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RAPID MONITORING OF INDICATOR COLIFORMS IN DRINKING WATER BY AN ENZYMATIC ASSAY

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ABSTRACT

Coliform group has been extensively used as an indicator of drinking water quality and historically led to the public health protection concept. Multiple tube fermentation technique has been currently used for assessment of the microbial quality of drinking water. This method, however, has limitations. Enzymatic assay constitute an alternative approach for detecting indicator bacteria, namely total coliforms and *E.coli* in various aquatic environments. This study compared the performance of LMX® broth as an enzymatic assay with the standard methods multiple tube fermentation technique and presence–absence test, for the detection of indicator coliforms in drinking water samples. In addition, the potential effect of water quality on the microbial detection method was assayed through measurement of some physicochemical parameters. From the 50 drinking water samples tested, 8 (16%) and 7 (14%) contained total coliforms and *E.coli* as indicated by all three techniques. Although on average the LMX recovered more total coliforms and *E.Coli* numbers comparing to multiple tube fermentation, but there was no significant difference. A significant difference existed between the level of residual chlorine for positive and negative samples. In conclusion, enzymatic assay showed a rapid and less labor method, allowing the simultaneous detection of total coliforms and *E.coli*. The method is particularly useful in the early warning of fecal pollution of drinking water.

Key words: Drinking water, coliform, enzymatic assay, multiple tube fermentation

INTRODUCTION

Continuous microbiological monitoring of drinking water is essential to ensure compliance with quality standards and to protect public health. Total (TC) and fecal coliforms (FC) have traditionally been regarded as indicators of microbial contamination of waters (Clark *et al*., 1991; Rompre *et al*., 2002). Recent reviews, however, have shown *E.coli* to be the best indicator for the assessment of fecal contamination (Clark *et al*., 1991; Edberg *et al*., 2000) and the possible presence of enteric pathogens (Geissler *et al*., 2000; US EPA, 2002).

The most commonly employed method for the detection of total and fecal coliforms in water is multiple tube fermentation (MTF) technique. A major limitation of MTF is the length of time (24-96 h) required to complete the testing (Edberg *et al*., 1988; George *et al*., 2000). This considerable delay in the assay response makes it impossible to take sanitary measures immediately after a fecal pollution has occurred. Moreover, it is labor intensive, uses several different types of media and two different incubation temperatures (Eckner, 1998; Rompre *et al*., 2002).

To overcome these limitations, enzymatic methods have been developed. These assays are specific, sensitive and rapid. The enzymatic assays for detection of total coliforms and *E.coli* are based on the hydrolysis of chromogenic or fluorogenic substrates by *β*-galactosidase and *β*-glucuronidase activity, two enzymes found in total coliforms and *E.coli,* respectively (Brenner *et al*., 1993; George *et al*., 2000; Rompre *et al*., 2002; Bitton, 2005; APHA, 2005). The assay provides a simultaneous analysis for total coliforms and *E.coli*, and provides a complete

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analysis in 24 h (Bitton, 2005; APHA, 2005). In addition, the method is easy to perform and interpret (Edberg *et al*.; 1990).

Various commercial kits based on these substrates are available and several comparisons between these commercial preparations and the standard methods MTF and membrane filtration (MF) techniques have been performed to enumerate TC and *E.coli* in various types of waters (Edberg *et al*., 1990; Olson *et al*., 1991; Clark *et al*., 1991; Palmer *et al*., 1993; Eckner, 1998). However, several factors including the source and physicochemical parameters of water samples may exert an effect on the performance of the detection method (Edberg *et al*., 1990).

Another important factor affecting the detection of coliforms in water is the occurrence of injured bacteria in samples (Bitton, 2005). For these reasons, this study was undertaken to determine the usefulness of LMX broth (as an enzymatic assay) in recovering chlorine stressed or damaged coliforms from drinking water in comparison with the standard methods multiple-tube fermentation (MTF) and presence-absence (P-A) in the both quantitative and qualitative formats, respectively. In addition, the potential effect of water quality on the microbial detection method was assayed through measurement of some physicochemical parameters.

MATERIALS AND METHODS

A total of 50 drinking water samples were collected aseptically from dinking water taps in various points in the city of Ahvaz, located in south-west of Iran. An effort was made to obtain water from locations most likely to yield positive samples, such as dead ends and known problem sites. All samples were collected in sterile glass bottles containing sodium thiosulfate to neutralize any residual disinfectant after the tap water was allowed to run for 2 min. Another sample was also collected for examination of physicochemical parameters.

MTF /MPN method: the MTF method was performed as a 10 tube MPN test. According to the *standard methods*, each tube containing 10 mL of double-strength lactose broth was inoculated with 10 mL of drinking water sample and incubated at 35ºC for 24-48 h. The tubes that were positive for total coliforms, as indicated by the production of acid or gas were then confirmed in brilliant green lactose bile (BGLB) broth and EC broth for the presence of total and fecal coliforms, respectively (APHA, 2005).

LMX broth (Fluorocult LMX, Merck) / MPN method

the test was performed with 100 mL of water sample. It was formatted in a 10 tube MPN arrangement. Water samples were added to the LMX tubes. The tubes were then placed in a 35ºC incubator for 24 h. Development of a blue–green color after incubation indicated the presence of TC in the test tube. Each positive total coliform test tube was exposed to a hand-held long-wave (366nm) UV light (Merck). Fluorescence in the test tube indicated the presence of *E.coli*. The number of coliforms per 100 mL was estimated from a 10-tube MPN table in both methods (APHA, 2005).

P-A test

Each water sample was thoroughly shaken and 100 mL of the water sample inoculated into a P-A culture bottle containing 50 mL of triple-strength lactose broth. After incubation at 37 ºC for 24-48 h, confirmation tests were performed on positive samples according to standard methods.

Negative and positive control samples were included in all assays using sterilized distilled water and wastewater effluent, respectively.

Physicochemical parameters

At the time of sampling the temperature, pH and free chlorine residual (DPD method) were measured. Turbidity and electrical conductivity were also measured in the laboratory.

Statistical analysis was performed by use of SPSS 13.0. Significant difference between the ability of the LMX broth and MTF method to detect total coliforms and *E.coli* was tested using *t*-test. To examine the effect of physicochemical parameters on microbial quality, samples were grouped into positive and negative categories, and then comparison was made by analysis of variance (ANOVA). A *P*-value of <0.05 was considered significant.

RESULTS

From 50 drinking water samples analyzed, 8 (16%) and 7 (14%) samples were positive for TC and *E.coli*, respectively, as determined by all three detection methods (Fig. 1). LMX test showed a mean of 2 and 1.63 MPN/100 mL for TC and *E.coli* enumeration respectively. Table 1 shows the results of MTF and LMX broth.

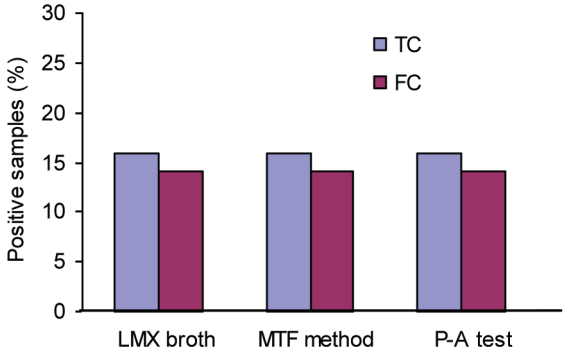

Fig. 1: Detection of total and fecal coliforms in drinking water by LMX broth, MTF method and P-A test.

Table 1: Comparison of MTF and LMX for detection of TC and *E.coli*.

	TC (MPN/100mL)		*E.coli* (MPN/100mL)	
	MTF	LMX	MTF	LMX
Average	1.4	2	1.38	1.63
SD	4.07	4.75	4.07	4.42
Min	0	0	0	0
Max	13.5	13.5	13.5	13.5

Out of the 50 samples, 5 (10%) of the samples contained no residual chlorine and 2 (4%) had a concentration of 0.1 mg/l. The results also showed that 12 (24%) of the 50 test samples had turbidity greater than 5 NTU. There was a significant difference between the level of residual chlorine for positive samples and negative samples (P=0.03 for total coliform and P=0.01 for *E.coli*). However, physicochemical parameters did not show any significant effect on the detection method. The results of physicochemical parameters are presented in Table 2.

Table 2: Summary of the results of physicochemical parameters

Parameter	Mean	Min	Max
Temperature (^{o}C)	21	15	32
pH		6.8	8.2
Turbidity (NTU)	4.26	0.5	20.7
Residual free chlorine (mg/L)	1.34	0	5
EC (µmhos/cm)	1330	823	1798
TDS (mg/L)[1]	798	494	1078

[1]TDS=EC*1.66

DISCUSSION

The results of this study indicated that LMX broth assay was comparable to the MTF method for the detection of total coliforms and *E.coli* in drinking water. This finding is in agreement with other studies which have compared the classical *standard methods* procedures with commercial kits and MI agar as enzymatic assay (Edberg *et al*., 1990; Clark *et al*., 1991; Brenner *et al*., 1993; Brenner *et al*., 1996; Eckner, 1998).

The overall results for total coliforms and *E.coli* tests showed that LMX recovered 1.4 and 1.18 times as many TC and *E.coli*, respectively, as the MTF method. However, statistical analysis demonstrated no significant difference between the two methods. A similar finding has been noted in the quantitative determination of TC and *E.coli* in marine waters by Geissler *et al*. (2000). However, higher level recovery of total coliforms and *E.coli* by the LMX could be explained by the presence of stressed cells which are unable to growth in culture media; but which maintain their viability (viable but non-culturable bacteria) and still capable of metabolic activity.

A number of chemical and physical factors involved in drinking water treatment, including disinfection, can cause sublethal injury to coliform bacteria (Rompre *et al*., 2002; Bitton, 2005). A free chlorine residual of 0.25 to 0.5 mg/l in drinking water was found to cause a >90% injury rate in coliforms (Brenner *et al*., 1996). Several studies have shown that bacteria could be metabolically active even they were not detected by the cultivation techniques commonly used (Pommepuy *et al*., 1996; George *et al*., 2000 ;Caruso *et al*., 2002). Comparison of an enzymatic assay (Colisure test) with standard methods techniques for detecting bacteria subject to chlorine stress by McFeters *et al*., (1997) showed more recovery of chlorine-injured TC and *E.coil* by enzymatic assay, resulting in a more realistic estimate of the actual population of indicator bacteria in public water supplies.

The results indicated that the LMX is an alternative approach that could provide better and more rapid information for the assessment of microbial quality of drinking water. It could simultaneously detect total coliforms and *E.coli* from a water sample within 24 h and hence, will

provide utilities an immediate measure of whither a sample has been subject to fecal contamination. However, some the LMX tubes needed more than 24 hours of incubation for fluorescence development (Geissler *et al*., 2000). Edberg & Edberg (1988) reported that chlorine injured *E.coli* required longer incubation times to produce fluorescence. Nevertheless, the test should not be extended beyond 28 h incubation period, because the increased incubation time may yield false positive results (Edberg *et al*., 1988).

In conclusion, the LMX is superior to the current MTF method for routine monitoring of drinking water. It is easy to perform and gives more rapid and more realistic estimate of total coliforms and *E.coli* than MTF. This method might be more expensive in terms of consumables than the classical methods when the latter require no additional confirmation steps. In all cases, however, enzymatic methods require less manpower and therefore their cost in terms of commercial value is lower.

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