

Revalidation of Testing Methods for Assessing Microbial Safety of Drinking Water

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Abstract: Revalidation of testing methods for assessing microbial safety of drinking water for risk assessment was performed by comprehensive bacteriological analysis. A total of 251 drinking water samples from 107 salinity-affected villages in Amravati district of Maharashtra State during June to December 2006 for potability of drinking water by standard accepted methods of water testing such as Multiple Tube Fermentation Technique (MTFT) for determination of Most Probable number (MPN), and Membrane filter techniques (MFT), Eijkman's test for Thermotolerant coliform (TTC) and Manja's Rapid H₂S test for detection of fecal contaminations in drinking water. The efficiencies of H₂S tests, MFT and TTC were 59 to 83%, 31% and 36% respectively when compared with MTFT. Efficiency of H₂S test varies with the source and decreased with the depth of the source of water. The study recommended that where there are laboratory facilities MFT and TTC and MTFT tests should be performed, however, in fields and in villages, rapid and cheap H₂S test should be used for detection of fecal contamination in drinking water, for places where time, man and laboratory facilities are very poor.

Key words: Water quality, MTFT, MFT, Rapid field test, TTC, MPN

INTRODUCTION

Drinking water is worldwide the most important single source of gastroenteric diseases and one of the major causes of morbidity and mortality worldwide, mainly due to the faecally contaminated raw water, failures in the water treatment process or recontamination of treated drinking water (WHO, 2003, 2004). The microbiological safety of water supplies is at present assured by monitoring for the absence of the faecal indicator organisms, total coliform bacteria and *Escherichia coli*. To reliably assess the level of faecal contamination of water and thus the possibility for occurrence of enteropathogenic microbes, numbers of indicators have been proposed, amongst which *E. coli* is considered to be superior as an indicator of faecal contamination and hygienic quality of drinking water (Ashbolt *et al.*, 2001, Edberg *et al.*, 2000). Detecting and counting of total coliforms and *E. coli* have traditionally been based either on the multiple-tube fermentation test (MTFT) or membrane filtration (MF) methods, or thermotolerant coliform (TTC) detection (International Organization for Standardization, 2000). In addition to coliforms and *E. coli* other organisms have also been proposed as suitable indicators of the hygienic quality of drinking water, e.g. faecal enterococci, sulphite-reducing fecal associated organisms, *Clostridium perfringens* and bifidobacteria (Barrell *et al.*, 2000).

Since traditional methods require a minimum of 24 h of incubation followed by a confirmation procedure lasting 24-48 h, the need for rapid test methods has increased (Hirulkar and Tambekar, 2006). During recent decades new chromomeric or fluorogenic defined-substrate based or hydrogen sulphide rapid test methods are developed (Tambekar *et al.*, 2007, Manja *et al.*, 2001). Due to differences in the test principles and apparent differences in sensitivity and specificity or procedures in the confirmation tests, the outcome of different test methods may vary in microbial quality of water (Tambekar and Hirulkar, 2007, International Organization for Standardization, 2004). Hence, it is important to understand the potentials and limitations of these testing methods before realistically implementing guidelines and regulations to safeguard our water resources. Therefore, the aims of the present study was to revalidate and compare some tests for detection of coliform bacteria and *Escherichia coli* with accepted microbial water quality testing methods and to assess the sensitivity and specificity of these tests for microbial quality and risk assessment in drinking water.

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MATERIALS AND METHODS

A total of 251 water samples were collected from regular drinking water sources from 107 salinity-affected villages from Amravati district of Maharashtra State during June to December 2006. These water samples were collected from rivers, lakes (Surface water, SW, 88 samples), open well (shallow ground water, SGW, 88 samples) and tube well water (deep ground water, DGW, 75 samples) in sterile sample bottles, date, time, source, collection villages were noted and immediately transported to laboratory. The bacteriological examination was performed within the 24 h of collection using standard Multiple Tube Fermentation Technique (MTFT) for determination of Most Probable number (MPN) index, nine multiple tube dilution technique using double and single strength Bromo-Cresol Purple MacConkey medium and Membrane filter techniques (MFT) by using M-EC test agar (Hi-media Lab. Mumbai), which detect only *E.coli* with production of yellow colour colonies on membrane filter; Eijekman's test for Thermotolerant coliform (TTC) by using Brilliant Green Bile broth (BGLB) for detection of gas production, and Tryptone broth for production of Indole and Manja's Rapid H₂S test for detection of fecal contaminations in drinking water (Manja *et al*, 2001). The MPN Index was calculated from MPN table and index of water more than 10 coliforms/dl is designated as polluted or unhealthy for drinking purpose or non-potable (APHA, 1998).

The isolation and identification of *E.coli* was made based on standard bacteriological tests such as morphological, cultural, biochemical and some special tests by subculturing the fermented (MTFT) tube culture in respective medium. The sensitivity, specificity and efficiency of accepted tests for quality of water compared with MTFT. The statistical analysis was performed with the Statistical Package for Social Sciences 15 for Windows (SPSS Inc.; Chicago, IL, USA) software.

RESULTS AND DISCUSSION

Results:

A total of 251 drinking water samples were analysed from 107 salinity-affected villages from Amravati district of Maharashtra State during June to December 2006 for potability of drinking water by standard methods of water testing. Out of total 251 water samples, 9 by MTFT/24, 2 by MTFT/48, 107 by H₂S/24, 31 by H₂S/48, 183 by MFT and 161 by TTC tests water samples were safe (Table 1). Out of 251 water samples, 9 samples were safe and 242 polluted by MTFT/24, whereas out of those safe by MTFT/24 were, 6 safe + 3 polluted= 9 by H₂S/24 and H₂S/48 and out of polluted by MTFT/24 were, 101 safe + 141= 242 polluted by H₂S/24 and 24 safe + 217 polluted= 242 by H₂S/48, all those samples safe by MTFT/24 were safe by MFT and TTC but those samples detected polluted by MTFT/24 were 174 safe + 68 polluted =242 by MFT and 152 safe + 90 polluted = 242 by TTC (Table 2). When the sensitivity, specificity and efficiencies of MTFT/24 were compared with H₂S/24 it was 58%, 67%, and 59% where as with H₂S/48 it was 83%, 61%, and 88% and with MFT 28%, 100%, and 31% and with TTC 37%, 100% and 36% respectively (Table 3). The water samples recorded safe by MTFT/24 and MTFT/48 and TTC showed total absence of *E.coli*. The water samples detected safe by H₂S/24 (24 samples), H₂S/48 (6 samples), and MFT (48 samples showed presence of *E.coli* (Table 4).

Table 1: Quality of water by various accepted tests

Water Quality	MTFT/24	MTFT/48	H ₂ S/24	H ₂ S/48	MFT	TTC
Safe	9	2	107	31	183	161
Polluted	242	249	144	220	68	90
Total	251	251	251	251	251	251

Discussion:

Water and sanitation are the primary driver of public health which means that once we can secure access to clean water and to adequate sanitation facilities for all people, irrespective of the difference in their living conditions, a huge battle against all kinds of diseases will be won. More than half of the world's population lives in villages in rural areas and most of those without access to safe drinking water supply (Tambekar *et al*, 2007, Howard *et al.*, 2003). The majority of diseases in developing countries are infectious in nature caused by bacteria, viruses and other microbes, which are shed in human faeces and pollute water supplies, which people use for drinking and washing purposes. Faecal indicator bacteria have been used to measure water quality and personal hygiene standards in a variety of settings (Kaltenthater *et al.*, 1996, Tambekar and Hirulkar, 2007). The aims of the present studies were to determine the prevalence of enteropathogens in water, evaluate the testing methods of water and the methods used for detection of

Table 2: Quality of water when compared with other accepted tests methods.

Test	Quality of water	Source	MTFT/24		Total
			Safe	Polluted	
H ₂ S/24	Safe	SW	0	26	26
		DGW	4	42	46
		SGW	2	33	35
		Total	6	101	107
	Polluted	SW	0	62	62
		DGW	2	27	29
		SGW	1	52	53
		Total	3	141	144
H ₂ S/48	Safe	SW	0	1	1
		DGW	4	15	19
		SGW	2	9	11
		Total	6	25	31
	Polluted	SW	0	87	87
		DGW	2	54	56
		SGW	1	76	77
		Total	3	217	220
MFT	Safe	SW		63	63
		DGW	6	51	57
		SGW	3	60	63
		Total	9	174	183
	Polluted	SW		25	25
		DGW		18	18
		SGW		25	25
		Total		68	68
TTC	Safe	SW		52	52
		DGW	6	44	50
		SGW	3	56	59
		Total	9	152	161
	Polluted	SW		36	36
		DGW		25	25
		SGW		29	29
		Total		90	90

Table 3: Sensitivity, specificity, and efficiency of accepted water quality testing methods compared with MTFT test.

Test	MTFT/24	
H ₂ S/24	Sensitivity	58%
	Specificity	67%
	Efficiency	59%
H ₂ S/48	Sensitivity	83%
	Specificity	61%
	Efficiency	88%
MFT	Sensitivity	28%
	Specificity	100%
	Efficiency	31%
TTC	Sensitivity	37%
	Specificity	100%
	Efficiency	36%

Table 4: Detection of *E.coli* in water when assess with accepted water quality testing methods.

Test	Water Quality	Source	<i>E.coli</i>		Total
			Absent	Present	
MTFT/24	Safe	DGW	6		6
		SGW	3		3
		Total	9		9
	Polluted	SW	60	28	88
		DGW	48	21	69
		SGW	57	28	85
		Total	165	77	242
MTFT/48	Safe	SGW	2	0	2
		Total	2	0	2
	Polluted	SW	60	28	88
		DGW	54	21	75
		SGW	58	28	86
			Total	172	77

Table 4: Continued

H ₂ S/24	Safe	SW	22	4	26		
		DGW	37	9	46		
		SGW	24	11	35		
		Total	83	24	107		
	Polluted	SW	38	24	62		
		DGW	17	12	29		
		SGW	36	17	53		
		Total	91	53	144		
		H ₂ S/48	Safe	SW	1		1
				DGW	17	2	19
SGW	7			4	11		
Total	25			6	31		
Polluted	SW		59	28	87		
	DGW		37	19	56		
	SGW		53	24	77		
	Total		149	71	220		
	MFT		Safe	SW	45	18	63
				DGW	42	15	57
SGW		48		15	63		
Total		135		48	183		
Polluted		SW	15	10	25		
		DGW	12	6	18		
		SGW	12	13	25		
		Total	39	29	68		
		TTC	Safe	SW	52		52
				DGW	50		50
SGW	59				59		
Total	161				161		
Polluted	SW		8	28	36		
	DGW		4	21	25		
	SGW		1	28	29		
	Total		13	77	90		

enteropathogens and indicators to obtain data for the assessment and management of microbial risks in drinking water. The present study will aid in developing practical plans to adopt, testing methods for assessing quality of drinking water.

When non-standard rapid H₂S test compared with standard or accepted MTFT, it was 58% to 83% sensitive and 59% to 88% efficient in 24 to 48 h of incubation. The 3 water samples detected polluted by H₂S test (false positive) were safe by MTFT; it may be due to soil containing H₂S producing bacteria. The water detected polluted by MTFT/24 (101 samples) and MTFT/48 (25 samples) were safe by H₂S /24 and H₂S /48, it may due to non-fecal originated coliform and do not contain fecal associated microbes. The water detected safe by MFT (174 samples) and TTC (152 samples) tests were polluted by MTFT, it may be due to non-fecal coliform and absence of fecal coliform or thermotolerant *E.coli*. The polluted water showed presences of *E.coli* were detected safe by H₂S /24 (24 samples), H₂S/48 (6 samples) and MFT (48 samples), whereas samples detected safe by MTFT and MFT showed total absence of *E.coli*.

MTFT and H₂S/48 tests were more or less equally sensitive to surface water as compare to ground or deep ground water. Efficiency of H₂S test varies with the source and decreased with the depth of the source of water and efficient for surface water. The study indicated that MFT and TTC tests are more reliable and highly specific for detection of fecal contamination in water but these tests required sophisticated laboratory and skilled personnel. However, the H₂S test, compared to other tests, was more suitable, reliable, inexpensive, easy to perform and useful to detect fecal contamination in drinking water within 24 h, for places where time, man and laboratory facilities are very poor. In principle, the test does not conform to the conventional standards of bacteriological testing of water samples and cannot replaces the conventional MTFT, MFT or TTC test. However, the H₂S test is easy to perform, user-friendly, screening test, suitable for handling by untrained personnel for community participation in monitoring of rural drinking water sources and low cost rapid test, hence recommended for the routine monitoring of water for recent faecal contamination in the field or villages where technical expertise, infrastructure and incubation equipment are not readily available. The study thus concluded that where there is laboratory facilities MFT and TTC and MTFT tests should be performed and in fields and in villages where manpower is inadequate rapid and cheap H₂S test should be used for detection of fecal contamination in drinking water.

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