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Research Article

BACTERIOLOGICAL EXAMINATION OF DRINKING WATER AFTER PURIFICATION BY DIFFERENT METHODS

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ABSTRACT

Water was tested after purification with methods like chlorination, iodination, boiling, cloth filtration, candle filtration, polyethylene membrane filtration, treatment with alum, treatment with activated carbon and treatment with ultra violet rays. Water was tested by using two microbiological methods namely Multiple tube Test and Membrane Filtration method. Of the methods of purification used chlorination, iodination, boiling, treatment with ultra violet and treatment with activated carbon disinfect water to make it potable. Methods like treatment with alum, candle filtration and cloth filtration reduce the bacterial content of the water but do not make the water potable. Of the methods which disinfect water to potable levels the cost effective method among those found to disinfect is chlorination. Membrane Filtration method is more sensitive than Multiple Tube Test for bacteriological assessment of water.

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INTRODUCTION

Water is one of the most important elements for all forms of life. It is indispensable in the maintenance of life on earth. Despite this, humans continue to pollute water sources thus causing water borne illnesses. The World Health Organization estimates that 88% of diseases are attributable to unsafe water supply, sanitation and hygiene (Chatterjee *et al*, 2007). Water borne diseases are caused by pathogenic microorganisms like bacteria (*Escherichia Coli*, *Klebsiella*, *Shigella*, *V cholera*, *Salmonella Typhi*), viruses (*Hepatitis A*, *Hepatitis D*, *Polio Virus*, *Rota Virus*) and parasites (*E histolytica*, *Giardia lamblia*, *Ascaris lumbricoides* etc). Most of the area of our country and many developing countries do not get treated water. Thus there is need to treat water at home before use to prevent water borne diseases. Various modalities available for purification includes various procedures like boiling, filtration, distillation, sedimentation, slow sand filters, activated carbon, chlorination, fluorination, flocculation, membrane filters, ultraviolet light(U.V.), reverse osmosis (R.O.) etc. Method of purification should be available at a low cost so that even the poor socio-economic can make use of it. So a study was undertaken to evaluate the different available methods of water purification.

MATERIALS AND METHOD

Type of Study: Prospective study

Study Design:

1. Sample collection and transport
2. Processing
3. Data collection

Duration of Study: 4 months

METHODOLOGY

Sample collection and transport-For this study drinking water was collected from various sources. A polluted source was thus identified from the collected samples. This water sample was tested for bacteriological examination by 2 methods namely multiple tube technique and Membrane filtration method the results were noted. Then water samples were purified by different methods of purification namely-

- Polyethylene membrane filtration
- Chlorination
- Treatment with alum
- Iodination
- Boiling
- Cloth filtration

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- Activated Carbon
- Candle filtration
- Treatment with Ultra Violet rays

Purified water samples were collected in sterile glass bottles. The collected water samples were preserved at a temperature between 4°C till further testing.

Processing and Data Collection

Water samples thus obtained on passing through various filtration methods were processed by two microbiological methods namely

1. Multiple tube technique (Senior BW *et al*, 1996)
2. Membrane filtration method (Senior BW *et al*, 1996)

Multiple Tube technique

In Multiple Tube Test method water was tested for presence of coliforms. Acid and gas production is tested by adding it to equal volume of MacConkey's double strength broth and incubating at 37°C.

1. 10 mL water was added to five test tubes containing equal amount (10mL) of MacConkey's double strength broth (HIMEDIA M539) with inverted Durham's tubes (for detection of gas production).
2. 50 ml of water was transferred in a bottle containing equal amount (50mL) of MacConkey's broth (HIMEDIA M 539) with inverted Durham's tubes.
3. The bottle and the test tubes were incubated in an incubator at 37°C for 24 h.
4. Test tubes and bottle showing acid and gas production were counted and the test tubes and bottle are further incubated for another 24 hours which totals to 48 hours.
5. After incubation, the number of tubes and bottle in which lactose fermentation with acid and gas production in Durham's tube has occurred was counted.
6. Tubes and bottles showing acid and gas productions were further tested for thermo tolerance. Thermo tolerance shows presence of *E.coli*.
7. 2 loop full each from the tube showing acid and gas production were transferred each to 5mL Peptone water with indole paper and 5mL Brilliant Green Lactose Bile Broth with inverted Durham's tubes.
8. These tubes were incubated in a water bath at 44°C for 24 hours.
9. Brilliant Green Lactose Bile Broth tubes showing acid and gas production and Peptone tubes showing indole positive were counted.
10. By referring to probability tables the MPN (Most Probable number) of coliform in 100 ml water sample was estimated.

MPN of coliform gives suitability of water fit for drinking purposes.

Membrane filtration

1. Under aseptic precautions sterile Cellulose Nitrate Membrane of diameter 47mm and having pore size 0.45µm (HIMEDIA SF95H) was placed in Millipore filter.

2. 100 mL of water was passed through the membrane with negative pressure created by vacuum.
3. The membrane thus retained all the bacteria present in the water.
4. The membrane was then placed on the petri dish containing MacConkey's agar upside up.
5. This agar plate was incubated at 37°C for 24 hours.
6. Each bacterium forms a colony on the Cellulose Nitrate Membrane. Thus number of colonies was counted which correspond to the number of bacteria present in 100mL of water.
7. If any colonies grow a single colony was used to identify whether the coliform is thermo tolerant. Thermo tolerance shows presence of *Escherichi coli*.
8. Single colony was inoculated each in 5mL Peptone water with indole paper and 5mL Brilliant Green Lactose Bile Broth with inverted Durham's tube each.
9. These tubes were incubated in a water bath at 44°C for 24 hours.
10. Brilliant Green Lactose Bile Broth tubes showing gas production and Peptone tubes showing indole reaction positive showed thermo tolerance and hence presence of *Escherichia coli*.

Based on both the methods water could be classified and certified safe or unsafe for consumption.

RESULTS

Table 1 Water Quality after different methods of purification

	Multiple Tube Test	Membrane Filtration Technique	Water quality
Untreated	>18	>200	Unsatisfactory
Polyethylene membrane	>18	>200	Unsatisfactory
Chlorine	0	0	Excellent
Alum	>18	50	Unsatisfactory
Iodine	0	25	Unsatisfactory
Boiling	0	0	Excellent
Cloth filtration	>18	70	Unsatisfactory
Activated carbon	0	0	Excellent
Candle	>18	130	Unsatisfactory
Ultra violet radiation	0	0	Excellent

Of all the methods used to treat water, boiling, chlorination, treatment with activated carbon and treatment with ultra violet gave water of excellent quality by reducing the bacteria count to '0'. Treatment with polyethylene membrane, treatment with alum, cloth filtration and candle filtration gave unsatisfactory results and did not reduce the bacteria count significantly. Iodination gave different results with different microbiological methods.

The results obtained by purifying water by different methods differed for Iodination. Water purified by iodination showed the 'most probable number' 0 and showed the presence of 25 colonies on Cellulose Nitrate membrane which indicates 25 coliforms in 100mL of water. The difference of counts can be due to high sensitivity of the Membrane Filtration technique and its ability to detect even the smallest quantity of bacteria.

Cost Effectiveness

These methods when compared for their cost effectiveness show the following results

Table 2 Cost of purification by different methods

No	Method	Cost in Rs (per L)
1	Chlorination	0.027
2	Iodination	0.040
3	Boiling	0.34
4	Activated carbon	0.4
5	Ultra Violet Radiation	1.00

Thus the cost effective method found to be was chlorination

DISCUSSION

Various attempts are made by each household to improve the quality of water it drinks and to prevent water borne diseases affecting the former. A method which is effective in clearing the bacterial load as well as available at a low cost can be used by the entire population. The study aimed to decide a cost effective method of purification which clears the bacterial load also.

Both the microbiological methods of water testing suggest that the water treated by polythene membrane filter, alum, candle filter and cloth filtration is not potable. The results of our study are matching with that of Olayemi *et al*, 2006 and Dies *et al*, 2003.

Amongst the different methods chlorination, boiling, iodination, treatment with activated carbon and treatment with ultra violet rays are effective methods to eliminate bacteria. Each method is having its own advantages and disadvantages. Referring to table 1,

Chlorination

Chlorination is a widely used method for treatment of water on both small and large scale. The water after chlorination showed no colonies of bacteria on the Cellulose Nitrate Membrane of the membrane filtration method and the Multiple Tube Test gave the Most Probable Number as 0. Thus the water is considered to be of excellent quality. As per the study done by Supriya P *et al*, 2006, chlorine is a bactericidal agent due to its strong oxidising property which affects its cellular property. Our study showed results similar to the study done as chlorine has killed the bacteria. Jeremy Parr *et al* suggested a contact time of 30 minutes best application of chlorine to water and consumption. In our study we allowed a contact time of 30 minutes and chlorine worked within this time by killing the bacteria.

Iodination

Iodination gave different results on two different methods of testing. As per study by Marchin G *et al*, 1987 bacterium *Escherichia Coli* was deactivated by iodine by attaching it to the cytoplasmic membrane when water containing it was passed through quaternary triiodide resin, tiocide (Marchin G *et al*, 1987). The results of the study done by Marchin G. L. and Fina L. R. are matching to results obtained on Multiple Tube Test. Study done by Supriya P. *et al*, 2006 states that iodination is 20 times costlier to chlorination. It also says that iodine is required in higher quantity than chlorine (Supriya P

et al, 2006). Iodination is costlier than chlorination though based on the same principle

Boiling

By both the methods boiled water was found to be of excellent quality. Though not tested for viruses and parasites boiling is known to be effective with these too (Williams L *et al* 2001). The World Health Organization (WHO) *Guidelines for Drinking Water Quality* says that rolling boiling indicates that a temperature for disinfection is achieved which is matching with results of our study (WHO guidelines, 2004). Thus boiling can be suggested as a method of purification subject to availability and affordability of fuels. In case of natural calamities boiling can be the dependable method. Also the cost of the fuel can be reduced by the use of cow dung cakes and biogas as fuels.

Activated carbon

Results by both the methods show that water is of excellent quality when treated with activated carbon. When the filter is saturated and can cause a higher coliform count than untreated water which is called 'breakthrough'. Hence the time of changing the cartridge should be kept track of (Dvorak *et al*, 2013). As per NEB guide activated carbon cannot remove bacteria, viruses, calcium, magnesium, fluoride, nitrate *et al*. This is contrary to our finding. As per NEB Guide, activated carbon can remove organic chemicals as well as harmless taste and odour producing compounds. It can remove chlorine, chloramines, and trihalomethanes (THM) which are by-products of chlorination which increase risk of certain cancers. It can remove lead which may be added during flow from old pipes (Dvorak *et al*, 2013) Thus it is a good method to be used in combination with chlorine. It is particularly good for household purpose as it removes lead which can be added during flow from old pipes. Ann Lemley *et al*, 1995 advocates slow flow of water through activated carbon so that the contact time is increased. Thus we can suggest the use of activated carbon for household purpose with slow flow of water through it and as a combination with other methods.

Ultra Violet

Both microbiological methods of water testing showed water treated by ultra violet rays was free of bacteria. UV light causes physical changes to the DNA structure preventing replication, whereas chlorination directly damages the cellular structure causing cell death. UV is effective in disinfecting water containing *E. coli* and other bacteria. Advantages of UV LEDs as the source of UV radiation resolve two of the major disadvantages of the vapour lamps listed above assuming that UV LEDs will have a longer life span than the lamps and will approach the lifetime of visible light LEDs (Crawford *et al*, 2005).

In treating water at homes, single or combination of methods can be used. A combination of filtration and disinfection can be used to remove physical and bacteriological contaminants respectively. Of the disinfecting methods Iodination and Chlorination have residual effects on the water treated. But they have to be used carefully to avoid under and over addition of chemical. Boiling does not have residual effect on the treated water. But their use is easier to teach on mass scale as it is not dose dependant.

Physical methods include candle filters, handkerchief, polyethylene filter, activated carbon, and sedimentation. Physical methods hold back the impurities due to contaminants' physical properties. Chemicals are better effective when added to clean water. A combination of a physical and a chemical method can give a better outcome.

Multiple Tube Test v/s Membrane filtration method

Samples on testing by Multiple Tube Test and Membrane Filtration methods, showed similar results by both methods. Difference in the readings among the two methods was found for Iodination. In Iodination, the count on Multiple Tube Test is '0' while on Membrane filtration method the colony count is 25. Hence we may say that, Membrane Filtration method is more sensitive.

CONCLUSION

Clearing of the bacterial load of water can bring down the occurrence of diarrhoea in the community. Diarrhoea being a major cause of death among children especially of the lower socioeconomic strata it can be prevented bringing down the mortality rate.

Simple methods of purification of water like boiling, iodination, chlorination, activated carbon work at par with sophisticated methods like UV treatment as far as bacteria are concerned. Chlorination of water is most cost effective method which can be used as method of purification on small and large scale. Care should be taken during adding chlorine to add it in the right amount to avoid its excess which leads to altered taste and by products or less which won't purify water adequately. A combination of a physical and a chemical method is better. During emergency situations like natural or man-made calamities boiling is the most reliable method of water treatment.

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