BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Bacteriological Assessment of Drinking Water Using H_2S Test

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ABSTRACT

Drinking water is an important resource that needs to be protected from pollution and biological contamination. Water borne diseases continue to be a dominant cause of water borne morbidities and mortality all over the world and helps in the spread of enteric diseases.

A total of 38 water samples were tested by the new H_2S test methods. According to the H_2S test, 24 (63.15%) of the 38 samples had fecal coliforms, while 14 (36.84%) had no faecal contamination. From the 24 positive samples 47 isolates were investigated for the identification of faecal contamination. Out of 47 isolates, 21 (44.70%) were *Pseudomonas*, 14 (29.78%) were *E.coli*, 6 (12.76%) were *Salmonella*, 4 (8.5%) were *Klebsiella* and 2 (4.25%) were *Staphylococcus* spp. It is evident from the data presented in this work that H_2S producing organisms are consistently associated with the presence of coliforms in water. Enteric bacteria such as *Salmonella*, and certain species of *Klebsiella* also produce H_2S. The predominant H_2S producing bacteria found in polluted drinking water in the present study were *Salmonella* spp., *Klebsiella*, *Staphylococcus*, and H_2S producing variants of *E.coli*.

Thus study indicated that the H_2S test is a reliable and alternative indicator of fecal contamination in drinking water quality surveillance and screening of large number of water samples in short duration in the field where laboratory facilities are limited.

Keywords: Bacteriological water quality, H_2S test, Coliform, enteric infection

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Received 2 June 2016, Accepted 20 July 2016

Please cite this article as: Bhutada SA *et al.*, Bacteriological Assessment of Drinking Water Using H2S Test. British Journal of Medical and Health Research 2016.

INTRODUCTION

On the earth human life is depends on water. Water is indispensible for the life for drinking as well as other daily purposes. The transmission of disease caused by pathogenic microorganisms in drinking water represents a health risk in all societies. Therefore, unpolluted safe drinking water is one of the primary requisites for healthy human life (WHO, 1993, Pillai *et al.*, 1999,)[1,2.] According to the WHO (2001)[3], drinking water should be free from *Salmonella* spp. and thermo tolerant coliforms (*E. coli*). It is difficult and expensive to test for the all pathogenic organisms that may be present in contaminated drinking water. Many limitation and complication have been associated with faecal contamination testing (Doyle and Ericson, 2006)[4]. Therefore indicator organisms are used to determine the risk that these organisms might be present in drinking water.

A comprehensive field investigation in several parts of India has revealed that the presence of coliforms in drinking water is associated with hydrogen sulfide producing organisms. The H_2S test is reliable and simple to perform, and will be especially useful for screening rural water supplies and for large scale screening of urban water supplies where resources, time, manpower, and laboratory facilities are limited (Manja *et al*., 1982; Gawthorne, *et al.*, 1996,)[5,6]. Thus modified H_2S field test was found to be more suitable, reliable inexpensive, easy to perform and most useful to detect FCo in drinking water within 24h. It also proved suitable to assess microbial quality of drinking water and useful in screening for large no. of sample for places where time, man, and laboratory facility are very poor. Therefore H_2S test is recommended for routine monitoring of water for Feacal Coliforms (Genthe, and Franck, 1999; Hirulkar and Tambekar, 2007)[7,8].

The aim of this work is analyse the microbiological quality (faecal contamination) of drinking water of MGM campus of Aurangabad. Thousands of patients comes everyday and they consume water, departmental staff also used this water sources for drinking purpose. There is more chance of bacterial contamination of drinking water of faecal and non faecal origin due to various activities of public. Therefore, it is necessary to checking out microbial quality (faecal contamination) of drinking water of the campus.

MATERIALS AND METHOD

Sample collection:

A total of 38 drinking water samples were collected between Januarys to march 2012 from different departments of MGM Campus. The study was done at lab of Microbiology in MGM institute. Each sample had a name, of combination of alphabets and number. All the samples were analyzed within 3h.

H_2S Test procedure:

H2S test medium was prepared as per the composition and the 20 ml of drinking water sample were inoculated in each bottle and incubated at room temperature for 24h, 48h and 72h. simultaneously samples were filter by membrane filter. Then filter were placed on Mac-Conkey agar plates. Bottles were observed for black colour development after every 24h. Faecal pollution is indicated if the contents of the bottle turn black within 24 to 72h and turbidity of water sample indicates non faecal contamination.

Isolation of organisms:

The various samples that developed black color were separately inoculated into 10mL of nutrient broths and incubated at 37°C for 24h. Mac-Conkey agar plates were prepared, using a sterile platinum wire loop, each culture was separately streaked onto the surface of Mac-Conkey agar plates, labeled and incubated at 37°C for 24h and observed for signs of growth and colony appearance. Colonies that appeared pink on the Mac-Conkey agar plates were picked up with sterile inoculating wire loop and separately streaked onto the surface of different selective medium i.e. *Salmonella* and *Shigella* (SS) agar, Cystine Lactose Electrolyte Deficient agar (CLED), Manitol Salt agar (MSA) plates. These were also incubated at 37°C for 24h. After incubation plates was observed for growth, isolated colony from all the plates were picked up with sterile wire loop and inoculated in to the different tubes of 10mL nutrient broth at 37°C for 24h. The various sub cultures were streaked onto nutrient agar slants, incubated at 37°C for 48h and then kept (in the refrigerator at -20°C) for further identification i.e. Gram staining and Biochemical testing.

RESULTS AND DISCUSSION

The MGM campus including 32 different institutions with different drinking water sources, almost all the staff of MGM are used this drinking water. As the medical microbiology aspect here a small project was done i.e. whether this drinking water is really safe to use or not. Hence the attempt of this work was carried out Modified Rapid Analysis of Bacterial Contamination of drinking water with the Help of H_2S Bottle Test.

Out of 38 samples H_2S had shown 20 positive samples and MFT had shown positive result in 24 samples. The H_2S test was shown positive result at 24h incubation in 6 samples named as DW, PT(1), CABT, A(3), JNEClib, JNECmech. At 48h incubation 10 samples shown positive result named as OPD, CAS, FBT, IBTcant, JNECcant, CS, CSC, YC, B(1), RM and 4 samples named as VM, PHTY, CN and IHM were positive at 72h incubation. All this samples were developed black colour in H_2S test which indicates presence of faecal contamination whereas 17 samples named as DB, DM, DO, DR, SV, A1, A(2), A(4), B(2), PT(2), CFA, GYP, GH(B), IBTdept, JNECMH, ACH and JNECchem & bt, had not

developed black colour but samples named as DR, A1, A4, and ACH had shown positive result for MFT.

Positive samples were inoculated on Mac-Conkey agar and incubate at 37°C for 24 hour and isolates (47) were obtained and inoculated on nutrient broth. Out of 47 isolates 21 isolates no. as CAS1, CAS3, FBT1, FBT2, CABT1, IBTcant1, IBTcant3, CS1, CS3, CS4, YC2, A(3)1, RM1, RM2, PT(1)1, PT(1)3, JNEClib, JNECcant2, DW1, ACH and IBTcant4 had developed pink colour on Mac-Conkey agar and 26 isolates had developed colourless colony OPD1, OPD2, CAS2, VM1, VM2, CABT1, IBTcant2, JNECcant1, CS2, CSC1, CSC2, CSC3, YC1, YC3, A(3)2, B(1)1, B(1)2, PT2, DR, PHTY, CN, JNECmech, IHM, DW2 and A4.

Out of 47 isolates inoculated in to the nutrient broth and observed the growth after 24h, turbid appearance of the broth in 34 isolates were named as OPD1, OPD2, CAS1, CAS2, CAS3, PT1, PT2, PT3, FBT1, FBT2, CABT1, CABT2, IBTcant1, IBTcant2, IBTcant4, CS1, CS2, CS3, CS4, CSC1, CSC2, CSC3, YC1, YC2, YC3, A(3)1, A(3)2, B(1)1, B(1)2, RM1, RM2, VM1, VM2 and JNECcant2, Smokey growth was observed in 14 isolates and named as DR1, PHTY1, CN1, JNEC lib1, JNECmech1, JNECcant1, IHM1, DW1, DW2, A1, A(4)1, ACH1 and IBTcant3

Selective Medium:

A total of 47 isolates have been inoculated on the selective medium from nutrient broth for further confirmation.

CLED (Cystine Lactose Electrolyte Deficient) agar:

Out of 47 isolates, 21 isolates name as OPD1, CAS2, VM1, VM2, CABT1, IBTcant2, JNECcant2, CS2, CSC2, CSC3, YC1, YC3, A(3)2, B(1)1, PT2, DR, CN, JNECmech, IHM, DW2 and A(4)1 had developed Blue-green colour, 14 isolates name as CAS1, FBT1, CABT2, IBTcant1, IBTcant3, CS3, CS4, YC2, RM1, RM2, PT1, JNEClib, JNECcant2 and IBTcant4 had developed yellow colour, 4 isolates name as CAS3, FBT2, PT3 and DW1 had developed yellow to white colour, 6 isolates name as OPD2, CS1, CSC1, B(1)2, PHTY and A1 had developed white colour colony and 2 isolates name as A(3)1 and ACH had developed deep yellow colour colony.

SS (*Salmonella* and *Shigella*) agar:

Out of 47 isolates, 21 isolates name as OPD1, CAS2, VM1, VM2, CABT1, IBTcant2, JNECcant2, CS2, CSC2, CSC3, YC1, YC3, A(3)2, B(1)1, PT2, DR, CN, JNECmech, IHM, DW2 and A(4)1 had developed irregular colourless colony, 14 isolates name as CAS1, FBT1, CABT2, IBTcant1, IBTcant3, CS3, CS4, YC2, RM1, RM2, PT1, JNEClib, JNECcant2 and IBTcant4 had developed red (rose) colour colony, 4 isolates name as CAS3, FBT2, PT3 and DW1 developed pink colour colony, 6 isolates name as OPD2, CS1, CSC1,

B(1)2, PHTY and A1 had developed black colour colony and 2 isolates name as A(3)1 and ACH had no growth.

MSA (Manitol Salt agar):

Out of 47 isolates only 2 isolates name as A(3)1 and ACH had grow and developed yellow colour colony and rest the isolates had not grow.

Correlation between H_2S test and MFT:

According to the membrane filtration test, 24 (63.15%) of the 38 samples had fecal coliforms, while 14 (36.84%) had no faecal contamination. In Table the results of the study are shown as a contingency table in which the results of both the fecal coliform test and of the detection of H_2S producing bacteria had shown.

The two tests agreed exactly in 90% of the samples. It is important to point out that where there was not agreement 0% sample was false positives, that is, samples that showed the presence of H_2S producing bacteria but with zero fecal coliforms and 4 samples that is, 10.52% of the total number of samples tested as false negatives, that is, negative for the presence of H_2S producing bacteria but positive for fecal coliforms (Chandrashekara, KV., 2001)[9].

Table 1: Correlation between H_2S test and MFT test

NO. of Samples	H_2S test result		MFT result	
	+ve	-ve	+ve	-ve
38	20(52.63%)	18(47.36%)	24 (63.15%)	14(36.84%)
false +ve and false –ve result	False +ve = 0 (0 %)			
	False –ve = 4 (10.52%)			

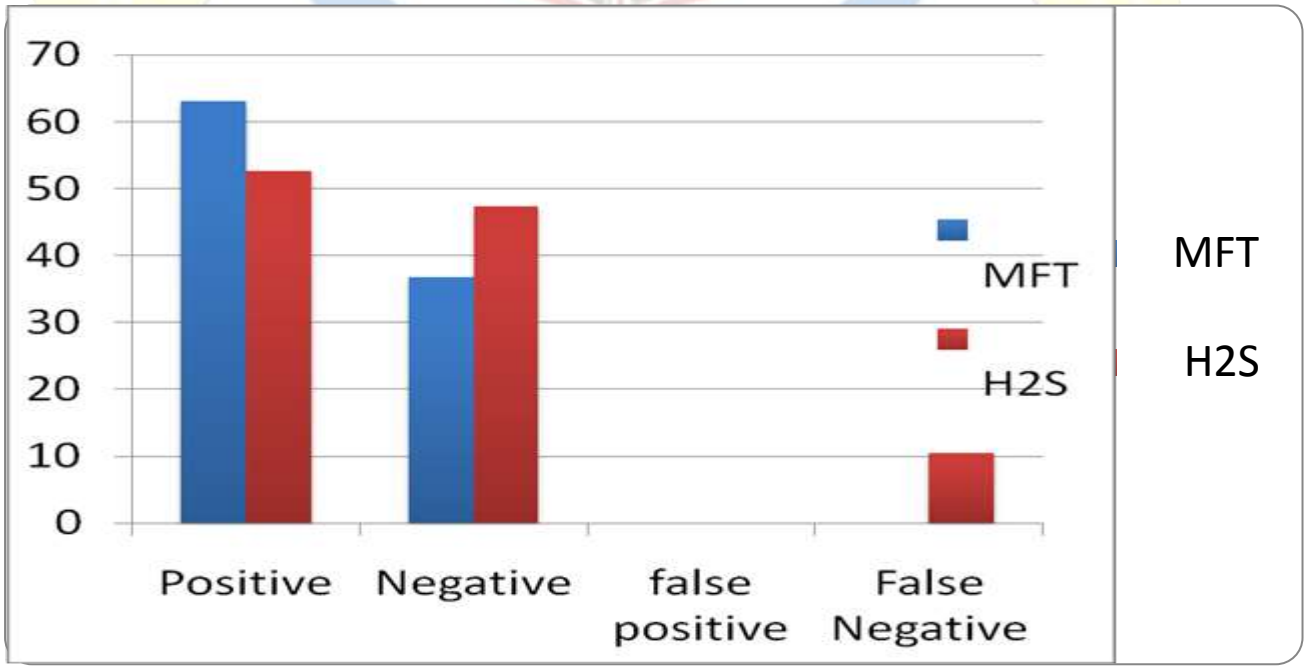

Figure 1: Correlation between H_2S test and MFT test

Organisms isolated from drinking water:

From the 24 positive samples 47 isolates were investigated for the identification of faecal contamination. Out of 47 isolates, 21 (44.70%) were *Pseudomonas*, 14 (29.78%) were *E.coli*, 6 (12.76%) were *Salmonella*, 4 (8.5%) were *Klebsiella* and 2 (4.25%) were *Staphylococcus* spp.

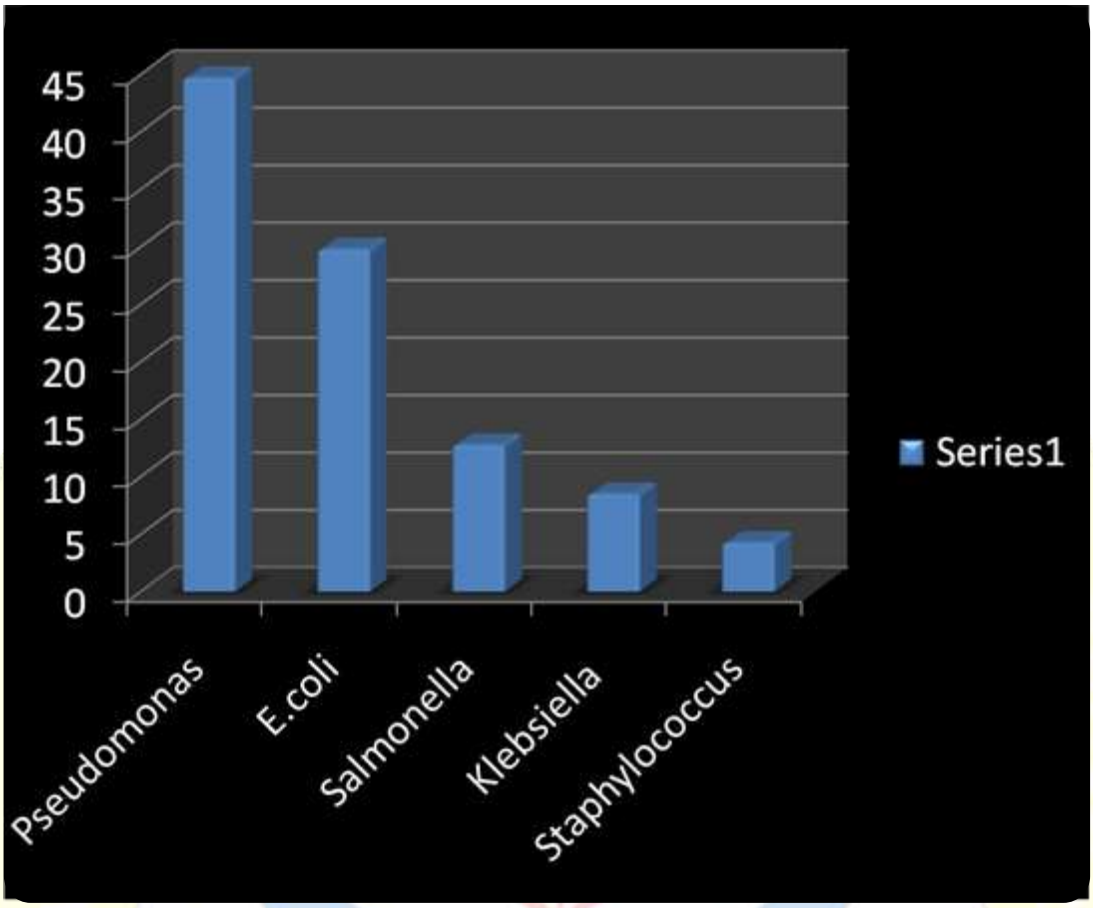

Figure 2: Isolated Organisms

DISCUSSION

It is evident from the data presented in this work that H_2S producing organisms are consistently associated with the presence of coliforms in water. Enteric bacteria such as *Salmonella*, and certain species of *Klebsiella* also produce H_2S. The predominant H_2S producing bacteria found in polluted drinking water in the present study were *Salmonella* spp., *Klebsiella*, *Staphylococcus*, and H_2S producing variants of *E.coli*. All previous studies were in concordance that the incubation period had significant effect on the efficiency of H_2S test. Hirulkar and Tambekar, (2006)[10] showed that as incubation period increased from 24 to 48 h, the efficiency also increased from 47% to 95% at room temperature and 63% to 96% at 37°C. In the present study the efficiency of H_2S were increased 30% to 50% at room temperature from 24h to 48h. Moreover the efficiency of H_2S test also increased to 83% at room temperature and 85% at 37°C with the increased in incubation period from 24 to 48h.

the efficacy of H_2S test was very high because total coliform detected only human thermotolerant *E. coli*, which may be low in the tested water samples. Thus study indicated that the H_2S test is a reliable and alternative indicator of fecal contamination in drinking water quality surveillance and screening of large number of water samples in short duration in the field where laboratory facilities are limited. Therefore, Manja's H_2S test, a simple and versatile test, can be carried out in the field within a broad range of incubation temperature and is recommended for the routine monitoring of water for detection of fecal contamination.

CONCLUSION

This study was confirmed that water supply of MGM campus was showing strong correlation between the presence of fecal coliforms and H2S producing bacteria. An analysis of the data showed that a strong, statistically significant correlation existed between the two methods; the strength of the correlation was 90%. So there is a need of suitable treatment method to maintain safe and unpolluted drinking water quality. Where there was not agreement between the two tests the false positives was 0% and 10.52% of the total number of samples tested for presence/absence of H_2S producing bacteria resulted as false negatives. Detection of H_2S producing bacteria cannot completely substitute for measurement of faecal coliforms by the membrane filter technique as a standard method for control of quality of potable water supplies. However the test can certainly play an important role in public health work. In rural areas where there is no laboratory method can serve very well as an alternative test to estimate the quality of potable water, a test which is simple, relatively cheap and very reliable.

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