SUITABILITY OF THE H_2S PAPER STRIP METHOD FOR TESTING RAINWATER

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ABSTRACT

Rainwater is the source of drinking water for many people in Australia and in other parts of the world. The water that is collected in the tanks may contain a variety of bacteria of bird and reptile faecal origin. The rainwater tanks are seldom treated and these bacteria can be of human health concern. The quality of rainwater is rarely tested due to multiple reasons. The availability of a cheap testing procedure, which could be conducted by the householders, would be advantageous. The H_2S paper strip method is an on - site bacterial testing method which detects the hydrogen sulphide producing bacteria in the water sample. A good correlation has been reported between the presence of hydrogen sulphide producing bacteria and the faecal indicators. In order to assess the suitability of the H_2S method for testing rainwater, 113 samples were analysed for total coliforms, *Escherichia coli* and *Salmonella* sp by the standard procedures and the results compared with the H_2S method. Coliforms were observed in 59 samples and 32 samples contained *E.coli*. The H_2S method gave more true results when the bottles were incubated at 24 hours than at 48 hours. Many false positive and false negative results were observed. The false positive results could be due to the presence of other Enterobacteriacea such as the *E.cloacae*, *Proteus* , *Citrobacter* and *Salmonella arizona* as well as the H_2S producing bacteria of plant origin which were isolated from some of the positive bottles. The presence of false negative results is a concern in using this method as an authentic test for testing drinking water but it could be used as a screening test for isolated drinking water sources.

KEYWORDS

Rainwater, H_2S method, coliforms, *E.coli* and *Salmonella sp.*

INTRODUCTION

Rainwater is a source of drinking water for many householders throughout Australia as well as other parts of the world. Rainwater is considered to be a pure source of drinking water and therefore good to drink even without treatment. However there are many sources of contamination which may affect the physical, chemical and microbial quality of the water stored in tanks. Reptile and bird droppings as well as the decayed plant and animal matter carried to the tank with the rainwater are the main sources of contamination. The presence of overhanging trees increases the risk of bird droppings. Therefore even if the tanks are properly covered the rainwater in the tank may get contaminated. This is often left unnoticed and rainwater tanks are seldom treated. The microbial quality of rainwater stored in tanks was found to be very poor by Yaziz *et al.*(1989), Thomas and Greene, (1993), Fujioka *et al.*(1995), Rijal and Fujioka (1995). As each tank has to be considered as an individual unit, water quality assessment and treatment are primarily the interest of the householders. The water quality in the tank changes according to the season. Therefore there is a need for frequent testing of water quality in the tanks. However frequent monitoring of the quality of rainwater tank is difficult, as the standard methods currently available are expensive, that demand

laboratory and technical support. The on - site testing methods such as the Colilert and the Colisure are expensive for routine testing especially by the householders. An affordable and easy method to test water by the householders is a requisite for testing rainwater tanks as well as for isolated drinking water sources.

The H_2S method developed by Manja *et al.* (1982) is an onsite bacterial testing method, which is simple and less expensive. This method detects the hydrogen sulphide producing bacteria rather than the faecal indicators or specific pathogens. The H_2S method was found to give a good correlation with the standard methods for detecting faecal pollution (Rijal *et al.*, 1995; Ziel *et al.*, 1995; Grant and Ziel, 1996; Martins *et al.*, 1996). Since a majority of *Salmonella* sp produce H_2S, the method was found to be good for testing the presence of *Salmonella* (Gawthorne *et al*, 1996; Pillai *et al.*, 1997). Many members of the Enterobacteriacea such as the *Citrobacter, Klebsiella and Proteus* produce hydrogen sulphide so their presence will also be detected by this method.

The present study was aimed at assessing the suitability of the H_2S method for testing rainwater. Results of the H_2S test were compared with the standard procedures for testing total coliforms, *Escherichia coli* and *Salmonella* spp. As rainwater from each tank is different, the samples taken from tanks formed a wide range to be analysed for testing the efficiency of the method. It was previously reported that addition of l-cystine to the H_2S medium improved the efficiency of the method (Venkobachar *et al.*, 1994; Pillai *et al.*, 1997). The study also compared the two media for testing rainwater.

MATERIALS AND METHODS

Rainwater samples (500ml) were collected from household tanks in and around Perth, Western Australia. Sterile bottles were used for collecting the samples. Samples were collected from the taps on the rainwater tank after running out the water for 10 seconds to prevent contamination from the taps. The samples were analysed within 24 hours of collection and were refrigerated if stored for more than 4 hours. The water was tested for total coliforms, *Escherichia coli* and *Salmonella* by the membrane filtration method described by the *HMSO* (1982). Each sample was simultaneously tested with the H_2S Bottles. The H_2S medium (M1) was prepared according to Manja *et al* (1982). A modification of the H_2S medium (M2) with 0.25g of L-cystine added to 100 ml of the medium was also tried. The bottles were incubated at 37°C. The bottles were examined after 24 and 48 hours for positive results which were indicated by the blackening of the bottle. The bottles that did not turn black after 48 hours were considered as negative. The data were analysed to find a correlation of the H_2S method with the presence of total coliforms, *E.coli* or *Salmonella* spp. Some of the bacteria that caused positive results were identified using 20E API strips.

According to WHO Guidelines (1996) treated drinking water should not contain any coliforms but the presence of coliforms in 5% of the total samples collected in a year is allowed. Australian drinking water guidelines NHMRC & ARMC (1996) recommend that 100ml of the sample should not contain any coliforms or *E.coli.*

RESULTS

The modified H_2S medium (M2) was tested with 113 rainwater samples and the original H_2S medium was tested for 53 of these samples. True positive results were taken as those that were positive for the H_2S test and positive for total coliforms or *E.coli.* True negative results are those which are negative for H_2S and total coliforms or *E.coli.* False positive are positive for H_2S and negative for total coliforms or *E.coli* while negative H_2S result for a positive total coliforms or *E.coli* are considered as false negative. Table 1 shows the percentage of true and false results with medium M1 and M2 at 24 and 48 hours of incubation. Out of the 113 samples analysed 59 samples

contained coliforms and 32 samples contained *E.coli*. The H_2S method gave more true results when the bottles were incubated for 24 hours. After 24 hours of incubation 48 samples were positive and after 48 hours, 67 bottles turned positive. High contamination with total coliforms (>10CFU/100ml) was noticed in 35 samples. Many false positive and false negative results were observed with the two media. The reason for the false positive and false negative results could not be identified but *Enterobacter cloacae, Proteus and Citrobacter* were isolated from some of the positive bottles. *Salmonella arizona* was present in two samples where the tank was never cleaned or treated. Four samples which were false negative contained *E.coli*. Table 2 shows the total coliform and *E.coli* counts of the samples which were false negative.

Table 1. Percentage of true and false results with M1 and M2 at 24 and 48 hours

	No. of Samples	True Positive (H_2S &TC or *E.coli*+ve)	True Negative (H_2S &TC or *E.coli*-ve)	False Positive (H_2S+ve &TC or *E.coli*-ve)	False Negative (H_2S-ve &TC or *E.coli*+ve)
M1(24 hrs)	53	19 (35.85%)	22 (41.51%)	2 (3.77%)	10(18.87%)
M1 (48 hrs)	53	24 (45.28%)	17 (32.07%)	7 (13.21%)	5 (9.43%)
M2 (24 hrs)	113	42 (37.17%)	48 (42.48%)	6 (5.3%)	17 (15.04%)
M2 (48 hrs)	113	49 (43.36%)	36 (31.86%)	18 (15.93%)	10 (8.85%)

Table 2. Data of total coliforms and *E.coli* in false negative samples

No	Total coliforms(CFU/100ml)	*E.coli* (CFU/100ml)	M2	M1
1	38	0	neg	
2	4	3	neg	
3	25	8	neg	
4	2	1	neg	neg
5	1	0	neg	neg
6	1	0	neg	neg
7	31	16	neg	neg
8	5	0	neg	neg
9	1	0	neg	neg
10	14	0	neg	neg

With the medium M2 the percentage of false results increased when the bottles were incubated for 48 hours. At a total coliform count >0 CFU/100ml M2 gave more true results than M1 after 24 hours of incubation. But when incubated for 48 hours M1 gave slightly more true results than M1. The percentage of true results and false results with M1 and M2 at 24 and 48 hours are shown in

Fig. 1. True results are the total of true positive and true negative results whereas false results are the total of false positive and false negative results.

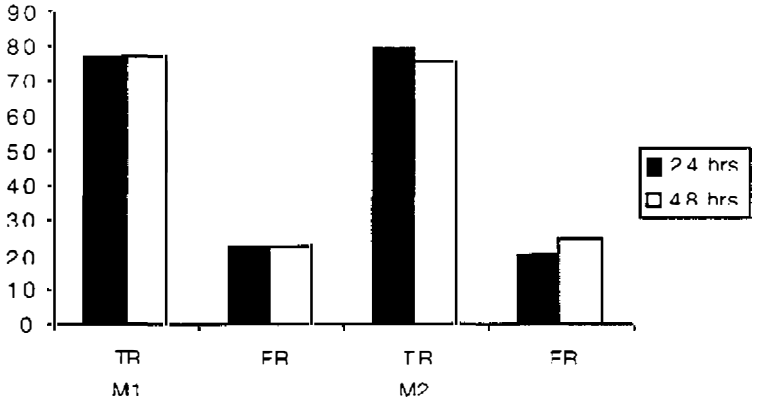

Fig.1. Percentage of true and false results for different media at 24 and 48 hours of incubation

DISCUSSION

Analysis of the water samples showed that about half of the rainwater tanks tested were contaminated with micro organisms of health concern. In medium M2, L-cystine accelerated H_2S production and therefore more positive results were observed at 24 hours whereas in M1 the H_2S production was slightly slower and therefore took 48 hours. Pillai *et al* (1999) observed that M2 was better than M1 for detecting faecal coliforms to the lowest concentration and at a wider range of incubation temperature. However the greater sensitivity of M2 is associated with a greater number of false positive results when compared to the total coliform and *E.coli* tests.

As the method detected H_2S producing bacteria, the false positive results could be due to the presence of other H_2S producing bacteria like *Enterobacter* sp., *Proteus* and *Citrobacter* which were isolated from some of the positive bottles. This was observed by Castillo *et al.* (1994). Although all samples were tested for *Salmonella* sp. only 2 contained *S. arizona*. Some phyto pathogens such as *Erwinia* sp and *Chromobacterium* were also isolated from positive bottles. The source of these bacteria could be leaves that were carried into the tanks. Many phytopathogens as well as the other bacteria identified from the positive samples are of human health concern. Therefore false positive may indicate greater risk of infection and the H_2S method may be a better indication of human health risk potential.

False negative results observed are a concern when using this method for testing rainwater. The results proved that false negative result is not dependent on the coliform count as even the sample with very high numbers of coliforms gave negative results. This showed that the method did not fully correlate to the presence of coliforms and some samples although contained coliforms did not contain the H_2S producing bacteria. Levett (1990) reported that in faeces H_2S producing bacteria occurred at a concentration of 10^{10}/g. In that case it seems likely that H_2S producing bacteria would be present in the samples contaminated with faeces. However the presence of *E.coli* in 4 samples that were false negative suggests that this may not always be the case. As to whether the negative results indicate a less human health risk potential is a question.

Ziel *et a.l* (1995) reported that the H_2S method had a 87.7 % correlation with the presence/absence (P/A) coliform test. Martins and Pellizari (1990) compared the H_2S Method with different coliform tests and reported a percentage agreement varying from 66.7-90% with raw waters and a 90-94 % with drinking water samples. More positive results were obtained with the H_2S test than the other tests as was found in the present work. According to the data given by Grant and Ziel (1996) out of 14 well water samples tested there was 1 false positive and 1 false negative result whereas 7.2% false negative results were observed by Castillo *et al.* (1994). The false positive and false negative results were also observed by Desmarcheliar *et al.* (1992).

Rijal and Fujioka (1995) tested 5 different sources of rainwater tanks for five weeks and observed that the concentration of total coliforms correlated well with H_2S producing organisms. They concluded that testing water for hydrogen sulphide bacteria is a reliable method for assessing the quality of water. But since the same five cisterns was tested for five weeks the bacterial population could almost be the same. Also the number of false positive and false negative results were not identified separately. As shown in Fig 1. with M2 79.64 % true results were obtained where as with M1 it was 77.35%. Therefore the addition of l-cystine to the medium would increase the correlation. M2 had 5.3% false positive and 15.03% false negative results while M1 had 3.77% false positive and 18.87% false negative results. Wallis (1991) reported that on testing rainwater tanks in Thailand 20 % false positive and 41 % false negative results were obtained. Hazbun and Parker(1983) recommended that false negative results could be reduced by increasing the incubation temperature to 37 °C for 24 hours and the numbers as done in the present work.

CONCLUSIONS

1. This method could be an initial screening test which could be carried out by the householders to asses the quality of rainwater.
2. The addition of l-cystine to the H2S medium would increase the correlation with the standard methods for testing coliforms.
3. The false positive results obtained could be due to Enterobacteriacea other than the coliforms or due to the presence of other H_2S producing bacteria of animal or plant origin.
4. The appearance of false negative results indicates that H2S producers may not always be present need to be investigated.

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