella* a *Streptococcus. Escherichia coli* is a gram negative, aerobic or facultative anaerobic organisms. This microbes have the important features of being highly specific for the faeces of man and warm blooded animals. For all practical purposes these bacteria cannot multiply in natural water environment and they are therefore used as specific indicator for faecal pollution of this drinking water. This microbe can be detectable by simple and inexpensive tests and widely used in water quality monitoring. Generally the four genera *Escherichia, K, enterobacter* and *Citrobacter* are considered as total coliform population. Estimation of the population of these four genera present in the water samples has been universally applied to document the sanitary quality of drinking water. These faecal indicator bacteria including total and faecal coliform and Enterococci have been used all over the world as a monitoring tool for microbiological impairment of water.

In the same well water the presence of *Salmonella typhi* was also detected. Isolation of pathogenic organisms specifically *Salmonella typhi* in conjunction with the coliforms group enhances the importance and validity of the coliform test as an indicator of faecal pollution

detrimental to health. The total number of *Salmonella typhi* was calculated as 1/100 ml of the water sample. *Salmonella* a very large group of rod shaped gram negative bacteria comprising more than 2000 known serotypes that are members of the family Enterobacteriaceae. These bacteria are virulent to humans and can cause a variety of symptoms from mild gastroenteritis to severe disease or death. *S. typhi* is a typical human pathogen. These microbes present in the drinking water may cause septicemia with high temperature without diarrhea, a condition known as enteric fever.

CONCLUSION

The study revealed that the load of microbial population in well water samples should be subjected for regular monitoring and purification of well water to ensure the drinking water quality.

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