

# DETECTION AND ENUMERATION OF FEACAL COLIFORMS AND OTHER MICRO ORGANISMS IN DRINKING WATER (A COMPARISON OF TWO TECHNIQUES)

Pages with reference to book, From 329 To 334

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## Abstract

Comparative efficacy of two water analysing techniques, based on bacterial analysis has been investigated. Method of Rand et al<sup>1</sup>. with some alterations has proved to be ideal as it provides information on presumptive coliform, fecal coliforms, standard plate counts and confirms the identity of the isolates. Moreover, the improvised method is less time consuming and is comparatively inexpensive (JPMA 35: 329, 1985).

## INTRODUCTION

Water sources used by rural and urban population in developing countries are usually, very prone to fecal contamination.<sup>2</sup> Majority of the population in the developing countries is not adequately supplied with potable water, thus it appears obligatory that they are to use unsafe waters for drinking and domestic purposes.

Pakistan is also facing such a problem. Assessment of fecal contamination and detection! enumeration of coliforms and other microorganisms in water is one of the measures to determine hygienic quality of water.

Although, there is much debate concerning the adequacy of the coliform indicator concept and its ability to accurately. determine the potability of drinking water, currently it is the microbiological standard mandated by law. The presence of any coliform organisms in drinking water is an indication of a contaminated source, inadequate treatment or post-treatment contamination.<sup>3</sup>

However, in developing countries, where some degree of fecal contamination of water sources is considered inevitable and where it would not be feasible to condemn such supplies simply due to mere presence of coliforms, a quantitative assessment of a more specific indicator is required.

The conventional methods of water microbiological analysis based on presumptive coliforms, fecal coliforms, standard plate counts and further confirmatory tests are cumbersome, time consuming and expensive. There is, therefore a need for a rapid and critical assessment technique for water quality testing in developing countries. This investigation was thus carried out to assess the comparative efficacy of two water analysing methods based on bacterial analysis.

## MATERIAL AND METHODS

### Sample collection:

One hundred and twelve water samples were aseptically collected in sterilized 800 ml capacity glass stoppered bottles from Rawalpindi and Islamabad. Samples were collected from May 60-October 84, from wells, springs, rivers and municipal tap water supplies. They were immediately transported to the laboratory and processed within 4 to 6 hours of collection.

pH of the samples was recorded by pH meter.

## **Culture media:**

The following culture media were used in this study:-For presumptive coliforms test Lactose broth LB (Oxoid) was used, Brilliant Green Lactose Bile Broth BGLB (Oxoid) and Eosine Nethylene Blue agar EMB (Difco) were used for confirmatory fecal coliforms, Ringers solution (Oxoid). Plate count agar PCA (Oxoid) and Mac Conkey's agar (Difco) were used for standard plate count and coliform counts, selenite broth (Difco), Peptone (Oxoid), Sodium Chloride NaCl (Merck), Xylose lysine dextrose, medium XLD (Oxoid) and Thiosulphate citrate bile salts sucrose agar TCBS (Oxoid) were used for special tests, systic system No. 1 (Eiken Ltd. Japan) and other biochemical tests were used for identification of the isolates.

## **Methodology**

Method No. 1. Standard Methods for the Examination of water and waste water<sup>1</sup>.

### **(i) Presumptive Test for coliforms (MPN)**

#### **(a) Untreated water samples.**

Following strict aseptic procedures, the sample was shaken vigorously and inoculated in 2 tubes of lactose broth, one with 1.0 ml and the other with 0.1 ml of sample, and 5 tubes of double strength (2N) lactose broth, each with 10 ml of sample and 10 ml of the medium with inverted Durham tubes. Inoculated tubes were incubated at 35°C for 48 hours. Following the incubation period results were recorded. If no gas was formed within 48 hours this constituted a negative presumptive test. The presence of 10% or more gas displacement in any of the Durham tubes was considered a positive presumptive test. The most probable number (MPN) for the water sample tested was recorded by number of positive tubes, containing gas in the series of 7 tubes and consulting MPN table (Rand et al., 1976).

#### **b. Treated water samples.**

In case of chlorinated or sand filtered waters, 50 ml of double strength lactose broth was inoculated with 25 ml of water sample and incubated at 35°C for 48 hours.<sup>4</sup> The rest of the procedure was same as for untreated water samples.

(ii) Confirmatory test for fecal coliforms. One ml from each positive tube was inoculated in BGLB broth tubes containing 10 ml of medium with one inverted Durham tube in each. They were incubated at 44.5°C in a water bath for 24 hours. Tubes with gas and turbidity after incubation were considered positive. Positive tubes were further cultured on EMB agar for isolation of fecal coliforms<sup>4</sup>. After 24 hours incubation at 35°C the isolated colonies were inoculated in the systic system No.1 for species identification.

### **(iii) Standard plate count for water (SPC).**

The standard plate count was done by pour plate technique. Prepared 10 fold dilutions, each tube containing 9 ml of ringers solution. Dilutions were made upto 10<sup>-6</sup>

Accurately measuring 1 ml from each dilution was inoculated in duplicate empty and sterilized petridishes. About 12 to 15 ml of plate count agar already kept at 45°C in a water bath was added to each plate. Plates were gently rotated after pouring the media and allowed to set and were incubated in an inverted position at 35°C for 24 to 48 hours. The surface and subsurface bacterial colonies thus received were counted by a colony counter. Plates showing 30-300 colonies were counted to determine the SPC per ml of sample tested<sup>5</sup>.

### **(iv) Identification of the isolates.**

Isolated colonies from the SPC plates were inoculated in the systic system No.1 for the identification of the isolates from the water samples. Results were recorded after 24 hours incubation at 35°C.<sup>6</sup>

Method No. 2 (Improvise technique).

### **(i) Presumptive Test for Coliforms (MPN)**

#### **(a) Untreated water samples.**

Following strict aseptic procedure, 1 ml of water sample was inoculated in a tube containing 9 ml of

double strength (2 N) lactose broth and further 10 fold dilutions were made upto  $10^{-6}$ . Each inoculated lactose broth dilution tube was placed with an inverted Durham tube.

The tubes inoculated were incubated at  $39^{\circ}\text{C}$  for 48 hours. Following the incubation period, the dilutions were observed for gas production. If no gas was formed within 48 hours a negative presumptive test was constituted. The presence of 10% or more gas displacement in any of the Durham tubes was considered a positive presumptive test and result recorded by positive tubes in series and interpreted in terms of the lowest positive dilution factor per ml.

**(b) Treated water samples.**

In case of chlorinated and sand filtered water samples, the same procedure as for untreated water was used except the addition of a flask containing 50 ml double strength (2N) lactose broth. This flask was then added with 25 ml of the water sample to be tested<sup>4</sup>.

**(ii) Confirmatory test for fecal coliforms.**

This was carried out by using one GBLG tube per sample on zero day by directly delivering 1 ml of vigorously shaken water sample to 10 ml BGLB tube containing inverted Durham tube.

The inoculated tubes were incubated at  $44.5^{\circ}\text{C}$  in a water bath for 24 to 48 hours. Turbidity and the presence of 10% or more gas displacement in the Durham tube was considered to be the positive test.

**(iii) Standard plate count for water (SPC).**

Duplicate petriplates each of platecount agar and MacConkey agar were taken for each water sample. All plates were divided in six portions and each portion was marked with the dilution factor, 0.05 ml of each dilution (already prepared in lactose broth for the MPN) was delivered in the respective portions in all the four plates already marked and was spread over.

All the plates used for total viable and differential plate counts were observed after 24 hours incubation at  $35^{\circ}\text{C}$ . Portions showing 5 - 50 colonies were counted and multiplied by 20 to determine the SPC and coliform counts per ml of the sample tested. The results were also checked and confirmed by comparison with dilution factor of the MPN positive tubes and the number of lactose fermenting colonies on the MacConkey agar plates.

**(iv) Identification of the isolates.**

Isolated colonies from the plate count agar plates and MacConkey agar plates were inoculated in the systic system No.1 for the identification of isolates. Results were interpreted after 24 hours incubation at  $35^{\circ}\text{C}$ <sup>6</sup>.

Special Tests

**(a) Salmonella sp.**

Fifty ml of selenite broth in 100 ml sterile flask was inoculated with 25ml of well mixed water sample. This was incubated for 16 - 18 hours at  $43^{\circ}\text{C}$ , then subcultured on Xylose Lysine Dextrose medium (XLD) incubated for 24 hours at  $35^{\circ}\text{C}$ . Suspected colonies were confirmed biochemically<sup>7</sup>.

**(b) Vibrio sp.**

Fifty ml of alkaline peptone water pH 8.6 in 100 ml sterile flask was inoculated with 25 ml of well mixed water sample which showed pH more than 8.2. This was incubated for 16 to 18 hours at  $35^{\circ}\text{C}$  then subcultured on TCBS agar and incubated for 24 hours at  $35^{\circ}\text{C}$ . Suspected colonies were confirmed biochemically<sup>5</sup>.

## RESULTS

pH of all the water samples varied between 7

Fifty water samples from different sources were examined by method No. 1.

(i) Presumptive test for coliforms (MPN).

Twenty nine untreated water samples were examined by this technique and 19 were found positive having MPN from 2.2 to 240 +1100 ml. Ten samples were negative.

Out of 21 treated water samples, 11 were positive having MPN from 2 to 240+/100 ml, whereas 12 samples were found negative.

(ii) Confirmatory test for faecal coliforms. Eleven water samples were found positive for faecal coliforms while 39 were negative.

(iii) Standard plate count for water (SPC).

Fourteen treated water samples and four untreated samples showed no growth on the plate count agar plates and the other 32 samples enumerated had counts between  $10^2$  and  $10^5$ . A few samples exhibited even higher counts upto  $10^6$  or more (Table - I).

Table - I

Comparison of Standard Plate count by Standard and Improvised Methods.

Standard plate count range/ml	Standard Method n = 50				Improvised Method n = 62				Total water samples No. %	
	Treated water samples		Untreated water samples		Treated water samples		Untreated water samples			
	No.	%	No.	%	No.	%	No.	%	No.	%
<10	3	10.3	0	0	0	0	2	5.5	5	4.5
$10 - <10^2$	2	6.9	1	4.8	1	3.9	7	19.5	11	9.8
$10^2 - <10^3$	6	20.7	6	28.5	3	11.5	7	19.5	22	19.6
$10^3 - <10^4$	5	17.2	3	14.3	3	11.5	5	13.9	16	14.3
$10^4 - <10^5$	0	0	5	23.8	3	11.5	6	16.7	14	12.5
$10^5 - <10^6$	1	3.4	1	4.8	3	11.5	4	11.1	9	8.0
$>10^6$	0	0	1	4.8	2	7.8	3	8.3	6	5.4
No growth	12	41.5	4	19.0	11	42.3	2	5.5	29	25.9
Total water samples	29		21		26		36		112	

(iv) Identification of isolates.

Nine different varieties of microorganisms were isolated by standard method, namely E. Coli, Kiebsiella sp., Enterobacter sp., Yeast sp., Pseudomonas sp., Alcaligenes sp., Acenitobacter sp., Streptococcus feacalis and Staphylococcus sp. (Table II and III).

Table II

Comparison of Bacterial Isolates identified by Standard and Improved Methods.

Isolates	Standard Method n = 50						Improved Method n = 62					
	Isolates from Treated water samples		Isolates from Untreated water samples		Isolates from Total water samples		Isolates from Treated water samples		Isolates from Untreated water samples		Isolates from Total water samples	
	No	%	No	%	No	%	No	%	No	%	No	%
E. Coli	6	54.5	9	31	15	37.5	4	18.2	7	19.4	11	19
Klebsiella sp.	0	0	5	17.2	5	12.5	4	18.2	1	2.8	5	8.6
Enterobacter sp.	0	0	3	10.3	3	7.5	0	0	2	5.5	2	3.4
Yeast sp.	0	0	1	3.5	1	2.5	0	0	2	5.5	2	3.4
Pseudomonas sp.	2	18.2	1	3.5	3	7.5	5	22.7	11	30.5	16	27.7
Alcaligenes sp.	2	18.2	1	3.5	3	7.5	5	22.7	6	16.9	11	19
Acinetobacter sp.	0	0	8	27.5	8	20	1	4.5	0	0	1	1.7
Streptococcus faecalis	0	0	1	3.5	1	2.5	2	9.2	3	8.3	5	8.6
Staphylococcus sp.	1	9.1	0	0	1	2.5	0	0	1	2.8	1	1.7
Proteus sp.	0	0	0	0	0	0	0	0	1	2.8	1	1.7
Bacillus sp.	0	0	0	0	0	0	1	4.5	2	5.5	3	5.2
Total bacterial isolates	11		29		40		22		36		58	

Table III

Bacterial Isolates Detected By Method No: 1 (Standard Method).

Type of water samples												
Positive samples of treated water	6	0	0	0	2	2	0	0	1	11	22%	
Positive samples of untreated water	9	5	3	1	1	1	8	1	0	29	58%	
Total Positive water samples n = 50	15	5	3	1	3	3	8	1	1	40	80%	

Complete results of presumptive coliforms, fecal coliforms, standard plate count and identification of isolates were available after 72 hours or more.

Sixty two water samples from different sources were examined by the improvised technique (method No.2).

(i). Presumptive test for coliforms (MPN) Thirty six untreated water samples were examined by the

MPN technique and 27 were found positive having MPN between 10 to 10<sup>6</sup> organisms/ml and nine were negative having no gas and turbidity.

Out of 26 treated water samples 10 were positive having MPN from 10 to 10<sup>4</sup> organisms/ml, whereas 16 samples were negative.

(ii). Confirmatory test for faecal coliforms.

Twelve water samples were positive for faecal coliforms and 50 were negative for the same.

(iii). Standard plate count for water (SPC) Eleven treated water samples and two untreated samples showed no growth on the plate count agar plates and the other 49 water samples enumerated counts between 10 to 10<sup>6</sup> (Table I).

(iv). Identification of the isolates.

Eleven different varieties of isolates were isolated using improvised method. The identified isolates were namely, E. coli, Klebsiella sp., Enterobacter sp., Yeast sp., Pseudomonas sp., Alcaligenes sp., Acinetobacter sp., Streptococcus faecalis, Staphylococcus sp., Bacillus sp, and Proteus sp. (Table II and IV).

**Table IV**

**Bacterial Isolates Detected by Method No: 2 (Improved Method).**

Type of water samples														
Positive samples of treated water	4	4	0	0	5	5	1	2	0	1	0	22	35.5%	
Positive samples of untreated water	7	1	2	2	11	6	0	3	1	2	1	36	58%	
Total Positive water samples	11	5	2	2	16	11	1	5	1	3	1	58	93.5%	
	n = 62													

Complete results for presumptive coliforms, faecal coliforms, standard plate counts and identification of the isolates were available after 48 hours.

Special Tests.

No water sample was found positive for Salmonella or Shigella sp. from the whole lot i.e. 112 water samples tested by both the methods.

All water samples tested for the Vibrio sp. were also negative.

## DISCUSSION

The period of this study was chosen in view of obtaining results over the transition period when dry weather changes to wet season and potentially, water sources are most prone to faecal contamination and microbial built ups. pH of all the water samples examined were between pH 7 to 8.5 i.e. more

towards the alkalinity.

Results for presumptive coliforms by standard method can be obtained per 100 ml, whereas by the improvised method exact coliform count/ml of the water sample can be obtained. The results can also be checked and confirmed by comparison with the dilution factor of the MPN positive tubes and the number of lactose fermenting colonies on the Mac Conkey agar plates.

For confirmatory faecal coliforms test, the time consumed by standard method is at least 4 days (i.e. 96 hours) and by the improvised method this result is available after 2 days (i.e. 48 hours), and the glassware and culture media required are also less in quantity.

By standard method first of all 10 fold dilutions of water sample have to be made in the ringers solution and at least twelve plate count agar plates are used for each sample. Whereas, by the improvised technique the 10 fold dilutions already made in lactose broth for the presumptive coliforms can be used for standard plate count. Although the coliform counts were not done in this study but the Mac Conkey agar plates can be used for coliform counts by counting lactose fermenting colonies and multiplying by the dilution factor on the portion where colonies were counted. Only two plate count agar and two Mac Conkey agar plates were used for both standard plate count and coliform counts by the improvised method. Thus, consuming less culture media, glassware and manhours. By adding lesser input through the improvised technique better results could be achieved in lesser time (Table I).

Nine different species were identified by standard method and eleven isolated by the improvised technique. Reason for this difference can be the isolated colonies picked from the Mac Conkey agar plates, because in the standard technique only plate count agar is used and it is very difficult to differentiate the isolates are from which family. But in the improvised technique the Mac Conkey agar plates are also used which is a differential media and inhibits the growth of microbes other than the family enterobacteriaceae and the lactose fermenting colonies can also be easily differentiated from non lactose fermenting colonies. Making it easier to identify more varieties of the microbes. Organism recovery was 13.5% more in the improvised method than the standard method (Table II, III and IV). Identical procedure was used in special tests for all the water samples examined and all were found negative for Salmonella, Shigella and Vibrio sp.

## REFERENCES

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