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(RESEARCH ARTICLE)

Toxicological examination of microbial isolate from sites located in freshwater ecosystem at Ugwuomu-nike, Enugu State

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Abstract

The isolation and identification of bacteria associated with influx from human residues located in freshwater ecosystem at Ugwuomu-nike in Enugu State was examined in this study. About eight (8) water samples were collected, four (4) from the lake and the other four (4) from the river close to the residual points. The samples were physiochemically examined for Temperature, pH, Turbidity and Conductivity. Samples were centrifuged and viewed under the microscope for any presence of parasites to be identified. Water culture was carried out using lactose broth to check for possible acid and gas production by the test organisms, and further cultured into eosin methylene blue agar and macConkey agar incubated at 37 ^{o}C for 24-48 hours. The isolates were finally cultured on nutrient agar and stored in a stock culture bottles for further analyses. Biochemical testing was also carried out. The microorganisms isolated and identified were *Proteus spp, Esherichia coli*, *Bacillus spp, Staphylococcus aureus*, *Legionella spp*, *Enterobacterspp* and *Micrococcus spp*. The *Bacillus spp* (rod shaped), *Staphylococcus aureus* (cocci in clusters) and *Micrococcus spp* (cocci in pairs) turned to be gram positive and non-motile, having *Bacillus spp* a spore former and the other two species a non-spore formers. *Proteus spp, Esherichia coli, Enterobacter spp* and *Legionella spp* as gram negative, non-spore formers and motile microorganisms. Results showed that *E.coli* isolated was the most frequent organisms and when compared to the remaining microbial isolates after the biochemical analysis, *E-coli* isolates produced its result as indole positive, oxidase negative, catalase positive, citrate negative, urease negative, coagulase negative. However, when tested on glucose, lactose, and galactose, it produced acid and gas. This study confirms the presences of bacteria at the freshwater ecosystem because of human residues, hence the need for water treatment should be employed to avoid water borne diseases by contact and possible consumption by citizens in that surroundings.

Keywords: Toxicity; Micro-organisms; Wastewater; Water analysis; Freshwater ecosystem

1. Introduction

Water is an essential resource for life. Water bodies such as streams, rivers, and lakes, are used by humans for multipurpose most importantly for human domestic purposes. An adequate, safe, and accessible supply must be available to all. Improving access to safe drinking water can result in significant benefits to health. Every effort should be made to achieve drinking water with good qualities as safe as possible [1]. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, like Nigeria access to both clean water and sanitation are not the rule, and waterborne infections are common. Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrheal diseases [2]. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place. When water becomes contaminated by microorganisms such as bacteria, parasite, viruses, protozoa and so

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on it causes a variety of illnesses. Wastewater discharges in fresh waters and costal seawaters are the major source of fecal microorganisms, including pathogens [2]. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water [3]. When the test of coliforms is carried out with environmental waters, several species of the four Enterobacteriaceae genera *Escherichia, Klebsiella*, *Enterobacter* and *Citrobacter* give positive results and therefore are coliforms. Coliforms are bacteria that are always present in the digestive tracts of animals, including humans and are found in their wastes. Recent studies carried out in temperate zones indicated that *E. coli* can persist in secondary, non-host habitats, outside the hot tropical areas, and become naturalized in these habitats [4]. The presence, persistence, and possible naturalization of *E. coli* in these habitats can confound the use of fecal coliforms as a reliable indicator of recent fecal contamination of environmental waters. The study aims to isolate and identify these microbial organisms because of human residues around the freshwater ecosystem in Ugwuomu-nike.

2. Material and methods

Water samples were collected from both river and lake freshwater ecosystem and transferred into eight (8) test tubes respectively and sponge using a centrifuge and further viewed under the microscope for parasites identification. The water samples collected from the lake were labelled as Lake 1 (L1), Lake 2 (L2), and Lake 3 (L3), while the samples collected from the river as River 1(R1), River 2 (R2), and River 3 (R3) were transferred into two tubes of lactose broth with 10 ml, two tubes with 1.0 ml and two tubes with 5 ml. Durham tubes were vertically inserted downwards and the test tubes were incubated at 37 ^{o}C for 24hours and were observed microscopically for bacterial growth by the presence of turbidity and production of gas or no gas [5][6]. For cultural examination of water samples through confirmed test eosin methylene blue agar plates six in number were inoculated with the material from a tube containing gas six test tubes in number from the presumptive test using the pour plate technical. These plates were inverted and incubated at 37 ^{o}C for 24-48hours. Also, organisms from the tube containing gas from the presumptive test was inoculated into an already prepared MacConkey agar plates six in number and incubated at 37 ^{o}C for 24hrs in order to identify other coliforms [7]. For cultural examination of water samples through completed test, organisms from the eosin methylene blue agar plates from the confirmed test it was inoculated into a lactose broth tube and nutrient agar slant (stalk culture). Incubation of the broth tube and agar slant were done at 37 ^{o}C for 24hours. For gram staining, about ten (10) slides of smear were made from the organism on the nutrient agar slant fixed unto the slides by passing over the flame at least three consecutive times. It was stained with Crystal violet for one minute and washed out with tap water. Then treatment with lugols iodine was done on the smear for one minute and washed out with tap water and decolorized with acetone; which was washed off immediately with water and lastly counter stained with safranin for one minute and washed out with tap water. The smear was allowed to dry and viewed under the microscope using x100 oil immersion of the objective lens. In conducting the spore staining, ten (10) slides of smear were prepared and heat fixed from the organism on the nutrient agar slant; the smear were then steamed with malachite green for 5minutes and washed with water and later counter stained with safranin for a minute; washed out and allowed to dry before viewing under the microscope. A semisolid agar medium was prepared in a test tube in conducting motility test. The inoculum of the organism from nutrient agar slant was collected using a straight wire and inoculated into the tube by making a single stab down the center of the tube to about half the depth of the medium and incubated at 37 ^{o}C for 24hours. The following biochemical tests were necessary for the organism's identification and classification. These include: Indole Test: The test organism was inoculated into peptone agar using a sterile wire loop and incubated at 37 ^{0}C for 48hours using ten test tubes. After incubation, 0.5 ml of Kova's reagent was added into each test tube and timed for ten minutes. (Note that a pink/red in top layer of the culture is indicative of a positive result). Urease Test: The test organisms were inoculated on agar slant of urea agar using ten bijoux bottles and incubated at 37 ^{o}C. After an overnight culture, formation of purple pink colour is indication of urease production and a positive result while the absence of colour change shows a negative result. Oxidase Test: The test organism is smeared on a filter using a sterilized wire loop, two drops of the oxidase reagent was added, after 5-10seconds a positive reaction was indicated by an intense deep-purple blue coloration, a "delayed positive" reaction by coloration in 10-60seconds and negative reaction by absence of coloration or by coloration later than 60 seconds. Catalase Test: Normal saline was dropped on a sterile grease slide and the colony of the test organism was smeared on the slide using a sterile glass rod, three (3) drops of hydrogen peroxide (H_2O_2) was placed on the smear, a positive result is the rapid evolution of oxygen (within 5-10sec) as evidenced by bubbling while a negative result is no bubbles. Coagulase Test: Normal saline was dropped on each end of a slide and with a sterile loop, a portion of the isolated colony was emulsified in each drop to make two thick suspensions. A drop of human plasma was added to one of the suspensions and mixed gently, clumping of the organisms within ten (10) seconds indicates positive result and vice versa while no plasma was added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping. Citrate Test: The test organism was inoculated into an agar slant of citrate agar and incubated at 37 ^{0}C for 24hours. A blue coloration at the top layer of the culture media indicates positive reaction while absence of colour change indicates a negative reaction. Sugar Fermention Test using Glucose, Lactose and Galactose: Peptone agar and 1gram of the sugar was prepared by heating over the flame to

mix properly, then phenol red was added in drops until red coloration was obtained, the Durham tube were inserted vertically downwards and incubated at 37 ^{0}C for 24 hours. (Note that air bubbles in the Durham tubes indicates gas production while yellow colour change indicates acid production).

3. Results and discussion

Table 1 shows the results of the physiochemical examination of lake (L) and river (R) freshwater ecosystem ranging from the slight differences in Temperature, pH, Turbidity, Conductivity and Total Dissolved Solid respectively.

Table 1 Physiochemical Examination of Freshwater Ecosystem

Samples	Temperature	pH	Turbidity	Conductivity	Total Dissolved Solid
L1	28 ^{0}C	8.8	0.60	0.200 μS/m	0.100mg/L
L2	28 ^{0}C	8.8	0.60	0.200 μS/m	0.100mg/L
L3	29 ^{0}C	8.9	0.70	0.210 μS/m	0.100mg/L
L4	29 ^{0}C	8.9	0.70	0.210 μS/m	0.100mg/L
R1	29 ^{0}C	7.45	83.0	0.113 μS/m	0.56mg/L
R2	29 ^{0}C	7.45	83.0	0.113 μS/m	0.56mg/L
R3	30 ^{0}C	7.55	84.0	0.114 μS/m	0.57mg/L
R4	30 ^{0}C	7.55	84.0	0.114 μS/m	0.57mg/L

Table 2 Gram's Reaction and Morphology of the Bacterial Isolates

Isolates	Gram Reaction	Motility	Shape	Spore formers
Proteus spp	-ve	Motile	Rod Shaped	Non spore former
E. coli	-ve	Motile	Rod Shaped	Non spore former
Bacillus spp	+ve	Non motile	Rod Shaped	Spore former
Staph. aureus	+ve	Non motile	Cocci (Clusters)	Non spore former
E. coli	-ve	Motile	Rod Shaped	Non spore former
Legionella spp	-ve	Motile	Rod Shaped	Non spore former
Staph. aureus	+ve	Non motile	Cocci (Clusters)	Non spore former
Enterobacterspp	-ve	Motile	Rod Shaped	Non spore former
E. coli	-ve	Motile	Rod Shaped	Non spore former
Micrococcus spp	+ve	Non motile	Cocci (pairs)	Non spore former

Table 3 Biochemical Analysis of the Various Bacterial Isolates

Catalase	Coagulase	Citrate	Urease	Indole	Oxidase	Probable Organism
+	-	+	-	+	-	*Proteus spp*
+	-	-	-	+	-	*E. coli*
+	-	+	-	+	-	*Bacillus spp*
+	+	+	-	-	-	*Staph. aureus*
+	-	-	-	+	-	*E. coli*
+	-	+	-	+	+	*Legionella spp*
+	+	+	-	-	-	*Staph. aureus*
+	-	+	-	-	+	*Enterobacterspp*
+	-	-	-	+	-	*E. coli*
+	-	+	-	+	+	*Micrococcus spp*

Table 4 Sugar Fermentation Test Using Glucose, Lactose and Galactose

Glucose Acid	Gas	Lactose Acid	Gas	Galactose Acid	Gas	Possible Organism
+	+	+	-	+	+	*Proteus spp*
+	+	+	-	+	+	*E. coli*
+	+	+	-	+	+	*Bacillus spp*
+	+	+	-	+	+	*Staph. aureus*
+	+	+	-	+	+	*E. coli*
+	+	+	-	+	+	*Legionella spp*
+	+	+	-	+	+	*Staph. aureus*
+	+	+	-	+	+	*Enterobacterspp*
+	+	+	-	+	+	*E. coli*
+	+	+	-	+	+	*Micrococcus spp*

Table **5** shows the result of the Presumptive test carried out using the Lactose broth to check for the production of gas, acid and macroscopic growth by turbidity of the organism in lake and river water samples. Gas production is indicated by air bubbles in the Durham tubes while acid production is indicated by colour (yellow) change.

Table 5 Presumptive Test

Samples	Gas Production	Acid Production	Macroscopic Growth (Turbidity)
L1	+ve	--ve	+ve
L2	+ve	--ve	+ve
L3	+ve	+ve	+ve
R1	+ve	--ve	+ve
R2	+ve	--ve	+ve
R3	+ve	+ve	+ve

Table 6 Confirmatory Test from Macconkey Agar and Eosin Methylene Blue Agar using Pour Plate

Samples	Colonies Mac-conkey Agar Plate	Colonies EMB Agar Plate
L1	One (1 Isolate)	No Growth
L2	Three (4 Isolates)	No Growth
L3	One (1 Isolate)	No Growth
R1	One (1 Isolate)	Growth (2 Isolates)
R2	No Growth	No Growth
R3	One (1 Isolate)	No Growth

The results obtained from this research showed the bacterial isolates present at Ugwuomu-Nike lake and river water bodies which includes *Proteus spp, E. coli, Bacillus spp, Staphylococcus aureus, Legionella spp, Enterobacter spp* and *Micrococcus spp*. Unlike [8] who stated that *Aeromonas hydrophila* has been isolated from human feces and from water sources*. Aeromonas spp* have been found to grow at temperature of about 5 ^{0}C and 45 ^{0}C [9]. Which is quite different

from the temperature of organisms found to grow at Ugwuomu-nike lake and river ranging from 28 ^{0}C – 30 ^{0}C, and [10], who was able to isolate Mac organisms from raw water along with [11] discovered Mac organism can proliferate in water temperature up to 51 ^{0}C, can as well grow in natural water over a wide pH range. Also the coliform bacteria isolated from this research work were able to produce acid and gas production from lactose broth which agrees with [12] who brought about the concept of acid and gas production (detected by the Durham tube) from lactose as a diagnostic feature. Most isolates obtained from this work is in line with [1] who stated that total coliforms are gram negative, oxidase negative, non-spore forming, rod shaped that ferments lactose with gas production, at 35 ^{0}C – 37 ^{0}C after 24 - 48hrs and species of two (2) Enterobacteriacae genera emerged *Escherichia* and *Enterobacter*. Though [13] and [14] stated that the presence of *E. coli* in environmental water is definitively not associated with fecal pollution and these situations were first detected in some African countries namely Nigeria, Ivory coast and New Guinea (although not in others, such as Uganda) but not in line with [15] who stated that *E.coli* and *Enterococci* can serve as fecal coliform and also [16] who stated that isolates from the several lake Michigan beaches was shown to harbor high densities of *E.coli*. According to the American legislation, total coliforms are the routine parameter to be determined, only when these determinations are repeatedly positive, it is mandatory to assess fecal coliforms.

4. Conclusion

This research revealed the presence of some microorganisms associated with human body contact sites of lakes and rivers at Ugwuomu-nike. It also revealed the presence of traces of fecal coliforms from the samples analyzed as indicator of fecal contamination and potential pathogens to humans. Although no visible parasite was seen after centrifugation and observation under the microscope of the raw water samples, but the humid environment was seen as a conducive for parasite host habitat, hence the need for proper measures to be taken in water treatment before drinking or other usage related purposes to avoid outbreak of water borne diseases. Future studies should consider a more extensive study using larger test samples for parasites associated with respect to the water bodies at Ugwuomu-nike. As the site is humid and can harbor the parasite intermediate host (snail). Advanced studies should consider other non-host habitats as potential source of fecal coliform in aquatic environment as regular human activities serves as proof of possible human fecal contamination.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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