Original Research Article

Physicochemical and Microbiological Assessment of Utaewa Estuary Water

Abstract

Most communities in the oil rich Niger Delta find it difficult to access to potable water due to constant pollution of their natural water sources. The sole aim of this study was to examine the microbiological and physicochemical qualities of the Estuary water at Utaewa to ascertain its suitability for potable use. Standard culture dependent techniques as well as metagenomics approach using Next Generation Sequencing (NGS) on Illumina Miseq platform was used to determine the microbiological characteristics while standard analytical procedures were employed to ascertain the physicochemical properties of Utaewa in Imo River. The average microbial counts ranged as follows: Total Heterotrophic Bacteria; $2.17\pm0.08x10^6$ to $2.23\pm0.08x10^6$ cfu/ml, Hydrocarbon Utilizing Bacteria; $1.03\pm0.06x10^5$ to $1.39\pm0.08x10^5$ cfu/ml, Total Heterotrophic Fungi; $1.23\pm0.13x10^5$ to $1.64\pm0.09x10^5$ cfu/ml, Hydrocarbon Utilizing Fungi; $7.9\pm0.21x10^3$ to $8.6\pm1.00x10^3$ cfu/ml. Bacterial isolates belonging to the genera *Escherichia, Citrobacter, Bacillus, Salmonella, Shigella, Proteus, Flavobacterium, Vibrio, Micrococcus* and *Pseudomonas*. were isolated and identified. The fungal isolates belonged to the genera *Aspergillus, Rhizopus, Fusarium, Penicillium, Saccharomyces* and *Candida.* On the other hand, Unknown (50.16%), Acinetobacter (8.34%), Ignatzschineria (4.73%), Planctomyces (3.28%), Anaerospora (3.27%), RS62 (2.73%), Methylophaga (1.64%), Wohlfahrtiimonas (1.31%), Myroides (1.23%) and Candidatus (0.95%) were captured by metagenomics analysis at the generic level. Cultural methods used in this study were able to identify many potential water borne pathogens, metagenomics captured more microbial groups and give a better insight to bacterial composition and diversity. The presence of theses pathogenic bacteria underlies poor water quality and can pose public health threat to man and aquatic fauna. There is need to adhere to good hygienic practices and minimize the direct discharge of waste without proper treatment.

Keywords: [pollution, estuary, metagenomics, water, counts]

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INTRODUCTION

One of the most important natural resources for the sustenance of life on earth is water. The usefulness of water be it ground water which serves as a source of drinking water or surface water used for different purposes such as transportation, recreation, sanitation (washing) and other domestic activities cannot be overemphasized [1].

Water is a source of life but poorly managed resources in the world today. Water though a source of life, has been poorly managed owing to increased human activities and other natural processes including erosion, flooding, runoff or seepages from waste decomposition. Urbanization has shown to be one of the major causes of contamination of water bodies and this poses a threat to all forms of life in the water environment [2]. Most sources of contamination may be described as point or non-point sources in which leachates from domestic wastes, agricultural wastes, Industrial wastes, sewage discharges, among other types of contamination find their way into water bodies [3]. Access to safe drinking water eludes millions

of people around the world especially those in sub-Saharan Africa. In Nigeria, there is scarcity of fresh water or potable water supplies as a result of increasing population due to migration of people to urban centres and increase in pollution of the available water resources [4].

Estuaries are ecologically and economically important aquatic systems, functioning as feeding/staging sites for migratory birds, as nurseries for marine fish, and as repositories of high biodiversity. They are also important for the tourism industry and serve as sites for productive fish and invertebrate fisheries and aquaculture. Over the past decade, there has been a dramatic increase in urban, agricultural and industrial development along the southern African coastline, particularly in the vicinity of estuaries, resulting in an escalation in anthropogenic stresses on these delicate ecosystems [5].

The Niger Delta mangrove ecosystem is the largest in Africa and third in the world. Its estuary waters play very important roles in the Niger Delta ecosystem. It serves as a source of fish, food, transportation and "portable" water in some areas and the cultural heritage of the people. Exploration and production activities since the late 1950's have brought enormous foreign exchange to the country and the region. However, this has come at the expense of the Niger Delta environment [6].

This is even made worst in the Niger Delta because inhabitants of its riverine communities are in the habit of disposing sewage directly into surrounding and nearby water bodies. The situation is further worsen by incessant crude oil spillage which usually have far reaching health and economic implications beyond the aquatic habitats. According to Forstinus *et al.* [7], Access to unclean water is very significant from a public health point of view as it remains the main transmission route of water washed, water based and water borne diseases. Waterborne diseases result in considerable morbidity and mortality amongst children under five years of age, elderly and immune-compromised persons [7].

The use of cultural based methods for the biological examination of water samples has been in existence for a long time but recent review of this method shows that it is plagued with problems. These challenges include being time consuming, non-sensitive to viral and protozoan communities that might be present, and also limited to small culturable minority [8, 9].

The introduction of metagenomics as a more sensitive method to the conventional methods plays a vital role in unearthing and monitoring the microbial communities by providing access to the taxonomic and functional gene composition. Most of the metagenomic analysis tools have opened new windows of opportunities for researchers to analyze the microbial community as a whole (whole-genome sequencing) and the genetic diversity, which facilitates active metabolic pathways in any given environment. With the advent of massive DNA sequencing technologies, several methods have been developed to assign shotgun reads to microbial taxonomic categories. These methods aim to perform a microbial community profiling that infers its relative structure, and they are very important to understand how microbiomes work in nature, their phylogenetic composition, and even their dynamics and evolutionary history. Microbial diversity is measured as a function that depends on the richness and abundance of distinct taxons among

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any community [10]. Previous studies in this location has used the conventional method in the examination of the microbiological diversity of the estuarine water.

Thus, the sole aim of this study was to examine estuary water quality using both cultural and metagenomic techniques as well as its physicochemical characteristics.

MATERIALS AND METHODS

Description of Study Area

Utaewa Estuary is located at latitude 4°32’ to 4°52’N and longitude 7°25’ to 7°45’E (Figure 1). The estuary lies on the western bank of Eniong creek about 12km from the mouth of Imo River, where drainage has broken the coastal area into a jigsaw of irregular shaped tidal flat. Characteristically, the area is typical of an estuarine tidal water zone with fresh water input from Imo River and with extensive mangrove swamps and intertidal mud flats. The elevation of the area is generally less than 30m above sea level. The estuary is bounded by thick mangrove dominated by *Rhizophora* species and interspersed by Nypa palm. The main occupation of the people occupying the areas is fishing; other activities such as trading on fish; both fresh and smoked, fire wood cutting and water transportation system are also carried out [11]. sampling stations with unique ecologically setting and human activities were established along the length of the estuarine. The sampling stations were about two kilometers apart from each other.

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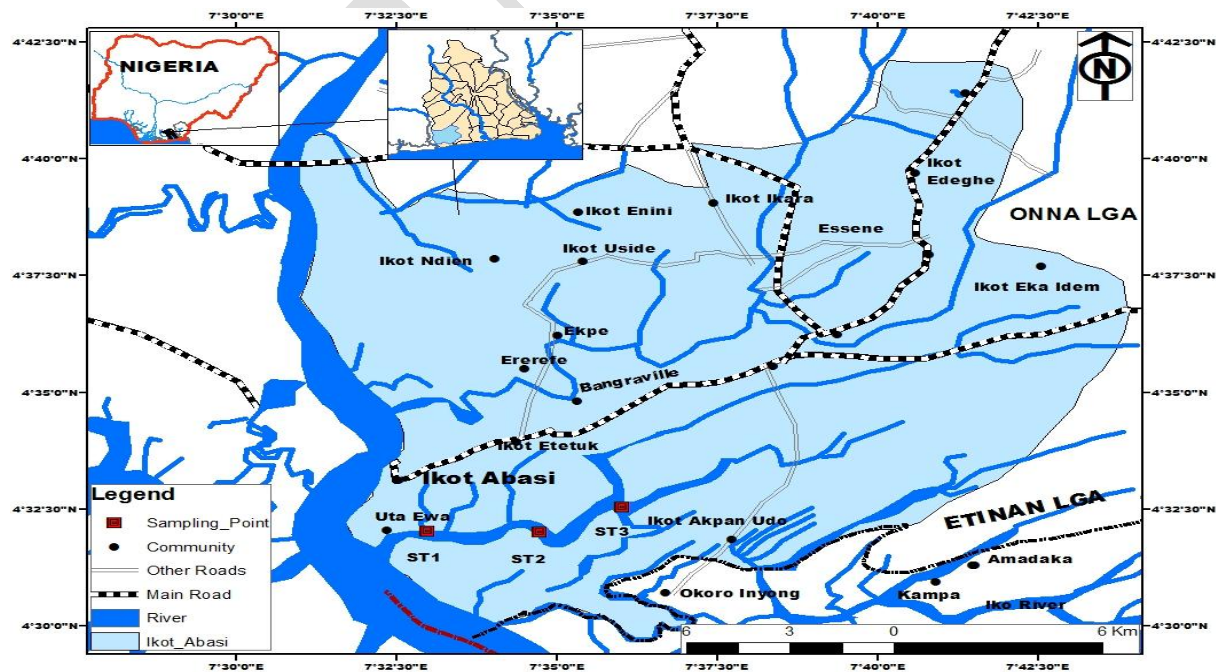

Figure 1: Map of Utaewa Estuary and their Sampling Points

Sample collection

Estuary water samples were collected from a total of 3 different locations. From each location, the samples were collected in triplicates using sterile sample bottles. The samples were transported immediately to the laboratory for microbiological, metagenomics and physicochemical analyses. These were done as previously described American Public Health Association, APHA [8].

Physiochemical Analysis

Water Samples from the location were made into composite samples subjected to physicochemical analysis. The various physicochemical parameters analyzed were pH, temperature, electrical conductivity, turbidity, total dissolved solids, total hardness, dissolved oxygen, biochemical oxygen demand, calcium, potassium, magnesium, N-nitrate, N-nitrite, copper, zinc, sulphide, N-ammonia. These were all carried out using standard methodologies described previously by APHA [12] and WHO [13].

Microbiological analysis

The water samples collected in triplicates were made into three composite samples of 1 litre each and used for the microbiological analyses. From each of the water samples, a ten-fold serial dilution were carried out (10^{-1} to 10^{-10}) as described previously by Antai *et al.* [14] and Udotong *et al.* [15]. For each of the water samples, dilutions from 10^{-2} and 10^{-3} were plated in duplicates on freshly prepared Nutrient agar and Sabouraud Dextrose agar (SDA) for the enumeration of total heterotrophic bacteria and fungi, respectively. The plates were then incubated for 24 and 48 hours, respectively.

The counts of hydrocarbon utilizing bacteria and fungi were enumerated by pour plate techniques [16] using vapour phase transfer technique of Amanchukwu *et al.* [17] on mineral salt medium (MSM). The oil agar plates were incubated at room temperature for 5 days before enumeration [18, 19]. After incubation, the plates were then observed for growth and the colonies counted. Distinct colonies were purified, maintained and identified as previously described by Martini *et al.* [20]; Barnett and Hunter, [21].

Identification of bacterial isolates by conventional phenotypic method

The discrete bacteria isolated from the samples were characterized based on their cultural morphology which includes colour, texture, shape, size, elevation, etc. of the isolate while, biochemical characteristics which include test include; Gram' reaction, motility, catalyse, oxidase, spore formation, indole production, methyl red, citrate utilization, Voges Proskauer test and sugar fermentation of the discrete bacterial isolates were compared using the Bergers's manual of determinative bacteriology for identification of the isolates [22, 23].

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DNA Extraction from Sample and Polymerase Chain Reaction (PCR)

The three composite water samples were further made into one composite sample for metagenomic analysis. From the prepared samples, metagenomic DNA was extracted using NORGEN BIOTEK CORP (Canada). The extraction was done by strictly following the instruction of the manufacturer. The process of DNA extraction is divided into 5 basic steps namely: Lysate preparation, binding to column, column wash, DNA elution and storage of DNA. Lysate preparation was done by first adding 1ml of lysis buffer E solution to the Eppendorf tubes holding the samples and vortexed briefly using the GmCLab Gilson table top centrifuge. This was followed by addition of 100μL of lysis additive A and the mixture vortexed using the GmCLab Gilson table top centrifuge briefly. The resulting mixture was then incubated suing a heating block (Dry bath incubator, Fisher Scientific) at 55°C for 300 minutes. Approximately 1mL of each resulting solution was transferred into a 2mL DNAase-free microcentrifuge tube. After transfer, they were then centrifuged for 20,000xg for 2 minutes and this resulted in the formation of white layer on top of the supernatants. From these tubes, 700μL was carefully transferred while avoiding the white layer into another DNAase-free microcentrifuge tube. Approximately 100mL of binding buffer I was added, mixture vortexed a few times (5 to 6) and incubated on ice for 10 minutes. The resulting Lysate was then spun at 20,000 x g to pellet the cell debris. Using a pipette, 700 μL of supernatant while avoiding the pellets were transferred into a fresh DNAase-free microcentrifuge tube. To the mixture, an equal volume of freshly prepared 70% ethanol was added and vortexed using a low speed centrifuge (Sartorius Centrifuge Stedim, Model Centristart A1-14). Following Lysate preparation, the next step was binding to column. This was done applying 700 μL of the clarified Lysate with ethanol onto the column and centrifuged for 1 minute at 10,000 x g. The flow through was then discarded and the column reassembled and the process repeated until the entire Lysate is passed through the column. The column wash step was carried out by applying 500μL of the binding buffer to the column and centrifuged for 1 minute at 10,000 x g. The flow through was discarded and the spin column reassemble with its collection tube. Approximately 500μL of wash solution A to the column and centrifuged for 1 minute at 10,000 x g and the flow through discarded. The column was then spinned for 20,000 x g for 2 minutes in order to thoroughly dry the resin. The next step was DNA elution. This was done by first placing the column into a fresh 1.7 mL elution tube and 50μL of elution buffer to the column. This was then centrifuged for 1 minute at 200 x g and then at 20,000 x g for 1 additional minute. The resulting purified genomic DNA was then stored at 2-8°C for further analysis. Following DNA extraction from the samples, the genomic DNA extracts were subjected to PCR amplification. The PCR was set at 30 cycles for 2 hours at 96, 72 and 65°C for denaturation, annealing and extension. The amplified genomic DNA (15 µl) were then subjected to 1.5% gel electrophoresis after mixing with 2 µl of loading dye. These were done using as previously described by Salaam *et al*. [24] and Anika *et al*. [25].

Next generation sequencing and bioinformatics analysis of samples

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DNA sequencing was performed using Next Generation Sequencing (NGS) with universal primer pair - 16S: F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and R338 (5'-TGCTGCCTCCCGTAGGAGT-3'). Cycling condition were same as those reported earlier [24, 25, 26]. The whole metagenome sequencing was done using Illumina Miseq Next Generation Sequencing platform. Overall bioinformatic analysis was performed using the online tool of National Centre for Biotechnology BLAST version 2.2.24 and CLC bioGenomics workbench version 7.5.1.

Results

Table 1 shows the mean results of the physicochemical analysis of the estuary water samples. The mean pH and temperature were 7.01 ± 0.17 and 26.87 ± 0.50 respectively. The mean value for Total dissolved solids, dissolved oxygen, biological oxygen demand, alkalinity, acidity and total hardness were 7.70 ± 1.06, 39.93 ± 1.46, 38.93 ± 2.55, 0.56 ± 0.08, 20.33 ± 3.21 and 23.60 ± 0.73 mg/l respectively. The salinity was 13.90 ± 1.61 (ppt) while conductivity had a mean value of 21.34 ± 0.60 (µs/cm). Calcium, sulphate, phosphate, nitrate and magnesium were all detected with mean values of 1.22 ± 0.04, 0.20 ± 0.04, 0.33 ± 0.02, 0.05 ± 0.02 and 3.11 ± 0.30 mg/l respectively.

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Table 1: Mean physicochemical characteristics of estuary water samples

Parameters	Mean Value	WHO	NIS
pH	7.01 ± 0.17	6.5-8.0	6.5-8.5
Temperature (^{0}C)	26.87 ± 0.50	20.0-30.0	Ambient
Total Dissolved Solids (mg/l)	7.70 ± 1.06	1000	500
Dissolved Oxygen (mg/l)	39.93 ± 1.46	≥5.0	-
Biological Oxygen Demand (mg/l)	38.93 ± 2.55	2.0-60	-
Alkalinity (mg/l)	0.56 ± 0.08	100-200	-
Salinity (ppt)	13.90 ± 1.61	-	-
Conductivity (µs/cm)	21.34 ± 0.60	1000	1000
Acidity (mg/l)	20.33 ± 3.21	-	-
Turbidity (NTU)	12.67 ± 3.06	5.10	5

Total Hardness	23.60 ± 0.73	100	-
Calcium (mg/l)	1.22 ± 0.04	7.5	-
Sulphate (mg/l)	0.20 ± 0.04	-	-
Phosphate (mg/l)	0.33 ± 0.02	3.50	-
Nitrate (mg/l)	0.05 ± 0.02	50	50
Magnesium (mg/l)	3.11 ± 0.30	0.01-0.20	0.2

Where WHO = World Health Organization

NIS = Nigerian Industrial Standards

NTU = Nephelometric Turbidity Unit

Table 2 shows the total heterotrophic bacterial and fungal (THB and THF) counts as well as the hydrocarbon utilizing bacterial and fungal (HUB and HUF) counts. The results show that THB counts were higher than the THF counts. The highest THB count was recorded at location ESW1 while the highest THF count was recorded at location ESW3. The count ranged from 2.17×10^6 to 2.32×10^6 cfu/ml for THB and 1.23×10^5 to 1.64×10^5 cfu/ml respectively. The hudrocarbon utilizing bacteria (HUB) and Hydrocarbon utilizing fungi (HUF) counts ranged from 1.03×10^5 to 1.39×10^5 cfu/ml and 7.9×10^3 to 8.6×10^3 cfu/ml respectively.

Table 2: Mean bacterial and fungal counts from the estuary water samples

	Sampling Locations		
Parameters (cfu/ml)	**ESW1**	**ESW2**	**ESW3**
THB	2.32×10^6	2.2×10^6	2.17×10^6
HUB	1.21×10^5	1.03×10^5	1.39×10^5
THF	1.23×10^5	1.64×10^5	1.36×10^5
HUF	8.4×10^3	7.9×10^3	8.6×10^3

Keys: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi) and ESW 1, 2 3 = Estuary water locations 1, 2 and 3

The results of the bacterial and fungal isolates are presented in table 4. The bacteria identified were dominated by members of the genera *Escherichia, Citrobacter, Bacillus, Salmonella, Shigella, Proteus,*

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Flavobacterium, Vibrio, Micrococcus and *Pseudomonas*. The fungal isolates belonged to the genera *Aspergillus, Rhizopus, Fusarium, Penicillium, Saccharomyces* and *Candida.*

Table 3: Bacterial and fungal isolates from the estuary water samples

Bacterial isolates	Fungal isolates
Escherichia coli	*Aspergillus* sp
Pseudomonas sp	*Penicillium* sp
Bacillus sp	*Rhizopus* sp
Citrobacter sp	*Fusarium* sp
Salmonella sp	*Saccharomyces* sp
Shigella sp	*Candida* sp
Proteus sp	
Flavobacterium sp	
Vibrio sp	
Micrococcus sp	

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Table 4 showed the top phyla has revealed by metagenomics analysis together with their read counts and percentage coverage. The water sample had Proteobacteria (68.57%) as the predominant group. Other dominant Phyla include; Bacteroidetes (17.40%), Planctomycetes (5.52%), Firmicutes (2.42%), Actinobacteria (2.27%), Verrucomicrobia (1.41%), Cyanobacteria (1.12%), Chloroflexi (0.27%), Acidobacteria (0.24%) and Gemmatimonadetes (0.20%).

Table 4: Top Phyla classification of the 16S rRNA metagenome from composite estuary water sample

Phyla classification	Read count	Percentage (%)
Proteobacteria	5073.0	68.57
Bacteroidetes	1287.0	17.40
Planctomycetes	408.0	5.52
Firmicutes	179.0	2.42
Actinobacteria	168.0	2.27
Verrucomicrobia	104.0	1.41
Cyanobacteria	83.0	1.12

Chloroflexi	20.0	0.27
Acidobacteria	18.0	0.24
Gemmatimonadetes	15.0	0.20

Table 5: Top Class Classification of the 16S rRNA metagenome from composite estuary water sample

Class	Read count	Percentage (%)
Gammaproteobacteria	2142.0	28.95
Alphaproteobacteria	1420.0	19.19
Flavobacteriia	916.0	12.38
Betaproteobacteria	858.0	11.60
Deltaproteobacteria	612.0	8.27
Planctomycetia	337.0	4.56
Bacilli	181.0	2.45
Cytophagia	156.0	2.11
Actinobacteria	132.0	1.78
Sphingobacteriia	108.0	1.46

The dominant Class as shown in table 5 were; Gammaproteobacteria (28.95%), Alphaproteobacteria (19.19%), Flavobacteriia (12.38%), Betaproteobacteria (11.60%), Deltaproteobacteria (8.27%), Planctomycetia (4.56%), Bacilli (2.45%), Cytophagia (2.11%), Actinobacteria (1.78%) and Sphingobacteriia (1.46%). The Genus as represented in table 6 were dominated by Unknown (50.16%), Acinetobacter (8.34%), Ignatzschineria (4.73%), Planctomyces (3.28%), Anaerospora (3.27%), RS62 (2.73%), Methylophaga (1.64%), Wohlfahrtiimonas (1.31%), Myroides (1.23%) and Candidatus (0.95%).

Table 6: Top Genus classification of the 16S rRNA metagenome from composite estuary water sample

Genus	Read count	Percentage (%)
Unknown	3711.0	50.16
Acinetobacter	617.0	8.34

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Ignatzschineria	350.0	4.73
Planctomyces	243.0	3.28
Anaerospora	242.0	3.27
RS62	202.0	2.73
Methylophaga	121.0	1.64
Wohlfahrtiimonas	97.0	1.31
Myroides	91.0	1.23
Candidatus	70.0	0.95

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Discussion

Water bodies be it oceans, seas, estuaries, fresh waters, lakes or streams do have very complex quality and it is very essential to the survival of humans, flora and fauna, as well as microorganisms [27]. Most of these natural water bodies are the only source of drinking water in most riverine communities though often threatened by anthropogenic activities such as oil and gas exploration, industrial activities and domestic sewage disposal [26]. The assessment of the quality of Estuary water bodies especially in the Niger Delta ecosystem becomes imperative as it sustains the economic activities of the inhabitants and are often exposed to receiving both legal and illegal crude oil activities and oil spillages that causes environmental pollution and degradation.

The results of the physicochemical analysis showed that some of the parameters failed to meet the standard of most regulatory bodies for water [28, 29]. The pH, temperature and conductivity were within the acceptable range while alkalinity, calcium, phosphate and nitrates were below the standards. The findings in this research agrees with that of Edet *et al.* [26] and Onojake *et al*. [30] who reported similar observations pH, temperature, DO, BOD and turbidity at Iko River Estuary and Bonny/New Calabar River Estuary respectively. The pH of water is very important in that changes in pH values may affect the toxicity of microbial poisons in the water [31]. In the present study, pH near neutrality of the water sample poses no health risk to consumers who use the water for cooking, drinking, washing, bathing etc. Acidic pH observed may be an indication of the contamination of the water sample [32].

The high microbial counts in the surface water and wastewater could be attributed to anthropogenic activities as well as flooding from rainfall, erosion or urban runoff from the activities around the Utaewa with beehive of industrial activities into the river. Increased in the population of heterotrophic microorganisms during this time of study may be due to changes in biological oxygen demand, dissolved oxygen levels, temperature and salinity aa these factors are known to influence microbial diversity [26].

The main reason for high abundance of microbial populations in estuaries is the high productivity rate because estuaries provide habitats for a large number of organisms and the presence of phytoplankton (mainly the diatoms and dinoflagellates) which are the primary producers in estuaries [33]. The bacterial and fungal counts in this work within the range reported by Unimke *et al.* [34] at Imo river estuary where the range was 2.23-2.39 $x10^6$ and 1.17-1.38 $x10^5$ cfu/ml, respectively for bacteria and fungi. Ogbonna *et al.* [35] reported higher total bacteria counts ($1.12\pm0.13x10^8$ to $1.28\pm0.09x10^8$ cfu/m) in surface water. The bacteria and fungi isolated and identified in this study are similar to those reported by several researchers in similar ecosystems [26, 36, 37]. The preponderance of coliform group of bacteria in the estuary water in our study is a calls for concern as it indicates recent faecal contamination [29, 38]. The presence of hydrocarbon utilizing microorganisms serves as a sensitive index of environmental exposure to hydrocarbons. This is in agreement with a number of reports [39, 40] that the number of hydrocarbon utilizing microorganisms and their proportion in the heterotrophic community increases upon exposure to petroleum or other hydrocarbon pollutants and that the levels of hydrocarbon utilizing microorganisms generally reflect the degree of contamination of the ecosystem.

Molecular assessment of the composite estuary waters sample showed that the dominant phyla were Proteobacteria, Bacteroidetes, Plantocmycetes, Firmicutes, Actinobacteria, Veruucomicrobia, cyanobacteria, Chloroflexi, Acidobacteria and Gemmatimonadetes. Earlier reports by Bobrova *et al.* [41] and Edet *et al.* [26] also found this group of bacteria at the phyla level of characterization. the top classes of bacteria reported in this work corroborates the findings of Lamendella *et al.* [42] and Edet *et al.* [26] who reported Alpha and Gammaproteobacteria and Alphaproteobacteria as the two most dominant classes. The generic level of classification was dominated by Unknown, Acinetobacter Ignatzschineria *planctomyces, anaerospora, RS62 methylophaga, wohlfahrtiimonas, Myroides* and *Candidatus* in descending order of abundance.

Conclusion

Most Estuaries in the Niger Delta are impacted and polluted due to anthropogenic interferences. This study provides information on the microbiology and physicochemistry including nutrient contents of Utaewa Estuary. The study revealed microbial counts with respect to physicochemical parameters, which can provide useful information in understanding the microbiology of crude oil impacted environments and inference can be made on the health of the environment as well. Culture dependent analysis revealed that members of the genera *Escherichia*, *Bacillus* and *Pseudomonas* while the fungi were dominated by *Penicillium* sp. and *Aspergillus* sp. Which provided insight to the level of contamination and the potentials of the isolated organism to utilize crude oil and degrade if properly harnessed. Metagenomics analysis provided a more robust identification of the microbial composition and diversity in the sampled ecosystem.

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The presence of pathogenic and non-pathogenic bacteria in the aquatic ecosystem may be potentially harmful to animal/human health has been revealed in this study. The identified bacteria are of public health significance and may be causative agent for zoonotic infections that may develop as a consequence of handling of aquatic biota and use of the water. If proper hygiene and implementation of aquatic water policy and regulations are not enforced to discourage anthropogenic pollution, it may pose challenges to both humans and aquatic fauna. There provision of potable water for the inhabitants of the community thus becomes a necessity.

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