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Environmental Hazard Evaluation of Fecal Indicator Bacteria and Hepatitis A Virus in River Owena

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Abstract This study was carried out to determine the suitability of fecal indicator bacteria to predict human health risks associated with hepatitis A virus in a river in Nigeria, representing a regional situation in comparison with other global aspects. Water samples were collected from River Owena weekly over a period of twelve weeks i.e., July-September, 2017. The concentration of *Escherichia coli*, fecal coliforms, *Salmonella* and *Shigella* were determined by standard microbiological method. The concentration of hepatitis A virus was determined using standard molecular detection technique. Physicochemical properties of the water samples were determined using standard methods. Results showed that the concentration of *Escherichia coli* in the water samples ranged from 4.11 to 4.35 $\log_{10}$ CFU 100 ml^{-1} whereas those of fecal coliforms ranged from 4.23 to 4.51 $\log_{10}$ CFU 100 ml^{-1}. Whilst the concentrations of the bacterial indicators correlated positively, there was no significant relationship between the concentration of hepatitis A virus and those of the bacterial indicators in the water samples. The findings from this study suggest that the sanitary quality of surface waters based on bacterial indicators may be inadequate in protecting human health from risks associated with hepatitis A virus.

Keywords: *fecal indicator bacteria, hepatitis a virus, human health, risk assessment, surface waters*

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1. Introduction

Hepatitis A virus (HAV) has been observed to pose the greatest risk to public health and it is the most common type of hepatitis virus associated with human hepatitis [1,2]. Five viruses are responsible for most cases of viral hepatitis, these are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV) [2]. All hepatitis virus can cause acute hepatitis, however, only HAV and HEV have been reported to be human pathogenic viruses that may be waterborne. HAV is a small (40-60 nm), positive-sense, non-enveloped, single stranded RNA virus that belongs to the *Picornaviridae* family [3]. The mode of transmission of the virus is the fecal-oral route and its incubation period is usually between 15 and 45 days. HAV infect the intestinal tract of humans through ingestion of water contaminated with viruses of fecal origin and they are excreted in enormous quantities in the feces of infected persons [4]. Viral contamination of surface waters may derive from untreated or partially-treated wastewaters discharged into the water body, or from surface run-off following open defecation by an infected person [5].

The burden of ill health, morbidity and mortality as a result of viral hepatitis is a major public health challenge and has a direct relationship with access to clean water, sanitation and the socioeconomic status of the population [1]. In 2015, viral hepatitis caused 1.34 million deaths, a number higher than those caused by human immunodeficiency virus (HIV) [2]. The World Health Organization (2017) estimated that worldwide, HAV caused approximately 11,000 deaths i.e., 0.8% of the mortality from viral hepatitis [2]. The Global Health Sector Strategy endorsed by the World Health Assembly in 2016 has the specific objective of eliminating viral hepatitis as a public health threat by 2030 [2]. The detection of HAV in clinical samples is based on demonstration of specific immunoglobulin M and G (IgM and IgG) antibodies by cell culture or enzyme-linked immunosorbent assay (ELISA). Molecular techniques using reverse transcription polymerase chain reaction have been described as a suitable method of detection after extraction and purification of viral RNA from food, water and other environmental samples [6,7]. However, in low-income countries, these molecular methods are relatively expensive and are less likely to be adopted for routine monitoring of environmental waters in low-resource settings. Another limitation is that the molecular methods do not distinguish between infective and non-infective viral particles [8].

Globally, *Escherichia coli* and fecal coliforms are widely accepted as bacterial indicators of fecal contamination. However, there is limited information on the predictive value of these fecal indicator bacteria for important viral pathogen such as HAV for low-income countries such as

in Nigeria. Studies have demonstrated that the presence of enteric viruses in water matrices does not always correlate with the detection of fecal indicator bacteria [7,9]. For instance, in a study that examined the bacteriological quality and the occurrence of enteric viruses in groundwater used for irrigation in Italy. The authors observed widespread fecal contamination and the inadequacy of fecal bacteria (*E. coli*, *Salmonella* and total coliforms) to predict the occurrence of viruses in the groundwater, although, hepatitis A virus was not detected in the water samples [10]. In another related study that investigated the presence of HAV and bacterial indicators of fecal contamination in spring and river water samples in Spain. The authors observed that there was no quantitative relationship between the bacterial indicators of fecal contamination and the presence of HAV in the river water samples [11]. Similarly, a study evaluated bacterial contamination as an indicator of viral contamination in a sedimentary aquifer in Uruguay. Findings from this study suggested that bacteriological indicators were not adequate to predict the presence of viruses in individual groundwater samples [12].

This study was aimed at determining the suitability and applicability of fecal indicator bacteria in predicting human health risks associated with HAV in a surface water in southwest Nigeria representing a regional situation in comparison with other global aspects. This hazard evaluation of the sanitary quality of the waters from the river as well as the prediction of risk of gastroenteritis and viral hepatitis as a result of contamination of the river with HAV is of great public health significance.

2. Materials and Methods

2.1. Sampling Site and Collection of Samples

The study area was River Owena situated in Owena, Nigeria. Owena is a rural community with a population size of about 53,274 people. The river has an approximate length of 43 km and was selected because of its close proximity to fecal contamination from humans (because there is no sewage treatment facility in Owena) and farm animals (such as cattle, goats, sheep, dogs etc.) and from other various anthropogenic activities (such as swimming, bathing, irrigation etc.) taking place in and around the river (Figure 1).

The water from the river is often used for domestic, recreation and agricultural purposes. Sampling activities were carried out weekly over a period of twelve weeks in the months of July, August and September, 2017 representing the peak period of wet season with high anthropogenic activities in the catchment area (n = 12). On each sampling occasion, a grab sample of approximately one litre of river water was collected at a depth of about 20 – 30 cm in a pre-sterilised plastic bottle in accordance with standard protocol [13]. The water samples were transported to the laboratory in a cool box with ice packs and processed immediately within less than one hour.

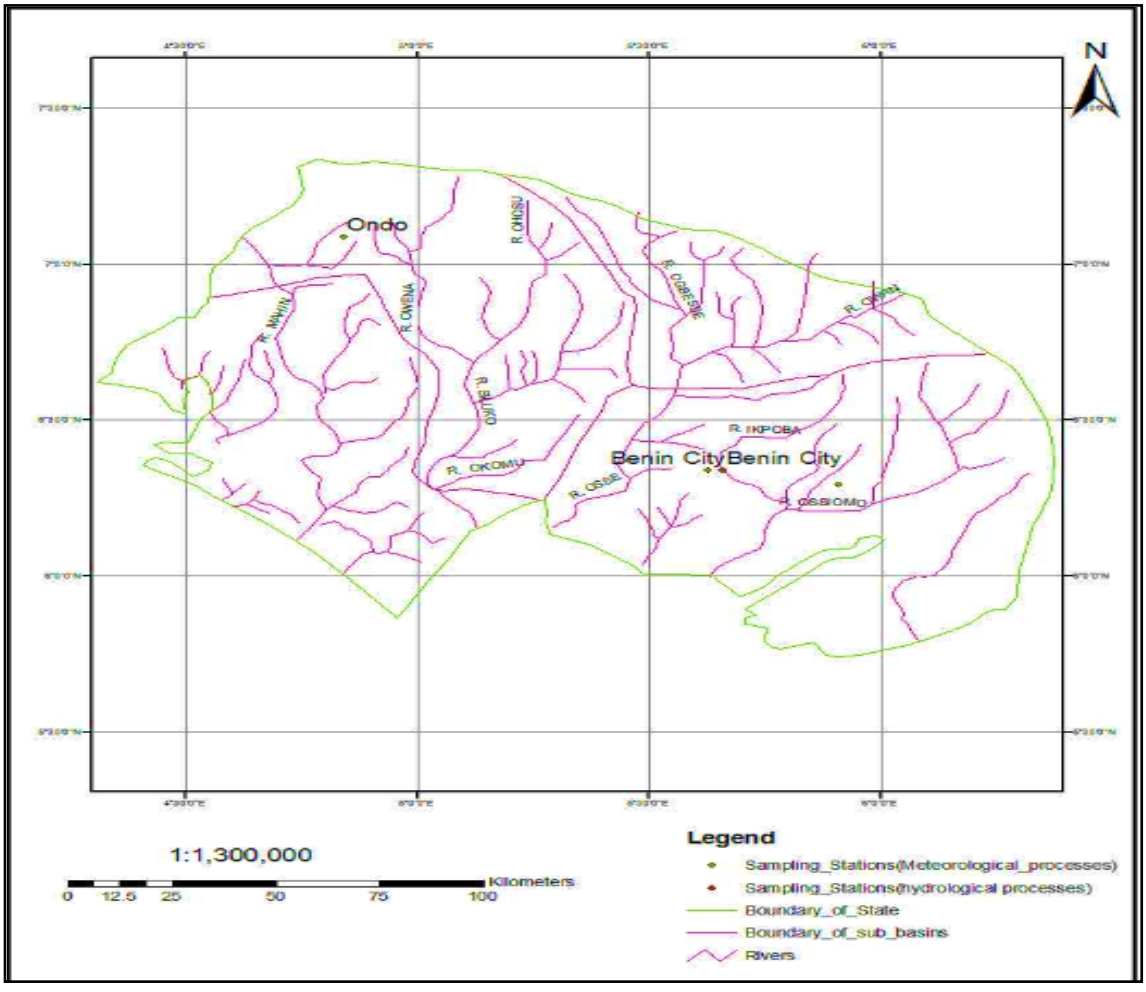

Figure 1. Locality map showing the River Owena in Nigeria

2.2. Enumeration of Fecal Indicator Bacteria in the Water Samples from River Owena

The concentrations of *E. coli*, fecal coliforms, *Salmonella* and *Shigella* in the water samples were determined using the membrane filtration method (ISO 9308-1) [14]. The membrane filters were placed on freshly prepared selective media (MLSA, EMB, *m*-FC and SSA). Agar plates were incubated at 37°C for 24 hours (MLSA, EMB), 44°C for 24 hours (*m*-FC) and 37°C for 24 hours (SSA) and colonies were counted, calculated and expressed as colony-forming units (CFU) 100 ml^{-1} of water.

2.3. Molecular Detection and Quantitation of HAV in Water Samples from River Owena

The water samples were prepared for analysis by concentration, nucleic acid extraction, molecular detection and quantitation [15]. HAV were concentrated from 300 ml of water samples filtered through 0.45 µm membrane cellulose nitrate filters. Prepared magnesium chloride (5 M $MgCl_2$) was added before filtration process to increase viral recovery by facilitating and enhancing virus attachment to the filters [16]. The filter was scrapped into a 1.5 ml microcentrifuge tube for nucleic acid extraction using QIAamp MinElute Virus Spin Kit (Qiagen GmbH) according to the manufacturer's protocol. HAV was enumerated by reverse transcription real-time polymerase chain reaction (RT-qPCR) on QIAGEN Rotor-Gene® 2.1.0.9 software (Qiagen, GmbH) Q thermocycler using PrimerDesign™ genesig HAV 5' Non-Coding Region (NCR) Advanced Kit (PrimerDesign, UK). Thermocycling conditions were RT step (42˚C for 10 minutes), enzyme activation step (95°C for 2 minutes), 50 cycles of denaturation step (95°C for 10 seconds) and data collection (60°C for 60 seconds). Data were analysed using Rotor-Gene 2.1.0.9 software with a threshold fluorescence value of 1.000. Standards were prepared, serially diluted and quantified to make standard curves following manufacturer's protocol. The highest concentration of HAV standard was 2×10^5 copies/µl. Standard curve was run in triplicate and the 'pooled' standard curve was then used to relate quantification cycles to copy numbers and quantity of HAV in samples. Again, all data were analysed with the comprehensive ROTOR-GENE® Q software, which enables quantification and enhances data security.

2.4. Determination of the Physicochemical Properties of the Water Samples from River Owena

The physicochemical properties of the water samples were measured using standard methods (Anon. 2012). These include temperature (˚C), pH, electrical conductivity (µS/cm), alkalinity (mg/l), turbidity (NTU), total dissolved solids (mg/l), dissolved oxygen (mg/l) and salinity (ppt).

2.5. Statistical Analysis

Data were transformed to $\log_{10}$, then examined using general descriptive statistics and checked for normality using the skewness and kurtosis statistic. Further analyses were undertaken using Statistical Package for Social Sciences (SPSS) Version 20.0, and all data were subjected to the Pearson's correlation analysis to determine whether there were positive correlations between the concentration of the fecal pollution markers, HAV and physicochemical properties of the water samples.

3. Results

3.1. Detection of Fecal Indicator Bacteria

The mean concentration of *E. coli* in the water samples ranged from 4.11 to 4.35 $\log_{10}$ CFU 100 ml^{-1} whereas those of fecal coliforms ranged from 4.23 to 4.51 $\log_{10}$ CFU 100 ml^{-1}. In addition, the mean concentrations of *Shigella* and *Salmonella* ranged from 4.04 to 4.45 $\log_{10}$ CFU 100 ml^{-1} and 4.01 to 4.42 $\log_{10}$ CFU 100 ml^{-1} respectively (Figure 2).

3.2. Detection of Hepatitis A virus

The mean concentration of HAV in the water samples from the river ranged from 5.85 to 6.38 $\log_{10}$ copies 100 ml^{-1} (Figure 3). The concentration of HAV appeared to be higher than those of fecal indicator bacteria in the water samples.

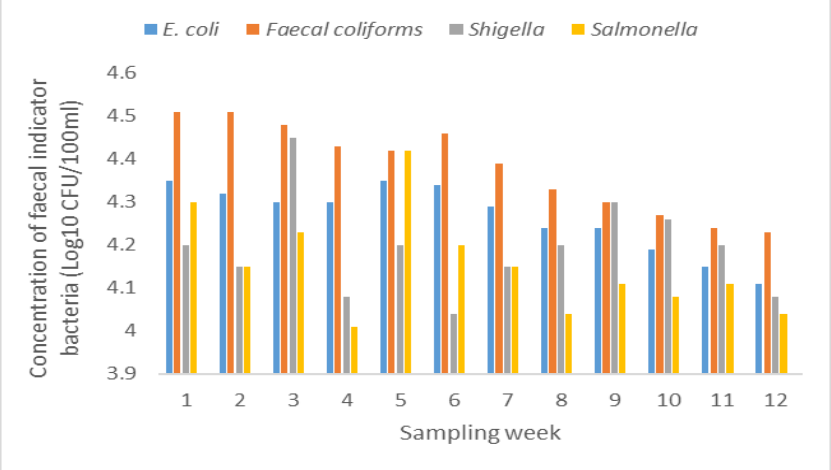

Figure 2. Concentration of fecal indicator bacteria in water samples collected from the river

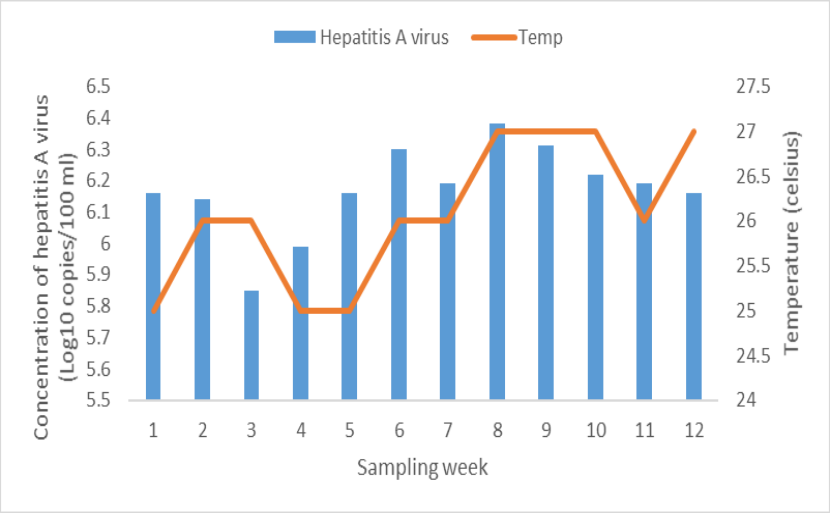

Figure 3. Concentration of HAV in water samples collected from River Owena and its the positive relationship with water temperature

3.3. Physicochemical Characteristics of the Water Samples from River Owena

The temperature of the water samples ranged from 25 to 27°C, while the pH values ranged from 6.0 to 7.5. The electrical conductivity ranged from 180 to 230 µS/cm, whereas turbidity values ranged from 0.02 to 0.45 NTU. Salinity ranged from 30 to 47 ppt and the total dissolved solids ranged from 0.05 to 0.28 mg/l. The amount of dissolved oxygen ranged from 2.03 to 8.24 mg/l (Table 1).

Table 1. Physicochemical characteristics and Pearson's correlation coefficient (r) with HAV in water samples from River Owena over the period of study

Physicochemical parameters	Mean ± Standard Deviation (Min. – Max.)	Correlation coefficient (r)
Temperature (˚C)	26.08 ± 0.79 (25-27)	0.50
pH	6.82 ± 0.52 (6.0-7.5)	-0.21
Salinity (ppt)	36.83 ± 5.98 (30-47)	-0.05
Total Dissolved Solids (mg/l)	0.18 ± 0.07 (0.05-0.28)	0.07
Dissolved oxygen (mg/l)	5.40 ± 2.27 (2.03-8.24)	-0.26
Electrical conductivity (µS/cm)	207.92 ± 14.06 (180-230)	0.05
Turbidity (NTU)	0.16 ± 0.12 (0.02-0.45)	-0.01

Key: Values are expressed as Mean ± Standard Deviation (n = 12) (Range: Min. 'Minimum' – Max. 'Maximum').

3.4. The Relationship between Fecal Indicator Bacteria and HAV in Water Samples from River Owena

The Pearson's correlation co-efficient demonstrated that the bacterial indicators *E. coli* (r = -0.19), fecal coliforms (r = -0.40), *Shigella* (r = -0.35) and *Salmonella* (r = -0.16) did not have any predictive value for the presence of HAV in water samples from the river (Table 2). Whilst the concentration of *E. coli* in the water samples from the river showed a positive correlation with the level of fecal coliforms (r = 0.92) and *Salmonella* (r = 0.65) (Table 2).

Table 2. Significant Pearson's correlation coefficient (r) between fecal indicator bacteria and hepatitis A virus in water samples from River Owena over the period of study

	E. coli	FC	*Shigella*	*Salmonella*	HAV
E. coli	1.00				
FC	**0.92**	1.00			
Shigella	0.01	0.02	1.00		
Salmonella	**0.65**	**0.55**	0.24	1.00	
HAV	-0.19	-0.40	-0.35	-0.16	1.00

Key: FC – Fecal coliforms; HAV – Hepatitis A virus.

4. Discussion

The water from the River Owena is often used for domestic, recreation, irrigation and agricultural activities. This may be responsible for the high concentration of *E. coli* in the water samples. Point and non-point sources are major contributory sources of fecal pollution in River Owena. For instance, farm animals (such as cattle, goats, sheep, dogs etc.) graze in and around the river and oftentimes defecate directly into the river or on the soils surrounding the river that are eventually washed-off into the river during rainfall or storm event. In addition, humans use the waters from the river for recreational activities and some tend to defecate directly into the river and this may likely be responsible for the high levels of *E. coli*, fecal coliforms, *Shigella* and *Salmonella* observed in the water samples from River Owena. *E. coli* being part of the fecal coliforms group in the family – *Enterobacteriaceae* exhibited significant positive relationship with the concentration of fecal coliforms (r = 0.92) (Table 2) and approximately 35-37% of total fecal coliforms were *E. coli*; 28-30% were *Shigella* and 26-28% were *Salmonella* in the water samples from River Owena (Figure 2).

The concentration of fecal coliforms in River Owena that ranged from 4.23 to 4.51 $\log_{10}$ CFU 100 ml^{-1} was lower compared to those obtained in a study where the authors observed high concentration of fecal coliforms (zero to 5.38 $\log_{10}$ CFU 100 ml^{-1}) in Poyang lake in China [7]. Similarly, the concentration of *E. coli* in River Owena that ranged from 4.11 to 4.35 $\log_{10}$ CFU 100 ml^{-1} was

higher than those obtained in a study where the authors observed that the concentration of *E. coli* in River Ouse, southeast England ranged from 1.55 to 4.00 $\log_{10}$ CFU 100 ml^{-1} [17]. The classification of fecal pollution levels into five microbiological water quality categories based on concentration of *E. coli* ($\log_{10}$ CFU 100 ml^{-1}) are Class I – low (≤ 2.0), Class II – moderate (>2.0-3.0), Class III – critical (>3.0-4.0), Class IV – strong (>4.0-5.0) and Class V – excessive (>5.0) [18,19]. Fecal pollution levels in River Owena may be classified as 'strong' and it exceeds the *E. coli* threshold level of 3.0 $\log_{10}$ CFU 100 ml^{-1} for 'good' bathing water [20]. This may pose a great risk to human health because the sources of pollution are from humans who use the water for bathing and other anthropogenic activities and a lesser risk from non-humans such as animals grazing in and around the river. Furthermore, the concentrations of *Shigella* and *Salmonella* were highest during the third and fifth sampling occasions respectively. These were periods of heavy rainfall when municipal wastes and sewage were washed-off into the river course, thereby increasing the levels of *Shigella* and *Salmonella* in the river. This observation is in agreement with many studies demonstrating that increase in rainfall and pollution events often lead to increased microbial population in surface waters [21,22].

Interestingly, of all the physicochemical parameters tested, only water temperature (r = 0.50) demonstrated a positive correlation with the concentration of HAV (Table 1 and Figure 3), although it has been shown that HAV has the ability to survive at low refrigeration temperature of 4 °C in cranberry-based juices [23], and thermal treatment may eliminate or reduce the infectivity of HAV in human feces and contaminated shellfish [24].

An ideal fecal indicator organism must be present in relatively high levels in all members of the population and are being shed at all times and must be present in fecally polluted waters in higher concentrations than actual pathogenic organisms [25,26]. There was no significant relationship between the targeted bacterial indicators and HAV. This is in agreement with studies that have highlighted the limitations to the use of traditional fecal indicator bacteria to predict the presence of enteric pathogens and that fecal indicator bacteria appear to have limited or no predictive value for many important human viral pathogens, such as enterovirus, norovirus, HAV [7,9]. Furthermore, studies have shown that enteric viruses persist longer than bacteria in environmental samples, probably as a result of their simple structure and lack of membrane that enhances their survival by making them resistant to stressful or unfavourable environmental conditions in the gastrointestinal tract and during their onward transmission through the fecal-oral route [4].

The occurrence of HAV in the environment is associated with human fecal contamination, since the virus is restricted to the human host and can only replicate within this host [4,9,11]. In addition, the prevalence of HAV in surface waters has been shown to depend on the extent of human activities, presence and the size of human population in close vicinity to the surface water [27]. Outbreaks of infection associated with drinking water, recreational water and raw or undercooked seafood harvested from polluted waters have been described [28]. Although, a study reported that the concentration of HAV in mussels and their overlying waters obtained from River Ouse in East Sussex, southeast England were below detection limit of 10 genome copies per 100 ml [5]. Similarly, another study reported that hepatitis A virus was not detected in water samples from groundwater sources used for irrigation in Italy [10].

5. Conclusions

The findings from the highlighted studies suggests that high-income countries with adequate wastewater treatment plants may have low endemic HAV levels. However, in this study, high levels of HAV were detected in water samples collected from River Owena, thus, demonstrating that low-income countries without adequate wastewater treatment plants may have high endemicity because endemic levels of HAV are related to hygiene and sanitary conditions. The findings from this study suggest that the assessment of microbiological quality of surface waters based on bacterial indicators may be inadequate in protecting human health from risks associated with enteric viruses such as hepatitis A virus.

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Statement of Competing Interests

The author has no competing interests.

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