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Research Article

A Pilot Study on Microbial After growths in the Municipal Water Supply from Aluva to West Kochi

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Abstract: Pollution of potable water is a very serious issue which has received world wide attention. Occasionally, it has been learnt through the media that out break of epidemics occur in the west Kochi area and the same is reported to be due to the microbial contamination of drinking water. The present project is an attempt to analyze the material facts of the reports and also to understand how the supply system gets contaminated before it reaches west Kochi area. Hence the present study deals with the microbial load of the water from various sources of supply system from different sampling points between Aluva and West Kochi. In the present study the quality of drinking water from Municipal water supply was assessed by analyzing the samples for various microbial after growths. Eleven sampling points between Aluva and West Kochi were identified to collect water samples for the analysis. Samples were collected under aseptic conditions at an interval of two weeks during August and September. These samples were analyzed for pH, BOD, COD, TPC, *E.coli*, *Pseudomonas* and iron reducing bacteria. It was found that water in West Kochi area alone was mostly contaminated with *E.coli*. It was observed that the contamination is mainly due to the extensive corrosion of the pipelines at West Kochi area, which is thought to cause microbial aftergrowth. The pipes are laid close to the sewage canals which may contribute to the water contamination by the microorganisms due to back siphoning. So the concerned authorities should take necessary steps for the renewal of the pipes to face the challenges of outbreak of water borne epidemics. It is advisable to take boiled water for drinking purposes in this area.
Keywords: Municipal water supply, Aluva, West Kochi, Microbial contamination

INTRODUCTION

Water, the universal solvent, has a large number of dissolved salts in it which largely govern its physico-chemical properties. These properties in turn have an indirect effect on flora and fauna of the aquatic habitat. Water is the basic and primary need of all vital life processes. With increasing industrialization and population growth, water sources available for various purposes, such as drinking, recreation, aquaculture, agriculture have been polluted with industrial as well as animal and human waste. As a result, polluted water has a formidable factor in transmission of several diseases. Polluted (sewage) waters contain solids and dissolved organic compounds that import an offensive odor and serve as an excellent medium for the growth and multiplication of microorganisms [1].

Sewage consists of approximately 99.9 percent water, 0.02-0.03 percent suspended solids and other soluble organic and inorganic substances. Though the amount of solids appears small on a percentage basis, the tremendous volume of material handled everyday by a major municipal plant contains about 100 tons of solids. BOD (Biological Oxygen Demand) is used as a parameter to express the strength of sewage. The BOD is a way of expressing the amount of organic matter in sewage as measured by the volume of oxygen required by bacteria to metabolize it. If there is more dissolved organic matter in sewage, more oxygen will be utilized by bacteria to mineralize it, in other words, the BOD of the sewage will be higher [1].

Typical organisms found in different types of water belong to fungi, protozoa, algae, bacteria, actinomycetes and viruses. According to 1980 study of World Health Organisation (WHO) at least 30,000 people die every day in developing countries because of unsanitary water supplies [1].

The purity of drinking water is evaluated by testing for the presence of coliforms as evidence of faecal contamination. The coliform bacteria include *E.coli, streptococcus faecalis* etc. Of these *E.coli* is the most predominant coliform. The only natural source of coliforms is the intestines of humans and other mammals. Although these bacteria are usually non-

pathogenic when ingested by healthy people, their presence in water indicates faecal contamination and thus possible presence of water borne pathogens [1].

The underground water is the alternate source of fresh water and is comparatively free from pollution. However this source also has been polluted to a great extent because of indiscriminate disposal of chemical waste for land filling, whereby, along with rain water, the chemicals and contaminated waste penetrate through the soil and pollute the ground water resource [2].

Microbial After Growths

Source of organism

Not all bacteria in water are indigenous. Some are derived from soil and sewage and a proportion of these will survive the water treatment processes. Other organisms gain access from the treatment process themselves or at the time of assembly or repair of pipelines. Service resources and water towers may add further pollution either as a result of structural defects or simply through contact of the water with air and the walls of such structures [3].

Factors controlling numbers of bacteria in supply

The multiplication of organisms within the system will depend on many factors such as (a) level and type of inorganic and organic nutrients including growth factors (b) environmental factors such as temperature, redox potential, pH (c) whether an adequate level of residual disinfectant is maintained throughout the system [4].

The relationship between increase of temperature, decline of chlorine residue and microbial growth is already reported by Palin [5].

Source of energy

Three distinct sources of energy are available to bacteria in distribution systems: (a) Inorganic compounds (b) soluble organic matter (c) particulate or colloidal organic matter [6].

In water supply, energy sources for chemoautotrophs include ferrous iron, hydrogen and reduced sulphur compounds, all derived from the process of corrosion. It may be argued therefore that corrosion of iron pipelines is an essential driving force for many biological processes which occur in water supply. The source of organic carbon present inwater supplies is particulate, and include algae and variously flocculated organic materials which may pass through treatment or even arise within the distribution system itself by adsorption on hydrated ferric oxide floc formed in the pipelines as a result of corrosion [7].

At Kochi, the ground water does not form a source of fresh water. Due to the geological peculiarities, the ground water available is mostly saline and polluted by mud and silt. Therefore the only source of fresh water is the public water supply scheme. Kochi city has been mainly dependant on river water from the Periyar for various purposes like drinking, industries, agriculture and so on. The ecosystem of Periyar has been destroyed by industrial effluents, rain water run off through chemically fertilized and pest controlled agricultural fields, untreated municipal wastes, plant wastes etc. The water so polluted has been captivated in tanks, treated and improved to make it fit for consumption and supplied through the public water supply system. The water is chlorinated and the pH of the water has been maintained between 6.2 and 7.5, thereby controlling the microbial growth.

METHODOLOGY

Sites of Sampling

The Periyar river water undergoes the primary treatments including chlorination at Aluva, where the water is made useful for household and industrial purposes. From here water is distributed to entire kochi city. We were interested in the route leading to west Kochi as shown in the map (Fig. 1). Water is also treated at the following points before it reaches west Kochi (Fig.2), they are Perumanoor, Thoppumpady and Karuvelipady, which includes only chlorination. Chlorinated water from Karuvelipady is distributed to the west Kochi area (Mattancherry and Fort Kochi). At all these chlorinating points water is stored in huge tanks. We have collected samples from all these tanks and a public tap/ household tap which is supplied by each tank sampled.

Sampling Method

Water samples from storage tanks and public taps from the above places were collected in sterilized bottles and stored at 4°C to prevent further contamination. Two sets of eleven samples at an interval of 10 days was taken for the analysis of pH, BOD, COD and microbiological analysis of TPC, *E.coli*, *Pseudomonas*, IRB were carried out.

Microbial Analysis

Identification of Microorganisms

Identification of *E.coli*, *Pseudomonas* and IRB were done at the microbiology lab of The Cochin College. Bacteriological estimation (MNP) was conducted at the Quality Control Lab, Indo Cargo Surveyers, Mattancherry.

Fig. 1: Map showing the site of sampling, the route through which treated water from river Periyar reaches West Kochi (Starred region in the inset shows the location in the map of India

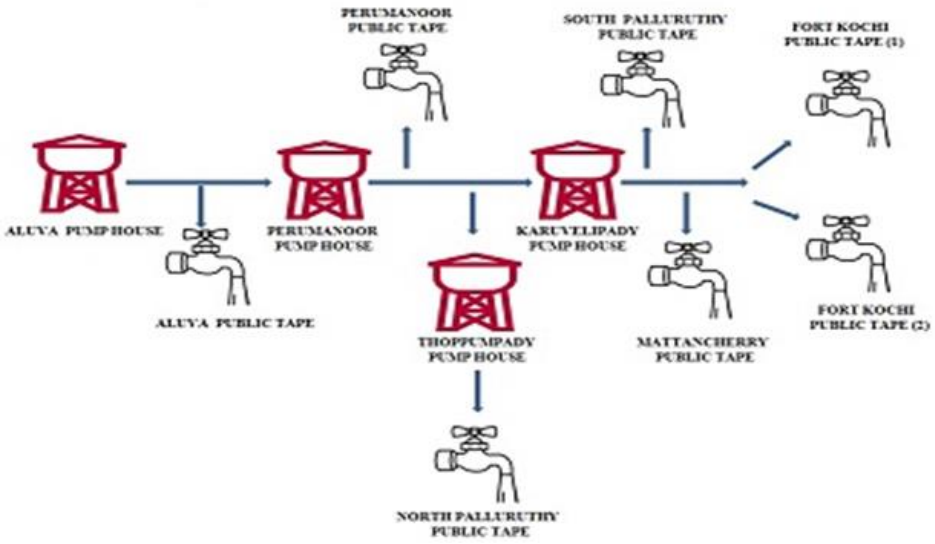

Fig. 2: Diagrammatic representation of sites selected for sampling

MATERIALS AND METHODS

Identification of bacteria was done by Gram staining procedure, motility by hanging drop method and cultural characteristics using specific selective media.

Gram Staining

- Prepare a smear on the slide with bacterial cultures as done for the sample staining method
- Stain for 1 minute with Crystal violet solution (Gram stain). Wash in tap water.
- Apply iodine solution for 1 minute, wash in tap water
- Decolorize with alcohol by adding it drop wise on the tilted slide until free of color. Wash in tap water
- Flood this slide with saffranin for 1 minute. Wash in tap water and air dry
- Examine the stained smear under the oil immersion objective to determine whether organism is gram negative or gram positive

Hanging drop method

Motility of the bacteria using concave slide technique

Cultural characteristics

The selective media like Mac Conkey agar, DCA and Iron oxidizing media were used to study the characteristics of the organisms.

Estimation of Microbial Population

Determination of pH

pH is determined using pH paper

Determination of Biochemical Oxygen Demand (BOD) of water

- Adjust the pH of the water sample to neutrality using 1 N acid or 1 N alkali solutions
- Fill the water sample in 6 BOD bottles without bubbling
- Add 1 ml of allylthiourea to each bottle
- Determine the dissolved oxygen content in three of the 6 BOD bottles by the titration method
- Take the mean of the three readings (D_1)
- Incubate the rest of the three BOD bottles at 27°C in a BOD incubator for 3 days
- Estimate the oxygen concentration in all the three incubated samples
- Take the mean of three readings (D_2)

Calculate the BOD of the water in mg/litre by applying the formula

BOD_3 in mg l^{-1} = D1 – D2.

Where D1 = initial DO in sample (mg l^{-1}), D2 = DO after 3 days incubation (mg l^{-1})

Determination of Chemical Oxygen Demand (COD) of water

- Take three 100ml conical flasks and pour 50ml of water sample in each (i.e. in triplicate)
- simultaneously run distilled water blank standards (also in triplicate)
- Add 5ml of $K_2Cr_2O_7$ solution in each of the six flasks
- Keep the flasks in water bath at 100°C (boiling temperature) for one hour
- Allow the samples to cool for 10 minutes
- Add 5ml of potassium iodide in each flask
- Add 10ml of H_2SO_4 in each flask
- Titrate the contents of each flask with 0.1M sodium thiosulfate until the appearance of pale yellow color
- Add 1ml of starch solution to each flask (solution turns blue)
- Titrate it again with 0.1M sodium thiosulfate until the blue colour disappears completely

Find out the COD (mg/l) per liter of the water sample by applying the formula:

COD of sample mg/liter = 8*C*(B-A)/S

Where C = Concentration of titrant (m Mol/liter), A = Volume of titrant used for blank (ml), B = Volume of titrant used for sample (ml), S = Volume of water sample taken (ml)

***E.coli* & Coliforms in Water (MPN method)**

Presumptive test for coliform bacteria: Arrange double strength (three 10ml tubes) and single strength (six 10ml tubes) and label appropriately as 10ml, 1ml and 0.1ml. Dispense 10ml of mixed sample aseptically to DS tubes, 1ml and 0.1ml tubes to triplicate of SS tubes. Incubate the tube at 37°C for 24 hours. Gas and acid producing tubes are recorded as positive.

Confirmed total coliforms

Subculture each positive tubes of presumptive test to BGLB tubes and incubate at 44.5±5°C for 24 hours. Tubes showing growth and gas production are recorded as positive. From this number the total coliform count is obtained from the 3 tube MPN table.

Faecal coliform & *E.coli*

From positive tubes of BGLB, inoculate one loopful each to EC broth and trytone broth. Label appropriately and incubate at 44.5±5°C for 24 hours. Number of tubes showing growth and gas production in EC broth are recorded. Compare this with MPN table to get MPN for faecal coliforms. Add 3-4 drops of Kova c's indole reagent to tryptone broth. A red layer at the top indicates a positive indole test. Compare this value with MPN table to get MPN value for *E.coli.* Coliforms which produce gas in EC broth and indole in tryptone broth at 44.5±5°C are *E.coli.*

Confirmation of *E.coli*

Subculture positive tubes to Eosin methylene blue agar. Colonies with greenish metallic sheen confirms *E.coli*. Subculture these colonies to TGA slants and can be further confirmed by IMViC test.

Aerobic Plate Count/ Total Plate Count of Water

Dilution of sample: 1ml of mixed sample is pippetted out to 9ml saline to give 10^{-1} dilution. 1ml from this to 9ml NS gives 10^{-2} dilution. Similarly prepare suitable to get 30 to 300 colonies on plating.

Plating: Dispense 1ml each of two appropriate dilutions to sterile marked petri plates in duplicate. Pour 15-20 of TGBE agar cooled to 45°C and mix well by back and forth motion of plates on flat surface. After solidifying incubate the plates for 24-48 hours at 37 ± 1°C. Count the number of colonies on duplicate plates.

Calculations: TVC = Average no. of colonies * dilution factor

Recorded as colony forming units (CFU) present per ml of sample

S.O.P. for *Pseudomonas*

Medium Used

Asparagine Proline broth, Milk Agar A, Milk Agar B and Ethanol

Procedure

Medium: Asparagine Proline broth (7.5ml of ethanol added/100ml broth)

a. 10ml of sample added with 10ml of Asparagine Proline broth
b. Keep it for incubation at 37°C for 48 hours

Positive: Illumination observation when Asparagine Proline broth kept in IJV cabinet

Negative: No illumination observed

Confirmed test

Milk Agar A (75g) + Milk Agar B (25g)
Dissolve the agar by boiling
After sterilization mix the agar A & B

Procedure: Pour plate method
Incubation: 37°C for 24 hours
Positive: Formation of pigmented colonies in Milk agar

***E.coli* and Coliforms in water (MPN Method)**

The corresponding MPN values for Total coliforms, Faecal coliform and *E.coli* are recorded as values per 100ml of sample (Table 1)

Table 1: MPN Table

Positive Tubes			MPN per 100ml	Positive Tubes			MPN per 100ml
10 ml sample	1 ml sample	0.1 ml sample		10 ml sample	1 ml sample	0.1 ml sample	
0	0	0	0	2	2	2	35
0	0	0	3	2	2	3	40
0	1	0	3	2	3	0	30
0	1	1	6	2	3	1	35
0	2	0	6	2	3	2	40
1	0	0	4	3	0	0	25
1	0	1	7	3	0	1	40
1	0	2	11	3	0	2	65
1	1	0	7	3	1	0	45
1	1	1	11	3	1	1	75
1	2	0	11	3	1	2	115
1	2	1	15	3	1	3	160
1	3	0	16	3	2	0	95
2	0		09	3	2	1	150
2	0	1	14	3	2	2	200
2	0	2	20	3	2	3	300
2	1	0	15	3	3	0	250
2	1	1	20	3	3	1	450
2	1	2	30	3	3	2	1100
2	2	0	20	3	3	3	1400
2	2	1	30				

RESULTS

Tables 2 & 3 show the results obtained during the identification and estimation of microorganisms. Identification tests like Gram staining, hanging drop for motility and cultural characteristics by selective media was carried out. The tests for *E.coli* were all positive. But in case of *Pseudomonas* and IRB, there was no result or negative results were obtained. This suggests that the water samples contained coliform bacteria. MNP method and serial dilution techniques were used for estimation of microbial after growths. The results show that there was a gradual increase of microbial contamination from Aluva to West Kochi. TPC count ranges from 10-35 CFU/ml and that of *E.coli* is 1-20 (MPN value) (Fig. 3).

These indicate high level of fecal contamination in the water samples. When the site was revisited, it was observed that the pipelines were rusted and worn out, through which the sewage water enters the pipe. It might be thought that the corrosion of the pipelines is a main reason for the high level of microbial contamination. The presence of suspended impurities in the water samples of West Kochi justifies the role of corrosion in contaminating the water.

Table 2: Shows the results obtained during the identification of microorganisms using gram staining procedure, motility by hanging drop method and cultural characteristics using selective media

	E.coil	***Pseudomonas***	**Iron reducing bacteria**
Gram staining	Gram negative Bacilli	No result	No result
Motility	Motile	No result	No result
Cultural characteristics	Mac conkey agar: Lactose fermenting, medium sized, flat, smooth	DCA agar: Non-lactose fermenting colonies, large, opaque, irregular colonies	Iron oxidizing medium: No result

Table 3: Shows the results obtained during the estimation of bacterial aftergrowths of the water samples collected by MPN method and serial dilution technique

Set 1								
Sl. No.	**Sites**	**pH**	**BOD mg/l**	**COD mg/l**	**TPC cfu/ml**	***E.coli* MPN value**	***Pseudomonas***	**IRB**
1.	Aluva PH	6.37	52	85	10	<1	Absent	Absent
2.	Aluva PT	6.67	51	82	20	<1	Absent	Absent
3.	Perumanoor PH	6.40	59	88	16	<1	Absent	Absent
4.	Perumanoor PT	6.50	60	91	18	<1	Absent	Absent
5.	Thoppumpady PH	6.95	53	84	20	3	Absent	Absent
6.	North Palluruthy PT	6.95	55	95	23	3	Absent	Absent
7.	Karuvelipady PH	6.41	51	83	15	3	Absent	Absent
8.	South Palluruthy PT	7.04	58	82	20	4	Absent	Absent
9.	Mattanchery PT	7.07	59	89	22	11	Absent	Absent
10.	Fort Kochi PT1	7.56	62	98	30	15	Absent	Absent
11.	Fort Kochi PT2	7.58	59	98	35	20	Absent	Absent
Set 2								
1.	Aluva PH	6.30	53	84	10	<1	Absent	Absent
2.	Aluva PT	6.61	50	85	23	<1	Absent	Absent
3.	Perumanoor PH	6.33	60	87	14	<1	Absent	Absent
4.	Perumanoor PT	6.52	59	90	20	<1	Absent	Absent
5.	Thoppumpady PH	6.80	54	85	22	2	Absent	Absent
6.	North Palluruthy PT	6.87	56	96	25	3	Absent	Absent
7.	Karuvelipady PH	6.39	50	84	17	4	Absent	Absent
8.	South Palluruthy PT	7.02	57	83	20	3	Absent	Absent
9.	Mattanchery PT	7.09	58	90	25	14	Absent	Absent
10.	Fort Kochi PT1	7.55	62	99	32	16	Absent	Absent
11.	Fort Kochi PT2	7.60	60	100	34	20	Absent	Absent

PH – Power House, PT – Public Tap

Sample description: 1 to 9 – clear, colourless and odourless water; 10 and 11 – colourless with suspended impurities

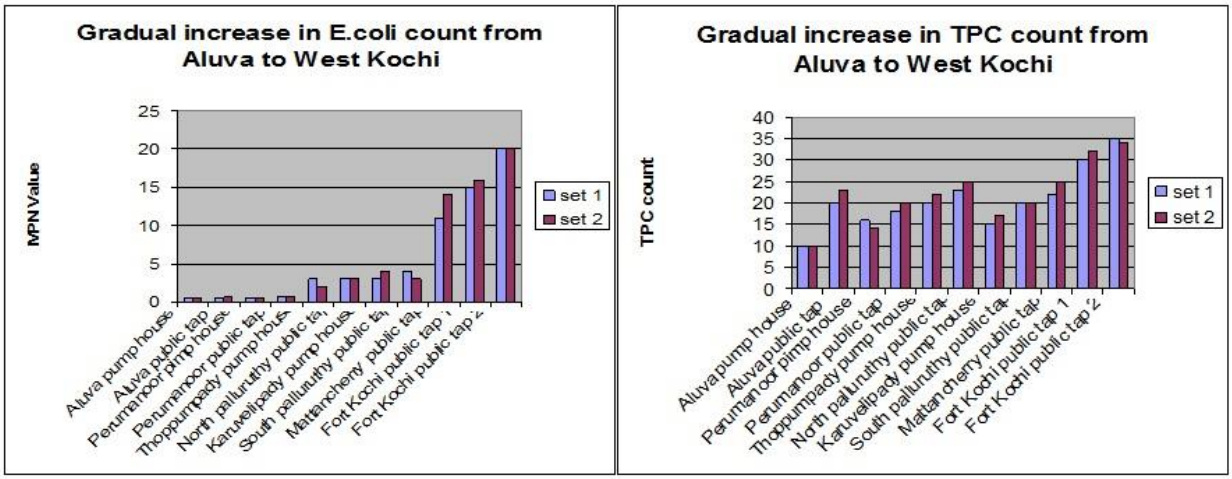

Fig. 3: Graphs showing the gradual increase in *E.coli* and TPC from Aluva to West Kochi

DISCUSSION

The study was aimed at investigating whether the water in the supply gets contaminated before it reaches Kochi area. From the results it was found that the intensity of contamination increases on reaching West Kochi. Water is chlorinated at the storage tanks at different points of the study area. After this treatment for disinfection, there is an increase in contamination in West Kochi area.

Analysis pH, BOD and COD, it was found that both BOD and COD were kept within their normal limits as per Geldreich *et al.* [8]. But a gradual increase of pH is observed from the initial point to the final point of study. This gradual increase in pH might be a reason for contamination observed at West Kochi. The same was observed by [4] that showed a relationship between the microbial after growth, temperature and decline in chlorine residue. With the increase in temperature there is more rapid decline of chlorine residue, which results in the increase of pH, due to the reaction with organic matter.

Result for TPC, *E.coli*, *Pseudomaonas* and Iron reducing bacteria showed that the latter two are absent. But in the case of TPC and *E.coli* there was a gradual increase from initial to final supply points, which indicate faecal contamination of water [9]. *E.coli* and coliform count was found to be 16 (MPN value) and 35 cfu/ml respectively. These values are far greater from the water quality standards, >10 coliforms/ml and >4 *E.coli* /100ml, suggested by Anon [10, 11] To analyze the reason, the site (West Kochi area) was revisited and it was found that the pipes laid were rusted, worn out and laid through, across and along sewage drains and canals. The corrosion of the pipes might be a reason for the increase in contamination at West Kochi. The relationship between corrosion of the iron pipes and microbial out growths were studied extensively by Hutchinson *et. al.* [12].

Corrosion in the interior effects greater then on the exterior of the pipes because the products of corrosion give rise to deposits and nodules, which interfere the carrying capacity of the main and also affect the quality of water conveyed [12]. It may be argued that corrosion of iron pipes is an essential drawing force of many biological processes, which occur in water supply. It is also reported that ferrous iron from iron mains and its oxidation to ferric state seriously affects the quality of water supplied to the consumer [13].

It is also observed that the suspended impurities such as algae and sediments in the water sample from West Kochi also show the extension of corrosion. The same is also reported due to the difference in the pressure inside and outside of the pipes. Dangers of pollution exists when reduced pressure occur in the public supply as a result of heavy draw off at times of peak demand, for fire fighting or serious leaks in water mains. Under these conditions pollutions can be drawn in. Many episodes of enteric diseases have occurred out of this reason [14]. In distribution systems, Collingwood [15] have shown the presence of algae and animals can be a major aesthetic problem in water supply.

As there is no spread of epidemics this year it is presumed that during the year attention should be given by the authorities to keep the microbial level within limits prescribed. This will help to overcome the above explained ill effects

CONCLUSION

In this projects, microbial after growth in the fresh water from the Municipal supply system of Kochi area is studied. Results show that the count of *E.coli* and TPC is comparatively high in West Kochi than other areas. The mingling of waste water due to corrosion of pipes should be avoided. Only renewing the pipelines or shifting it from the sewage canals can avoid it. The renewal processes should be done before the spread of epidemics this year. Maintaining adequate chlorine residue throughout the distribution system, which in turn maintain the pH and also reduce the organic matter becoming available to the bacterial population, controls the bacterial growth. It is advisable to boil the water up to prescribed limits (up to 20 minutes at 100°C) before drinking, so that all microbes are destroyed.

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