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Determination of Seasonal Dynamics of Microbiological Quality in a Hospital Wastewater, Zaria, Nigeria

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Abstract: The microbiological examination and monitoring of water sources is commonly used worldwide to ensure safety where by contamination with human and animal excreta could pose serious risks to the community. The sources of microbial contamination of water are numerous and have severe implications for public health. This study determines the seasonal dynamics of microbial counts from a hospital wastewater in Zaria. The Microbial analyses was determined within the hospital using the effluent from the wastewater treatment plant to quantitatively ascertain the total coliform count by presumptive, confirmatory and completed test methods while heterotrophic count was assessed by Spread plate method. Multiple Tube Fermentation technique using Most Probable Number table was used for enumeration of coliform count. The result shows significant difference between dry and wet seasons. At P>0.05, Coliform counts was (35.17± 9.58 / 50.50 ± 9.85 dry and 10.50 ± 1.75b / 27.17± 2.70b wet seasons), Heterotrophic count was (304.00±26.00 / 300.17±34.20 dry and 277.67±30.99 / 234.67±46.61wet seasons) for two seasons each respectively, highest concentrations was obtained in dry seasons. High counts of Coliform in this study indicates fecal/sewage contamination and was found to be above acceptable threshold of WHO, FAO and NESREA while heterotrophic count were within acceptable standards and guideline for both NESREA and FAO but above acceptable limits for WHO. Therefore the microbial load (Coliform) is highly contaminated in the wastewater and will cause health implications to humans and the environment.

Keywords: Coliform Count, Heterotrophic Count, Hospital, Wastewater, Seasonal

1. Introduction

Clean water is essential for nature and humans alike. Estimates indicate that developing countries surface waters are subjected to enormous pressures and may already be affected by severe pollution due to easy accessibility for disposal of waste-water [1].

Pollution is a major environmental issue in the world due to its adverse effect on living organism. In the past few decades, uncontrolled urbanization has caused serious pollution problem due to the disposal of sewage, industrial and hospital effluents to water bodies [2].

Water contamination poses harmful risks to the whole environment. The continuous use of untreated wastewater has long-term impacts on Plant, aquatic and associated biota [3].

Hospital effluents consist of both organic and inorganic substances including pathogenic microorganisms. Their presence in such effluent, especially in high quantity could sometimes pose grave problem for the populace if untreated [4].

Therefore the quality of water should be checked at regular interval, because in the use of contaminated water, human population suffers from various water borne diseases [5].

Microbial pathogens are among the major health problems associated with water and wastewater. The microbial density

and diversity in water sources is a reflection of contamination. Occurrence of coliform in potable water sources could be due to the presence of human and animals' excreta in such water. [6]. Classical indicators of feacal contamination include total coliforms, *Escherichia coli*, and *Clostridium perfringens*. These feacal indicators are monitored in order to obtain information regarding their evolution during wastewater treatment processes and they can survive for a long duration in the environment and have a high potential for waterborne transmission, making them reliable contaminant indicators [7].

Some of the major health risks are caused by microorganisms such as bacteria or pathogen because it may survive reproduce and disperse in water systems [8]. The use of untreated or inadequately treated wastewater contaminated with microbes for domestic/irrigation purposes has been responsible for waterborne diseases including gastroenteritis, cholera, Hepatitis, typhoid fever and giardiasis [9].

Microbiological examination and monitoring is commonly used worldwide to ensure the safety of a range of water sources where by contamination with human and animal excreta could pose serious risks. Many potential pathogens could be associated with contaminated water however; it is both time consuming and expensive to test for all possible pathogens present. Hence, representative indicators associated with human and animal contamination are used as a means to detect such pollution [10]. Total coliforms include those microorganisms that can survive and proliferate within the water environment and includes several species of the Enterobacteriaceae family, belonging to the genera *Escherichia*, *Citrobacter*, *Klebsiella* and Enterobacter. These bacteria live in the human and animal intestine, and have also been found to occur in both sewage and natural water sources with some of these bacteria being excreted in the faeces of humans and animals. In addition, they are far more sensitive to disinfection than enteric viruses and protozoa and thus should be absent immediately after disinfection indicating that their presence serves as an indication of inadequate wastewater treatment. Furthermore, discharge of improperly treated effluent often results in an increased number of bacterial, viral and protozoan pathogens which may result in a range of waterborne related diseases such as giardiasis and gastroenteritis [11].

2. Materials and Methods

2.1. Sampling and Microbial Examination of Wastewater

Wastewater samples were aseptically collected for coliform characterization using 200ml sterile glass bottles. The sample was stored in ice pack (4±2°C) and transferred to the microbiology laboratory, for analysis. A standard total coliform and faecal coliform was performed by Multiple-Tube Fermentation Technique which is based on the principle of dilution by using Most Probable Number (MPN) to enumerate bacterial form [12].

2.2. Total Heterotrophic Bacterial Count

The spread plate was used. Water sample was serially diluted using sterile 1ml pipettes and 9ml sterile physiological saline as diluent. Aliquots of 0.1 of undiluted water samples and water at dilutions of 10^{-1} and 10^{-2} was plated on Nutrient agar (oxoid) plates in duplicates. The plates were incubated at 37°C for 24h, before enumeration.

2.3. Total Coliform and Faecal Coliform Count

2.3.1. Presumptive Test

Coliform counts were obtained using the three-tube assay of the Most Probable Number (MPN) technique [13]. Presumptive coliform test was performed using MacConkey broth (oxoid). The first set of three tubes will have sterile 10ml double strength broths and the second and third sets will have 10ml single strength broths. All the tubes contained Durham tubes with bromocresol blue before sterilization. The three sets of tubes will receive 10ml, 1ml and 0.1ml quantities of water samples using sterile pipette. The tubes were incubated at 37°C for 24-48h for total coliform and 44.5°C at 24-48h for faecal coliforms. It was examined for acid and gas production. Acid production was determined by color change of the broth from reddish purple to yellow and gas production was checked by entrapment of gas in Durham tube.

The MPN will then be estimated from the MPN table for three tube tests.

2.3.2. Confirmatory Test

From each of the fermentation tubes with positive results, one loopful was transferred to: 3ml Brilliant Green Lactose Broth (BGLB) fermentation tube, then to an agar slant and to 3ml tryptone water. The inoculated lactose-broth fermentation tubes were incubated at 37°C and gas formation was inspected after 24hrs. The agar slants were incubated at 37°C for 24hrs and gram-stained preparations was made from the slants and examined microscopically for gram-negative, non-spore-forming bacilli. The tryptone water was incubated at 44.5°C for 18-24hrs after adding 0.1ml of Kovacs reagent and mixed gently. The presence of indole indicated by a red color in the Kovacs reagent, was observed.

2.3.3. Completed Test

Completed test was carried by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates was incubated at 37°C for 24-48h. Colonies developing on EMB agar was identified as coliforms or faecal coliforms (*Escherichia coli*) using cultural characteristics, morphology and biochemical tests. For faecal coliforms, colonies with green metallic sheen was Gram stained and the IMViC test was carried out on Nutrient agar stock cultures and used to identify the colony as *E. coli*. The MPN per 100ml water was calculated using the completed test.

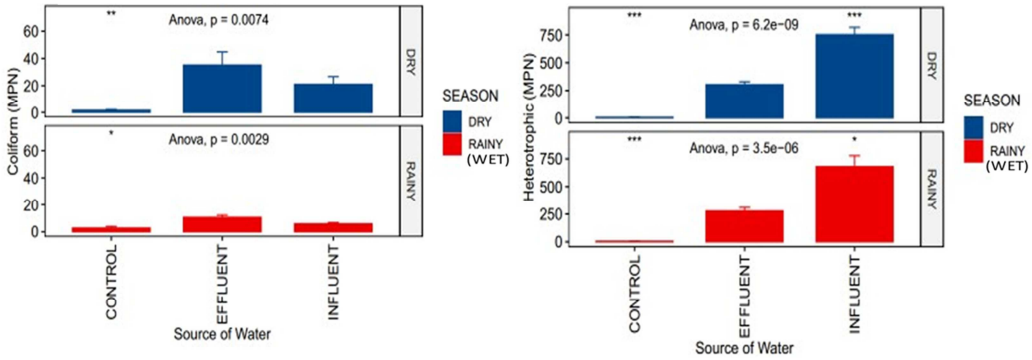
2.4. Determination of Coliforms Count

Number of positive test tube with acid (yellow coloration) and gas production were matched with the McCrady's statistical table, and MPN of coliform present in 100ml of sample was determined.

3. Result and Discussion

3.1. Seasonal Variation of Coliform and Heterotrophic Count Determined In 1st Season

There was significant variation at P<0.05 of coliforms between dry and wet seasons with highest coliform count of (35.2.17±9.58) in dry season while wet season is having counts of (10.50±1.75). Also heterotrophic count was higher in dry season too with (304.00±26.00) in dry season and (277.67±30.99) in wet season.

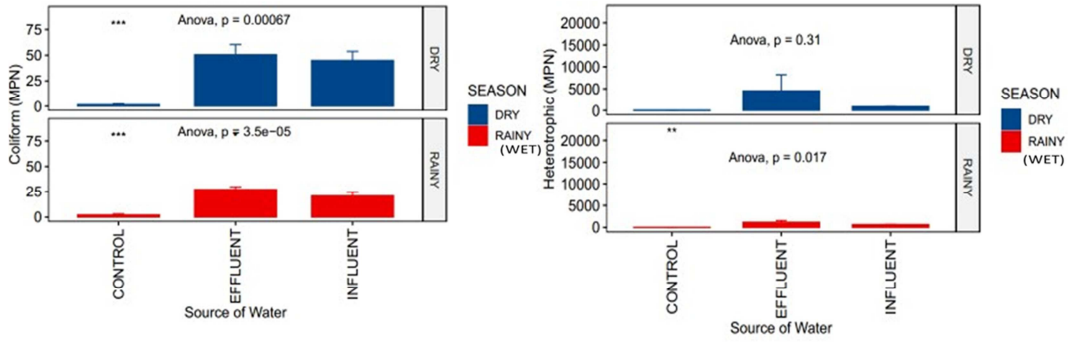


Coliform (MPN) Count dry and wet season 1st year Heterotrophic Count, dry and wet 1st year

Figure 1. *Seasonal Variation of Coliform and Heterotrophic Count Determined In 1st Season.*

3.2. Seasonal Variation of Coliform and Heterotrophic Count Determined In 2nd Season

Both Coliform and Heterotrophic counts were significantly different in dry and wet seasons in Effluent there was significant difference between coliform in dry and wet season with highest concentration of coliform (50.50±9.85) and heterotrophic counts of (300.17±34.20) both in dry season while coliform counts of wet season was (27.17±2.70) and heterotrophic counts of (234.67±46.61).



Coliform Count, dry and wet season 2nd year Heterotrophic Count, dry and wet season 2nd year

Figure 2. *Seasonal Variation Of Coliform and Heterotrophic Count Determined In 2nd Season.*

Microbial loads (Coliform and Heterotrophic counts) were higher in the Effluent during dry season in this study for both first year season and second year season. This is due to increase in degree of decomposition in dry season than wet season due to increase in Temperature which facilitates microbial activities. Coliform count was above limit standard for drinking (WHO), Irrigation (FAO) and national standard (NESREA) in dry season but within limit FAO/NESREA in wet season. Heterotrophic count was above limit for WHO but within limit for FAO/NESREA. This assessment conforms to the findings of [14] on the influence of hospital wastewater and food samples grown within ABUTH Zaria on its receiving environment where the microbial loads was also high in the wastewater. As also studied by [15] that the microbial count of coliform and fecal coliform exceeds the standard limits for WHO/FAO in "a review of the microbial quality of potable water sources in Nigeria. The result in this study relate to the findings of [16], which observed higher amount of total viable count and total coliform counts in sample studied from industrial wastewater and stated this is due to low level of sanitation and contamination with fecal sources.

4. Conclusion

Microbial assessment in this study proves contamination with fecal material where microbial load was higher with coliforms and Heterotrophs in dry season due to increase in metabolic activities during dry season more than in wet season caused by increase in Temperature during the season.

Pathogenic microorganism such as bacteria found in intestinal tracts of humans and warm blooded animals, enters the water due to poor sanitation and insufficient water quality control, therefore, microbial analysis is the most effective way and important aspect of testing water to detect fecal contamination and should be on frequent basis to prevent injurious effects for our health and the environment.

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