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Research Article
Evaluation of Parasites/biological Indices as Veritable Indicators of Faecal *Enterococcus* Contamination of Surface Waters: Case Study of Adada River, Nigeria

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Abstract

Background and Objective: There is need for alternate quick-search of pathogens' distribution in community water sources, instead of the cumbersome "*Escherichia coli*" detection. Parasites were evaluated as veritable indicators of faecal *Enterococcus* contamination of surface waters, using Adada River in Nigeria as case-study. **Materials and Methods:** Seventeen parasites of human biotic origin (in dry season) and 13 (in the rainy season) isolated from the river (at specific geographical coordinates) were analyzed for their quality and quantity (using Stoll's Counting Technique) and connected (using Pearson's correlation analysis) with the distribution of the river's isolated *Enterococcus* sp. The parasites consist of: *Taenia* sp., *E. coli*, *E. histolytica*, *B. coli*, cercaria/miracidia, *S. mansoni*, *S. haematobium*, *A. lumbricoides*, *Giardia* sp., hookworm, *T. trichiura*, *S. stercoralis*, *I. butschlii*, *C. mesnili*, *E. nana*, *B. hominis* and *H. diminuta*, while the 13 consist of: *Taenia* sp., *E. coli*, *E. histolytica*, *B. coli*, *S. mansoni*, *A. lumbricoides*, *Giardia* sp., hookworm, *T. trichiura*, *S. stercoralis*, *I. butschlii*, *C. mesnili* and *E. vermicularis*. **Result:** Biological indices from the analysis, revealed very strongly significant correlation relationship of *Enterococcus* sp. with the presence of *Taenia* sp. and cercaria/miracidia, in the dry season and *C. mesnili* in the rainy season. **Conclusion:** From the evaluation, potential index analysis indicated that *Taenia* sp., cercaria/miracidia and *C. mesnili* could serve as markers for the faecal bacteria indicator and possible index for future monitoring of the potability of this surface water. The methodology is straight forward, cost effective, less cumbersome than other currently existing approaches.

Key words: Water analysis, pearson correlation analysis, *Enterococcus* sp., parasites of human biotic origin, Adada River

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is the common name assigned to the liquid state of a naturally occurring hydrogen-oxygen compound with the molecular formula H_2O, chemical structure of H-O-H and IUPAC name of hydrogen/hydroxonium ion (depending on the oxidation state), the solid state is known as ice, while the gaseous state is called steam. It is the most important natural resource, second only to air[1]. Also, of the many substances on earth, it is one of the most important for maintenances of life[2]. Despite occupying more than 75% of the earth surface, it is hardly found naturally in potable form owning to many biological, physical and chemical pollutants[3]. So, it must be treated before consumption and must be certified potable before consumption. There are standard methods laid down by the World Health Organization (WHO) for this as well as various nationals: United State Environmental Protection agency (USEPA), Nigerian Standard Organization (NSO), National Agency for Food and Drugs Administration and Control (NAFDAC), etc, biologically, it is by detection of *Escherichia coli* as evidence of faecal pollution, absence of which mean potability. The first of such method was the detection of *Bacillus coli*, later renamed the *Escherichia coli* by Castellani and Chalmers[4]. But over many decades, this method has been found wanting as a universal indicator due to some limitations. Quest for other mean therefore ensued. Currently, there are 4 emerging microbiological methods as follows: Fast detection using chromogenic substances, application of monoclonal and polyclonal antibodies, Immuno-magnetic separation and gene sequencing methods[5] but all these methods have the problems of costs: affordability, speed, high-technology, etc. that will definitely not be easy for routine laboratory and to developing and under-developed countries where more than 65% of the world population reside. Hence, though still undergoing development, they are currently not an acceptable universal indicator of faecal contamination in quest.

Besides, Ashobol *et al.*[5] pointed out that water sanitary engineers require simple and rapid methods for the detection of faecal indicator bacteria which is oblivion of the cumbersome culture and high-tech. Notwithstanding, it has long been recognized that artificial culture media lead to only a very small fraction (0.01-1%) of the viable bacteria present being detected[6]. Further, these authors agreed with Vivian[7] suggestion that using more than one methods of determining the degree of sewage pollution would be prudent and advantageous. And, also are particularly in support of Ashobol *et al.*[5] suggestion that substances can be used to avoid the need for isolation of pure culture and confirmatory tests, such as the use of faecal sterol biomarkers Therefore, the uses of alternative indicators offer a new way to distinguish sources of faecal contamination and monitor river health as suggested by Leeming *et al.*[8], which could be in conjunction with existing microbiological indicators or in isolation. Most importantly, the quest for a universal faecal indicator of human biotic origin as a microbial risk assessment in potable, agricultural or recreational water must put into consideration, among many factors: cost, afford ability and sustainability. This is in lieu of the fact that it must not only be something within the reach of routine laboratories but also those of 2nd and 3rd World countries where greater than 65% of the world population resides. That was one of the reasons why for more than 2 centuries now, the ability to reach a consensus on the matter has been an enigma.

It was on this premises for a cost-effective means and on the tripod that certain elements, ions and parasites has been associated with the distribution of certain bacteria and parasites in water[1,9,10], that this project was borne. Such association has never been linked to *Enterococcus* sp. faecal bacteria. This project therefore aimed to assess parasites (biological indices) as veritable indicators of faecal *Enterococcus* contamination of surface water as a means of qualitative microbial risk assessment factor. This study then brought forth a case of Adada River, used untreated by more than 16 communities of more than 1 million populations in Nsukka area of Eastern Nigeria. There is need to assess its microbial risk factors, using the river as a case study. The specific objective of this study were to (i) Examine Adada River and determine its distribution, quality and quantity of the isolated parasites of human biotic origin in specific 6 stations (of specified geographical coordinates) , (ii) To along this, also determine the distribution, quality and quantity of *Enterococcus* in the 6 stations, (iii) To then determine whether some parasites (biological factors) can be connected with the distribution of detect *Enterococcus* faecal bacteria indicators, using Pearson's correlation analysis, (iv) If so, such properties connected will be assessed further for true affinity and avidity using Pearson's possible paired correlation analysis, (v) Such affinity and avidity connected will reaffirm the use of that parameter as marker for the respective faecal bacteria contamination in water.

If biological/parasites indices of *Enterococcus* faecal bacteria indicators' distribution can be assessed, it will not only serve as a marker for the potability of Adada River but those of such water resources in the region. Not only could it be a cheaper and simpler approach to qualitative microbial risk assessment but may be the best affordable.

MATERIALS AND METHODS

Sampling site: Water samples were collected in duplicates at 6 different sites (stations 1-6) along the Aku bank of the Adada River flow, at about 6 km from Aku, a village located at Igbo-Etiti Local Government Area in Enugu State of Nigeria on 6°40″ N and 7°18″ E on the geographical map (6°42′7″ N 7°19′56″ E on Infinix Hot 7 Smartphone-compass, measured at the Post Office). The sampling areas were selected according to the vegetation's cover and river use as follows:

- **Station 1:** Geographical coordinate: 6°42′2″N 7°17′19″E (Infinix Hot 7 Smartphone-compass). It was upstream, towards the water source where there is limited human activity, the vegetation was originally rainforest but in the distant past slightly disturbed by water tanker drivers that created a part to the river from where they were then fetching water they sold to the local communities
- **Station 2:** Geographical coordinate 6°44′20″ N 7°16′50″ E. It was ways downstream from station1, at the beginning of where the river water was diverted for an ongoing Adada River Dam construction, the vegetation is only still slightly virgin and disturbed by Fulani herdsmen that occasionally graze cattle along the bank of the river and it is the camping site of the construction workers
- **Station 3:** Geographical coordinate 6°44′25″ N 7°16′49″ E. It was about the foot of the embankment where the Adada River water was diverted for the ongoing construction of the dam and heavily disturbed by the ongoing construction work and tanker driver that come to fetch water they sell to the local communities and beyond
- **Station 4:** Geographical coordinate 6°44′17″ N 7°16′37″ E. It was down-stream, a bit from the tail of the dam proper where from far and wide there are human activities, such as washing of clothes, soaking of cassava for fermentation, swimming, picnics, farmland at both banks and point where Fulani herdsmen occasionally bring their cattle to drink water
- **Station 5:** Geographical coordinate 6°44′13″ N 7°16′32″ E. It was the temporary run-off point downstream for the diverted water flow from the dam and also heavily disturbed on both banks of the river by heavy human activities, such as farmlands, etc.
- **Station 6:** Geographical coordinate 6°44′11″ N 7°16′29″ E. It was a little way downstream from station 5, before a former animal husbandry established by Eastern Nigeria Development Corporation (ENDC/ADP), also where Adada Secondary School [site of the re-proposed satellite Adada Campus of Enugu State University of Science and Technology (ESUT)] students fetches water, bath, wash clothes, swim, fishing, etc

All the stations environments were formerly typical rainforest, gradually converted as described above into agricultural, grazing, fishing, recreational and now the N2.6 billion Naira dam in progress. The climate is typical tropical rainforest, with average temperature of 25°C (range18-37°C) and average rainfall of 156.89 mmHg.

Collection of water for analysis: At each of the 6 sampling stations, water samples were collected in duplicates at some distance from the shore with clean pre-sterilized 500 mL bottles with stoppers. The bottles were aseptically opened five centimeters (5 cm) below the water surface, rinsed with the first set of water samples, then filled with the required water sample and the bottle aseptically closed. These were done between 10.00 am to 12.00 pm (late morning to early afternoon by which human activities have resumed) and done in 2 different sampling periods, June 13, 2016 (rainy season) and February 27, 2017 (dry season), precisely at the geographical coordinates. The samples were transported to the laboratory under ice and stored at about 4°C until they were ready for analysis. However, some physico-chemical properties: odour (manual), taste (manual) and temperature (mercuric thermometer) were done at source. A total of 24 water samples were collected (6 stations × duplicates samples = 12 × 2 seasons = 24 total). Total of 26 sample analysis were done (24 water samples plus control × 2 seasons).

Detection and enumeration of parasites: Parasites were isolated using a slightly adjusted Finch[11] method (but this was later discarded when it was discovered that the very careful methodical counting process was more adequate) and detected by their various morphological characteristics. Enumeration of parasites were as molecular methods could not have discerned, through their potentials (ova, cysts, larvae, oocyst and adults) by Stoll's Counting Technique for parasites in fluid and watery specimen after Cheesbrough[12], except that normal saline were used in the dilutions.

Identification of parasites: Parasites were microscopically detected and identified by their various morphological properties after Cheesbrough[12] in each water samples as follows:

Parasites	Descriptive identification
S. stercoralis	Microscopically identified as large, unsheathed, active mobile rhabditiform larva, measuring about 250×16 µm, showing characteristics large bulbed oesophagus, differentiated from hookworm larvae by shorter mouth cavity
Hookworms (identified as ova and larvae)	Ova, which are colourless, thin-shell (which appear as black line around an ovum), oval in shape and about 65×40 µm in diameter, usually segmented with 4-8 cell-stage and distinguished from the ova of *Trichostrongylus* spp., *Ternidens deminitus*, *S. fuelleborni* and *Oesophagostum* spp. and Larvae, distinguished from S. *stercoralis* larvae by its characteristic deeper buccal cavity
T. trichiura	Identified by a characteristic yellow-brown, barrel-shaped ovum, about 25-50 µm in size with colourless protruding mucoid plug at each end
A. lumbricoides	Identified by decorticated, fertilized and unfertilized eggs: fertilized eggs that were about 50-70×30-50 µm in length and breath, respectively, yellow-brown in colour, oval in shape and containing a central granular mass covered by a shell with uneven albuminous coat, unfertilized eggs which were darker in colour and contains a central mass with larger granules that is covered by a thinner wall with more albuminous coat and more elongated (90×45 µm in size) than the fertilized one
S. mansoni	Identified by eggs that were oval in shape, pale yellow-brown in colour and measuring about 60-150 µm with, at times, fully visualizable internal fully developed miracidium and with the characteristic single lateral spine
S. haematobium	Identified by large eggs (145-45 µm in length and breath, respectively), that are pale yellow-brown in colour and oval in shape, each containing a fully developed miracidium and the characteristic single terminal spine
Taenia spp.	Identified by round eggs of about 30-40 µm in diameter, containing barely visible onchosphere that is surrounded by thick, brown radially striated wall
E. vermicularis	Identified by colourless eggs measuring about 30-50 µm that were oval in shape and flattened on one side and containing barely visualizable larva
E. histolytica (identified by trophozoites and cysts)	Trophozoites with active unidirectional amoeboid movement, unit nucleus that has barely discernible central karyosome and measuring about 20-25 µm in size and cysts that were round (10-15 µm in diameter), containing 1-4 nuclei with barely discernible central karyosome and having some chromatoid bodies in immature ones and distinguished from larger *E. coli* (15-30 µm in size), with 1-8 nuclei and at times needle-like chromatoid body
I. butschlii	Identified by small cysts (9-15 µm in sizes) with only one nucleus that has compact mass of glycogen inclusion and no chromatoid body
G. lamblia (identified by trophozoites and cysts)	Trophozoites were small, pear-shaped flagellates (12-15×5-9 µm in length and breath, respectively) with rapid its tumbling and spinning motions, having some discernible structures like four pairs of flagella, 2 axonemes, two discernible nuclei, large concave sucking disc located on the ventral surface, one or two curved median bodies and by cysts that are also very small (7-12 µm in diameter) with some discernible internal structures in the saline medium (e.g. four nuclei, remain of flagella, axonemes and median body Both cysts and trophozoites were carefully differentiated from those of other flagellates of medical importance: C. *mesnili*, *Retortamonas intestinalis*, *E. hominis* and *Pentatrichomonas hominis* by their trophozoites with single nucleus, fewer flagella, shapes and smaller sized cysts that have not the characteristic appearances of *G. lamblia* (i.e. remains of flagella, four nuclei grouped to one end and peculiar shape)
C. mesnili	Identified by lemon-shaped cysts that are smaller in size (<8um) than those of other medical important flagellates (*R. intestinalis* = pear-shaped) and containing no remains of internal structure like *G. lamblia*
B. coli (identified by trophozoites and cysts)	Trophozoites seen as large ciliates (50-200×40-70 µm in length and breath, respectively), with rapidly revolving movement, well discernible macro-nucleus, two contractile vacuoles, discernible cilia beating at the region of the funnel-shaped cytostome when carefully focused and by the round and thick-walled cysts that are also large (50-60 µm in diameter) with discernible cilia lining the wall of the cyst
B. hominis	Identified as small round protozoa (about 15-30 µm in size), with peripheral cytoplasm, a central vacuole, no discernible nucleus even at ×40 and a granule which form a ring around the periphery

Bacteriological study: This was done by the "Standard methods of bacteriological analysis of water," after Cheesbrough[13] and as specified by Ashobol *et al.*[5] for *Enterococcus* sp resistant to 60°C for 30 min (and confirmed by molecular tests using 16s rRNA gene.

Molecular testing: The molecular testing was performed at Bioformatic Services, Ibadan, Nigeria. DNA was extracted using ZR Fungal/Bacterial DNA Miniprep (Zymo Research Cat Number: D6005). Then Agarose gel was prepared for electrophoresis of DNA (1gm of agarose) and PCR (2g of agarose) and allowed to completely solidified. Then, after all the necessary protocols, the eluted DNA and PCR product were loaded on wells in the gel, then the gel was run at 80-150 V for about 1-1.5 h. Thereafter, power was turned off, electrodes disconnect from the power source and then the gel was carefully removed from the gel box, after which DNA fragments and PCR product were visualize under UV transilluminator. The PCR mix was made up of 12.5 µL of Taq 2X Master Mix from New England Biolabs (M0270), 1 µL each of 10 µM forward and reverse primer, 2 µL of DNA template and then made up with 8.5µL nuclease free water. Primer sequences were: 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA. Cycling conditions were: Initial

denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 45 sec, followed by a final elongation step at 72°C for 7 min and hold temperature at 10°C forever. After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. The amplified fragments were sequenced with a Genetic Analyzer 3130×L sequencer from Applied Biosystems using manufacturers' manual, while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Genomic composition of the sample was determined by mapping its sequence read against viral, bacterial and eukaryotic sequences data bank.

Statistical analysis: Results obtained in the parasitic analysis of the river were summarized in tables. Pearson's correlation relationship analysis was used to determine correlation of *Enterococcus* sp. with the distribution of the isolated parasites. The statistical analysis was done in bits of the 2 seasons. Null hypothesis (H_o) is: Correlation does not exist between *Enterococcus* sp and any parasites of human biotic origin in the rainy and dry seasons.

RESULTS

Of all the 13 parasites (Tables 1) detected in the rainy season and analyzed, *C. mesnili* showed statistically significant positive correlation relationship to the distribution of *Enterococcus* sp. in the rainy seasons. And, of all the 17 parasites (Table 2) detected in the dry season and analyzed, *Taenia* sp. and cercaria/miracidia showed very strong statistically significant positive correlation relationship to the distribution of *Enterococcus* sp. in the dry seasons.

Results in Table 3 of the significant test of Pearson's correlation relationship showed that *C. mesnili* exhibited very significantly positive correlation relationship ($r = 0.8959$) with the distribution of *Enterococcus* sp. in the rainy season ($p<0.05$). Also, result in Table 4 of the significant test of Pearson's correlation relationship showed that *Taenia* sp. exhibited significant positive correlation relationship ($r = 0.8531$) with the distribution of *Enterococcus* sp. in the dry season ($p<0.05$), while cercaria/miracidia exhibited very strongly significant positive correlation relationship ($r = 0.9798$) with the distribution of *Enterococcus* sp. at 99% confidence level ($p<0.01$).

However, *B. coli*, *T. trichiura* and *H. diminuta* that exhibited very strong significant positive paired correlation to cercaria/miracidium (Table 5) were not found to have had significant Pearson's correlation Relationship with *Enterococcus* sp. but this does not matter much since they were analysis on different parlance.

Table 1: *Enterococcus* faecal bacteria indicators' distribution with parasites (biological indices) in Adada River in the dry season

Species	Types quantity (mL g^{-1})	Variable/stations											
		1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Enterococcus	0/100 mL	0	0	0	0	0	0	0	0	0	0	9.0×10^3	2.55×10^4
Taenia sp.	33500/25.5%	500	2500	1500	1500	4000	4500		2500	1000	2500	4000	9000
Entamoeba coli	12500/9.5%	500				500		5000	1000	3000		2000	500
E. histolytica	17000/12.9%	1000				4500		3500	500	2000		5500	
B. coli	2500/1.9%											2500	
Cercaria/miracidia	3000/2.9%									500		2000	500
S. mansoni	6500/5.1%		1000			500	500	1500	500	1000		500	1000
S. haematobium	7000/5.3%		1500	500	3000			500		500	1000		
A. lumbricoides	5000/3.8%	500	1000				500				1500		1500
Giardia sp.	23000/17.5%			1000		4000	3500		2500	1500	3000	6000	1500
Hookworm	10000/7.6%		500	5000	500		1000	500	1000	500		500	500
T. trichiura	500/0.4%											500	
S. stercoralis	500/0.4%			500									
I. butschlii	2000/1.5%				500	1000						500	
C. mes*nili*	1000/0.8%						1000						
E. nana	5000/3.8%		5000										
B. hominis	500/0.4%											500	
H. diminuta	2000/1.5%												2000
Total parasites	131500	2500	11500	8500	5500	14500	11000	11000	8000	10000	8000	24500	16500

WHO STD: World health organization standard, 1-6: Stations, A and B: Duplicates, Table 1 showed the distribution of *Enterococcus* sp. with detected parasites (biological indices) in quality and quantity in the 6 stations (1- 6) in duplicates (A, B) along the Adada River water flow in the rainy seasons, From this table, the Pearson's correlation statistical analysis was done for the rainy season (Tables 3)

Table 2: *Enterococcus* faecal bacteria indicators' distribution with parasites (biological indices) in Adada River in the rainy season

Species	Types quantity (mL g^{-1})	Variable/Stations 1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Enterococcus	0/100 mL	0	0	0	0	0	3.22×10^4	0	1.48×10^4	0	0	0	0
Taenia sp.	45000/23.1%	1000	8000	1000	1000	10000	4000	6000	2000	1000	2000	4000	5000
Entamoeba coli	6000/3.1%		3000	1000			1000						1000
E. histolytica	66000/33.9%	11000	8000	5000	14000		15000	2000	2000	3000	2000	4000	
B. coli	2000/1.0%	2000											
S. mansoni	11000/5.6%	1000	1000					1000	1000	1000		4000	2000
A. lumbricoides	5000/2.6%		1000	1000	1000					2000			
Giardia sp.	23000/11.8%		2000	2000	2000		3000		5000	5000	1000	2000	1000
Hookworm	25000/12.8%			2000	11000	4000	2000	1000	2000	1000		2000	
T. trichiura	1000/0.5%								1000				
E. vermicularis	1000/0.5%											1000	
S. stercoralis	3000/1.5%												3000
I. butschlii	6000/3.1%		6000										
C. mesnili	1000/0.5%					1000							
Total parasites	195000	15000	29000	12000	29000	15000	25000	10000	13000	13000	5000	17000	12000

1-6: Stations, A and B: Duplicates, Table 2 showed the distribution of *Enterococcus* sp. with detected parasites (biological indices) in quality and quantity in the 6 stations (1-6) in duplicates (A, B) along the Adada River water flow in the rainy seasons, from this table, the Pearson's correlation statistical analysis was done for the rainy season (Tables 3)

Table 3: Results for the significant test of Pearson's correlation relationship between *Enterococcus* sp. and 13 parasites in rainy season

Variables (mL or g)	Pearson r-values	95% CI	R^2	p-value	p-value summary	n
E*nterococcus* vs. *Taenia* sp.	0.7366	-0.1864 to 0.9689	0.543	0.0949	ns	6
Enterococcus vs. *Entamoeba coli*	-0.2028	-0.8710 to 0.7287	0.041	0.7000	ns	6
Enterococcus vs. *E. histolytica*	0.5275	-0.4967 to 0.9377	0.278	0.2821	ns	6
Enterococcus vs. *B. coli*	-0.288	-0.8913 to 0.6833	0.083	0.5799	ns	6
Enterococcus vs. *S. mansoni*	-0.381	-0.9109 to 0.6233	0.145	0.4562	ns	6
Enterococcus vs. *A. lumbricoides*	-0.5979	-0.9490 to 0.4150	0.358	0.2100	ns	6
Enterococcus vs. *Giardia* sp.	-0.09755	-0.8424 to 0.7754	0.010	0.8541	ns	6
Enterococcus vs. Hookworm	0.1308	-0.7616 to 0.8519	0.017	0.8049	ns	6
Enterococcus vs. *T. trichiura*	0.2561	-0.7012 to 0.8840	0.066	0.6242	ns	6
Enterococcus vs. *E. vermicularis*	-0.288	-0.8913 to 0.6833	0.083	0.5799	ns	6
Enterococcus vs. *S. stercoralis*	-0.288	-0.8913 to 0.6833	0.083	0.5799	ns	6
Enterococcus vs. *I. butschlii*	-0.288	-0.8913 to 0.6833	0.083	0.5799	ns	6
Enterococcus vs. *C. mesnili*	0.8959	0.3089 to 0.9886	0.803	0.0157	ss	6

ns: Not significant, ss: Strongly significant, significant test showed that the correlation of *C. mesnili* to *Enterococcu*s sp. was very strongly significant, while that of the other parasites were not significantly correlated

Table 4: Results for the significant test of Pearson's correlation relationship between *Enterococcus* sp. and 17 parasites in dry season

Variables (mL or g)	Pearson r-values	95% CI	R^2	p-value	p-value summary	n
Enterococcus vs. *Taenia* sp.	0.8531	0.1348 to 0.9836	0.7277	0.0308	ss	6
Enterococcus vs. *Entamoeba coli*	0.09002	-0.7784 to 0.8402	0.008104	0.8653	ns	6
Enterococcus vs. *E. histolytica*	0.6047	-0.4062 to 0.9500	0.3657	0.2035	ns	6
Enterococcus vs. *B. coli*	Perfect line				na	6
Enterococcus vs. Cercaria/miracidia	0.9798	0.8213 to 0.9979	0.96	0.0006	vss	6
Enterococcus vs. *S. mansoni*	0.3071	-0.6719 to 0.8955	0.09434	0.5538	ns	6
Enterococcus vs. *S. haematobium*	-0.43	-0.9204 to 0.5861	0.1849	0.3947	ns	6
Enterococcus vs. *A. lumbricoides*	0.4339	-0.5830 to 0.9211	0.1882	0.39	ns	6
Enterococcus vs. *Giardia* sp.	0.5579	-0.4635 to 0.9427	0.3113	0.25	ns	6
Enterococcus vs. Hookworm	-0.1706	-0.8627 to 0.744	0.02909	0.7466	ns	6
Enterococcus vs. *T. trichiura*	Perfect line				na	6
Enterococcus vs. *S. stercoralis*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
Enterococcus vs. *I. butschlii*	-0.2928	-0.8923 to 0.6805	0.08571	0.5734	ns	6
Enterococcus vs. *C. mesnili*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
Enterococcus vs. *E. nana*	-0.2	-0.8703 to 0.7301	0.04	0.704	ns	6
Enterococcus vs *B. hominis*	Horizontal line				na	6
Enterococcus vs. *H. diminuta*	Perfect line				na	6

ns: Not significant, ss: Strongly significant, significant test showed that the correlation of *Taenia* sp. and cercaria/miracidium to *Enterococcu*s sp. was very strongly significant, while that of the other parasites were not significantly correlated

Table 5: Results of the significant test of all possible pairs of Pearson correlation relationship for the parasites during rainy and dry seasons

Species	Results
Rainy seasons	
B. coli and *Entamoeba coli*	Significant positive paired correlation relationship (r = 0.894)
Giardia sp. and *Entamoeba coli*	Significant negative paired correlation relationship (r = -0.868)
I. butschlii and *Entamoeba coli*	Significant positive paired correlation relationship (r = 0.894)
I. butschlii and *B. coli*	Significant positive paired correlation relationship (r = 1)
E. vermicularis and *S. mansoni*	Significant positive paired correlation relationship (r = 0.916)
S. stercoralis and *S. mansoni*	Significant positive paired correlation relationship (r = 0.916)
S. stercoralis and *E. vermicularis*	Significant positive paired correlation relationship (r = 1)
Dry seasons	
C. mesnili and *I. butschlii*	Significant positive correlation relationship (r = 0.8783)
S. mansoni and *Entamoeba coli*	Significant positive correlation relationship (r = 0.824)
S. haematobium and *E. histolytica*	Significant negative correlation relationship (r = -0.9287)
S. haematobium and *S. stercoralis*	Significant positive correlation relationship (r = 0.86)
S. stercoralis and Hookworm	Hookworm showed significant positive correlation relationship (r = 0.9807)
Giardia sp. and *E. histolytica*	Significant positive correlation relationship (r = 0.8291)
Cercaria/miracidia and *B. coli*	Coli showed significant positive correlation relationship (r = 0.9798)
Cercaria/miracidia and *T. trichiura*	Significant positive correlation relationship (r = 0.9798)
Cercaria/miracidia and *H. diminuta*	Significant positive correlation relationship (r = 0.9798)

I. butschlii that exhibited very strong significant positive paired correlation to *C. mesnili* (Table 5) was not found to have had significant Pearson's correlation relationship to *Enterococcus* sp.

DISCUSSION

Laboratory analysis of water supplies from Adada River showed that *C. mesnili* exhibited very significant positive correlation relationship (r = 0.8959) with the distribution of *Enterococcus* sp. in the rainy season ($p < 0.05$). The analysis also showed very strongly significant positive correlation relationship with *Taenia* sp. (r = 0.8531) and cercaria/miracidia (r = 9798) in the dry season. In addition, the correlation with cercaria/miracidia is at 99% confidence level ($p < 0.01$). These correlations indicated high levels of affinity or relativity that can be extrapolated as indices of affiliations. Therefore, the hypothesis that correlation does not exist between *Enterococcus* sp. and any parasite of human biotic origin in the rainy/dry seasons has to be rejected, to imply significant specified correlation relationship of *Enterococcus* with *Taenia* spp., *C. mesnili* and cercaria/miracidia.

Evaluation went this way: The hypothesis (H_o) that correlation does not exist was drawn. To test the hypothesis, Pearson's correlation relationship analysis was calculated on the collected data (Table 1 and 2). The results were Pearson r values (Table 3 and 4). The nearer the Pearson r value is to one as a unit, the greater the affinity. Thus it measures magnitude of affinity. The magnitude could also be either positive (+) or negative (-). Whichever the sign, the p-value of the Pearson r needs to be calculated to determine its statistical significance, $p < 0.05$ indicates statistical significance to the Pearson r obtained, meaning there is correlation relationship, which therefore rejects the hypothesis that correlation relationship does not exist. But, $p > 0.05$ indicates no statistical significance correlation relationship from the calculated Pearson r and so accepts the stated hypothesis that there is no correlation relationship (as was determined for all the other cations/metals as well as other parameters analyzed in Table 1 and 2). Further, positive sign indicates direct correlation relationship, while negative sign indicates inverse relationship, both can be discerned from Table 3 and 4. Pearson's correlation analysis is the best statistical yardstick to test affinity/relativity

Thus, Pearson's correlation matrix (Pearson r) interprets both signs (+ or -) and magnitude, the closer the values to one, the greater the affinity. The plus sign (+) indicates direct relationship in many senses, such as if either of the comparing factors increases, the other also does, the negative sign (-) indicates inverse relationship and opposite of what the plus sign interprets.

Further, normally, for river water analysis of this nature, replicate samples from four stations are usually taken and analyzed in duplicates[3,4,12] but 6 duplicate samples (12 number total) was specially taken in this work for better sample size and statistical significance.

This study indicated that only *Taenia* sp., *C. mesnili* and cercaria/miracidia showed significant positive correlation to the distribution of *Enterococcus* sp. This was in spite of total 17 different parasites isolated in the river and analyzed. Though, numerous correlations were not, however, really expected from the blind search. Selection of the chosen parasites analyzed were simply based on the most common ones of human biotic origin isolatable from surface water and

on hope of finding just one, probably more, that can tag *Enterococcus* sp. and as such to be a substitute indicator. In something similar to this, according to Kikwawila Study Group[14] snail ecologists, for an instance, have tried to correlate snail distribution with physical and chemical factors and to discover the range of these factors within which the snail thrives. Besides, according to Sures *et al*.[15], parasites are attracting increasing interest from parasite ecologists as potential indicators of environmental quality because of the variety of ways in which they respond to anthropogenic pollution. They also suggest how environmental science and parasitology might profit from each other in the near future. Interest on this study aligned with this assertion.

There has as well been an un-validated similar observation in snail population with organic matters as a reflex of suspended matters which to an extent also agrees with the view of some other snail ecologists. Snail ecologists had as well found some physical, chemical and environmental correlation to snail distribution. These physical and chemical factors include: temperature, DO, TS, UDS, TDS, pH, conductivity, electrolytes, calcium, organic matters, vegetation and osmotic stress[16,17]. The environmental factors, also not validated include: rain, season, climatic condition, slow flowing streams and topography. These investigations were similar to the investigations with parasite, except that biological indices (parasites) were rather correlated in this study and correlation relationships were the same.

Another near assertion to this fact is from the works, not validated, which stated that vegetation as a reflex of suspended solids (TSS/UDS) is a positive index of aquatic life in general. However, it was biological indices that were investigated in this study. The only other case in literature where chemical property was related to a biological index was in an invalidated study, whereby nematodes were related mostly to soil chemicals [pH, P, K, etc rather than physical (sand, silt)] parameters. This study as earlier indicated, is a new dimension to QMRA. So, there was paucity of literature for correlation of biological indices on bacteria or faecal indicators, there was consequently paucity of comparative analysis in that `direction. It is more so because this is a new dimension clearly different from the other 4 approaches mentioned in the introductory chapter that were trying to replace the use of "detection of *Escherichia coli*" as a way of QMRA.

Further, biological indices in terms of correlation (tag) to bacteria distribution are different dimension from normal standards of water analysis in the determination of the potability or levels of pollution or degree of contamination of water sample. Further, although there have been many works on water analysis such as Ekundayo *et al*.[2]. No literature ever looked at evaluation of biological/parasites indices as index of water contamination as was established in this study. Consequently as earlier pointed out, there was paucity of literature for correlation of biological indices on bacteria or faecal indicators, there was therefore paucity of comparative analysis in that 'direction.

Under normal conditions, biological factors are exposed to a wide range of varying and often interacting environmental factors which produces collective effects on them and it is usually difficult to separate the effect of any 1 factor from the other[18]. There is, therefore, no immediate explanation for these correlations apart from some parasites' specificity for a particular niche due to either physiological factor or environmental factor or need for a special ecological niche[19,20]. For instance, *Plasmodium* spp. specificity for the red blood cells' ecological niche is due to its affinity for iron that is best found in the required abundance in the heme proteins in the blood. Thus, this study is also in line with observed speculation that certain physico-chemical index has been found to correlate with some bacteria's ecological niches[11], though never before investigated in faecal bacteria's indicators.

It cannot be explained off-hand why *B. coli*, *T. trichiura* and *H. diminuta* that exhibited very strong significant positive paired correlation relationship to cercaria/miracidium (Table 5) were not found to have had significant Pearson's correlation relationship to *Enterococcus* sp. too. Thus Pearson possible pair correlation test here was not clear as was established for *Escherichia coli* in another unpublished study. Though, this study was not however expecting large number of correlation, so reason might be related to the limits of experimental designs in this study.

Lastly as is with this research venture, none of the currently four emerging microbiological methods for qualitative microbial risk assessment (QMRA) of water potability as enumerated above (after Ashobol *et al*.[5]) in the introductory chapter, including the one on the horizon and of immediate future development (microarrays and biosensors) are strictly abiding by Bonde[21] requirements for an indicator organism. Bonde[21] outlined (for indicator organism) that: (a) It must be present whenever the pathogens concerned are present, (b) It must be foreseen only when the presence of pathogenic organisms is an imminent danger, (c) It must occur in greater number than the pathogens, (d) It must grow readily on relatively simple media, (e) It must be more resistant to disinfectants and to aqueous environment than the

pathogens, (f) It must yield characteristic and simple reactions enabling as far as possible, an unambiguous identification of the group or species, (g) it should preferably be randomly distributed in the sample to be tested or it should be possible to obtain uniform distribution by simple homogenization procedure and (h) Its growth in artificial media must be largely independent of any other organism present, that is, the growth indicator bacteria should not be seriously inhibited by the presence of other species. All current approached deviates from these.

Thus, from this evaluation, biological/parasites index analysis indicated that *C. mesnili*, *Taenia* sp. and cercaria/miracidia in the results could serve as markers for *Enterococcus* sp. These may as well serve as indices for future monitoring of the potability of this widely used water source in the community. Further, the method is a straight forward, cost effective, has lower risk exposure and is less cumbersome than all the other currently existing approaches.

SIGNIFICANT STATEMENT

This study discovered the method that is more straight forward, cost effective, has lower risk exposure and is less cumbersome than all the other currently existing approaches and which can be beneficial for quicker water analysis. This study will help the researchers to uncover the critical areas of qualitative microbial risk assessment (QMRA) of surface water that many researchers were not able to explore. Thus a new theory on water analysis may be arrived at

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