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Comparative Study of Two Conventional Methods Used for Coliform Enumeration from Port Harcourt Waters

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Abstract

This study compares two conventional methods: Membrane Filtration (MF) and Multiple Tube Fermentation (MTF) used in the analysis of two Port Harcourt natural water sources, Abonnema Wharf (A) and Tourist Beach (B). However, these methods are generally used in the determination of the sanitary condition of the waters and its suitability for general use. It is observed from the study that the MF gives a more reliable and precised data than that of the MTF which is time consuming, labour intensive and less precised. Bacterial isolates such as the *Enterococcus faecalis* not present with the use of MF method may be as a result of seasonal changes, spatial distribution of organism, and varying degree of human/animal and industrial activities/influence on the ecosystem. Studies on the effects of physiological stress especially stress by chlorination should be carried out on the waters in order to substantiate the effectiveness of each method and also identify the best conventional method to be applied during analysis of water.

Keywords

Membrane Filtration, Most Probable Number, Coliforms

Subject Areas: **Environmental Sciences, Microbiology**

1. Introduction

Novel protocols used in the detection and characterization of microorganisms have helped to advance research in the field of Microbiology. Although, rapid methods (molecular and immunological based) for microbial identification and characterization have led to appreciable remarkable progress in science by its peculiar fast throughputs of results and accuracy (*i.e.*, its sensitivity, specificity and limit of detection), the International Organization for Standardization (ISO) accredited conventional methods are very important in that they are rela-

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tively easy to use and cost effective. Both the Multiple Tube Fermentation (Most Probable Number, MPN) and Membrane Filtration (MF) methods for Coliform enumeration are permitted [1] [2].

The analyses of water samples, for enumeration of Coliforms, require two major convectional techniques also known as culture-dependent methods: the MF and the MPN techniques. The screening of the water samples are important because water as a universal solvent is an essential requirement for all forms of life, be it prokaryotes or eukaryotes. Roohul-Amin *et al.* [3] estimated that over 250 million cases of waterborne diseases are reported worldwide and over 25 million deaths are blamed due to waterborne diseases. It is therefore necessary that the supply of water for human consumption should be of good quality—free from disease causing micro-organisms, harmful chemicals, poisonous elements and other toxic substances.

The MF technique which was developed for routine examination of water has the advantages of being able to examine large volumes of water than with MPN [4], as well as having a high precision and reliability and requiring significantly reduced time, labour, equipment, space, and materials. But it cannot be used on highly turbid water samples (see **Table 1**). It has also been observed that inhibitory materials (particulate matter) in the water can interfere with colony development when they stick to the filters. Also, high population of bacteria other than Coliforms may cause confluent growth.

In the MPN method, there is statistical bias and lack of precision inherent in it [5]. Thus, it gives only a statistical estimate of the number of bacteria that would give the observed result; not an actual count of the bacteria present despite the longer time it takes to produce results [4]. However, the MF is not feasible, such as with turbid or grossly contaminated water, the MPN procedure is found to be useful.

The comparability of the MPN method with the MF method in unchlorinated and natural waters by so many researchers has favoured the use of MF to MPN. Reports have showed that chlorine-stressed Coliforms are not as efficiently enumerated with the MF method as with the MPN method [6]-[8]. Contrary to this stance, Tobin *et al*. [2] have demonstrated that the MF method gives results equivalent to those given by the MPN method even with chlorine-stressed Coliforms.

Aims/Objectives

To compare two conventional methods used in the enumeration of Coliforms from natural water samples obtained from Abonnema Wharf (A) and Tourist Beach (B) in Port Harcourt.

2. Materials and Methods

The enumeration of faecal and total Coliforms from Abonnema Wharf (A) and Tourist Beach (B) waters in Port Harcourt involves aseptic collection of water samples from the two sample stations. A total of eight water samples were collected from eight sites (A_0, A_1, A_2, A_3, B_0, B_1, B_2 and B_3) and bacteriologically analysed via MPN method as described by Abu and Nnadozie [10], and another total of eight water samples were collected with a sterile screw-capped glass bottles from the same sites as described by Nnadozie [11].

Table 1. Comparison of coliform analysis methods.

Multiple Tube Fermentation Method	Membrane Filter Method
Slower: requires 48 hours for a positive or presumptive positive	More rapid: quantitative results in about 18 hours
More labour intensive	Less labour intensive
Requires more culture medium	Requires less culture medium
Requires more glassware	Requires less glassware
More sensitive	Less sensitive
Low precision	High precision
Difficult to use in the field	Can be adapted for field use
Applicable to all types of water	Not applicable to turbid waters
Consumables readily available in most Countries	Cost of consumables is high in many Countries
May give better recovery of stressed or damaged organisms	

Source: [9].

2.1. Membrane Filtration Procedure

In the Membrane filtration (MF) procedure membrane filter absorbent pads were each placed inside a sterile Petri-dish, and saturated with 2 ml of Endo agar lactose-based medium. 100 ml of water sample, each for the sample stations, was filtered through a membrane filter (0.45 µm pore size). After which the filters (bearing the residue) was placed on the medium with aid of sterile forceps. The Petri dishes were inverted and incubated for 24 - 48 hours at 35˚C ± 0.5˚C for total Coliform and at 44˚C ± 0.5˚C for faecal Coliform. After 48 hours of incubation, colonies (20 - 200) were observed and counted.

The Coliform density of the water samples are based upon the membrane filter count within the 20 - 200 Coliform colony range and calculated thus [12]: Total Coliform or Faecal Coliform per 100 ml is equal to the ratio of the average Coliform colonies counted at 35˚C or 44.5˚C respectively to the volume of water samples filtered (in mililiter), and multiplied by 100.

2.2. Multiple Tube Fermentation Method

The method as described by Abu and Nnadozie [10] was adopted in the enumeration of coliforms. This method involves the enumeration of Coliform (bacteria) from water samples. The use of 5 test-tubes containing a lactose-based media Lauryl Tryptose Broth (LTB) with Durham's gas tubes is aimed at monitoring presumptively the fermentation of media by Coliforms and observing the acidification and gas production during growth in the lactose broth at either 35˚C or 44˚C ± 0.5˚C for 24 - 48 hours. The growth was confirmed by subculturing from the fermentation tubes on Eosine-Methylene Blue (EMB) agar plates, MacConkey agar plates and Brilliant Green Lactose Broth (BGLB). The presence of gas and acid in the lactose based broth within 48 hours at 35˚C or 44.5˚C constitutes a positive confirmation test. Positive cultures grown on EMB plates with metallic sheen colonies confirm the presence of faecal Coliform (specifically *Escherichia coli*). The MTF method for the Coliform check is completed when a lactose-based medium and agar slant (nutrient agar slope) were inoculated with positive cultures from the confirmed culture wares. The growth of Gram's negative rods and non-spore forming bacteria on the agar slant, and presence of gas production in the lactose medium constitutes a positive completed test.

3. Results/Discussion

The results from both methods showed the presence of faecal Coliforms in the two water bodies. Both methods supported the null hypothesis indicating no significant difference between the aquatic systems. The water indices contradict the World Health Organizations (WHO) standards for potable water. Tourist Beach water (A) contains the highest faecal Coliform than Abonnema Wharf water (B). This implies that B is more polluted than A.

Abu and Nnadozie [10] have showed that by MTF procedure, it was revealed that the faecal Coliform count is between 11- >17 MPN/100 ml and 26 - 46 MPN/100ml in A and B respectively; whereas through the MF method, Nnadozie [11] reported that it was 2.25×10^1 - 1.48×10^2 CFU/100ml and 7.30×10^1 - 1.88×10^2 CFU/100 ml microbial loads respectively. The total Coliform counts via MF in the A and B ranges from 4.05×10^1 - 1.77×10^2 CFU/100 ml and 5.60×10^1 - 1.96×10^2 CFU/100ml [11] respectively. Eleven (11) bacterial species were isolated through MPN method [10]; while through the MF technique, a total of twelve (12) bacterial genera were isolated by Nnadozie [11] and mostly, which are of great public health concern, and are the cause of enteric diseases in humans.

The use of MF method revealed the presence of *Vibrio* spp. and *Serratia* spp. which were not found through the use of MTF method. This is because unlike the MTF method, the MF method allows an appreciable volume of water to be filtered; and it has been recommended for its accuracy and speed of result [13]. The presence of *Enterococcus faecalis* which was found only at site B_3 of Tourist Beach by MPN method may be as a result of seasonal changes, spatial distribution of bacteria, and varying degree of human/animal and industrial activities/ influence on the ecosystem as suggested by Nnadozie [11]. The design of the study is limited to bacteriological analysis of water samples. With the exception of pH, it does not cover other physico-chemical parameters such as the Dissolved Oxgyen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Dissolved Solids (TDS), salinity as chloride content, etc. that could also be used in the determination of water quality. Studies on the effects of physiological stress especially stress by chlorination should be carried out on the waters in order to substantiate the effectiveness of each method and also identify the best conventional method to be applied during analysis of water (**Figure 1**).

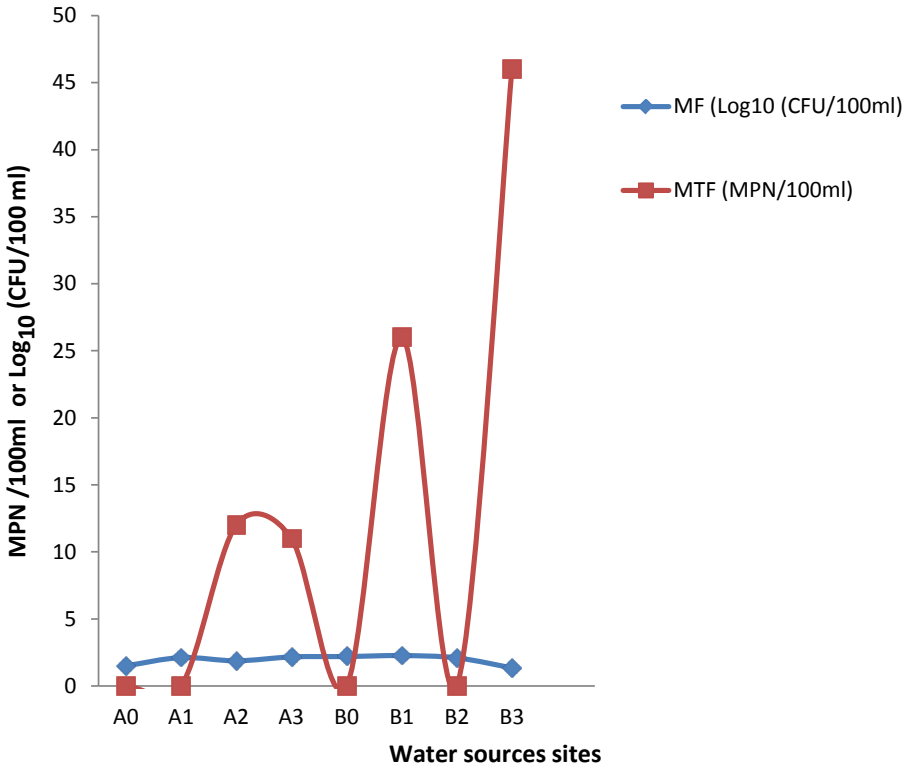

Figure 1. Graphical representation of faecal coliform counts of water samples enumerated via MF and MPN methods.

4. Conclusion

The analyses of natural water samples from the same source using two different methods have revealed that the MF method is the best conventional technique for water analysis as it gives the accurate microbial population present in a given sample. The varying degree of human and animal wastes, industrial activities, seasonal changes and spatial distribution of bacteria may have direct effect on the number of bacterial species present in any water system.

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