

## Microbial Analysis of Drinking Water And Water Distribution System

T.SUBRAMANI<sup>1</sup>, N.JAYAGOBU<sup>2</sup>

<sup>1</sup>Professor & Dean, Department of Civil Engineering, VMKV Engineering College, Vinayaka Missions University,

<sup>2</sup>PG Student of in Environmental Engineering, Department of Civil Engineering, VMKV Engineering College, Vinayaka Missions University, Salem,

### ABSTRACT

The discharge of wastes from municipal sewers is one of the most important water quality issues world-wide. It is of particular significance to sources of drinking-water. Municipal sewage contains human faeces and water contaminated with these effluents may contain pathogenic (disease-causing) organisms and, consequently, may be hazardous to human health if used as drinking-water or in food preparation. Faecal contamination of water is routinely detected by microbiological analysis. It is impractical to attempt the routine isolation of pathogens because they are present in relatively small numbers compared with other types of micro-organism. Moreover, there are many types of pathogen and each requires a unique microbiological isolation technique. The approach that has been adopted is to analyse for indicator organisms that inhabit the gut in large numbers and are excreted in human faeces. The presence of these indicator organisms in water is evidence of faecal contamination and, therefore, of a risk that pathogens are present. If indicator organisms are present in large numbers, the contamination is considered to be recent and/or severe. Bacteria in water are, in general, not present individually, but as clumps or in association with particulate matter. When enumerating bacteria in water it is not the number of individual bacteria present which are counted, but the number of clumps of bacteria or the particles and their associated bacteria. Each clump or particle may have many bacteria associated with it.

**KEYWORDS:** Microbial Analysis, Drinking Water, Water Distribution System

### I. INTRODUCTION

Water is the second essential factor for life after oxygen. If this essential factor is not available some organism dies early, some from resistance stage, while some other dies late. Human beings are not excluded from this marvelous factor. Peoples obtain their drinking water from surface and underground sources. However both surface and ground water sources could become contaminated by biological and chemical pollutants arising from point and non point sources. Surface water sources are lakes, river and stream.

The qualities of surface water rapidly alter as a response to alteration in the surrounding environment. For example high level of nutrients [Nitrogen and Phosphorus] cause eutrophication in surface water sources and massive growth of algae, leading the growth of other microorganisms and cause turbidity in surface water. Other contamination sources of surface water are domestic waste water and sewage, storm water runoff, cattle feedlots runoff etc., (Geldreich, 1990).

Pollution originates from these sources cause to raise the level of turbidity, suspended solids, BOD, disease causing microbes and parasites in surface water sources. On the other side, quality of ground water is remaining consistent throughout in aquifer. In quality of ground water changes occur slowly because it is not directly exposed to pollution sources. Due to natural filtration process ground water free from microbial contamination.

In most cases ground water contamination results due to improper dwelling of well and waste disposal (American Water Works Association, 1971). Different types of pollutant emit from waste water discharge which include household chemicals such as insect repellents, surfactants and pharmaceuticals.

Toxic organic and inorganic compounds, heavy metals, salt, pathogenic microorganism may also be present in water which find their way through industrial and agricultural waste. Improper management of human and livestock waste also their disposal and treatment (Singhl and Mosley, 2003) infiltration of effluent from septic tanks can cause the contamination of ground water.

Sewage is one of the most common sources of drinking water pollution gain entry the distribution

system of drinking water through leakage, alter the physiochemical properties of drinking water spread disease causing microorganism. In term of human health the most dangerous water pollutants are pathogenic microorganism (Cunningham, 2005).

Since from the bagging of the recorded history water play vital role in the spreading of various bacterial, viral and protozoan diseases. Drinking water contaminated by a wide variety of microorganism. Some of these microbes may be pathogenic and cause different diseases in human. It is also an established fact that drinking water if focally polluted can spread dangerous disease like hepatitis, cholera, dysentery, typhoid and diarrhea.

## II. STUDY AREA:

**2.1 Salem** is a city and a Municipal Corporation in Salem district in the Indian state of Tamil Nadu. Salem is located about 160 kilometres (99 mi) northeast of Coimbatore and about 340 kilometres (211 mi) southwest of the state capital, Chennai. Salem is the fifth largest city in Tamil Nadu in terms of population after Chennai, Coimbatore, Madurai, and Tiruchirappalli respectively and fourth in terms of urbanisation. The area of the city is 100 km<sup>2</sup> (39 sq mi). Cheralam, Shalya, Sayilam and Sailam are found in inscriptions referring to the country around the hills, Nagarmalai in the north, Jeragamalai in the south, Kanjanamalai on the west and Godumalai on the east. Local tradition claims Salem as the birthplace of Tamil poetess Avvaiyar. Salem and the hilly region around it were part of the Chera and the city was ruled by kings who were known as the Kurunila Mannargal of ancient Tamil Nadu. Later, it was ruled by Gatti Mudalis Poligars who built some temples and forts in and around the city. In the early 18th century, after the Mysore-Maduraiwar, it came under the rule of Hyder Ali. Subsequently, Salem was taken from Hyder Ali by Colonel Wood at the beginning of 1768. It was recaptured by Hyder Ali towards the end of the year 1772. Under Lord Clive in 1799, it was again occupied by a detachment of the regiment stationed at Sankagiri Durg and remained a military station until 1861 when the troops were withdrawn.

Salem is located at 11.669437°N 78.140865°E. The average elevation is 278 m (912 ft). The city is surrounded by hills on all sides, namely, Nagaramalai in the north, Jarugumalai in the south, Kanjamalai in the west, Godumalai to the east and the Shevaroy Hills to the north east. The Kariyaperumal Hill is situated within the city to the southwest. Thirumanimuthar river flows through the city, dividing it into two parts. The fort area is the oldest part of the town. Salem local planning area extends up to 100 km<sup>2</sup> (39 sq mi) within the city.

In Salem District alone, 34000 hectares of land is under tapioca cultivation which is the raw material for the sago industries and there are 650 units engaged in tapioca processing. In 1981, Salem Starch and Sago Manufacturers Service Industrial Co-operative Society Ltd (popularly called as SAGOSERVE) was established to promote the growth of sago industries. Nearly 80% of the national demand for Sago and Starch is being met by the Sagoserv. In and around Salem the yield of tapioca is about 25-30 t/ha, highest in the World. National average is 19 t/ha and World average production stands at 10 t/ha. The region around Salem is rich in mineral ores. Salem has one of the largest magnesite and bauxite deposits in India. It has many magnesite factories operated by private and public sectors such as Burn Standard & Co, Dalmia Magnesites and Tata Refractories <sup>[43]</sup> The Leigh Bazaar market in Salem is the biggest regional market for agro products. Narasus coffee one of the famous coffee in Tamil Nadu, Nandhi Dall Mills the oldest flour mill company, BSP refineries (Usha Refined Sunflower Oil) are other few companies have their presence in Salem.

## 2.2 CHARACTERISTICS OF INDICATOR ORGANISMS

### 2.2.1 Total coliforms:

The term “total coliforms” refers to a large group of Gram-negative, rod-shaped bacteria that share several characteristics. The group includes thermotolerant coliforms and bacteria of faecal origin, as well as some bacteria that may be isolated from environmental sources. Thus the presence of total coliforms may or may not indicate faecal contamination. In extreme cases, a high count for the total coliform group may be associated with a low, or even zero, count for thermotolerant coliforms. Such a result would not necessarily indicate the presence of faecal contamination. It might be caused by entry of soil or organic matter into the water or by conditions suitable for the growth of other types of coliform. In the laboratory total coliforms are grown in or on a medium containing lactose, at a temperature of 35 or 37 °C. They are provisionally identified by the production of acid and gas from the fermentation of lactose.

### 2.2.2 Thermotolerant (faecal) coliforms.

The term “faecal coliform” has been used in water microbiology to denote coliform organisms which grow at 44 or 44.5 C and ferment lactose to produce acid and gas. In practice, some organisms with these characteristics may not be of faecal origin and the term “thermotolerant coliform” is, therefore, more correct and is becoming more commonly used. Nevertheless, the presence of thermotolerant coliforms nearly always indicates faecal

contamination. Usually, more than 95 per cent of thermotolerant coliforms isolated from water are the gut organism *Escherichia coli*, the presence of which is definitive proof of faecal contamination. As a result, it is often unnecessary to undertake further testing to confirm the specific presence of *E. coli*. In the laboratory thermotolerant coliforms are grown on media containing lactose, at a temperature of 44 or 44.5 °C. They are provisionally identified by the production of acid and gas from the fermentation of lactose. Nutrient-rich environments may encourage the growth or persistence of some species of thermotolerant coliform other than *E. coli*. This possibility should be considered when, for example, an unusually high result is obtained from water that was thought to be relatively clean. In such a case, the advice of a microbiology laboratory should be sought for the determination of the more specific indicator, *E. coli*.

### 2.2.3 Faecal streptococci

The presence of faecal streptococci is evidence of faecal contamination. Faecal streptococci tend to persist longer in the environment than thermotolerant or total coliforms and are highly resistant to drying. It is, therefore, possible to isolate faecal streptococci from water that contains few or no thermotolerant coliforms as, for example, when the source of contamination is distant in either time or space from the sampling point. Faecal streptococci grow in or on a medium containing sodium azide, at a temperature of 37-44 °C. They are usually detected by the reduction of a dye (generally a tetrazolium-containing compound (Table.2.1)

**Table-2.1 Faecal Streptococci**

Multiple fermentation tube technique	Membrane filter technique
Slower: requires 48 hours for a positive	More rapid: quantitative results in or presumptive positive about 18 hours
More labour-intensive	Less labour-intensive
Requires more culture medium	Requires less culture medium
Requires more glassware	Requires less glassware
More sensitive	Less sensitive
Result obtained indirectly by statistical approximation (low precision)	Results obtained directly by colony count (high precision)
Not readily adaptable for use in the field	Readily adapted for use in the field
Applicable to all types of water	Not applicable to turbid waters
Consumables readily available in most countries	Cost of consumables is high in many countries
May give better recovery of stressed or damaged organisms in some circumstances	

### 2.2.4 Heterotrophic plate count

The heterotrophic plate count includes all of the micro-organisms that are capable of growing in or on a nutrient-rich solid agar medium. Two incubation temperatures and times are used: 37 °C for 24 hours to encourage the growth of bacteria of mammalian origin, and 22 °C for 72 hours to enumerate bacteria

that are derived principally from environmental sources. The main value of colony counts lies in comparing the results of repeated samples from the same source. If levels increase substantially from normal values, there may be cause for concern.

### 2.2.5 Selecting a bacteriological analytical technique

Two techniques are commonly used to detect the presence of coliforms in water. The first of these is called the “multiple fermentation tube” or “most probable number” technique. In this method measured portions of a water sample are placed in test-tubes containing a culture medium. The tubes are then incubated for a standard time at a standard temperature. In the second technique, a measured volume of sample is passed through a fine filter that retains bacteria. The filter is then placed on culture medium and incubated. This is called the “membrane filter” technique. Features of the two techniques are compared in Table 10.1.

### 2.2.6 Multiple fermentation tube technique

The technique has been used for the analysis of drinking-water for many years with satisfactory results. It is the only procedure that can be used if water samples are very turbid or if semi-solids such as sediments or sludges are to be analysed. The procedure followed is fundamental to bacteriological analyses and the test is used in many countries. It is customary to report the results of the multiple fermentation tube test for coliforms as a most probable number (MPN) index. This is an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the test. It is not a count of the actual number of indicator bacteria present in the sample.(Table-2.2)

#### Principle

Separate analyses are usually conducted on five portions of each of three serial dilutions of a water sample. The individual portions are used to inoculate tubes of culture medium that are then incubated at a standard temperature for a standard period of time. The presence of coliforms is indicated by turbidity in the culture medium, by a pH change and/or by the presence of gas. The MPN index is determined by comparing the pattern of positive results (the number of tubes showing growth at each dilution) with statistical tables.

The ampoules of ready-to-use media are the most convenient form but are the most expensive and have the shortest shelf-life. Pre-weighed packages are easy to use and reduce the risk of error in making up a batch of medium. Media are inevitably expensive when packaged in small quantities. However, they are not a major component of the cost of bacteriological analysis, and the extra cost may be negligible when

compared with the greater convenience and reduced wastage. Large bottles containing dehydrated media must be tightly resealed after use to prevent spoilage; this is especially important in humid environments. Media should be stored in a cool, dark, dry place. After a medium has been prepared by dissolving the powder in distilled water, it should be distributed into culture tubes or bottles and sterilised. Batches of media should be tested before use, using a known positive and negative control organism. If the appropriate reactions are not observed, the media and the control organisms should be investigated and the tests repeated. Media should be used immediately but may be stored for several days provided that there is no risk of their becoming contaminated.

A stock solution of buffered dilution water is prepared by dissolving 34.0 g of potassium dihydrogen phosphate,  $KH_2PO_4$ , in 500 ml of distilled water. The pH is checked and, if necessary, adjusted to 7.2 by the addition of small quantities of 1 mol l<sup>-1</sup> NaOH solution. Distilled water is added to bring the final volume to 1 litre. The buffered water is stored in a tightly stoppered bottle in the refrigerator. To prepare bottles of dilution water, 1.25 ml of stock solution is added to 1 litre of distilled water, mixed well and dispensed into dilution bottles in quantities that will provide, after sterilisation, 9 or 90 ml. The bottles are loosely capped, placed in the autoclave, and sterilised for 20 minutes at 121 °C. After the bottles have been removed from the autoclave, the caps should be tightened and the bottles stored in a clean place until needed.

Medium	Uses	Incubation temperature	Remarks
<i>Isolation media</i>			
Lactose broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	Prepare single strength medium by diluting double strength medium with distilled water. Each tube or bottle should contain an inverted fermentation (Durham) tube
MacConkey broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	
Improved formate lactose glutamate medium	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	Available commercially in dehydrated form as Minerals Modified Glutamate Medium
Lauryl tryptose (lactose) broth	Total or thermotolerant coliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermotolerant coliforms	
<i>Confirmatory media</i>			
Brilliant green lactose bile broth	Total or thermotolerant coliforms (gas production)	44.5 ± 0.25 °C for thermotolerant coliforms	
EC medium	Thermotolerant coliforms (indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	Addition of 1% (m/m) L- or DL-tryptophan may improve performance of the medium
Tryptone water	Thermotolerant coliforms (gas + indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	
Lauryl tryptose mannitol broth with tryptophan	Thermotolerant coliforms (gas + indole production)	44.5 ± 0.25 °C for thermotolerant coliforms	

### Procedure

The procedure described below is for five tubes at each of three sample dilutions and provides for confirmation of both total and thermotolerant (faecal) coliforms. If fewer tubes (e.g. three of each of three sample dilutions or one 50-ml and five 10-ml portions) are inoculated, the MPN index must be determined from tables specific to the combination of tubes and dilutions used.

### Apparatus

Table-2.2 Culture media for most probable number (MPN) analyses

- Incubator(s) or water-baths capable of maintaining a temperature to within  $\pm 0.5$  °C of 35 and 37 °C and to within  $\pm 0.25$  °C of 44 and 44.5 °C. The choice of temperature depends on the indicator bacteria and the medium.
- Autoclave for sterilising glassware and culture media. The size required depends on the volume of work to be undertaken. A capacity of 100-150 litres would be required for a medium-size laboratory undertaking work on a routine basis.
- Distillation apparatus, with storage capacity for at least 20 litres of distilled water.
- Laboratory balance, accuracy  $\pm 0.05$  g, with weighing scoop. This may be omitted if culture media and potassium dihydrogen phosphate are available in pre-weighed packages of the proper size.
- Racks for tubes and bottles of prepared culture media and dilution water. These must fit into the autoclave.
- Pipettes, reusable, glass, 10-ml capacity graduated in 0.1-ml divs, and 1-ml capacity graduated in 0.01-ml divs.
- Test-tubes, 20 × 150 mm for 10 ml of sample + 10 ml of culture medium, with metal slip-on caps.
- Bottles, with loose-fitting caps, calibrated at 50 and 100 ml, for 50 ml of sample + 50 ml of culture medium.
- Measuring cylinders, unbreakable plastic or glass, capacity 100, 250, 500 and 1,000 ml.
- Test-tube racks to hold tubes in incubator and during storage.
- Phosphate-buffered dilution water.
- Autoclave tape.

Table-2.3 gives Typical sample volumes and number of tubes for multiple fermentation tube analysis

Table-2.3 Typical sample volumes and number of tubes for multiple fermentation tube analysis and Fig 2.1 and Fig.2.2 shows test procedure

Sample type	Sample volume (ml)				
	50	10	1	0.1	0.01
Treated drinking-water	1	5			
Partially treated drinking-water		5	5	5	
Recreational water		5	5	5	
Protected-source water		5	5	5	
Surface water			5	5	5

### Procedure

1. Prepare the required number of tubes of culture medium. The volume and strength (single or double) of medium in the tubes will vary depending on the expected bacteriological density in the water and the dilution series planned. For most surface waters, 10 ml volumes of single-strength medium are appropriate.
2. Select and prepare a range of sample dilutions; these will normally be suggested by experience. Recommended dilutions for use when there is no experience with samples from that station are given in Table 10.3. To prepare a 1/10 dilution series, mix the sample bottle well. Pipette 10 ml of sample into a dilution bottle containing 90 ml of phosphate-buffered dilution water. To prepare a 1/100 dilution, mix the 1/10 dilution bottle well and pipette 10 ml of its contents into a bottle containing 90 ml of dilution water. Subsequent dilutions are made in a similar way. Alternatively, 1 ml of sample may be added to a bottle containing 9 ml of dilution water.
3. Pipette the appropriate volumes of sample and diluted sample into the tubes of medium, as shown
4. Label the tubes with the sample reference number, the dilution and the volume of sample (or dilution) added to the tube. Shake gently to mix the sample with the medium. Place the rack in an incubator or water-bath for 48 hours at  $35 \pm 0.5$  °C or  $37 \pm 0.5$  °C.
5. After 18 or 24 hours, note which tubes show growth. The reactions are listed in Table. Tubes that show turbidity and gas production, or a colour change indicating the production of acid (if the medium contains a pH indicator), are regarded as positive. Record the number of positive tubes at each dilution, as shown in Figure. Return the tubes to the incubator and re-examine after a total of 48 hours of incubation. Continue with the next step of the procedure.

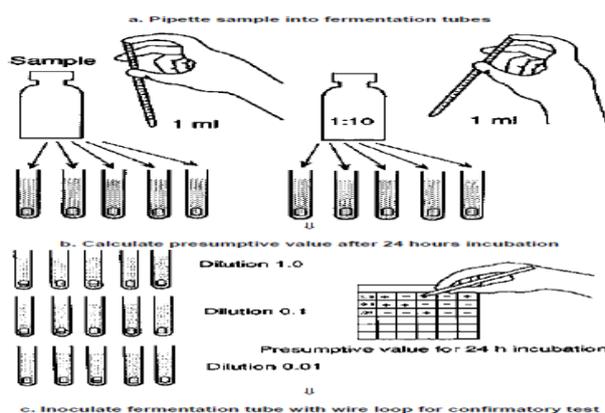


FIG: 2.1 TESTING PROCEDURE-1

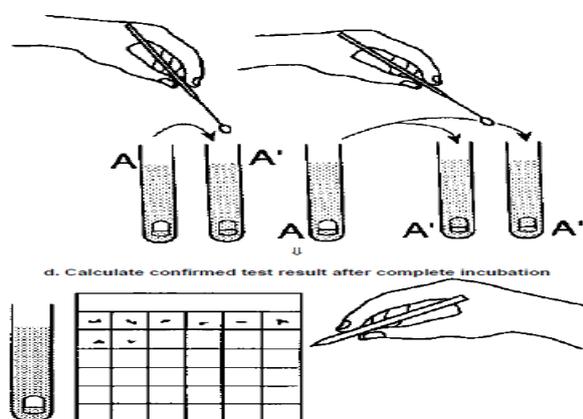


FIG:2.2 TESTING PROCEDURE-2

## 2.3 MATERIALS AND METHODS

### 2.3.1 Material:

A total 10 locations or areas for drinking water samples were selected and Thirty (30) water samples were collected from water supply, distribution system and storage tanks with 10 samples each. Physiochemical analyses, i.e., pH, turbidity and Total Suspended Solids (TSS) was determined by standard ASTM Methods Whereas, microbial analyses was conducted for Total & fecal coliform and E. coli (APHA, 2005).

For bacteriological analysis, the samples were collected in a 500 mL sterilized Schott Duran Bottle, the bottles were properly sterilized before the sample collection and for Physio-chemical analysis water samples were collected in plastic bottles.

When the sample were collected the bottle were tightly capped and properly labeled. Water samples collected for bacteriological analysis kept in ice box then transported to environment microbiology section of PCSIR Laboratory complex Peshawar with in 1 h to prevent from any Physio-chemical alteration and unwanted growth of microbes.

### 2.3.2 Analytical quality assurance and quality control

Standard methods for drinking-water analysis should be tested under local conditions for accuracy and precision, agreed at national level, and applied universally by both water-supply and regulatory agencies. However, the use of standard methods does not in itself ensure that reliable and accurate results will be obtained. In the context of analytical work, the terms quality assurance and quality control are often treated as synonymous.

In fact, they are different concepts. Analytical quality control is the generation of data for the purpose of assessing and monitoring how good an analytical method is and how well it is operating. This is normally described in terms of within-day and day-to-day precision. Analytical quality assurance, by contrast, comprises all the steps taken by a laboratory to assure those who receive the data that the laboratory is producing valid results.

- Equipment review. A commitment to decentralized testing with field test kits and other portable equipment normally results in a larger quantity of equipment being in use. Regular review of the equipment (e.g. temperature checking of incubators) is essential. To ensure standardization, this should be undertaken by supervisory staff from a control laboratory.

### 2.3.3 Microbial and source tracking analyses

Culture-based enumeration of TCs and E. coli was performed using the IDEXX Colilert 2000 method. The Colilert method has been proven to offer detection comparable to that of simple membrane filtration and growth on selective media, but with faster results. Most probable number (MPN) per 100 mL concentrations was determined using a statistical formula provided by.

Water samples that were positive for E. coli were confirmed by growth on eosin methylene blue (EMB) agar. Between 24 and 36 hours of sample arrival, 250 mL of sample was captured on a sterilized 0.4 µm Isopore membrane filter (Millipore, Billerica, MA) for later molecular analysis (MST). Many MST methods involve the use of molecular tools (commonly PCR) to target and amplify genetic sequences from a source-specific host.

DNA probes have been designed to target 16S rRNA gene sequences because these sequences are highly conserved and contain sourcespecific information. Bacteroides spp. have been used as indicators of human fecal contamination as: (i) the most prevalent Bacteroides spp. found in the human gut are specific to humans; and (ii) obligate anaerobes should theoretically indicate recent contamination.

Organic compounds in the environment that have been known to fluoresce naturally can obscure the

results of this test. As naturally fluorescing compounds are not degraded by sunlight, all positive samples were held under UV light for 4 hours to allow anthropogenic optical brighteners to degrade as confirmation. A positive result was confirmed if the reading decreased by approximately 30% of the original value, i.e. 30% of the fluorescence was attributable to degradable, likely anthropogenic, fluorescing compounds.

#### 2.3.4 Chemical analysis

As part of a VAHWQP drinking water clinic, all samples were tested for pH, conductivity [proxy for total dissolved solids], nitrate-N, chloride, fluoride, calcium, magnesium, sodium, manganese, copper, iron, sulfate and hardness in the Virginia Tech Biological Systems Engineering (BSE) Water Quality Lab and the Virginia Tech Crop and Soil Environmental Sciences (CSES) Soils Testing Laboratory using appropriate standard methods.

The cluster analysis revealed that the only two appropriate levels were 'zero' and 'nonzero'. This was not surprising, as the concentration data for TCs were also somewhat zero-heavy, given that almost 60% of the 538 total samples were negative for the presence of TCs. Therefore, 'contamination' was considered as a binary response with 'present' representing any concentration of TCs in the water samples other than zero.

This analysis also permitted the inclusion of all samples analyzed per Figure. A logistic regression model was used to combine several predictive factors into one equation that predicted the presence or absence of TCs. This type of regression allowed for the mean response ( $\pi$ ) to be constrained by  $0 < \pi < 1$ , in order to obtain a result in the form of a probability of contamination.

The regression equation is given as follows:

$$\pi = \frac{e^{\beta_0 + \beta_1 x_1 \dots + \epsilon}}{1 + e^{\beta_0 + \beta_1 x_1 \dots + \epsilon}}$$

where  $\beta_0$  is the intercept,  $\beta_1$  is the estimate for the coefficient of the predictor  $x_1$ , and  $\epsilon$  is the error term. A response value ( $\pi$ ) below 0.5 was considered to be a prediction of an uncontaminated well, while a response value above 0.5 was considered to be a prediction of a contaminated well. Selection procedures were used to determine which variables were appropriate to include in the regression model based on a significance level of  $\alpha \leq 0.05$ .

Forward, backward and stepwise selection procedures all resulted in the inclusion of the same variables in the regression model. Two separate logistic regression procedures were carried out – one for the prediction of the presence/absence of TCs

based on chemical data, and one for the same prediction based on survey data.

#### 2.3.4 Source tracking

Of the 372 samples tested for the presence of optical brighteners via fluorometry, three were positive [i.e. values were between 50 and 100 units]. After 4 hours of exposure to UV light to degrade anthropogenic optical brighteners, the samples showed declines in their readings of approximately 30% or greater, indicating that these readings were not completely the result of natural fluorescing compounds (i.e. anthropogenic sources were present). All three positive samples were collected from shallow dug or bored wells, were positive for both E. coli and TCs, and all three homeowners noted discoloration of water. Data collected for two of the three wells revealed that wells were constructed in 1945 and 1956, both considerably older than the broader dataset average of 1981. The age of the third well was not provided. In order to further investigate possible sources of contamination, PCR targeting Bacteroides genes was performed on 26 E. coli-positive samples. One sample was positive for general Bacteroides (Bac32F). No samples were positive for the human specific marker (HF183). The sample that was positive for general Bacteroides was not analyzed on the fluorometer because it was taken from the February 2011 clinic before fluorometry analysis was initiated. It was, however, sourced from a spring on a farm, and had a TC concentration of 128 MPN/100 mL and an E. coli concentration of 95MPN/100 mL. It is worth noting that no internal processing control was used during PCR, which means that there is no way to determine quantitatively whether any inhibition occurred,

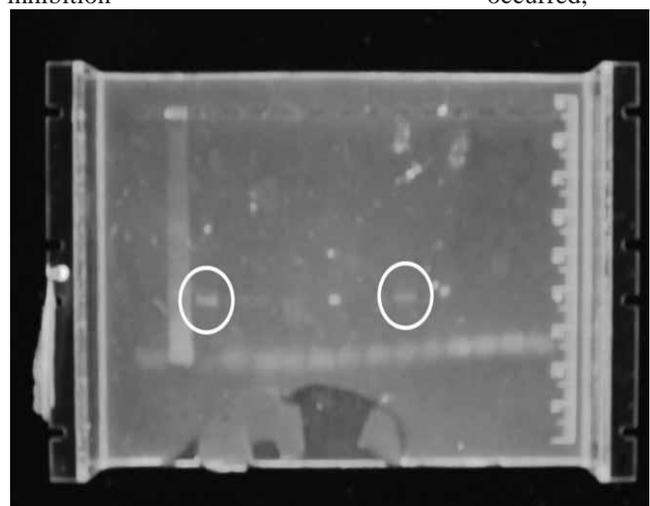


Figure Agarose gel showing control in well three and positive sample (Bac32F, general Bacteroides marker) in well ten.

It is also important to consider that negative source tracking results do not necessarily discount the possibility of fecal contamination from humans or animals, given that Bacteroides die off more rapidly than E. coli in the environment. Additional complications arise in characterizing human-specific Bacteroides because the human-specific marker (HF183) is not present in 100% of the human population.

### 2.3.5 Statistical analysis

Analysis of chemical data A backwards selection procedure was performed to identify chemical water quality parameters appropriate for inclusion in a logistic regression model to predict the presence/absence of TCs in a water sample. All variables (e.g. pH, nitrate, etc.) were initially entered into the model, and variables were removed one at a time based on a significance threshold of  $\alpha \frac{1}{4} 0.05$ .

At the conclusion of the selection procedure, no variables remained in the model (i.e. no parameters were significant predictors of microbial contamination). Construction of an initial scatter plot matrix (for basic observation of trends) in JMP 9 revealed no evidence of potential correlations. The scatter plot matrix did reveal expected correlations (e.g. between hardness and calcium and magnesium).

### III. MICRO BIOLOGICAL ANALYSIS OF DRINKING WATER

The most common and widespread health risk associated with drinking water is contamination; wither directly or indirectly, by human or animal excreta, particularly faeces. If such contamination is recent, and if those responsible for it include carriers of communicable enteric disease, some of the pathogenic microorganisms that cause these diseases may be present in the water.

Drinking the water, or using it in food preparation, may then result in new cases of infection. The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhea, dysentery, hepatitis, or typhoid fever, most of them are widely distributed throughout the world. Faecal contamination of drinking water is only one of several faeco-oral mechanisms by which they can be transmitted from one person to another or, in some cases, from animals to people.

Other pathogen cause infection when water containing them is used for bathing of for recreation involving water contact, rather than by the oral route. Some may also cause infection by inhalation when they are present in large numbers in water droplets, such as those produced by showers and some air-

conditioning systems or in the irrigation of agricultural land.

Ideally, all samples taken from the distribution system including consumers' premises should be free from Coliforms organisms. In practice, this is not always attainable. To control purity of water the following microbiological parameters for water collected in the distribution system is therefore recommended. Indian standard IS 1622 : 1981

- Throughout any year, 95 % of samples should not contain any coliform organisms in 100 ml.
- No sample should contain E.Coli in 100 ml.
- No sample should contain more than 10 coliform organisms per 100 ml.
- Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

### IV. E.Coli

E.coli is a gram-negative, non-spore forming, rod-shaped bacterium which can be either motile or nonmotile (motile cells are peritrichous); growth is aerobic or facultatively anaerobic. Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose.

- E.coli is found in large numbers in the faeces of humans and of nearly all warmblooded animals; as such it serves as a reliable index of recent faecal contamination of water.
- E.Coli is abundant in human and animal faeces, in fresh faeces it may attain concentrations of 10<sup>9</sup> per gram. It is found in sewage, treated effluents, and all natural waters and soils subject to recent faecal contamination. Whether from humans, wild animals, or agricultural activity.
- E.Coli may be present or even multiply in tropical waters not subject to human faecal pollution. However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded, because animals can transmit pathogens that are infective in humans, the presence of E.coli must not be ignored.

### 4.2 Total Coliform

The term "coliform organisms (total coliforms)" refers to gram negative, rod shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and able to ferment lactose at 35 – 37°C with the production of acid, gas, and aldehyde within 24 – 48 hours. They are also oxidase-negative and non-spore-forming. These definitions have recently been extended by the development of rapid and direct enzymatic methods for enumerating and confirming members of the coliform group. The existence both of non-faecal bacteria that fit the definitions of coliform

bacteria and of lactose-negative coliform bacteria limits the applicability of this group as an indicator of faecal pollution. The coliform test can therefore be used as an indicator both of treatment efficiency and of the integrity of the integrity of the distribution system. In our study for microbiological water quality we followed next methodology: Sample collection procedure for Bacteriological analysis of drinking water.

1. Remove any attachment from the tap.
2. Using a clean cloth outlet of the tap wipe to remove any dirt.
3. Turn on the tap for maximum flow and the water may run for two minutes.
4. Outlet of the tap is sterilized by means of flame from cigarette lighter.
5. Tap again opened to flow for 1 to 2 minutes at medium flow rate.
6. Sterile 250 ml plastic bottle is taken for sample collection.
7. Carefully unscrew the cap and immediately hold the bottle under the water jet and fill.
8. Water filled up to 200 ml and a small air space is left to make shaking before analysis.
9. Collected sample delivered to laboratory within 20 to 30 minutes and inoculated immediately.

#### 4.3 Method for testing Total Coliform & E.coli

Name of the method: Multitube fermentation technique/MPN method

##### Presumption test:

1. Inoculated in 10 ml tubes containing Macconky broth and Durham tubes.
2. Tubes are kept in the incubator 37°C for 24 to 48 hours.
3. Any presence of bacteria will show gas production or colour change of the broth from violet to yellow.

#### 4.4 Conformation test for total coliform:

1. Inoculate from the positive tube from presumption test in Brilliant green broth which contains Durham tubes.
2. The temperature is  $37 + 0.50$  C.
3. Presence of gas production confirm the presence of bacteria..

#### 4.5. Conformational test for E.coli

1. Inoculate from the positive tube from presumption test in EC broth which contains Durham tubes.
2. The temperature is  $44 + 0.50$  C.
3. Presence of gas production confirm the presence of bacteria.

Completed test for E.Coli

1. Inoculate from the positive tube from conformation test for E.coli in EMB agar.

2. Presence of metallic sheen confirms the presence of E.coli.

Totally from the period of time: 08.01.02 – 14.12.02 were analyzed 372 samples for microbiological quality of water. Water was collected from the Autryville places: guest houses, schools and kindergarten, public places, production units, communities and four villages located within Autryville.

- Sources of collection:
- Tanks – untreated water
- Filters – different types of purification system

## V. MICROBIAL POPULATIONS IN A MODEL DRINKING WATER DISTRIBUTION SYSTEM

The composition of microbial populations in drinking water distribution systems is important in understanding bacteriological processes, including the fate of pathogens. In low nutrient environments it may be assumed that bacteria adapted to oligotrophic conditions are the first colonizers of the system whereas potential pathogens like E. coli or eukaryotic organisms are considered to be secondary colonizers feeding on remnants and excretions.

Therefore, to perform a risk analysis for the pathogens in water supply systems, it is necessary to understand the ecology of the indigenous population including their interactions with the surroundings (Szewzyk et al., 2000). At present, the composition of microbial populations in potable water is mostly treated as “a black box” and at best addressed with heterotrophic plate count or total count enumeration.

Another aspect of the microbial ecology in water supply systems is the fact that several microorganisms are used as indicators for various parameters of the hygienic quality. For example, a *P. fluorescens* P17 is used in the assimilable organic carbon test assay (AOC) as an indicator of the accessibility and amount of organic nutrients in water (van der Kooij, 1992).

To evaluate if the metabolic potential of *P. fluorescens* is representative for the indigenous microflora, a deeper insight into the composition and physiology of the autochthonous bacterial population is needed.

Using a standard flow cell set-up supplied with drinking water one can visualize the microbial distribution as well as their structural composition using fluorescent in situ hybridization combined with microscopical techniques. With this approach we wanted to analyze which microorganisms that colonized the surface and their spatial distribution and compare it with the free-flowing community.

### 5.1 Flow cell set-up

The biofilm was grown in small flow cells made of Plexiglas, with a microscope coverslip attached on top with silicone-glue (Wolfaardt et al., 1994). This construction made it possible to follow the biofilm build-up and microbial diversity by transmission light microscopy without destruction of the sample.

The system was operated with laminar flow ( $v = 15.5$  mm/s  $NRe = 15.5$ ) at 23°C. For a more detailed description of the set-up, see Christensen et al. (1999). Water from a household tap at the Technical University of Denmark was continually fed to the system and the indigenous microbial population served as inoculum for the flow cells.

### 5.2 Biofilm analysis

The formation of biofilm was followed for 81 days with transmission light microscopy. The biofilm was also examined with fluorescent in situ hybridization (FISH) followed by scanning confocal laser microscopy (SCLM) (for a detailed description, see Christensen et al., 1999). Prior to hybridization, the biofilm was fixed with cold 4% formaldehyde and embedded in 20% polyacrylamide to preserve the hydrated structure (Møller et al., 1998). Hybridizations were done at 46°C and 30% formamide (final concentration after addition of probe) with the Eub338 (Amann et al., 1990), ALF1b, and BET42a probes (Manz et al., 1992). The hybridized biofilm was analyzed with scanning confocal laser microscopy and multichannel simulated fluorescence projection (SFP) images were generated using IMARIS software.

### 5.3 Isolation and phylogenetic analysis of culturable strains

Bacterial strains were isolated on R2A medium (10 d, 23°C). Selected colonies were restreaked twice and harvested on plates since many strains did not grow in liquid culture. DNA from the harvested cell-cultures was extracted according to Grimberg et al. (1989). All strains were hybridized with Eub338, ALF1b, BET42a and Gam42a to get a broad phylogenetic affiliation.

### 5.4 Biofilm structure

The biofilm formation in the flow cells was followed for 81 days with transmission light microscopy to give a first impression of the biological system without interrupting it. Figures show the microbial colonization and biofilm development in a flow chamber. Many single cells were immobilized at the surface after 4 days, and a few microcolonies had been formed.

The bacteria were mostly cocci or rod shaped. A few bigger microcolonies had developed after 12 days, and protozoans were grazing on these larger

microcolonies. By day 17, the microcolonies had developed further, however, there were still large areas of a monolayer of cells surrounding the microbial stacks.

At day 24, larger microcolonies with protozoans attached was observed. This biofilm structure was commonly observed during the rest of the study. Several models predicting the biofilm structure have been proposed by different researchers (Wimpenny and Colasanti, 1997).

The heterogeneous mosaic biofilm model proposes that biofilms consist of microcolonies attached to substratum at the base, but well separated from their neighbors (Walker et al., 1995). Between the microcolonies individual cells attach to the surface forming a very thin film layer.

The main difference to the water-channel model proposed by Costerton et al. (1994) is sparseness of the observed microcolonies, which stand as unconnected towers surrounded by the water phase. Using a cellular automaton model, Wimpenny and Colasanti (1997) showed that a structure closely related to the heterogeneous mosaic biofilm model could be formed at low substrate concentration.

The observations in this study are consistent with this model, indicating that the low carbon concentration in the inlet water results in a biofilm consisting of microcolonies scattered around the surface separated by large areas colonized only by single cells.

## VI. CONCLUSION

EM are often isolated from potable water and can be considered as common inhabitants of public and hospital drinking water distribution systems. It is important to realize that each DWDS harbors its own (myco) bacterial flora as a result of the specific physico-chemical and microbiological properties of the water (including raw water and treated water), and the specific construction and condition of the DWDS. The complex cell wall is responsible for the relative resistance of EM to disinfectants such as chlorine, and some *Mycobacterium* spp. are resistant to or even multiplies at high temperatures. In combination with other properties such as association or growth in biofilms and protozoa, EM seem difficult to eradicate from water distribution systems. Although most attention has been paid to *M. avium*, the ecology and health risk of other EM should not be neglected.

There are different water purification systems available, microbial removing process can be based on the micro filtration, UV radiation, chlorination, reverse osmosis. Each of the systems has advantages and disadvantages but none of them is absolutely reliable. The microbiological examination is still remaining an important way to control the purity of the water and it is suitable for drinking purposes.

Drinking water in the distribution system is not sterile, regardless of the degree to which the water is treated. The water contains microbes that survive the treatment process or enter the distribution system through the pipe network. Many of these microbes can attach to the pipe wall and become part of a biofilm. Once biofilm development begins, subsequent material, organisms and contamination introduced to the distribution system can become entrained in the biofilm and may be subsequently released into the flowing water under various circumstances.

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