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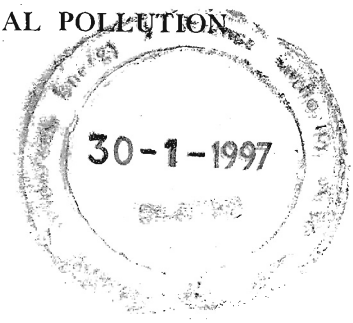
STUDIES ON BACTERIAL INDICATORS OF FAECAL POLLUTION IN DRINKING WATER



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Abstract: The objective of the study was to compare the routinely used bacterial indicator systems of faecal pollution, the coliforms and faecal coliforms with faecal streptococci and spores of sulphite reducing anaerobes (Clostridia) to assess their importance in determining the bacterial quality of water. A total of 84 samples of water from the Kandy distribution system of water were tested. Percentage of samples that were found to be contaminated by the three indicators, coliforms, faecal coliforms and faecal streptococci are 37%, 15% and 54% respectively. It was found that the use of faecal streptococci to determine faecal pollution of treated tapwater has additional advantage over the presently used coliform, faecal coliform indicator systems. Results of the use of *Clostridium* spores in determining the hygienic quality of water was found to be unsatisfactory.

1. Introduction

The search for an adequate indicator of faecal contamination in water has logically been associated with organisms common to the microbial flora of faeces Prerequisites for the ideal indicator have restricted the probable candidates to total coliforms, faecal coliforms, faecal streptococci and *Clostridium perfringens.*[7] Of these, coliforms and faecal coliforms are routinely used.

There are well known epidemiologic histories of the presence of bacterial pathogens when the coliform index was low. Boring *et al*[1] reported that *Salmonella typhimurium* outnumbered coliforms by a factor of 10 in the River Side California Outbreak. Similarly a report by Sleigman and Reitter[16] showed that index organisms can be low in the presence of pathogens. Isolation of *Salmonella* from drinking water which fullfilled the bacterial standards with regard to coliforms have been reported.[12] The reports of the failure of the index organism concept emphasize the need for more research that compare different indicator systems that could be used as indices of faecal pollution of drinking water.

Different opinions exist worldwide with regard to the question whether streptococci should be regarded as an indicator of faecal pollution. This poor acceptance of faecal streptococci as a measure of pollution from human and warm-blooded animal excreta has been a result in part of low recovery rates, of the multiplicity of detection procedures of poor agreement between the detailed and systematic studies of the sources, survival and interpretation of streptococci in various kinds of water. [5, 6, 14, 15] The use of *Clostridium perfringens* as a supplementary indicator in addition to the routine examination in potable water has also been proposed,[4] although it is not used routinely.

This paper reports the results of the application of faecal streptococci together with the routinely used indicators of faecal pollution coliforms and faecal coliforms in the determination of bacterial quality of drinking water. In addition, detection and enumeration of the spores of sulphite reducing anaerobes has been attempted in the present study, based on the working paper for a draft proposed for a draft international standard of *Clostridia* spores by the International Standardisation Organisation (ISO/TC/147/SC4/WG/5N41E).

2. Experimental

2.1 Collection of Samples

A total of 84 samples of tap water from the Kandy distribution system were examined during the period March 1982 to June 1983. All the samples were collected from street standposts which are directly connected to the mains and transported in accordance with the methods recommended by the WHO.[17] Examination of the samples were carried out within 3 hours of sampling.

2.2 Bacteriological Analysis

2.2.1 Enumeration of faecal streptococci

Hundred ml of the sample was concentrated on a 0.45 μ millipore filter and the filter was placed on the surface of a petridish containing KF streptococcus agar (Merck 10707). The plates were incubated at 37° C for 40 hours and the maroon or pink colonies were counted.

2.2.2 Enumeration of Clostridium perfringens spores

The samples of water were heated for 10–20 minutes at 70° – 75°C. Hundred ml of this preheated sample was filtered through a 0.45 μ millipore

filter and the filter was transferred with face upwards to the bottom of a petridish. Ten ml of liquefied sulphite-glucose-iron agar[3] which has been cooled to about 50°C was carefully poured over the membrane filter. After this layer of agar has set, incubated aerobically at 37°C for 24—48 hours and all the black colonies were counted.

2.2.3 Enumeration of total and faecal coliforms

Most probable number technique was used according to the WHO recommendation.[17]

2.3 Isolation of *Salmonella*

Ten litre samples of tap water were concentrated using the membrane filtration technique. The filters were incubated in a pre-enrichment broth[8] at 37°C for 16 hours. From this a drop was transferred to Preuss K tetrathionate broth (Merck No. 5173) and lactose saccharose agar (Merck No. 7237) at 37°C was followed from Preuss tetrathionate broth. Suspicious colonies were subjected to biochemical reactions according to Cowan and Steel [3] and serology was performed.

3. Results

3.1 Faecal streptococci

Of the 84 samples of water that were tested 46 samples (54%) were found to be positive for faecal streptococci. The count of faecal streptococci ranged from 1— 360/100 ml.

3.2 *Clostridium perfringens*

Growth of *Clostridium perfringens* was observed only in 3 samples out of 84 samples of water that were tested.

3.3 Total and faecal coliforms

Thirty-one samples (36.9%) were positive for total coliforms by the most probable number technique. Twenty samples (23%) contain more than 10 coliforms/100 ml. Thirteen samples out of the 31 (41.9%) that were positive for total coliforms were confirmed for the presence of faecal coliforms. Of the samples, 15% were positive for faecal coliforms from the total samples examined. Both the total and faecal coliform densities range from 2 to 1600/100 ml.

3.4 Salmonella species

Salmonella was isolated from 4 samples out of the 84 samples that were examined (4.8%). The counts of coliforms, faecal coliforms and faecal streptococci in the 4 samples in which *Salmonella* was recovered were 79, 70, 12, 2/100 ml, 33, <2, <2, <2 and 45, 8, 11 and 3/100 ml respectively.

3.5 Safety of water with regard to different indicator systems

The proportions of samples that can be considered safe by the indicator coliforms (53/84) and faecal coliforms (71/84) are significantly higher (at the 5% level) than that obtained by faecal streptococci (38/84).

4. Discussion

The method used in the present study in determining the number of faecal streptococci has been to isolate streptococci from Lancefield's serological group D (ISO/TS/147/5C, 4G4). It has been shown that these faecal streptococci are more persistant than coliforms under natural conditions.[13] In the present study too, the proportion of samples that can be considered Safe by the coliform faecal coliform indicator system is significantly higher than faecal streptococci. This shows that the recovery rate of faecal streptococci by the use of the method recommended by the International Standardisation Organisation is much higher than coliforms with the most probable number technique. This could be due to the greater resistance of faecal streptococci to the purification processes as reported by Cohen and Shuwal.[2] The faecal coliform measurement is said to relate more precisely to faecal contamination by warm blooded animals.[7] In the present study, *Salmonella* was isolated from 10 litre samples of water without the detection of faecal coliforms by the routinely used most probable number technique. But faecal streptococci was detected in all 4 samples in which *Salmonella* was recovered. Therefore by having a specific standard for faecal streptococci a higher degree of purity and sense of security could be attained than the presently used indicator system coliform/faecal coliforms.

Results obtained in the use of *Clostridium* spores as an indicator of faecal pollution of water was not satisfactory in the present study.

In conclusion it can be said that it is advantageous to use faecal streptococci in addition to total and faecal coliforms in determining the pollution of treated water.

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