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Full Length Research Paper

Identifying a cost effective method for thermo tolerant coli forms enumeration in raw water resources used for domestic consumption and irrigation

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The present study is descriptive and analytical in nature, aimed to determine the correlation and compatibility rates of the detection methods of multiple tube fermentation (both traditional and direct techniques) using *Escherichia coli* (EC) Broth and plate count in special fecal *Coli form* culture media. The objective was to identify the one method among these which is economical, effective, rapid and easy to use under emergency conditions. Pour plate count using Eosin-methylene blue (EMB) was evaluated in comparison with the traditional, and direct multiple tube fermentation (MTF) as techniques used in enumerating thermo-tolerant bacteria and *E. coli* in water resources. Fourteen samples were required, based on a pilot study. However, 70 samples were analyzed to obtain a higher level of accuracy through three microbial laboratory methods, in evaluating raw water collected from wells, springs, and rivers as well as the effluent of wastewater treatment plants. A high Pearson correlation was found for enumerating thermo-tolerant bacteria. The results revealed that majority of the samples contained thermo-tolerant *coli form*. Based on the correlation coefficients, calculated among the three methods, economical considerations, test duration, and time to yield results, the plate count, the direct MTF, and the traditional MTF may be ranked in a descending priority order from the viewpoint of bacterial quality assessment of water resources for the emergency conditions and irrigation purposes. Conventional, direct MTF and plate count methods were powered in detection *E. coli* in rate of 95, 90 and 80% respectively.

Key words: Multiple tube fermentation, pour plate count, thermo-tolerant *Coli form*, agreement rate, water resources.

INTRODUCTION

Polluted drinking water is an essential cause of the diarrhea diseases that results in 2.5 million childhood deaths per year. The International water-quality standards allowed no detectable level of injurious pathogens at the point of distribution. In spite of this, microbiological water quality can deteriorate in the course of collection, transport, and home storage. Thus, access to a safe source alone does not ensure the quality of water that is consumed. Furthermore, a better water source does not lead to full health benefits in the absence of improved water storage and sanitation (Oswald et al., 2007). *Coli form* bacteria are the recommended hygienic indicator organisms for both raw and potable water. However, many bacteria in the genera of *Enterobacteriaceae* meet the definitional criteria for *coliform* although they are not of fecal origin (Kuang Chao et al., 2004). Regarding changes and reconstruction of technology, evaluation of detection and determination in microbial indices, such as fecal *coli form* to determine the water resources quality is important and necessary. Many states are replacing microbiological water quality standards based on “fecal” or thermo-tolerant *coliform* with new standards that

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employ *Escherichia coli* as the indicator organism (Hamilton et al., 2005). Determination of quality of water used for hygienic purposes like drinking and swimming and also in agriculture consumption has specific importance. Water quality assessment methods are investigated taking into account the economic concerns and the discrimination efficiency of each method, specifically for emergency conditions (Eccles et al., 2004, Chihara et al., 2005). Researchers consider and compare the aspects of economical effectiveness and rapid and easy to access results in the shortest time (Reasoner et al., 1979; Richards, 1978; Bwciebin et al., 1995; Ononu et al., 1999; Bitton, 1999; Board-Andrew et al., 1998; Alonson et al., 1999; Schets et al., 2002; Anon, 2000a, b; Filius et al., 2003; Ahmed et al., 2009). Precision, accuracy and quick generation of results from water quality experiments is important and because of current multiple tube fermentation (MTF) method, direct method and plate count (PC) are used. Evaluation of those methods is important in the aspect of efficiency and time to be concluded. However, in the detection of *coli form* at plate count (PC) method, conclusion is near multiple tube fermentation (MTF), plate count can be chose as the superior option if direct methods do not have conspicuous difference, it can be used at the optimum decision (World Health Organization, 1993; Board-Andrew et al., 1998). In this study, compare the experimental program in corresponding with polluted water samples in the aspect of determiner, fecal *coli form*. Based on economical concerns and time for access the result of case study specifically in emergency conditions and with corresponding the result for using it in the agriculture or recreation purposes it is better to use the method that have economical effectiveness furthermore, in considering the time limitation and access ability of results and spending expanses for each method, necessity of comparing to test the specific methods is more than previous time. Up to now, discrimination of mentioned ways is not determined exactly. Therefore, in this research with statistical analyzing, compare the result with each other until if direct method and plate count method don't corresponding with current way in detection and determine fecal *coli form* used one of simpler and cheaper way which might be cost-effectiveness.

MATERIALS AND METHODS

This study carried out with doing experiments corresponds on standard methods. For detection the number of samples, forehead do at the pilot scale that in this way apply 4 sets test in each method at detection microbial quality of water in the aspect of fecal *coli form*. In result of this study, number of samples for MTF is 19 ± 1 and for PC is 21 ± 2. By using the comparison of two averages with 95% confidence limits, 90% power and assumption of 0 (zero) correlation, forty samples would be enough, but for being more precise and accurate, 70 samples were applied as the volume of sample. For increasing the correctness and precession in each test at MTF methods and plate count, with at least 2 times repetition, it should be doing in every MTF and plate counts for 28 times 70 samples are considering for this current study that it uses results of further study. It doesn't repeat the MTF also, 70 samples recount in the Mc Farlane method and cultivation. Result of tests recorded in the table and analyzed with SPSS software that used for agreement *kapa* coefficient and compared their average with the analysis of variance ANOVA, independence (t) test and Levene's test. Polluted water samples collected from different resources like: rivers, springs and home-made wells. Further more, some of the samples provided from watery and deposit sewages in 10^{-5} scale that those samples were liquefy with sterile dilution water. The methods of tests were taken from APHA and water quality guidance of world health organization (1999) (Board-Andrew et al., 1998). Incubation temperature for MTF 44.5 ± 0.2ºC for the time 24 to 48 ± 2 to 3 h and agars (cultivation environments) that used include: lactose Broth, production of Merck company with serial number: 1/07661/0500. Brilliant Green Bile Broth with serial number: 1/05454/0500 and EC Broth the production of Merck with serial number 1/10765. Eosin methylene blue agar the production of Merck with serial number: 1/01342. For the plate count (PC), used 44 and 35 ± 0.5ºC temperature in 24 to 48 ± 2 to 3 h. Differentiation tests were carried out, according APHA 2005. For evaluating the economical effectiveness record time for responding the supervision and expert in the laboratory, duration of performance the activity and maximum and minimum time for conclusions and expenses for each test account with the attention of time and consumed material in the final price.

RESULTS AND DISCUSSION

Generally 70 polluted water samples tested and show current MTF, direct MTF and plate count that orderly A, B, C. Analyzing the data shows that most coefficient correlation is exist between A-B, A-C, C-B methods. That in order is: 0.92, 0.59 and 0.56 with P- value < 0.001 and most agreement coefficient in order is 0.88, 0.25, and 0.56 with P < 0.001 (Table 1).

Table 2 shows that average of counting *coli form* in the plate count (PC) is more than two other methods. But those differences are not meaningful. Two methods of direct MTF and plate count orderly have 80, 100, 20, 96/9 percent sensitivity and feature (Table 3). Result of pure cultivation shows agreement coefficient 0.83 and 0.16 for two methods of direct MTF and plate count in the current way. As seen in the Table 5, current methods have performance time, conclusion time and more expensive than two other methods.

In the conventional method, 19 samples (95%) of picked up colonies had positive motility and were positive response to Indole and Methyl red indicator. This notes, that these micro-organisms are probability *E. coli* at level of 95 percent. In direct MTF, 18 cases (90%) was the same as conventional method. But in the plate count method with Eosin methylene blue agar, 16 cases (80%) of picked up colonies was *E. coli* (Table 6).

The statistical analysis of data show that results of conventional MTF and direct MTF have agreement coefficient (*Kapa*) 0.88 and have a significant correlation (r = 0.92) and Predictive Positive Value (PPV) equal 100% high amount of PPV, which facilitate the access ability to more precise results(Table 4). This adjustability

Table 1. Comparison of Conventional MTF (A), Direct MTF (B), and Plate Count (C) with P-value ≤ 0.001.

Interactions In method	Coefficient correlation (%)	Coefficient agreement (%)
A,B	92	88
A,C	59	25
B,C	56	56

Table 2. Average counting of the fecal *coli form* between 3 direct MTF, current MTF and plate count methods based on Log number.

Method	Mean (SD)
Conventional MTF	6.5798 ± (6.7404)
Direct MTF	6.4914 ± (6.7160)
Plate count	5.8451 ± (6.9445)

Table 3. Sensitivity, feature and informative value -/+ in two methods of MTF and plate count in the fecal *coli form* based on current method.

Methods	Precision (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
MTF	100	80	100	100	98.5
Plate count	91.4	20	96.9	33.3	94

Table 4. Results of statistical analysis of the methods (direct MTF and plate count in fecal *coli form* enumeration) and compare with conventional MTF, using defined specific type and certain population.

Method discriminator	Pearson correlation coefficient	P-value	*Kapa* agreement coefficient	P-value
MTF	0.62	0.004	0.83	0.03
Plate count	0.69	NS	0.16	0.01

Table 5. The results of performance and conclusion time and expense of three methods (the number of sample 70).

Method	Conclusion time (h)	Expense ($)*	Performance time (min)
MTF	52 ± 34	5.75 ± 0.87	42 ± 17
Direct MTF	24 ±20.5	2.5 ± 1.2	29.5 ± 11.25
Plate count	22 ±3	2 ±0.6	16.25 ± 6.2

* Change by time.

with World Health Organization instruction and is the same with Bredie and Deboer study, that correspond with direct method in the emergency conditions (Bredie and Deboer, 1992). This study is adjusted with Rompre et al., study, that though many innovative bacterial detection methods have been developed, few have the potential for becoming a standardized method for the detection of *coli form* in drinking water samples (Rompre et al., 2002). Schraft and Watterworth illustrated the high specificity of the Petri film EC plates for enumeration of both fecal *coli form* and *E. coli* in water (Schraft and Watterworth, 2005). Khan et al., compared two different methods of CF and MF for detecting *Campylobacter* species in surface water samples from agricultural watersheds across Canada and that CF methods can have significantly different recovery efficiencies for *Campylobacter* species (Khan, et al., 2009). In mentioned conditions with considering the economical concerns and short time of conclusions if fecal *coli form* consider, direct enumerating method of fecal *coli form* is better than the conventional method

Table 6. Result of Indole, Methyl red, Voges-Proskauer and citrate (*IMViC*).

Method	Samples corresponded with detection of *E. Coli* (20 samples)	(+)%
Conventional MTF	19	95
Direct MTF	18	90
Plate count	16	80

(Bredie and Deboer, 1992). Result of plate count method has weak harmony with conventional multiple fermentation tube and direct multiple fermentation tube. Agreement coefficient 0.25 with predictive positive value (PPV) equal 33.3% show relative adjacent and result of the test, show no difference between the result of plate count and conventional MTF. Plate count with direct MTF have better agreement coefficient (*kapa* coefficient = 0.56). Considering the difference of conclusion time, expense and time and needed facility in three methods, show that plate count in comparing with direct MTF and conventional MTF is easier, shorter and cheaper, which is preferable in the emergencies and the other extensive demand such as microbial assessment and agricultural irrigation. Therefore, with observing the beach economical concerns and emergency conditions, plate count, specific agar is prefer to direct method and direct method is prefer to conventional method due to several levels of evaluations. Plate count method is preferred rather than direct MTF and this method is preferred rather than conventional MTF and conclusion time. Result of this study in the aspect of accessibility rather than *E. coli* in accounting colonies use three methods that correspond with Gonzales, Ruben, Tamagini, Lucia, and Chihara et al. findings (Lucia et al., 2001, Chihara et al., 2005). The important point in this study is that in the fecal *coli form* recognition and enumerating, *E. coli* has the most part existence resources consider more than 90% of it in the polluted water (World Health Organization, 1993, Board-Andrew et al., 1998). But in this survey conventional MTF determined 95% and direct MTF 90% and plate count 80% show micro-organism with distinguishing features of *E. coli*. Low enumerated of this microorganism in plate count method could be because of vulnerability and disability of weak micro-organisms in this method. In tube method reproduction and alive of *E. coli* is more than plate count method but high amount of number in micro-organisms and interference with another micro-organisms without fecal in this method is another reason for low 15 percent that this is indicator of microbial quality in the plate count.

Conclusions

This study shows that plate count is orderly used rather than direct MTF and the second method in the aspect of economical effectiveness prefer rather than conventional MTF. In the emergency conditions when the shortage of resulting time is important for evaluation of water sources as a fecal index, plate count can orderly be used than direct MTF. The end suggested that this study is applied with more specific media, until it can be judge based on trust and sureness of result.

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