IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 11, Issue 7 Ver. II (July. 2017), PP 57-64
www.iosrjournals.org

Bacteriological Analysis of Drinking Water by MPN Method from Chennai, India

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Abstract: *Bacteriological analysis of drinking water – corporation supplied water, mineral water sold in cans and well water were analyzed by multiple tube fermentation test to find the total or presumptive coliform count, whose results were expressed as most probable number (MPN) index. Of the 20 water samples from three different sources tested, 3 were satisfactory and 17 were unsatisfactory. Of the 3 samples, which were graded as satisfactory, one was mineral water supplied in can and 2 were corporation supplied drinking water to houses through taps/hand pumps. Of the 17 samples that were unsatisfactory, 10 were mineral water in cans, 5 were corporation supplied water and 2 were well water. MPN index of water samples which tested satisfactory was < 2 per 100 ml and MPN index of water samples which were graded as unsatisfactory ranged from 38 to > 1600 per 100 ml. Coliforms isolated were Eschericia coli, klebsiella sps and Citrobacter freiundiii. Thus 85 % of the water tested (17/20) were unsatisfactory for drinking which highlight the poor quality of drinking water available to the population of Chennai and necessary measures has to be taken to improve the quality of drinking water as well as awareness among the people about the enteric diseases spread by contaminated waters and its preventive measures.*

Keywords: *drinking water, coliforms, enteric diseases, contamination, Chennai*

Date of Submission: 25-07-2017 Date of acceptance: 05-08-2017

I. Introduction

Water is the second most essential factor for the survival of human beings next to oxygen. Though 71% of earth's surface is covered with water, availability of safe drinking water to human population is becoming scarce. India being the second most populous country in the world, with more than 1.2 billion citizens, experiences water shortage with its increasing population and economic growth, and lack of adequate infrastructure. According to World Health Organization (WHO), 2.6 billion people lack access to clean water and water-related diseases leads to 3.4 million deaths mostly in children every year [1,2]. According to United Nations Children's Fund (UNICEF) assessment, contaminated water leads to 4000 deaths in children each day [3]. Global disease burden can be reduced approximately by 4% by improving water quality[1]. Diarrhea is the third most common cause of death in children under-five years age group and contributes to 13% of death in this age group, killing 300,000 children each year in India [4]. More people in India have access to mobile phones than to safe drinking water, which is of utmost importance, to maintain the health of the community. Groundwater represents an important source of drinking water and its quality may be altered due to over – extraction, microbiological and chemical contamination [5]. Effluent from septic tanks can infiltrate and cause the contamination of ground water. Factors that affect the microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements and contamination by faecal waste of human and animal origin [6]. Water is said to be contaminated when it contains infective and parasitic agents. Faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution and hence used to assess the microbiological quality of drinking water [7]. As per WHO standards, any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution should not be present in drinking water [6].

Bacterial flora of water includes:- Natural water bacteria such as Micrococcus, Pseudomonas, Serratia, Flavobacterium, Chromobacterium, Acinetobacter and Alcaligenes, soil bacteria such as *Bacillus subtilis*, *B.megaterium*, *B. mycoides*, *Enterobacter* aerogenes and *E. cloacae* and sewage bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Clostridium perfringens*, *Salmonella typhi*, *Vibrio cholerae*, *Proteus vulgaris*, *Zoogloea ramigera*, *Sphaerotilus natans*, *Haliscomenobacter hydrossis*, *Nostocoida limicola*, *Microthrix parvicella*, Flexibacter, Microscilla and Nocardia [7].

Chennai, which is located on a flat eastern coastal plains, features a tropical wet and dry climate with hot and humid weather and average annual rainfall of about 1,400 mm (55 in) from north-east monsoon winds. Cyclones in the Bay of Bengal sometimes hit the city. The Cooum River (or *Koovam*) in the central region and the Adyar River in the southern region which meander through Chennai are polluted with effluents and trash from domestic and commercial sources. The Buckingham Canal which travels parallel to the coast, link the two rivers. The Otteri Nullah, an east-west stream runs through north Chennai and meets the Buckingham Canal at Basin Bridge. Several lakes such as Red Hills, Poondi, Sholavaram and Chembarambakkam with a combined capacity of 11,057 mcft are located on the western fringes of the city and supply Chennai with potable water. Groundwater sources are mostly brackish. It has two seawater desalination plants at Minjur and Nemmeli with a capacity of 100 mld each. Telugu Ganga project brings water from water-surplus river, such as the Krishna River in Andhra Pradesh.

Chennai Metropolitan Water Supply and Sewage Board (Metro Water) controls the supply of drinking water to the residents. As of 2012, It supplies about 830 million litres of water every day through pipelines. Chennai with a population of 8.24 million as per 2011 census, suffers from water stress since, the entire basin is dependent on rainfall.

There are several newspaper reports that metro water supplied to residents are contaminated with disease causing bacteria. Broken pipes, leaking sewer lines and inadequate maintenance of old pipe networks are the primary causes of contamination. Majority of residents purchase packed drinking water (mineral water) in 20 litre cans for cooking and drinking purpose, whose genuineness again is not monitored. With this background, we wanted to study the bacteriological analysis of drinking water by doing “multiple fermentation tube test” or “most probable number (MPN)” technique, by collecting water samples (metro water, packed mineral water in cans and well water) from various locations in Chennai.

II. Materials And Methods

2.1 Collection of water samples*:* Sterile glass stoppered bottles were used to collect water samples. If water was chlorinated, to neutralize the effects of chlorine 1.8% aqueous solution of sodium thiosulphate was added to the bottles (0.23ml). Using a clean cloth, outlet of the tap was wiped to remove any dirt. Tap was turned on, for maximum flow for two minutes and then 200ml water was collected under medium flow, cap was replaced and sample brought to the laboratory for processing within 6 hours [6]. Water samples were properly labeled with full details of the source, time, place and date of collection.

2.2 Sources of water samples: Total of 20 samples were analyzed. Among these, 12 were purified mineral water sold in 20 litre cans, 7 were drinking water supplied by Chennai Corporation which was collected from hand pumps/taps at homes and railway station, syntex tanks kept at streets and 2 were well water.

2.3 Bacteriological examination: It was done by multiple tube fermentation test, to find the total or presumpive coliform count. Results of multiple tube fermentation test for coliforms has been reported as most probable number (MPN) index, since it was an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the test. It was not a count of the actual number of indicator bacteria present in the sample [8].

Double and single strength macConkey broth with Durhams tube was used for tube fermentation test. Measured amount of water samples were added using graduated pipettes. Five numbers of 10 ml volumes of water added to 10 ml double strength macConkey broth, five numbers of 1 ml water added to 5 ml single strength macConkey broth and 5 numbers of 0.1 ml water was added to 5 ml of single strength macConkey broth (table 1). The tubes were incubated at 37^0C for 48 hours. The presumptive coliform count per 100 ml of water was determined from the tubes showing acid and gas production using the probability table (Table 2). The results were interpreted as excellent, satisfactory, suspicious and unsatisfactory as given in table 3.

Table: 1 - Sample volumes and number of tubes for multiple tube fermentation test

. Volume and strength of MacConkey broth	10 ml double strength	5 ml single strength	5 ml single strength
Number of tubes included	5	5	5
Volume of water samples added	10 ml	1 ml	0.1 ml

Table: 2 - MPN index and 95% confidence limits for various combinations of positive results when 5 tubes are used per dilutions (10 ml, 1.0 ml and 0.1 ml) [9]

Combination of positives	MPN index/100 ml	95% confidence limits		Combination of positives	MPN index/100 ml	95% confidence limits	
		Lower	Upper			Lower	Upper
0-0-0	<2	-	-	4-3-0	27	12	67
0-0-1	2	1.0	10	4-3-1	33	15	77
0-1-0	2	1.0	10	4-4-0	34	16	80
0-2-0	4	1.0	13	5-0-0	23	9.0	86
1-0-0	2	1.0	11	5-0-1	30	10	110
1-0-1	4	1.0	15	5-0-2	40	20	140

1-1-0	4	1.0	15	5-1-0	30	10	120
1-1-1	6	2.0	18	5-1-1	50	20	150
1-2-0	6	2.0	18	5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7	40	5-4-4	350	160	820
4-0-0	13	5	38	5-5-0	240		940
4-0-1	17	7	45	5-5-1	300	100	1300
4-1-0	17	7	46	5-5-2	500	200	2000
4-1-1	21	9	55	5-5-3	900	300	2900
4-1-2	26	12	63	5-5-4	1600	600	5300
4-2-0	22	9.0	56	5-5-5	≥1600	-	-
4-2-1	26	12	65				

Table: 3 - Interpretation of results

Class	**Grade**	**Presumptive total coliform count (per 100 ml)**
I	Excellent	**0**
II	Satisfactory	1-3
III	Suspicious	4-10
IV	Unsatisfactory	>10

2.4 Identification of coliforms: Tubes that showed turbidity, along with acid and gas production were sub-cultured to macConkey agar and were incubated at 37^0C overnight. Colonies that grew were identified based on lactose fermentation on macConkey agar, gram stain and biochemical reactions such as oxidase test, mannitol fermentation test, motility of bacteria on mannitol motility medium, triple sugar iron (TSI) agar test, citrate utilization test, production of indole in peptone water, Methyl red test, Voges Proskauer test, nitrate reduction test etc.

III. Results And Observation

Tubes were looked for acid and gas production (fig 1). The number of tubes with acid and gas production for each volume of water added i.e. 10 ml, 1 ml and 0.1 ml (each volume in 5 tubes) were noted and the results obtained were compared to the probability table (Table 2). Of the 20 water samples from three different sources (mineral water from 20 litre cans, corporation water and well water) tested, 3 were satisfactory and 17 were unsatisfactory by presumptive coliform count by MPN method (Table 4). Of the 3 samples, which were graded as satisfactory, one was mineral water supplied in can and 2 were corporation supplied drinking water to houses through taps/hand pumps. Of the 17 samples that were unsatisfactory, 10 were mineral water in 20 litre cans, 5 were corporation supplied water and 2 were well water.

MPN index of water samples which tested satisfactory was < 2 per 100 ml and MPN index of water samples which were graded as unsatisfactory ranged from 38 to > 1600 per 100 ml. It was 38 (1 no), 50 (1 no), 90 (1 no), 240 (3 no), 500 (1 no), 1600 (1 no) and >1600 (9 no) per 100 ml of water tested. Of the 11 samples of drinking water supplied in 20 litre cans and supposed to be purified by reverse osmosis method, 10 were unsatisfactory for drinking among which 7 showed an MPN index of >1600 per 100 ml and remaining three showed an MPN index of 240 per 100 ml. Among the 7 corporation water samples which were purified by chlorination, 5 were unsatisfactory for drinking among which, two showed an MPN index of >1600 per 100 ml and the remaining three showed an MPN index of 38, 50 and 90/100 ml respectively. Among the two water samples taken from well, both were unsatisfactory and one showed an MPN index of 500 and another 1600 per 100 ml (table 5).

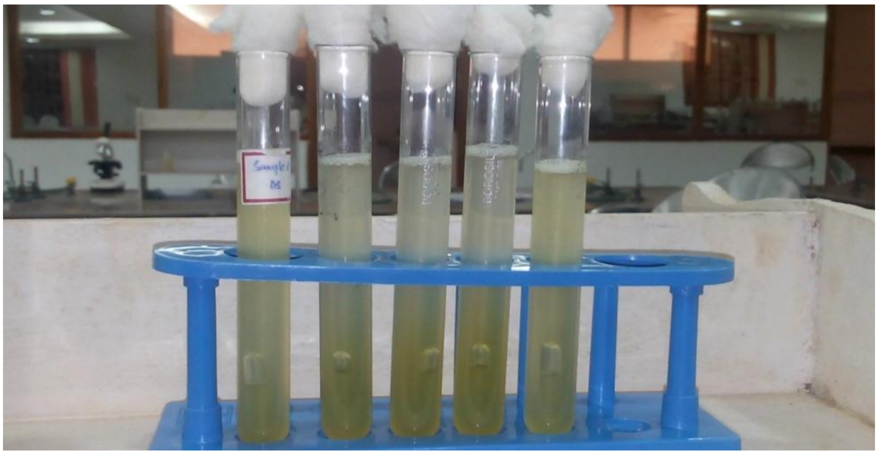

Figure. (1): MacConkey broth (10 ml double strength) showing acid and gas production

Table: 4 - Quality of water samples

Class	Grade	No. of water samples (total no – 20)	Source of water sample
I	Satisfactory	3	Mineral water in can (1) Corporation water (2)
I1	Unsatisfactory	17	Mineral water in can (10) Corporation water (5) Well water (2)

Table: 5 - MPN index of water samples when 5 tubes are used per dilutions (10 ml, 1.0 ml and 0.1ml) and coliforms identified.

S.No	Source of water	Place of collection	Combination of positives	MPN index/100 ml	Interpretation	Coliform isolated
1	Mineral water in Can	Shollinganallur	0-0-0	<2	Satisfactory	nil
2	Mineral water in Can	Nochikuppam	5-5-0	240	Unsatisfactory	*E.coli* & Klebsiella
3	Mineral water in Can	Arcot road	5-5-0	240	Unsatisfactory	*E.coli*
4	Mineral water in Can	Alwarpet	5-5-5	≥1600	Unsatisfactory	*E.coli* & Klebsiella
5	Mineral water in Can	kelambakkam	5-5-5	≥1600	Unsatisfactory	*E.coli*
6	Mineral water in Can	Saidapet	5-5-0	240	Unsatisfactory	*E.coli* & Klebsiella
7	Mineral water in Can	Porur	5-5-5	≥1600	Unsatisfactory	*E.coli* & Klebsiella
8	Mineral water in Can	Royapuram	5-5-5	≥1600	Unsatisfactory	*E.coli*
9	Mineral water in Can	Thiruvanmiyur	5-5-5	≥1600	Unsatisfactory	E.coli & *C.freundii*
10	Mineral water in Can	Thiruvottiyur	5-5-5	≥1600	Unsatisfactory	*E.coli* & Klebsiella
11	Mineral water in Can	kellys	5-5-5	≥1600	Unsatisfactory	*E.coli* & Klebsiella
12	Corporation water - syntex tank	Thiruvanmiyur	4-0-0	38	Unsatisfactory	*E.coli*
13	Corporation water - hand pump	Thiruvanmiyur –	0-0-0	<2	Satisfactory	nil
14	Corporation water – tap water	Egmore Railway station	5-2-2	90	Unsatisfactory	*E.coli* & Klebsiella
15	Corporation water – hand pump	Adyar	5-5-5	≥1600	Unsatisfactory	Klebsiella & *C.freundii*
16	Corporation water – hand pump	Palavakkam	0-0-0	<2	satisfactory	
17	Corporation water - hand pump	Thiruvottiyur	5-5-5	≥1600	Unsatisfactory	*E.coli* & Klebsiella
18	Corporation water - tap water)	Chennai central Railway station	5-2-0	50	Unsatisfactory	*E.coli* & Klebsiella
19	Well water	Thiruporur	5-5-4	1600	Unsatisfactory	E.coli
20	Well water	hriruvottiyur	5-5-2	500	Unsatisfactory	*E.coli* & klebsiella

Figure. (2): Mucoid lactose fermenting colonies of Klebsiella sps on macConkey agar

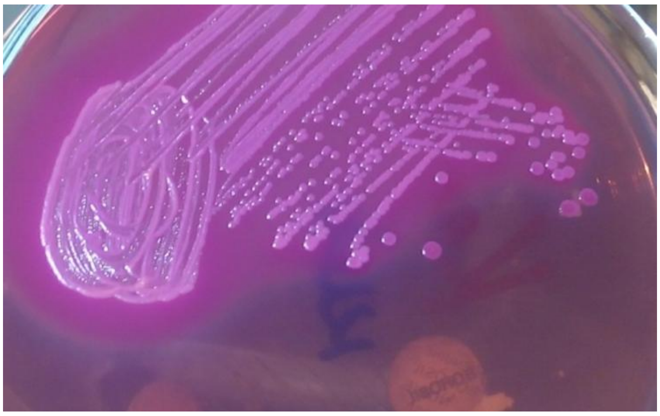

Figure. (3): Non mucoid lactose fermenting colonies of *E.coli* on macConkey agar

Figure. (4): Non mucoid lactose fermenting colonies of *C. freundii* on macConkey agar

Lactose fermenting colonies that grew from tubes positive for acid and gas production when sub-cultured to macConkey agar, were identified as *Escherichia coli* (*E.coli*), Klebsiella and *Citrobacter freundii (C. freundii*) (Table 5, fig 2, 3, 4). *E.coli* was isolated from 16 of the 17 unsatisfactory water samples. It was a sole isolate in 5 of the samples, whereas in 10 samples, it was accompanied by klebsiella and in one sample, it was accompanied by *C. freundii*. A combination of Klebsiella and *C. freundii* grew in one sample. *E. coli* was mannitol fermentation positive, it was motile in mannitol motility medium, it gave acid slant, acid butt with gas production in triple sugar iron agar test, it was negative for citrate utilization test, indole test positive, oxidase negative and nitrate reductase test positive. Klebsiella was positive for mannitol fermentation, it was non motile in mannitol motility medium, it gave acid slant, acid butt with gas production on triple sugar iron agar, indole test negative, citrate utilization test positive, oxidase test negative and nitrate reductase test positive. *Citrobacter freundii* was mannitol fermentation test positive, motile in mannitol motility medium, it produced acid slant, acid butt with gas and hydrogen sulphide production on triple sugar iron agar, indole test negative, citrate utilization test positive, oxidase test negative and nitrate reductase test positive (table 6, fig 5,6,7).

Table: 6 -Biochemical reactions of coliforms isolated from water samples

S.No	Biochemical test	Biochemical results		
		E.coli	**Klebsiella**	***C. freundii***
1	Lactose fermentation on macConkey agar	Positive	Positive	Positive
2	Oxidase test	Negative	Negative	Negative
3	Mannitol fermentation test	Positive	Positive	Positive
4	Motility on mannitol motility medium	positive	Negative	positive
5	Reaction on triple sugar iron agar medium	Acid slant/acid butt with gas	Acid slant/acid butt with gas	Aicd slant/acid butt with gas and hydrogen sulphide production
6	Indole test in peptone water	Positive	Negative	Negative
7	Citrate utilization test	Negative	Positive	Positive
8	Methyl red test	Positive	Negative	Positive
9	Voges proskauer test	Negative	Positive	Negative
10	Nitrate reductase test	Positive	Positive	Positive

Figure. (5): Biochemical reactions for *E.coli*

(A) Mannitol motility medium - mannitol fermentation positive, bacilli motile, (B) TSI – Acid slant, acid butt with gas production (C) Citrate utilization test – negative (D) Indole test - positive

Figure. (6): Biochemical reactions for Klebsiella sps

(A) Mannitol motility medium - mannitol fermentation positive, bacilli non-motile, (B) TSI – Acid slant, acid butt with gas production (C) Citrate utilization test – positive (D) Indole test – negative

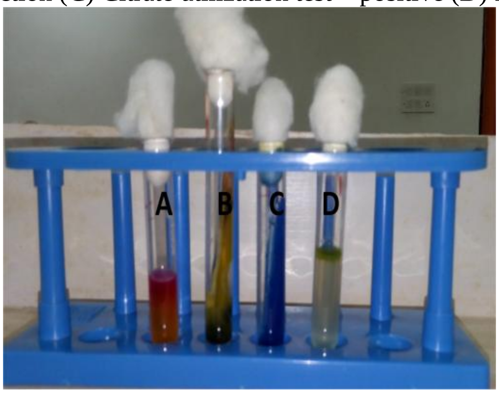

Figure. (7): Biochemical reactions for *C. freundii*

(A) Mannitol motility medium - mannitol fermentation positive, bacilli motile, (B) TSI – Acid slant, acid butt with gas production and hydrogen sulphide production (C) Citrate utilization test – positive (D) Indole test – negative

IV. Discussion

Water is indispensible for life. Urbanization, overpopulation, environmental pollution and ever increasing demand, pose a risk for the availability of safe drinking water. The study results shows that 90 % (10/11) of mineral water supplied in 20 litre cans which are purchased by people at cost ranging from Rs. 35 to 45, are unsatisfactory for drinking. Among the 7 samples of Corporation supplied water collected from different places of Chennai, 71 % (5/7) were unsatisfactory for drinking. We included 2 well water samples in the study to know the bacteriological status and both were found to be unsatisfactory. Though the sample size is small, the results highlight the poor quality of drinking water available to the population of Chennai. Among the 10 mineral water samples, which were unsatisfactory for drinking, 7 samples showed an MPN index of more than

1600/ 100 ml of water tested, whereas only 2 among the 5 corporation supplied water showed such high MPN index. This shows that mineral water being attested for its sterility is more unreliable than corporation water. In Pakistan, MPN count of coliforms/ 100ml, was 2.2-16 for tube well water samples, 1.1 - > 23 for water from distribution network and 2.2 to >23 for stored tank water [10]. According to a study done by Murugesan *et al* in 2015, 70% of water supplied in bubble top cans in Chennai are within exceptional boundary of WHO[11] whereas in our study 90 % (10/11) of water supplied in 20 litre bubble top cans (mineral water) are unsatisfactory for drinking. Out of ten well water sample processed in Kottayam district of Kerala state, 3 grew *Salmonella typhi* and six grew *Vibrio cholera*[12]. In western Maharastra 49.8% of the 313 samples which were collected from wells, tank, community standpost, handpumps, percolation lakes, and streams, and from households were polluted, and water from piped supply showed a pollution rate of 45.9%[13]. In patiala, from the state of Punjab, 28% of ground water supplied through taps contained unsatisfactory microbiological quality[14]. Twenty three out of the 24 samples collected from wells in Ethiopia showed presumptive bacterial count above the permissible limits for drinking water[15]. In Nepal, 87.5 % of the bottled water samples were found to be contaminated with heterotrophic bacteria[16]. All the 36 water samples sold as packed drinking water from road side vendors in Delhi, were contaminated with coliform organisms in the range of 14 to 1600 per 100 ml of sample. The bacteria isolated were *E. coli* (61 %), Salmonella (25 %) *S. aureus* (14 %) and *P. aeruginosa* (53 %)[17]. *E.coli*, Enterobacter, Klebsilla, salmonella and Shigella were isolated from water samples tested at Indore in Madhyapradesh[18].

Enteric pathogens such as salmonella, shigella, vibrio, rota virus, *Entamoeba histolytica*, *Giardia lamblia*, cryptosporidium spp, and several worms such as *Ascaris lumbricoides Trichuris trichiura* are transmitted through drinking water and are predominantly faecal in origin[19]. Globally more than 250 million cases of waterborne diseases are reported, which leads to over 25 million deaths[20] . Inadequate drinking water contributed to 5,02,000 diarrhoea deaths and inadequate sanitation lead to 2,80,000 deaths according to a retrospective data analysis collected from 145 countries[21]. People constantly exposed to poor quality of drinking water may develop resistance to infection, but those of extreme age groups like infants and elderly may suffer, due to under developed and waning immunity respectively. Other high risk group individuals, are those whose immunity is compromised either by immune deficiencies or by diseases such as malignancies and AIDS or transplant recipients who are on immunosuppression. Also people travelling from a developed country to a developing country where public sanitation is not good will also suffer from diarrheal disorders.

Nearly one tenth of the city households still drink water directly from taps. Broken water pipelines, overflowing sewage, open defecation, corroded pipelines and inadequate maintenance of old pipe network, whose average age is 50 years, are the primary causes of contamination. Rusty pipelines run through the city's filthy underbelly, facilitating seepage of sewage and muck. These reasons may nullify the effect of chlorination done at the supply point. Two well waters which are positive for coliforms, indicates the status of ground water which in turn reflects the activities on the surface. Well water may be contaminated from activities on the land surface, such as releases or spills from septic systems, open defecation, moving of pollutants through plant roots and animal burrows.

Actually speaking the mineral water available in cans and bottles as the name implies, is the purified water fortified with requisite amounts of minerals such as Barium, Iron, Manganese, etc. which can be absorbed by human body. It can be obtained from natural resources like springs and drilled wells or it is fortified artificially by blending and treating with mineral salts. It should be manufactured and packed under hygienic conditions. Due to unavailability of potable drinking water or due to its bad taste or color, or due to lack of faith in the water supplied by the Corporation, majority of population in urban areas irrespective of whether rich or poor started using the so called mineral water or packed drinking water supplied in bottles, cans etc. It is widely accepted practice to use them at homes, offices, restaurants, railway stations, airport, bus stands and hospitals. There is a great demand, since every house hold use them for even cooking, and hence, explosion of companies marketing "safe drinking water" in bottles and cans. This leads to, mushrooming of fraudsters, across the country to make quick bucks by flooding the market with polluted and harmful water. They may just fill bottles or cans with water from wells or taps and palm them off as "bottled water". Water purifying plants may be set in garages or cowsheds or in the backyards of homes without trained staff. The Bureau of Indian Standards (BIS), which regulates the industry, has laid down that water must be subjected to processes called pressure sand filtration, activated carbon filtering, reverse osmosis, ozonisation and ultraviolet treatment before it’s packaged and sold. Many function without the mandatory approvals from the BIS as well as Food Safety and Standards Authority of India (FSSAI). One needs an investment of up to Rs 20 lakh to run a legal and hygienic plant, but many jump into the business with just Rs 2.5 lakhs. Consumers should check whether the container is properly sealed, has manufacturing date and batch number and ISI mark which the BIS awards. If liable, BIS or local health authorities should be alerted.

V. Conclusion

The study highlights that drinking water available to the people of Chennai, whether, it is supplied by the corporation or sold as mineral water in cans by various private distributors, is highly contaminated. Chennai Metropolitan Water Supply and Sewage Board which is responsible for the supply of safe drinking water to the community of Chennai, should be stringent towards adequate chlorination, safe distribution from storage point to the receiving end through the use of non corrosive pipelines, replace broken pipelines and plug leakages of sewage pipes for the safe delivery. Mushrooming of mineral water companies should be checked by BIS and FSSAI, and those failing in maintaining the standards should be heavily fined and license cancelled. Quality of the well water can be improved by practicing proper waste disposal, creating storm water drains, controlling sewage spills and avoiding open defecation. Drinking water sources should be tested regularly to determine its microbiological quality to prevent outbreak of enteric diseases. People should be educated about the spread of water borne diseases and its control measures. The outcome of our study clearly reveals the deprived quality of water available to the population of Chennai, though the study sample is small.

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Balaraman Ravindrran Malathy. "Bacteriological Analysis of Drinking Water by MPN Method from Chennai, India." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 11.7 (2017): 57-64.