PHYSICAL AND BACTERIOLOGICAL QUALITY OF WELL WATER SAMPLES FROM KANAKKARY PANCHAYATH, KOTTAYAM DISTRICT, KERALA STATE, INDIA

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ABSTRACT: Water is one of the nature's most important gifts to mankind. Groundwater supposed to be the safest source of water can also be a carrier of many diseases. Its quality depends on the goodness of recharged water, atmospheric precipitation, inland surface water and sub-surface geochemical processes. In the present study, a comparative analysis were carried out on the physical and bacteriological quality of well water samples collected from ten different locations of Kanakkary Panchayath, Kottayam district, Kerala state. The pH of water samples collected ranged from 5.24 to 7.13. The results showed that the MPN values of samples collected from five areas exceeds the World Health Organization (WHO) standards and these when subjected to confirmatory and biochemical tests showed that *Escherichia coli* was present only in one sample. Out of ten well water samples, three samples showed the presence of *Salmonella typhi* and six showed the presence of *Vibrio cholerae*. The Biological Oxygen Demand (BOD) values of all samples except 2 exceed the WHO standards whereas the Total Dissolved Solids (TDS) values were all as per the standards.

Keywords: *Salmonella typhi*, *Vibrio cholerae*, Coliforms, Most Probable Number, Heterophilic Plate Count, Total Dissolved Solids.

INTRODUCTION

Increased human population and increased demands on water, pose a risk in maintaining acceptable water quality. Government agencies oversee environmental management to maintain water quality, assure the public health and preserve the environment [1]. Groundwater represents an important source of drinking water and its quality is currently threatened by a combination of over-abstraction and microbiological and chemical contamination [2]. Change in the origin and constitution of the recharged water, hydrologic and human factors may cause periodic changes in ground water quality. Depending on the source, raw water may contain a wide variety of harmless heterotrophic microorganisms such as *Flavobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., *Chromobacterium*, *Achromobacter* spp. and *Alcaligenes* spp., as well as numerous unidentified or unidentifiable bacteria [3].

Disease-causing microorganisms transmitted via drinking water are predominantly of faecal origin and are referred to as enteric pathogens [4, 5]. The World Health Organization (WHO) estimates that about 1.1 billion people globally drink unsafe water [6] and the vast majority of diarrhoeal disease in the world (88%) is attributable to unsafe water, sanitation and hygiene [7]. Poor water quality, sanitation and hygiene accounts for 1.7 million deaths a year worldwide (3.1% of annual deaths) and 3.7% of the [8] annual health burden (disability adjusted life years [DALYs]) world-wide (54.2 million) mainly through infectious diarrhea and nine out of ten such deaths are in children and virtually all of the deaths are in developing countries.

As per World Health Organization [9] standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. The concept of faecal indicator bacteria (FIB) in determining the sanitary quality of water was first proposed in the 1880's when workers began to use bacteriologic media to assess microbial presence in water and food commodities [4].

Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment and it is not possible to examine water for every possible pathogen that might be present.

Coliforms are the major microbial indicators of monitoring water quality [10]. Coliform bacteria are a natural part of the microbiology of the intestinal tract of warm-blooded mammals including man can be found in their wastes. Blachstein coined the term "coliform" in 1893 to include bacteria resembling *E. coli* that are present in faeces and fit the description above [11]. Total coliform and faecal coliform counts are the most widely used bacteriological procedures for assessment of the quality of drinking and surface waters [12]. Total coliforms are classically defined as "All facultative anaerobic, gram-negative, non spore-forming, Oxidase-negative, rod-shaped bacteria that ferment lactose to acid and gas within 48 h at 35ºC or members of *Enterobacteriaceae* which are *β*-galactosidase positive [13]. Furthermore, the efficacy of drinking water treatment (traditionally by filtration and chlorination) to remove the bacterial pathogens responsible for cholera (*Vibrio cholerae*) and typhoid fevers (*Salmonella typhi* and *S. paratyphi*), is well indexed by the common faecal indicator bacterium *Escherichia coli* (*E. coli*), which is excreted in the faeces of all warm-blooded animals and some reptiles [14].

The introduction of water treatment plants and various disinfection processes and frequent bacteriological analysis of water quality ensured the delivery of safe drinking water and this have drastically reduced the occurrence of water borne illness. The occasional occurrence of waterborne disease outbreaks, however, points out the continuing importance of strict supervision and control over the quality of public and private water supplies. In the present study a physical and bacteriological analysis was carried out for the well water samples from ten different locations of Kanakkary Panchayath, Kottayam district, Kerala.

MATERIALS AND METHODS
Collection of samples
Drinking water samples were collected from ten wells in Kanakkary panchayath during December 2011. The sites were Pattithanam, Retnagiri, Hospital junction, Cheruvil junction, Sankaramukk, Muthirakala, Manalel junction, Chumaduthangi, Parappuram, Kanakkary of Kottayam district, Kerala State, India. Water samples were drawn in sterile 500 ml Erlenmeyer flasks at normal atmospheric temperature and transported to the lab as per the standard methods as mentioned in APHA [13]. Wells were the sources of ground water.

Physicochemical parameters
The pH and total dissolved solids were determined as described by APHA [15]. The Biological Oxygen Demand (BOD) was also determined using the Winklers titration method as described by APHA [15, 16].

BACTERIOLOGICAL ANALYSIS
Heterophilic plate count
Heterophilic plate count serves as an indication of general microbial population. Samples to be analyzed for quantitative bacterial analysis were plated on glucose tryptone agar [13] and the plates were then incubated at 37^0C for 24 hours and the total plate count was performed.

Multiple-Tube Fermentation Test or Most Probable Number (MPN)
Most probable number test is the most often used technique for the sanitary analysis of water for the detection of coliforms. This was carried out by multiple tube fermentation technique [13] for members of the coliform group. Coliforms were detected by presumptive inoculation into tubes of Lactose broth and their incubation at 37^0C for 24 hours. The positive tubes were sub cultured into Eosin methylene Blue (EMB) agar for confirmation. MPN of coliforms were found in terms of index/100 ml by using standard tubes.

### Detection of *Salmonella typhi* and *Vibrio cholerae* from water samples
For the isolation of *Salmonella typhi*, 1 ml of water sample was inoculated into 10 ml of Selenite enrichment broth and incubated at $37^{\circ}C$ for 12-18 hours and swabs from the Selenite broth was spread over Bismuth Sulfite (BS) Agar which is widely used for isolation of *S. typhi* and other *Salmonella sp*. from food, faeces, urine, sewage, and other infectious materials. Before plating into nutrient medium water samples suspected to contain *Vibrio cholerae* were enriched by adding 10 ml water sample in 100 ml of double strength alkaline peptone water (pH 8.6) at 37ºC for 24 hours and swabs from the alkaline peptone water was streaked on selective plating media, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar and further incubated at 37ºC for 24 - 48 hours.

BIOCHEMICAL ANALYSIS

The biochemical characterization of bacteria was determined by IMViC test [17].

RESULTS AND DISCUSSION

The odour and taste of ten well water samples from the different locations were unobjectionable and agreeable. The pH of well water samples were in the range of 5.24 -7.13. The values of five samples like those from Hospital junction, Cheruvil junction, Sankaramukku, Muthirakal and Manalel were not as per the accepted Bureau of Indian Standards [18]. This may be due to the elevated levels of metal ions in the water which could be overcome by the application of a neutralizer like soda ash. The pH values of the samples are given in (Fig 1).
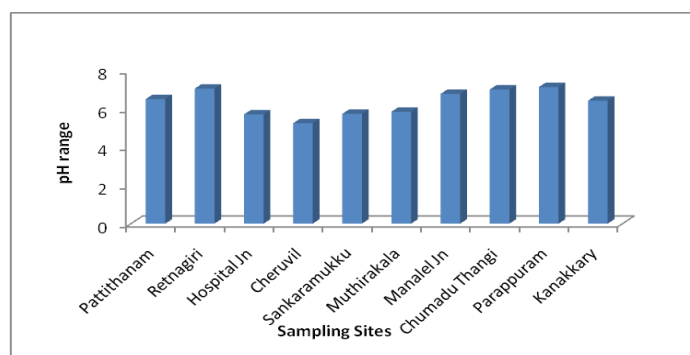
Fig 1: pH of well water sample

The heterophilic plate count or standard plate count of four samples like those from Pattithanam, Retnagiri, Kanakkary and Parappuram were above WHO standards and are unfit for human consumption. The lowest HPC count was observed in Cheruvil (7 CFU/ml) and the highest was observed in Parappuram (423 CFU/ml). The corresponding HPC values of the samples are given in (table 2). A water sample containing less than 100 microorganisms per milliliter is considered to be of good quality and is used for drinking purposes. HPC can provide an indication of the level of the general population in the system and is considered as a good general indicator of overall water quality [19].

Heterotrophic microorganisms include both members of the natural (typically nonhazardous) microbial flora of water environments and organisms present in a range of pollution sources. They occur in large numbers in raw water sources. The actual organisms detected by HPC tests vary widely between locations and between consecutive samples [20].

Table 1: Parameters of water quality

Sampling sites	Odour	Taste	Temperature (oC)	BOD (mg/l)	DO (mg/l)	TDS (mg/l)
Pattithanam	Unobjectionable	Agreeable	27.5	4.4	8.4	0.02
Retnagiri	Unobjectionable	Agreeable	27	4.6	8.2	0.02
Hospital junction	Unobjectionable	Agreeable	28	4.2	6.4	0.04
Cheruvil	Unobjectionable	Agreeable	27.6	4.8	8	0.01
Sankaramukku	Unobjectionable	Agreeable	27.4	2.8	7.2	0.01
Muthirakala	Unobjectionable	Agreeable	28	4.2	7.4	0.10
Manalel junction	Unobjectionable	Agreeable	27.7	0.8	4.8	0.15
Chumadu Thangi	Unobjectionable	Agreeable	27.6	4	8.8	0.07
Parappuram	Unobjectionable	Agreeable	27.8	3.8	9	0.21
Kanakkary	Unobjectionable	Agreeable	27.5	0.2	7	0.04

The MPN values of all the water samples were found out using multiple tube tests and out of the ten samples tested, five samples like those from Retnagiri, Manalel, Hospital junction, Sankaramukku, and Parappuram exceed the WHO standards and this may be due to the mixing of faecal matter with the waterways and also attributed to improper disinfection. The water sample from Cheruvil showed the least MPN value of 7 and this water is safe for drinking. This may be due to the efficiency of chlorination. According to the Central Pollution Control Board, India, the total number of coliforms shall not exceed 50/100ml in untreated or non-disinfected drinking water. The highest MPN was observed in the samples collected from Pattithanan and Parappuram (Fig 2).
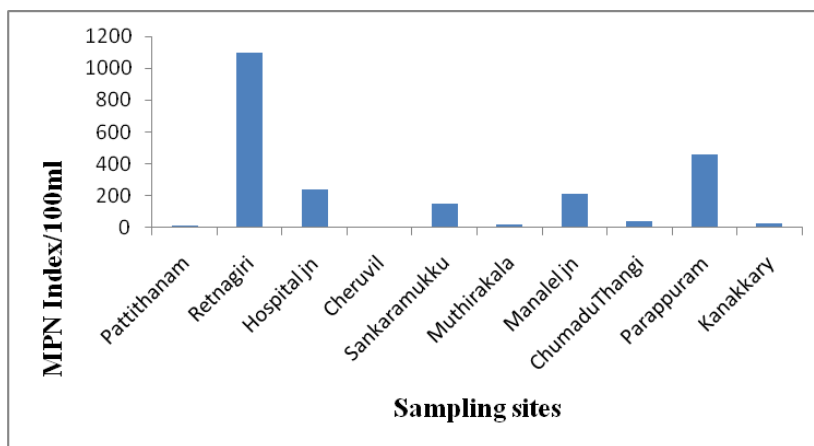

Fig 2: MPN of well water sample

The cultivation and enumeration of coliform indicator bacteria (e.g., total coliforms, fecal coliforms, and *Escherichia coli* (E. coli) remains the primary method for testing the biologic quality of fresh waters for drinking, recreation, fishing, and industry [21]. The sample positive for MPN tubes plated on Eosin Methylene Blue (EMB) agar showed a green metallic sheen, which confirmed the presence of *E. coli*. Fresh samples spread on BS agar and TCBS agar showed brown and yellow colonies respectively this clearly indicated presence of *S. typhi* and *V. cholerae*. *E. coli* was present only in one sample collected from Sankaramukku. *S. typhi* was present in six water samples and *V. cholerae* was present in three samples (table 2). And all the other samples were negative for *E. coli*.

Table 2: Microbiological parameters

Sampling sites	Heterophilic plate count (CFU/ml)	Presence (+) or absence (-) of		
		E. coli	*V. cholerae*	*S. typhi*
Pattithanam	371	-	-	-
Retnagiri	123	-	+	+
Hospital junction	38	-	-	+
Cheruvil	7	-	-	-
Sankaramukku	31	+	-	+
Muthirakala	40	-	-	+
Manalel junction	65	-	-	+
ChumaduThangi	20	-	-	-
Parappuram	423	-	+	+
Kanakkary	104	-	+	-

The IMViC test was done to differentiate the Gram-negative bacteria of the family Enterobacteriaceae. The result showed a positive indole test, methyl red test, a negative Voges-proskauer test and a negative citric acid test which confirms the presence of *E. coli*. BOD indicates the concentration of biodegradable organic matter in water. The measurement of BOD involves the measurement of dissolved oxygen of the sample immediately after collection. BOD values of all samples except 2 samples, those from Retnagiri and Cheruvil exceed the WHO standards and this may be due to the mixing of faecal matter with the waterways. The water sample from Kanakkary showed the least BOD value of 0.2 mg/L. Unpolluted waters typically have BOD values of 2 mg/l or less, whereas water bodies receiving wastewater may have BOD values up to 10 mg/l or more, particularly near points of discharge. Total dissolved solids (TDS) are the term used to describe the inorganic salts and small amounts of organic matter present in solution in water. The principal constituents of total dissolved solids are usually calcium, magnesium, sodium, and potassium cations and carbonate, hydrogen carbonate, chloride, sulfate, and nitrate anions and their presence also increase TDS. The TDS of all samples were as per the WHO standards.

The Dissolved Oxygen of well water samples in this study were in the range of 6.4 to 9 mg/l. The lowest value of 6.40 mg/l was that from hospital junction while the highest value of 9 mg/l was that from Parappuram. Dissolved oxygen is used as an indicator of the health of a water body, where higher dissolved oxygen concentrations are correlated with high productivity and little pollution (13).

CONCLUSION

In this study, ten well water samples taken for the analysis of physical and bacteriological quality from Kanakkary Panchayath, Kottayam showed the presence of enteric pathogens. Almost all samples showed the signs of contamination either by the presence of microbes or organic matter. The presence of *E. coli*, *S. typhi* and *V. cholerae* in certain well water samples indicates the faecal contamination in ground water. Therefore it is necessary to take serious attention to kill these microbes either by boiling or by the use of disinfection methods before human consumption, to prevent water borne illnesses. Consistent and periodical examination of drinking water samples and disinfection process should be done periodically to prevent the spread of pathogenic microbes.

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