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Evaluation of bacteriological water quality, Bangalore- in view of public health.

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From a public health perspective, access to sufficient amounts of clean and safe drinking water is a crucial issue. The provision of good quality household drinking water is often regarded as an important means of improving health. The study was undertaken to analyze the bacterial water quality in Bangalore district, Karnataka. The bacteriological analysis was carried out using the multiple tube technique (MPN- Most Probable Number) for detection of faecal coliform and subsequently organisms present in the sample of water were identified following standard methods. The identified organisms include *E. coli*, *Staphylococcus* species, *Citrobacter* species, *Enterobacter*, *Klebsiella pneumonia*, *Proteus* species, *Pseudomonas*, *Serratia* species. 60% of the isolated organism being of the family Enterobacteriaceae. The total heterotrophic plate count (THPC) gave a range of $1.5*10^4$ to $2.2*10^4$ CFU/ml while the total coliform plate count (TCPC) gave a range of $2*10^3$ to $8.3*10^3$ CFU/ml. The presumptive faecal coliform ranged between 0-180 coliform per 100 ml. *E. coli* / fecal coliform were detected in 60 % of the water sample.

Keyword: MPN, heterotrophic plate count, total coliform, presumptive faecal coliform, Colony forming Units (CFU).

1. Introduction

Drinking water quality has become a critical issue in many countries, especially due to concern that freshwater will be a scarce resource in the future, so a water quality monitoring program is necessary for the protection of freshwater resources [1]. Water is vital to our existence in life and its importance in our daily life makes it imperative that through microbiological and physicochemical examination be conducted on water. Bacteriological assessment particularly for coliforms, the indicators of contamination by faecal matters is therefore routinely carried out to ascertain the quality and potability of water to ensure prevention of further dissemination of pathogens. Potable water is the water that is free from disease producing microorganisms and chemical substances that are dangerous to health [2]. Water is a common resource quite abundant in nature but unfortunately not readily available to man in the form desired. Water is fundamentally important to all plants, animals and man [3]. The key to increase human productivity and long life is good quality water [4]. The provision of good quality household drinking water is often regarded as an important means of improving health [5]. Since the beginning of recorded history, water has been recognized as a potential carrier of diseases. The connection between a freshwater supply and the health of an urban population was recognized by the time of the Roman Empire (27 BC) [6]. The need for determining the suitability of water for drinking and bathing purposes has been recognized since 1855 when Snow and Budd related outbreaks of typhoid fever and cholera to water contaminated with faecal wastes [7]. It is estimated that upto 80% of the ill health in developing countries are water and sanitation related [8]. In the year 2000, the estimated global burden of disease with an annual death toll of 2.2 billion [9].

Recently, according to united nation (UN) more than 5 million people die annually first from diseases caused by unsafe drinking water and lack

of sanitation. The major problems of safe drinking water are those of availability and quality [10]. Though to many people, the quality of water can only be assessed in terms of its characteristics that are clarity, color, turbidity, taste and odour. Water may meet such aesthetic requirements, yet still be unsafe in terms of its bacteriological and/ or chemical quality [11]. Therefore the study was carried out to identify the possible source of contamination of drinking water in the study area and thus proper meaningful solution.

2. Material and methods

2.1 Sample collection

The water samples from public taps intended for drinking purpose were collected using sterile 250ml borosilicate glass bottles that were sterilized in hot air oven 160 ^{0}C for 1 hour and were covered tightly until used. The samples were aseptically collected after the taps were sterilized using 99% ethanol soaked in cotton wool. The water was allowed to run to waste for 3-5 min before collection. The water samples were kept in the ice box (4-10 ^{0}C) and transported to the laboratory < 4 hours of collection. All samples were analysed within 24 hours of collection [8, 12, 9].

2.2 Enumeration of bacteria

A fourfold serial dilution was made on nutrient agar for heterotrophic plate count while total coliform count on Mac Conkey agar [12].

2.3 Identification and characterization of isolates: The pure isolates were identified following a four step analysis. The step employed is cultural examination, microscopic examination, biochemical reaction and carbohydrate utilization test [13].

2.4 Test for coliforms (multiple tube technique/ most probable number) The standard method of analysis for the test of presence of coliform in water sample was followed in the three stages namely; presumptive, confirmed and completed tests as prescribed by [8, 13].

3. Results and discussion

Bacteria isolated from the fifteen selected drinking water sample are shown in Table. 1, while the results of THPC (total heterotrophic plate count) and total coliform plate count (TCPC) are shown in Table 2. The distribution and densities of the microbial groups enumerated in 15 selected drinking water taps are shown in table 3. The results of the MPN tests for the occurance of the presumptive coliform shown in table 4. The graphical representation of the classification of the tested drinking water is indicated in table 5.

Fifteen selected water samples was analysed for microbiological qualities. Several bacterial isolates majority of the Enterobacteriaceae were identified *E. coli*, *Enterobacter aerogenes*, *Serratia*, *Klebsiella* and *Citrobacter* (Table 1). Bacterial proliferation in drinking water distribution networks and the presence of fecal contamination indicator bacteria pose the problem of compliance to water quality health regulations [14].

Although microbial growth and /or regrowth phenomena in distribution networks certainly depend on multiple factors, natural organic matter in treated water has a determining effect because it provides a carbon and energy source for heterotrophic bacteria, including the coliform bacteria [15]. Heterotrophic bacteria are largely responsible for the process of organic matter decomposition. The recycling of minerals in aquatic ecosystem is also made possible by heterotrophic bacteria. The presence of heterotrophic bacteria in public water supplies is seldom considered as public health threat. According to WHO standard, every water sample that has coliform must be analyzed for faecal coliforms (*E. coli*) [16] with a view to ascertaining contamination with human or animal waste and possibly pathogenic bacteria. Many pathogenic (disease causing) bacteria are also abundant in the environment. The important pathogens identified were *Staphylococcus* Sp., *Bacillus* Sp. These organisms have been variously implicated in gastrointestinal disorders such as diarrhea and other associated symptoms [6] and upper respiratory tract infection. Opportunistic pathogen such as *Klebsiella pneumonia*, *Serratia marcenses*, *Proteus* Sp. *Citrobacter* Sp. *Enterobacter aerogenes* were also identified. (Hospital acquired infections) [12].

Table 1: Identified Bacterial Isolates

Colony characters	Morphology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Bacteria
Smooth & circular translucent	Short rods in single	–	+	+	–	+	–	–	–	AG	AG	AG	A	AG	AG	*E coli*
Swarming colonies creamish colour	Short rods	–	+	+	–	+	–	–	+	AG	A	A	AG	AG	A	*Proteus* Sp.
Pigmented red colonies with irregular edges	Short rods	–	+	+	–	+	+	–	–	AG	A	AG	AG	AG	A	*Serratia marcensens*
Pigmented fluorescent green smooth colonies	Small rods	–	+	+	+	+	–	–	+	AG	–	A	AG	A	A	*Pseudomonas aeruginosa*
Raised small smooth edge	Long rods	+	–	+	+	+	–	–	–	AG	A	–	A	–	–	*Bacillus* Sp.
Cream medium size circular	Short rods	–	+	+	–	–	+	–	+	AG	AG	AG	AG	AG	AG	*Citrobacter*
Large smooth circular cream irregular edge	Cocci in clusters	+	–	+	–	+	–	+	–	AG	A	AG	A	AG	AG	*Staphylococcus* Sp.
Small colourless circular and elevated	Short rods Coccobacilli	–	–	+	–	–	+	–	–	AG	–	–	–	–	–	*Acienitobacter* Sp.
Large circular smooth mucoid colony	Short rods Coccobacilli	–	–	+	–	+	–	–	+	AG	AG	AG	AG	A	AG	*Klebsiella pneumonia*
Circular creamy with wavy edge	Short rods	–	+	+	–	+	+	–	–	AG	AG	AG	AG	AG	AG	*Enterobacter aerogenes*

1. Gram stain 2. Motility 3. Catalase 4. Oxidase 5. MR 6. VP 7. Coagulase 8. Urease 9. Sorbital 10. Lactose 11. Sucrose 12. Mannitol 13. Celliobiose 14. Glucose (A-acid G-gas)

Table 2: Showing the total viable bacterial count (CFU/ml)

Code	Heterotrophic plate count	Total coliform plate count
1	$3.8*10^4$	$2.5*10^3$
2	$3.65*10^4$	$2.2*10^3$
3	$4.2*10^4$	$4.5*10^3$
4	$5.1*10^4$	$6.2*10^3$
5	$2.7*10^4$	$2.0*10^3$
6	$1.06*10^4$	$8.5*10^3$
7	$5.05*10^4$	$3.0*10^3$
8	$4.44*10^4$	$2.5*10^3$
9	$2.8*10^4$	$2.5*10^3$
10	$1.09*10^4$	$8*10^3$
11	$2.0*10^4$	$2.5*10^3$
12	$6.2*10^4$	$2.3*10^3$
13	$4.3*10^4$	$4.5*10^3$
14	$2.8*10^4$	$2.0*10^3$
15	$1.1*10^4$	$7.2*10^3$

Table 3: Showing the distribution and density of bacteria in selected drinking water samples

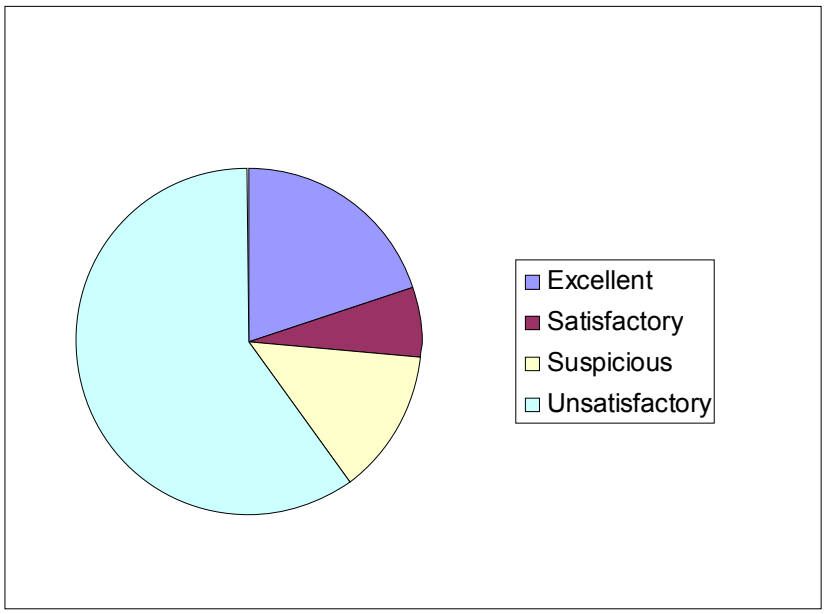
Microbial Group	Distribution			Density
	No. of water sample examined	No. of drinking water with group present	Percentage	AM
Presumptive coliform	15	12	80%	14.93
Fecal coliform	15	9	60%	
Heterotrophic plate count (aerobic) bacteria	15	15	100%	$3.35*10^4$
Total Coliform plate count (on Mac conkey agar)	15	15	100%	$4.03*10^3$

Table 4: Showing the occurrence of presumptive coliform in the tubes of the MPN

Sample	3 of 10ml	3 of 1ml	3 of 0.1ml	MPN Index per 100ml
1	1	0	0	4
2	1	0	0	4
3	1	1	1	11
4	0	0	1	3
5	2	2	1	28
6	3	1	1	75
7	2	0	1	14
8	2	1	1	20
9	1	1	1	11
10	1	1	1	11
11	0	0	0	0
12	0	0	0	0
13	2	1	0	15
14	0	0	0	0
15	2	2	1	28

Table 5: The graphical representation of the classification of the tested drinking water.
Classification of drinking water according to bacteriological tests (HMSO. 1970)

	Presumptive coliform count per 100ml	***E coli* count per 100ml**
Class I Excellent	0	0
Class II Satisfactory	1-3	0
Class III Suspicious	4-10	0
Class IV Unsatisfactory	>10	0,1 or more



A graph showing the classification of tested drinking water (in %)

Proteus Sp. belongs to the intestinal flora but is also widely distributed in soil and water [17]. *Enterobacter aerogenes* isolated from the water samples are examples of non fecal coliforms and can be found in vegetation and soil which serves as sources by which the pathogens enter the water [17]. Typical examples; such infections and their causative agents are lower respiratory infection (*Klebsiella pneumonia*) gastroenteritis (*E. coli*) septicaemia burns and wounds (*E. coli*) and urinary tract infections (*Enterobacter aerogenes* and *E. coli*). Another interesting organisms identified is *Acienitobacter* Sp. which has been known to give high coliform counts in sanitary analysis of bathing and portable water and is implicated in many infections of immuno- compromised hosts [18]. Apart from having faecal origin, some of these coliforms are natural inhabitants of soil, water, plants, human skin and animal. Some of the selected water supply pipes are situated in environments close to the contamination sources such as refuse dumps soak away pits, live stock grazing. The presence of bacteria can be attributed to the possibility of leakage in the manholes of the water supply. A gross contamination of the tanks and distribution system may be have accounted for the high counts of aerobic bacteria. Contamination by surface infiltration and percolation of rain and surface water, regrowth within pipes, detachment of the bacterial film following variation in pressure

and velocity, high anthropogenic that is human activities in the area could have contributed to the high counts obtained.

From the statistical analysis using the arithmetic mean measures, the density of THPC bacteria was as high $3.35*10^4$ with the TCPC giving $4.03*10^3$ while presumptive fecal coliform gave 14.93. This can be interpreted to mean that the coliform and presumptive fecal coliform are inclusion fractions of the THPC. The members of the heterotrophic bacterial population can grow at low nutrient level, whereas coliform have high nutritional requirement, coliform are susceptible to competition for nutrients in oligotrophic environments such as drinking water.

4. Conclusion: Safe drinking water for all is one of the major challenges of the 21st century. Microbiological control of drinking water should be the norm everywhere. In this study the bacterial group decrease in their distribution and densities thus; Heterotrophic bacteria> Total coliform>presumptive coliform> fecal coliform. Further maintaining good sanitary condition along the distribution system will help in reduction of the bacterial load in the water supply.

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