ISSN 2449-8947 MicroMedicine *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.2796152

Assessment of Bacteriological Quality of Drinking Water in Belagavi City, India

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Received: 07 February 2019; **Revised submission:** 05 April 2019; **Accepted:** 10 May 2019

ABSTRACT: With the growing population and industrialization, the potability of drinking water has been decreased due to pollution and improper sanitization. In this context the present study was aimed to check the potability of drinking water by Multiple Tube method and Membrane Filter technique and to identify bacteria from Membrane Filter technique. 100 samples from 10 different wards of Belagavi City were collected. Bacteriological analysis was done for the presence of fecal coliforms, fecal Streptococci, *Salmonella* and *Shigella* by presumptive coliform test and multiple tube method (MPN) and membrane filter (MF) techniques were used for estimation of coliform bacteria. Twelve out of 100 samples were found to be unsatisfactory for drinking purpose. A total of five different strains were isolated from 100 samples. Genera isolated were *Corynebacterium* species, Micrococci, Gram positive Bacilli, *Staphylococcus aureus*, *Citrobacter freundii*. 88% of the sources were hygienic for drinking purposes and for the rest of the sources, proper measure should be taken to maintain sanitary of the drinking water by regular check on the equipment, maintaining structural faults and proper disinfection of equipments.

Keywords: Potable water; Most probable number; Membrane filter; Fecal coliforms; Water sources.

1. INTRODUCTION

Water is a precious natural resource and one of the most essential requirements for all kinds of life, as it could not be replaced by any other known natural or man-made compound, it has its unique physical and chemical properties. After air, potable water is second essential need for existing of human life on this planet earth. Life itself originates in ocean water, about 3.2 billion years ago. Groundwater, which makes up about 20% of the world's fresh water supply and is about 0.61% of the entire world's water, so it is most important sources of potable water throughout the world [1]. Groundwater is used for domestic and industrial water supply and also for irrigation purposes in all over world. In the last few decades, there has been a tremendous increase in the demand for fresh water due to rapid growth of population and the accelerated pace of industrialization. According to WHO organization, about 80% of all the diseases in human beings are caused by water. Once the groundwater is contaminated, its quality cannot be restored back easily and to device ways and means to protect it [2-5]. Indicator organisms are commonly used to assess the bacteriological quality of

water. Fecal coliforms and fecal streptococci are the most commonly used bacterial indicators of fecal pollution. They are found in water that is contaminated with fecal wastes of human and animal origin. The coliforms are indicative of general hygienic quality of the water and potential risks of infectious diseases from water [6]. In India more lives are lost to unsafe drinking water than the wars and terrorism combined. About 85% of rural population in India is solely depended on groundwater, which is depleting at a fast rate. In the urban areas though about 60% of the population is depended on surface water sources, the availability and quality are questionable. In Belagavi city, corporation area also, the drinking water problem is noticed during summer seasons. A case of study of Nanded city was carried out to monitor the suitability of raw ground water for safe drinking purposes and to investigate the status of those sites for bacterial contamination throughout the year. The results of this study revealed significant increase in the concentration of indicator organisms in all the samples during monsoon season. As a result the potability of ground water has become a serious concern in such localities [6]. Singh et al. investigated the suitability of ground water for drinking by considering bacterial parameters like total coliform (TC) and fecal coliform (FC) as higher TC and FC counts are demonstrated indicating the presence of enteric pathogens in water [7]. Shaji et al. emphasize that ground water has long been considered as one of the purest forms of water available in nature and meets the overall demands of both rural and suburban people. Large scale industrial growth has caused serious concern regarding the susceptibility of ground water contamination due to waste materials. The water from four open wells in the industrial area was analyzed to assess suitability for domestic purposes. The levels of mineral, heavy metal analysis, coliform tests were performed. The study inferred that use of waters of open wells in and around industrial area may cause health hazards to nearby inhabitants [8]. Chauhan et al. in his study the virus is transmitted by the fecal-oral route, often through water or food contaminated by feces [9-12].This outbreak occurred in slum area receiving tap water supply from municipal corporation. The present outbreak was water born as in this study. Hepatitis E outbreak has been reported in urban areas when there is break in quality of water supplied including water chlorination [13-15]. Fecal contamination of the source of drinking water was documented in many of the epidemics [14-19]. The most likely source of the outbreak was drinking water contaminated with sewage due to leakages aggravated by overflowing drains and intermittent water supply.

Aims and objectives:

1. To test the potability of drinking water by Multiple Tube Method and Membrane filter Technique.
2. To identify bacteria from Membrane Filter Technique.

2. MATERIALS AND METHODS

Ten wards of Belagavi city had been identified for collection of water samples. Sample size was 100. Samples were collected from the areas where people are using for drinking purpose. Water samples were collected during from January to June, 2015.

2.1. Collection of samples

For collection of samples autoclaved/sterilized bottles of 250 ml capacity was used. While collecting the samples from tap extreme care was exercised to avoid contaminating it from bacteria from the environment. Water was allowed to run to waste for 2-3 minutes before collecting into the bottle. The bottle was closed with stopper, labeled with full details of the source of the water. The bottles with the samples was delivered to the laboratory as quickly as possible keeping in cool container and protected from light.

Two methods were used for this purpose:

1) The multiple tube method
2) The membrane filtration method

2.2. Methods of analysis

The standard tests usually used in water bacteriology were:

(a) Presumptive coliform test

(b) Two methods for estimation of probable number of coliform bacteria in water.

Multiple tube method: by this test the most probable number (MPN) of coliform organisms was detected in 100 ml of water sample. Media: double strength and single strength MacConkey's broth containing bromocresol was sterilized in test tubes containing Durham's tube for indication of gas production. Procedure: Measured amount of water sample was added by sterile pipettes as follows:

- 50 ml water sample, to 50 ml double strengths medium
- 10 ml water to 10 double strength medium
- Five 1 ml water sample quantities each added to 5 ml single strength medium.

The tubes were incubated at 37 centigrade degree for 48 hours. An estimate of coliform count was made from the tubes showing acid and gas production by the help of a statistical table probability table according to McCrady.

After 48 hours of incubation, the test tubes were checked for the number of cultures of acid (color change) and gas (a bubble large at the top Durham tube) formed in tubes. This acid and gas producing cultures was considered as presumptive positive growth of coliform bacilli cultures not showing production of both acid and gas at 48 hours were considered negative. The number of such tubes was analyzed using the table for determination of MPN.

Membrane filter technique: In this method, a measured (100 ml) volume of the water sample was filtered through a filter membrane with the help of syringe. The membrane filter then folded carefully and was transferred with help of forceps into sterile Eppendorf tube containing 1 ml autoclaved saline. It was vortexed for approximately 2 minutes, 100 microlitres filtrate was transferred to each of MacConkey agar plates. The filtrate was spread on the plates with help of glass spreader. The plates were kept overnight at 37 degree centigrade so that bacteria grow into colonies. These colonies were recognized by their color, morphology, and ability to grow on the selective medium and were counted.

Filter paper preparation: The membrane filter was autoclaved in a plastic filter keeping the lid slightly open. The filter paper was transferred aseptically into membrane filter folder and the lid was closed tightly. For each sample new filter paper was used. For every new water sample, different membrane filter was used after autoclaving.

Plate count: The plate count expresses the number of colonies forming bacteria. In MacConkey agar - two types of colonies seen:

1) Lactose fermenting (LF): pink color colony

2) Non-lactose fermenting (NLF): cream/ white color.

After 24 hours colony count was done.

Various media used: HiMedia MacConkey Agar-SM082 which also favours growth of gram positive bacteria, blood agar and special media for the isolation and identification of the organisms. Gram stain was done to differentiate gram positive and gram negative bacteria. Species identification was done by using various biochemical tests.

3. RESULTS

One hundred samples of water from different provenance; open well, bore, municipal, corporation, household kitchen, taps, coolers from different colleges and hostels, stored water tanks were tested. We analyzed its bacteriological quality and integratedly studied various bacteriological characteristics like isolation of different bacteria, their properties and identification of isolates given proper category based on their acceptance for potability. The samples taking in account of multiple tube method were categorized

according to McCardy probability rule as (Table 1):

- Category A: Samples are excellent.
- Category B: Acceptable; but make regular sanitary checks on equipment.
- Category C: Unacceptable; look forward for and correct structural faults and poor maintenance of pump plinth then disinfect equipment and source.
- Category D: look for alternative source or carry out necessary repairs and disinfect well.

According to the above data, 31% of sample falls under category-A, 57% under category-B and 7% and 5% under category-C and D respectively.

Table 1. Grading the sample according to McCardy's probability rule.

Grade	Category	Sample size (out of 100 samples)
0	A	31
1-10	B	57
10-50	C	7
More than 50	D	5

Taking into account of membrane filter technique, the lactose fermenter and non-lactose fermenter colonies were Gram stained. Furthermore, standard biochemical tests were done to identify the species and special media was used for further isolation and identification of organisms. The organisms isolated were:

1. *Staphylococcus aureus:* Gram positive cocci arranged characteristically in grape like clusters.
   - Catalase test- positive
   - Slide coagulase test- positive
   - Tube coagulase test- positive
   - Urease test- urea hydrolysed
   - Mannitol motility test- fermented and non-motile.

   On Mannitol Salt Agar: Yellow colonies were seen. On MacConkey's Agar: Smaller colonies, pink due to lactose fermentation. On Blood Agar: Colonies were large, circular, convex, smooth, shiny, opaque, golden yellow pigment, beta-hemolytic.
2. *Citrobacter freundii:* Gram stain shows long rod shaped pink bacilli.
   - Oxidase test- negative
   - Catalase test- positive
   - Indole- not produced
   - H_2S- produced
   - Citrate- utilized
   - Manitol motility test- fermented and motile
   - TSI- Acid/Acid with gas production

   On MacConkey's Agar- lactose fermenting pink colonies.
3. *Micrococcus*: Gram stain shows purple larger cocci mostly in pairs, tetrads and irregular clusters.

   On MacConkey's Agar: Pink colonies. On Blood Agar: cream colored colonies were seen.
4. *Corneybacterium species:* On gram stain, purple bacilli with Chinese letter or cuneiform arrangement was seen. On MacConkey's Agar: colorless wrinkled colonies. On Blood Agar: small, greyish colonies with granular appearance, translucent but with opaque centres, convex, with continuous borders were seen.
5. *Gram positive bacilli (GPB)*: On gram stain long filamentous purple bacilli seen. On MacConkey's Agar: Colorless large colonies. On Blood Agar: raised, dull, opaque, greyish white colonies were seen.

The isolated organisms were categorized based on considering both the techniques as in Table 2.

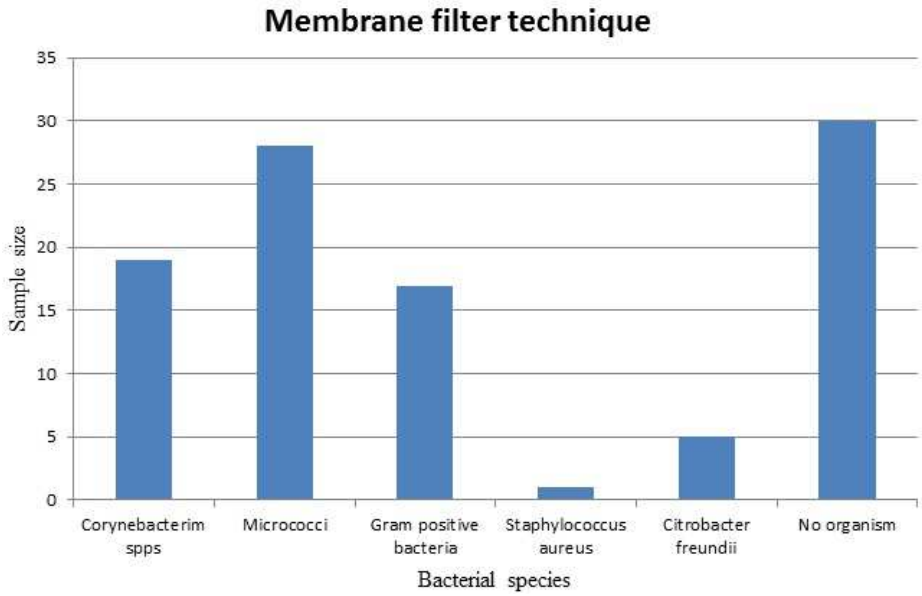

Figure 1. Organisms isolated based on membrane filter techniques.

Table 2. Spectrum of bacteria isolated from membrane filter techniques.

Organisms isolated from Membrane Filter techniques	Category (A/c to McCardy's Rule)			
	A	**B**	**C**	**D**
Corynebacterium spp.		✓	✓	
Micrococci		✓	✓	
Gram positive bacilli		✓	✓	✓
Staphylococcus aureus				✓
Citrobacter freundii			✓	✓
No organisms	✓			

4. DISCUSSION

Majority of people of new housing colonies uses ground water for domestic purpose in Belagavi City. In this context, the present study was aimed to check the suitability of ground water as well as surface water for drinking purpose by considering various bacteriological parameters like total coliforms, fecal coliforms, fecal streptococci and presence or absences of salmonella by using multiple tube method and membrane filter techniques.

Ten wards namely ward no. 1, 2, 3, 4, 5, 6, 9, 10, 19 and 43 were chosen for collection 100 samples from January to June to unite two seasons; summer and monsoon. Among these wards, out of hundred samples, twelve samples were categorized among category C and D according to McCardy probability rules and are given in the Table 3.

The remaining samples were categorized under category A and category B. From these data, 88% of water on these wards was potable while 12% were at risk. These unacceptability for drinking might be either by structural faults or poor maintenance of pump plinth or by improper disinfection of equipment.

According to data collected from membrane filter technique, the bacteriological analysis of water revealed the identification of *Corynebacterium*, Micrococci, Gram positive bacilli, *Staphylococcus aureus* and *Citrobacter freundii*. These organisms were categorized according to McCardy probability rules and are given in the charts above. Whilst the presence of indicator coliforms, like *E. coli*, pathogenic fecal coliforms, fecal streptococci, diarrheal *Salmonella* and *Shigella* were not seen.

Table 3. Grading of samples from different wards and sources according to McCardy's rule.

S. N	Ward no.	Sources	Grade	Category
1.	2	Open Well	50	C
2.	2	Municipal water	90	D
3.	5	Corporation water	35	C
4.	5	Corporation	50	C
5.	5	Open Well	90	D
6.	6	Open Well	35	C
7.	9	Open Well	50	C
8.	43	Cooler I	90	D
9.	43	Cooler II	90	D
10.	43	Cooler III	90	D
11.	43	Cooler IV	50	C
12.	43	Cooler V	40	C

The bacteriological analysis of water determines the potability of water [20]. According to Indian Standard (BIS 1981) throughout the year 95% of samples should not contain any coliform organisms or should not be detectable in 100 ml of any two consecutive samples and no sample contains E. coli [21]. The desirable limit of coliforms in water is 10MPN/100 ml (ISI) [20]. And our result shows less than 10MPN/100 ml. From the results, the isolated organisms; Micrococci, *Corynebacterium* and Gram positive bacilli are bacterial contaminants of water. While *Staphylococcus aureus* and *Citrobacter freundii* are the possible pathogens but again they might be the contaminants as *Staphylococcus* is a common skin commensals and *Citrobacter* is common coliforms. Out of 100 samples only one sample came to be *Staphylococcus aureus* and five samples were *Citrobacter freundii*. The result has shown that less than 95% of samples showed no pathogenic coliforms and no sample had *E. coli*.

5. CONCLUSION

The present study indicates that the water sources of Belagavi City are mostly potable. By comparing the membrane filter and MPN methods, 88% of water sources are potable and no pathogenic coliforms or fecal streptococci or *Salmonella* or *Shigella* were found. Most organisms found were bacterial water contaminant. Hence, it is necessary to make regular sanitary checks on equipments, look for and correct structural faults and proper maintenance of pump plinth and make proper disinfection of equipments.

Author Contributions: VPM: conceptualized the study, developed the methodology, result interpretation, sample collection and field documentation. VPM, ST and SK: introduction, discussion, conclusion and references. ST and VPM: analyzed the result. SAH: study supervision. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflicts of interest.

Acknowledgments: The authors acknowledge the management of Jawaharlal Nehru Medical College, Belagavi- 590010, Karnataka, India, and shows gratitude to all who helped and supported in completing this research.

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