Bacteriological Quality Of Drinking Water in Punjab: Evaluation of H_2S Strip Test

Pages with reference to book, From 237 To 241
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Abstract

Objective: To assess bacteriological quality of drinking water in Punjab and to evaluate usefulness of H2S strip test in comparison with multiple tube test,
Method: Samples of water were tested using H2S strips and multiple tube test.
Results: Maximum bacterial contamination was observed in water from domestic pumps (95.83%). Followed by tap water in rural areas of Punjab (91.30%) and tap water in Lahore (42.85%). Bacterial contamination was significantly higher ($p<0.001$) in rural areas as compared to urban areas.' Comparison of results of testing water samples by H2S strip test and multiple tube test revealed that H7S strip is 87.24% sensitive and 100% specific for detection of bacterial contamination with a positive predictive value of 100%. It was also observed that 100% water samples negative for total coliforms were also negative by H2S strip method. Moreover, with increase in number of total coliforms in the water samples, positivity by H7S strip method also increased (samples with more than 10 total coliforms/lOOmi were 100% positive by H2S strip method). Therefore, H2S strip test can be used as alternative to multiple tube test for detection of bacterial contamination of water supplies.
Conclusion: It is concluded that bacterial contamination of water is a significant problem in Punjab. it can be improved by regular monitoring of water supplies. For this purporse use of H2S strip test is advocated at house hold level (JPMA 49:237, 1999).

Inroduction

It is well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta particularly faeces[1]. Out breaks of water borne diseases continue to occur throughout the world but are especially serious in developing countries[2,3]. The human pathogens that present serious risk of disease whenever present in drinking water include Salmonella species, Shigella species, pathogenic Escherichia coli, Vibrio cholerae, Yersinia entercolitica, Campylobacter species, various viruses such as Hepatitis A, Hepatitis E, Rota virus and Parasites such as Entamoeba histolytica and Giardia species and so on1[3-5].
The recognition that microbial infections can be water borne has led to the development of methods of routine examination to ensure safety of drinking water. it is impracticable to monitor drinking water for every possible microbial pathogen[6]. Therefore, normal intestinal organisms are used as indicator of faecal polloution[7,8]. These include coliform group of organisms as a whole[9]. They are recognized as suitable indicator because they are easy to detect and enumerate in water[10]. Multiple tube test for estimation of total coliforms and faecal coliforms is a standard test used as indicator of sewage contamiiiation of water supplies[1].
Multiple tube test has several disadvantages for routine use in developing countries. They are not easily portable for use in rural areas[11]. They require trained technicians, sophisticated laboratory equipment and/or expensive reagents. These limitations seriously inhibit the effectiveness of most water quality control programmes[12]. To overcome these problems H2S paper strip method for screening

bacteriological contain ination of drinking water was developed[2]. Siiîce then many reports[13-15] have shown that H_2 S strip test is a sensitive, simple and inexpensive procedure to test water for bacterial contamination.

In Pakistan, water microbiology is a neglected subject. A few studies have been carried out in different parts of Pakistan[16-20]. These studies generally show high contamination of drinking water samples. They have been performed by multiple tube test. The present study was planned to determine the bacteriological quality of drinking water in different areas of Punjab. At the same time H7S strip test was used to assess its usefulness as an alternative method to conventional multiple tube test in areas away from main cities where facilities of microbiological studies are not available.

Material and Methods

Water samples were submitted to the bacteriology laboratory from different areas of Punjab from April to July, 1998. These were tested for their bacteriological quality. They were sent to the laboratory in sterilized sealed bottles and persons responsible for collection of water samples were asked to strictly follow the standard guidelines.

In the laboratory, all the samples were subjected to multiple tube test for determination of most probable number (MPN) of total coliforms and faecal coliforms as well as H2S strip method.

Multiple Tube Test

Multiple tube test was performed according to standard procedure[22]. After inoculation of water samples in different bottles and tubes of MacConkey's broth with inverted Durham's tubes, all the bottles and tubes were incubated at 37°C for upto 48 hours. The bottles or tubes with acid and gas were considered as positive for coliforms. From the distribution of these positive bottles and tubes MPN of total coliforms was determined by referring to standard probability table for estimation of MPN of coliforms[21]. All the tubes/bottles positive for total coliforms were subcultured into 10 ml of single strength MacConkey's broth with inverted Durham's tube and 5 ml of peptone water. These tubes were incubated at 44°C for 24 hours. The tubes showing acid and gas and indole production were taken as positive for faecal coliforms (Ejikman test positive). From the number of these positive tubes, MPN of faecal coliforms was estimated by referring to the table as for total coliforms.

H_2S Strip Test

This test was performed following the procedure used by previous workers[2,32]. MacCartney glass bottles with 28 ml capacity were used in this test. A concentrated medium was used in the test according to the following formula:

Bacteriological peptone 40.0 grams, Dipotassium hydrogen phosphate 3.0 grams, Ferric Ammoniurn Citrate 1.5 grams, Sodium Thiosulphate 2.0 grams, Teepol 2.0 ml and Distilled water 100.0 ml.

All the chemicals were dissolved in water in 250 ml flask. Then non-toxic filter paper was cut into 10x10 cm size, folded to suit the mouth of MacCartiiey bottle and placed in the bottle, To this paper. .0 ml of the concentrated medium was added carefully so as to absorb it into the paper. The bottles were loosely capped and autoclaved at 121°C for 15 minutes. Then they were placed in oven at 55°C to dry. After drying the caps were screwed tightly and marked to the volume of 20 ml. The bottles were kept at room temperature for use in the test.

One H_2S bottle was used for each water sample. Proper labelling of bottle with test number and date was done. After removing the cap, neck of the bottle was gently flamed and water sample was added upto 20 ml volume mark. The bottles were recapped and gently shaken. Then they were incubated at 37°C for 24 hours. After incubation, the bottles were examined. Any blackening of the paper strip was considered positive for presence of bacterial contamination. Statistical analysis was done byChi square

test and determination of sensitivity and specificity of H_2S strip test with reference to multiple tube test.

Results

In the present study 210 water samples were tested from different areas of Punjab. Out of these, 70 samples were from piped water in distribution (tap water) of Lahore, while 140 samples were from other areas of Punjab, (48 water samples obtained from domestic hand/motor pumps and 92 from tap water). Maximum bacterial contamination (presence of total coliforms) was observed in case of water from domestic pumps (46/48;95.83%) followed by tap water in rural areas of Punjab (84/92; 91 .30%) and tap water in Lahore (30/70:42.85%). Break up of water samples into urban (Lahore) and rural areas (other than Lahore) revealed significantly higher ($p<0.001$) bacterial contamination in rural areas (8 1.42%) as compared to urban areas (42.85%). It may be noted that in the present study approximately 100% of samples positive for total coliforms were also positive for faecal coliforms (Table 1).

Table 1. Results of H_2S strip test and multiple tube test on water samples (n=210).

Area tested	H_2S strip test		Multiple tube test			
			Total coliforms		Faecal coliforms	
	Positive		Positive		Positive	
	No.	%	No.	%	No.	%
Urban (n=70)	28	40.00	30	42.85	30	42.85
Rural (n=140)	102	72.85	114	81.42	112	79.45

$P<0.001$

Comparison of results of testing all water samples by H_2S strip and multiple tube methods revealed that H_2S strip test is 87.24% sensitive and 100% specific for detection of bacterial contamination of water with a positive predictive value of 100%. However, it has a negative predictive value of 76.25%. In all, H_2S strip test was found to have an accuracy of 90.45% (191/210) in detecting bacterial contamination of water (Table. 2).

Table 2. Comaparison of H_2S strip test with multiple tube test for determination of bacteriological quality of water.

		Multiple tube test (Total coliforms) Positive	Negative	Total
H_2S Strip Test	Positive	130	0	130
	Negative	19	61	80
Total		149	61	210

Sensitivity = 87.24% Positive Predictive Value = 100.00%
Specificity = 100.00% Negative Predictive Value = 76.25%

Further analysis of results revealed that samples which were negative for total coliforms by multiple tube method were also negative by H_2S strip method. With an increase in the density of total coliforms (MPN/100 ml of water), there was rise in percentage of H_2S strip test positivity. It was observed that 80.70% water samples with density of 4-10 total coliform were positive by H2S strip test while samples with density of >10 total coliforrns were found to be 100% positive by H_2. S strip test (Table 3).

Table 3. H_2S strip test positivity in relation with density (MPN/100 ml) of total coliforms.

Density of Total coliforms		H_2S Strip Test Positive No. (%)	Negative No. (%)
0	(n=61)	0 (0.00)	61 (100)
1-3	(n=16)	4 (25.00)	12 (75.00)
4-10	(n=57)	46 (80.70)	11 (19.30)
>10	(n=76)	76 (100)	0 (0.00)

Discussion

Pressure of urbanization and industrialization in the world led to increased demand for pure water supplies[24]. As Pakistan is a developing country therefore the need for providing safe water to its urban

and rural population is more important. Population-wise, Punjab is the largest province of Pakistan. In the present study, an effort was done to assess the bacteriological quality of drinking water in some parts of Punjab including its capital, Lahore. Residents of Lahore (urban population) as well as many other areas of Punjab (rural population) are served with piped water supplies using ground water as a source. This water is supposed to be treated with chlorine before it is introduced into the distribution system. However, in a recent study conducted at Lahore[25], only 28.96% of water samples from distribution system were found to be chlorinated.

According to WHO[26], there should be no coliforrns/l00 ml of treated water in distribution. In the present study, out of 162 tap water samples, 60.49% (98/162) were positive for total coliforms (unfit for human consumption). However, when tap water samples from Lahore city are only taken into consideration, the figure in the present study (42.85%) is lower than that observed in a previous study from Lahore[25]. This may mean some improvement in the bacteriological quality of drinking water in the city. At the same time these figures indicate that bacteriological quality of water in Lahore is far from satisfactory. In another small scale study on bacteriological quality of water supply of Mayo Hospital/King Edward Medical College, Lahore, out of 12 samples, 8 were found to be bacteriological iy contaminated[27].

Water in distribution is contaminated while it passes along in pipe lines. This contamination can occur due to defective joints, back siphonage, rusted pipe lines crossing over sewage pipes and low or high pressure in pipe lines[1,28,29].

Considering the results of water samples from rural areas (areas other than Lahore), the situation is very alarming. Both the tap water samples as well as samples from domestic pumps showed heavy bacterial contamination. In fact the figures are much higher than those in Lahore. This may be attributed to low depth of bore of domestic pumps. Such shallow bore gets easily contaminated by nearby polluted water as it percolates through earth. Proximity of water pipes to the main sewage pipe lines also leads to contamination of water during distribution[19].

In India, 68.9% of samples from rural piped water and in Indonesia 45.7% samples were found to be unfit for drinking[30,4]. In Pakistan, 38 (56.2%) water samples in Rawalpindi/Islamabad have been reported to be bacteriological ly contaminated in different studies[18,31,32]. These figures are similar to those observed in the present study. In Karachi, much higher contamination (87%) of tap water samples have been repoted[28]. All this data indicates that bacteriological contamination of water is a significant problem not only in Pakistan but other neighbouring developing countries.

Use of H_2S paper strip method for screening of potable water for bacterial contamination was first reported in 1982 by Manja et al2. This was based on the fact that presence of coliforms in water was also associated with H_2S producing organisms[32]. Since, 1982, may studies have been carried out using H_2S strip test to evaluate bacteriological quality of water. These studies show a good agreement between results of multiple tube test and H2S strip test[2,11-15,34]. In Lahore also, H_2S strip test was used and found to be as useful as multiple tube test[25].

In the present study, H_2S strip test was evaluated for its usefulness in assessing the bacteriological quality of drinking water in the rural areas. H_2S strip test showed 90.45% agreement with multiple tube test. It was found to be 100% specific with a positive predictive value of 100%. Therefore this test can be safely used in the field studies as an alternative to multiple tube test. In far off areas, away from proper laboratory facilities, this method can be adopted to monitor water supplies. This method was found to be less sensitive (87.24%) as compared to multiple tube test in the present study. However, the sensitivity of H_2S test can be increased by using 100 ml water samples instead of 20 ml, alongwith

proportional increase in the amount of concentrated medium[11]. This 100 ml volume will then correspond to the volume of water samples used in multiple tube test.

It has also been noted in the present study that water samples which were grossly contaminated (total coliform count of >10/100 ml) were 100% positive by H2S strip test, water samples with MPN of 4-10 were 80.70% positive while water samples containing very low number of coliforms (MPN 1-3/100 ml) were only 25% positive. It means that H_2S strip test detects almost all the water samples which are unfit for human consumption according to WHO criteria[1].

Conventional tests for determination of bacteriological quality of water (multiple tube test) has several disadvantages for routine use in developing countries. It is not easily portable for use in rural areas, requires trained technicians and sophisticated laboratory equipment. While H_2S strip test is cheap, does not require trained persons, is easily portable and has unlimited shelf life[11,13]. Keeping in view the high level of contamination of drinking water in our country, it is essential that water is examined regularly and frequently using a simple test as contamination may be intermittent[8,35].

It is concluded that bacterial contamination of piped water (tap water) as well as water from domestic pumps is a significant problem in Lahore and other areas of Punjab. It can be improved by regular chlorination of water and monitoring of water supplies. Keeping in view the simplicity and low cost use of H2S strip test, its use is advocated at household level in urban as well as rural areas.

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