

Microbial Diversity, and Structure of Coastal Water Bodies from Some Cholera-prone Communities in Rivers State, Nigeria

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Received March 10, 2024; Revised April 11, 2024; Accepted April 18, 2024

Abstract Background: This study aimed to determine the microbial diversity and Community Structure of Coastal water bodies from some cholera-prone communities in Rivers State. **Methods:** Water samples were collected from water bodies located in six different cholera prone coastal communities in Rivers state three times each with the determination of coordinates each time using Global Positioning System (GPS). At each collection point, in-situ physicochemical parameters of water sources were determined using H198194, H198195, and H198196 multiparameter meters (Hanna Instrument Inc, USA). The water samples were collected using a sterilized container of 750ml capacity (75cl). DNA was extracted from each water sample collected. The extracted DNA from the water samples were sequenced by Illumina HighSeq by InqabaBiotec, South Africa. The sequencing library was prepared by random fragmentation of the DNA, followed by 5' and 3' adapter ligation. Adapter-ligated fragments were then PCR-amplified and gel purified. For cluster generation, the library was loaded into a flow cell where fragments were captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment was then amplified in to distinct, clonal clusters through bridge amplification. When cluster generation was complete, the templates were sequenced using Illumina MiSeq SBS technology. **Results:** The microbial communities in the coastal water studied are highly diversified. The microorganisms are generally classified into kingdoms containing bacteria, protozoa, viruses, archaea, fungi, and Plantae. For all the coastal waters, kingdom bacteria is common with varying percentage reads which ranged from 45835 reads (46.64%) in Bonny river to 263985 reads (99.71%) in Kaa river. Protozoa were identified in addition to other microbial communities in Andoni and Kaa rivers, both in the South-east senatorial district of Rivers State. The number of viruses detected is more in the Chukwu-Ama river in Ogu/bolo with total reads of 384 (0.25% of microbes present). In addition to other kingdoms present in the Okirika River, fungi were also detected. Alpha diversity of bacterial species across the three regions investigated showed no significant difference while that of individual samples was significantly different at $P \leq 0.05$ using observed features, Shannon diversity index, Faith's phylogenetic diversity and Pielou's evenness tools. Bray-Curtis dissimilarity between the bacterial communities' structures in the three zones investigated did not reveal distinct differentiation in multivariate space. The observed clustering pattern of samples within the PCoA (Principal coordinate analysis) plot was found to be non-significantly (FDR-adjusted $p > 0.05$) different among the zones in Rivers State (PERMANOVA $R^2 = 43.3\%$, $p = 0.07$). The ASVs detected in all zones of Rivers state spanned 15 phyla (Rivers East = 13; Rivers South-east = 13; Rivers West = 10). Actinobacteriota was the most relatively abundant phyla in both Rivers East (43.9%) and Rivers South-east (35.5%) while Firmicutes (36.2%) was the most abundant phyla in Rivers West. **Conclusion:** This study has shown that microbial communities present in coastal waters are highly diversified with unknown and unassigned microorganisms detected from all the coastal water examined, showing that these waters are hubs for highly potential microorganisms that could be inimical to human health.

Keywords: microbial diversity, Microbial Structure, coastal water, cholera, community

Cite This Article: Chidimma Anthonia Azike, Vivian Nkemkanma Agi, Ollor Amba Ollor, Easter Godwin Nwokah, Chinyere Ihuarulam Okoro, and Confidence Kinikanwo Wachukwu, "Microbial Diversity, and Structure of Coastal Water Bodies from Some Cholera-prone Communities in Rivers State, Nigeria." *American Journal of Epidemiology and Infectious Diseases*, vol. 12, no. 1 (2024): 1-13. doi: 10.12691/ajeid-12-1-1.

1. Introduction

Cholera is most likely to be found and spread in places with inadequate water treatment, poor sanitation including an improper sewage system, and inadequate hygiene as such places are termed cholera-prone communities. The coastal environment can be immensely biodiverse because the activities of man result in contamination even with the use and expansion along coastlines, notwithstanding, can put weight on these conditions that may restrict the rates of development and availability of living organisms. This can thus lessen the overall biodiversity. Microbial diversity is an essential property of common complexes which decides the working and the origins of a specific indigenous microbial community in a particular environment. Hence, variety analysis turns into the initial phase in portraying the systems. It involves variations in the types of microbes present in an environment and their relative abundance [1]. There have been numerous imaginative, and valuable endeavors being advanced as different strategies to decide the structure and degree of the variety of such environments. While discussing microbial variability, the significant constraint has been most organisms' non-culturability. Procedures grew at first were reliant upon cultures and thus could deliver just those species which could be developed in the laboratory set-up. The need to catch microorganisms' historical taxonomy incited the creation of culture-independent procedures like metagenomics [2]. Notwithstanding, techniques like isolating the DNA, trailed by cloning and sequencing, were extremely bulky albeit valuable in giving extra data about the greater part of the species which couldn't be portrayed before without culture-independent technique. At that point came the innovation which was less work escalated and is getting less expensive step by step—the cutting edge sequencing (Next-generation sequencing). It changed the entire situation and permitted total mechanization with the chance of sequencing entire complexes in a day's time and with costs the same or lesser than the past innovations.

An operational taxonomic unit (OTU) is used to analyze population diversity. It indicates the cluster of similar sequences in a specimen under investigation and defines species. Microbial diversity is measured and expressed as alpha, beta, and gamma diversities at different scales when operational taxonomic units are known. Knowledge of microbial population diversity is critical because studies have shown that disease conditions are linked to microbiome diversity [3].

Phylogenetic distribution-based diversity considers the evolutionary distances and relationships of a variety of microorganisms within the microbial community. It describes the depth of the phylogenetic tree shared by microbes in a given microbial community in a sample. Phylogenetic diversity can be measured using a tool called Unifrac. There are two forms of Unifrac viz Weighed Unifrac and Unweighted Unifrac. While unweighed unifrac utilizes the presence and absence of operational taxonomic units and phylogeny, the weighed Unifrac uses abundance information of OTU and phylogeny [4].

The most common organized genetic marker critical in

the study of Phylogeny and taxonomy of bacteria is 16SrRNA gene sequencing [5]. It has been reported that microbial identification at the taxonomic level by 16SrRNA is no longer satisfactory [6], thus it becomes important to employ other molecular procedures in order to identify and characterize both culturable and non-culturable microbes at species/ strain levels, such technique is called metagenomics. Metagenomics involves the extraction of microbial genome directly from the specimen without growing the organism(s) in the artificial laboratory medium (culture). It is independent of microbial culture and has helped to resolve the limitations of the genomics study. Metagenome sequencing strives to obtain every genetic information directly from the samples. This technique has been used to characterize microorganisms from both environmental and clinical samples [2]. All extracted DNA are amplified and sequenced so that the sequence produced are used to determine the taxonomy and the function of all genomes present in a microbial community. By this technique, novel organisms are identified. The reason for metagenomics is to determine microorganisms present and the role they play. Therefore, sequencing alone is not ideal because there are a lot of genes whose functions are not known. The genes help in identifying and studying the organisms more when they are expressed and also aid in enriching the database, hence, providing more opportunities for the development of biotechnology. Metagenomics provides information regarding the functional properties of microbes in microbial communities and their genetic contents. It also reveals microbes not captured by 16SrRNA. The availability of reference genomes in the genebank makes metagenomics studies easier. This study aimed to determine the microbial diversity and Community Structure of Coastal water bodies from some cholera-prone communities in Rivers State.

2. Materials and Methods

Study Design

The research design used in this study is descriptive. The study was done seasonally from February to November 2020. This period covers wet and dry seasons. The reason is that cholera is usually influenced by season, hence seasonal determination of *Vibrio* species is very crucial. Water samples were collected from coastal communities. The water bodies were situated in coastal communities, two (2) water bodies from each Senatorial District in Rivers State. The water bodies include Marine Base Jetty, Chuku-Ama River, Kaa waterside, Andoni River, Egbolom River, and Bonny River. Water samples were collected in each of these water bodies three times with the determination of coordinates each time using Global Positioning System (GPS). At each collection point, in-situ physicochemical parameters of water sources were determined using H198194, H198195, and H198196 multiparameter meters (Hanna Instrument Inc, USA). The water samples were collected using a sterilized container of 750ml capacity (75cl).

Sample Collection

Sterile plastic containers of 250ml capacity were used to collect water samples from each water source in the six Coastal Communities. Water samples were collected three times from each source. The first collection was done during the dry season at the beginning of the year (February/March). The second collection was done during the middle of the rainy season (June/July) and the third collection was done at the end of the rainy season (October/November 2020). Surface water was collected 300m below the water by tilting and creating a current away from the shore, aseptically to avoid any form of contamination. After the collection, water samples were transported immediately in a cold chain to the laboratory for analysis. The analysis was done seasonally. The water containers were filled up and corked while still under water [7].

DNA Extraction from Water Samples

Each 75cl bottle of water sample was duly mixed and dispersed into a corresponding Eppendorf tube of 1.5ml capacity, centrifuged for 5 minutes at 2000rpm. The supernatant was decanted and tubes refilled with corresponding water samples respectively. This process was severally repeated until each water sample became adequately concentrated. Zymo Research bashing bead tubes were arranged and labeled with water sample codes and into each tube was added 250 μ l of concentrated water sample and 750 μ l bashing bead buffer and capped tightly.

Bead beaters filled with a 2ml tube holder were used to secure the tubes and centrifuged at 2000rpm for 5 minutes. Another set of tubes known as zymo-spin III filler in collection tubes were arranged and labeled and 400 μ l of supernatant was transferred accordingly into these zymo-spin III-F filter tubes and centrifuge at 8000g for one minute. The filter tubes were discarded leaving the collection tubes. The volume 1200 μ l of genomic lysis buffer was added to the filtrate on the collection tube.

Another set of tubes called Zymo-spin IC column filled in a collection tubes were arranged and 800 μ l of the mixture was transferred accordingly into these tubes and centrifuged at 10,000xg for one minute. The flow through from collection was discarded and the step repeated.

200 μ l of DNA prewashed buffer was added to the Zymo-spin IC column in a new collection tubes and spun for one minute at 10,000xg. The DNA wash buffer was added 500 μ l into each Zymo-spin IC column and centrifuged again for one minute.

The zymo-spin IC columns were transferred to a clean microcentrifuge tube of 1.5ml capacity and DNA elution buffer was directly added to the column matrix, centrifuged at 10,000xg for 3 minutes to elute the DNA. The elute DNA was transferred to another set of tubes Zymo-spin II-uHRC filter in a clean collection tube, centrifuged at 16,000xg for 3 minutes.

The filtered DNA was quantified and examined for purity using Nanodrop spectrophotometer and then sent to South Africa for metagenomics and biodiversity analysis.

Metagenomics sequencing (Next Generation Sequencing)

The extracted DNA from the water samples were sequenced by Illumina HighSeq by InqabaBiotec, South Africa.

Library Preparation

The sequencing library was prepared by random fragmentation of the DNA, followed by 5' and 3' adapter ligation. Adapter-ligated fragments were then PCR-amplified and gel purified.

Cluster Generation

For cluster generation, the library was loaded into a flow cell where fragments were captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment was then amplified in to distinct, clonal clusters through bridge amplification. When cluster generation was complete, the templates were ready for sequencing.

Sequence processing

Method of sequencing was used by applying Illumina MiSeq sequence of processing known as QIIME2 v2018.11 [8]. In addition, chimeric Sequences, marginal sequence errors and noisy sequences were filtered while picking amplicon sequence variants (ASVs) using DADA2 [9]. A trained naïve Bayes classifier (SILVA 132) for 16S rRNA V3-V4 hyper variable regions using the q2-feature classifier plugin was carried out using representative sequence taxonomy. Operational Taxonomy Units (OTU) count table was elevated to an even sampling depth of 3845 reads per sample and singleton discarded before determination of alpha-diversity and beta-diversity using the version 2.5-6 Vegan [10] and R-package (R-Core Team, 2019).

Sequencing was carried out by Illumina SBS technology using a proprietary reversible terminator-based that detected single bases as they were incorporated into DNA-template strands. As all four reversible terminator-bound dNTPs were present during each sequencing cycle, natural competition minimized incorporation bias and greatly reduced raw error rates compared to other technologies.

Statistical Analysis

Statistical analyses for metagenomics study were done using R vs3. 6.1 (R Core Team, 2019). Other parameters such as Alpha diversity differences and physicochemical were determined using the agricolae R package (de Mendiburn, 2020). Permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of the differences in multivariate space; while the comparison of microbial community differences (Beta-diversity) in the three zones of Rivers State was based on Bray-Curtis dissimilarities and Principal Coordinate Analysis (PCoA) using Vegan [10] R packages. The influence of physicochemical parameters on the community level differentiation was achieved by constrained redundancy analysis (RDA), which was performed using Hellinger transformed bacterial, and environmental data for Vegan R package functions. The significance of the constraining variables was estimated based on a permutation test using the Vegan function.

3. Results

Classification of Microbial Diversity in Coastal Waters

From the metagenomics results obtained, the microbial communities in the coastal water studied are highly diversified. The microorganisms are generally classified into kingdoms (Table 1a) containing bacteria, protozoa, viruses, archaea, fungi, and Plantae. For all the coastal waters, kingdom bacteria is common with varying percentage reads which ranged from 45835 reads (46.64%) in Bonny river to 263985 reads (99.71%) in Kaa river. Protozoa were identified in addition to other microbial communities in Andoni and Kaa rivers, both in the South-east senatorial district of Rivers State. The number of viruses detected is more in the Chukwu-Ama river in Ogu/bolo with total reads of 384 (0.25% of microbes present). In addition to other kingdoms present in the Okirika River, fungi were also detected. There were also microbes of unknown kingdoms detected in each of the coastal water. These also vary but more of such were detected in Chukwu-ama River in Ogu/bolo.

Table 1b showed top Phylum classification of microbial diversity in the coastal waters. The common phyla across the samples are Actinobacteria and Proteobacteria. All the samples showed unknown phyla. Some phyla are also unique to some coastal rivers. Such phyla include Cyanobacteria and Fusobacteria detected in Bonny, Phylum Gemmatimonadetes peculiar to Andoni, and phylum Nitrospira unique to Kaa River. Top-class classification of microbial diversity was also considered in all the water samples (Table 1c). The microbial communities cut across 17 classes with Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria present in all the coastal water samples. The samples also demonstrated microorganisms of unknown class. The

Abua water contained Verrucomicrobiae and Thermomicrobia unique to it while Bonny River contained microorganisms belonging to classes Clostridia, Bacteroidia, and Fusobacteria. Top ten order was considered and presented in Table 1d with a total of 21 microbial orders identified. The order Actinomycetales is common to all the water samples. The order Bacillales was found in Kaa River, Okirika River, and Bonny River with the highest reads recorded against Kaa River. The order Clostridiales was seen only in Bonny River. Pseudomonadales and Flavobacteriales were found only in Abua/Odual River while Pasteurellales, Neisseriales and Lactobacillales were found in Bonny River only. Moreso, Spingobacteriales were seen in Andoni and Okirika while the order Chlamydiales was detected only in the Kaa River. The order Enterobacteriales was identified in Okirika and Bonny River. At the same time, Rubrobacterales and Caudovirales were identified only in the Chukwu-ama River.

Table 1e showed the top families of members of microbes from studied coastal waters. A total of thirty-five families were identified in addition to the unassigned and unknown families. The top ten families identified include: Nocardioideaceae, Intraperangiaceae, Acidobacteriaceae, Mycobacteriaceae, Streptomycetaceae, Micrococcaceae, Planctomycetaceae, Methylobacteriaceae, Burkholderiaceae, and Enterobacteriaceae. Other families of the microbial communities of Medical importance identified are Podoviridae, Spathidiidae, Pseudomonadaceae, Clostridiaceae, Bacillaceae and Neisseriaceae.

Table 1a. Kingdom Classification of Microbial Diversity in All Coastal Waters

Kingdom	CW 23 Reads (%)	CW 25 Reads (%)	CW 24 Reads (%)	CW 26 Reads (%)	CW 27 Reads (%)	CW 28 Reads (%)
Bacteria	186249(99.04)	263985(99.71)	153462(99.58)	262917(99.77)	212816(99.2)	45835 (46.64)
Protozoa	1794 (0.95)	736 (0.28)	226 (0.15)	205 (0.08)	134 (0.06)	-
Virus	-	1 (0.00)	384 (0.25)	14 (0.01)	36 (0.02)	-
Archae	3 (0.00)	2 (0.00)	-	-	2 (0.00)	35 (0.04)
Fungi	-	-	-	6 (0.00)	-	-
Plantae	1 (0.00)	12 (0.00)	2 (0.00)	38 (0.02)	1 (0.00)	-
Animalia	-	-	-	5 (0.00)	-	-
Unknown	5 (0.00)	4 (0.00)	40 (0.03)	-	4 (0.00)	52411(53.33)

Table 1b. Phylum Classification of Microbial Diversity of All the Coastal Waters

Phyla	CW23 Reads (%)	CW25 Reads (%)	CW24 Reads (%)	CW26 Reads (%)	CW27 Reads (%)	CW28 Reads (%)
<i>Actinobacteria</i>	16947(9.01)	46425(17.54)	30373(19.71)	51022(19.36)	57195(26.85)	448 (0.46)
<i>Proteobacteria</i>	13268(7.06)	17099 (6.46)	4167 (2.70)	23069(8.75)	23191(10.89)	4933(5.02)
<i>Planctomycetes</i>	2130 (1.13)	639 (0.24)	534 (0.35)	2285(0.87)	690 (0.32)	-
<i>Acidobacteria</i>	1925 (1.02)	1194 (0.45)	1746 (1.13)	1866(0.71)	126 (0.06)	-
<i>Ciliophora</i>	1794 (0.95)	736 (0.28)	226 (0.15)	205 (0.08)	134 (0.06)	-
<i>Chloroflexi</i>	851 (0.45)	4019 (1.52)	709 (0.46)	1216 (0.46)	836 (0.39)	-
<i>Bacteroidetes</i>	593 (0.32)	18 (0.01)	120 (0.08)	-	456 (0.21)	52 (0.05)
<i>Chlamydiae</i>	115 (0.06)	585 (0.22)	29 (0.02)	80 (0.03)	125 (0.06)	-
<i>Gemmatimonadetes</i>	79 (0.04)	-	-	-	-	-
<i>Firmicutes</i>	-	8563 (3.23)	141 (0.09)	583 (0.22)	63 (0.03)	14712(14.97)
<i>Nitrospira</i>	-	62 (0.02)	-	-	-	-
<i>Verrucomicrobia</i>	-	45 (0.02)	-	100 (0.04)	402 (0.19)	-
<i>Cyanobacteria</i>	-	-	-	-	-	16 (0.02)
<i>Fusobacteria</i>	-	-	-	-	-	10 (0.01)
Unknown	150275(79.91)	185338(70.01)	116040(75.29)	182977(69.43)	129365(60)	78075(79.47)

Table 1c. Top Class Classification of Microbial Diversity in All Study Area

Class	CW23 Reads (%)	CW25 Reads (%)	CW24 Reads (%)	CW26 Reads (%)	CW27 Reads (%)	CW28 Reads (%)
<i>Actinobacteria</i>	16947(9.01)	46305(17.49)	30373(19.71)	50873(19.30)	57188(26.85)	439 (0.45)
<i>Alphaproteobacteria</i>	7043 (3.75)	11665(4.41)	3729 (2.42)	14555 (5.52)	14935(7.61)	104 (0.11)
<i>Betaproteobacteria</i>	4677 (2.49)	4259 (1.61)	-	3380 (1.28)	1100 (0.52)	113 (0.12)
<i>Gammaproteobacteria</i>	1251 (0.67)	689 (0.26)	246 (0.16)	4478 (1.70)	7002 (3.29)	4278 (4.36)
<i>Planctomycetacia</i>	2130 (1.02)	639 (0.24)	534 (0.35)	2285 (0.87)	690 (0.32)	-
<i>Acidobacteria</i>	1925 (0.95)	1194 (0.45)	1746 (1.13)	1866 (0.71)	-	-
<i>Gymnostomatea</i>	1794 (0.95)	733 (0.28)	207 (0.13)	-	-	-
<i>Chloroflexi</i>	805 (0.43)	4019 (1.52)	707 (0.46)	1121 (0.43)	834 (0.39)	-
<i>Sphingobacteria</i>	520 (0.28)	-	-	-	-	-
<i>Bacilli</i>	-	8551 (3.23)	139 (0.09)	566 (0.21)	-	2049 (2.09)
<i>Deltaproteobacteria</i>	-	-	121 (0.08)	-	-	-
<i>Flavobacteria</i>	-	-	-	-	429 (0.20)	26 (0.03)
<i>Verrucomicrobiae</i>	-	-	-	-	402 (0.19)	-
<i>Thermomicrobia</i>	-	-	-	-	362 (0.17)	-
<i>Clostridia</i>	-	-	-	-	-	12652(12.88)
<i>Bacteroidia</i>	-	-	-	-	-	18 (0.02)
<i>Fusobacteriia</i>	-	-	-	-	-	10 (0.01)
Unknown	150455(80.01)	185404(70.03)	116072(75.32)	183593(69.67)	129468(60.79)	78517(79.94)
Not assigned	-	-	-	149 (0.06)	-	-

Table 1d. Top Order Classification of Microbial Diversity of Coastal Waters

Order	CW23 Reads (%)	CW25 Reads (%)	CW24 Reads (%)	CW26 Reads (%)	CW27 Reads (%)	CW28 Reads (%)
<i>Actinomycetales</i>	16388 (8.71)	45619(17.23)	8562 (5.56)	37486(14.22)	33967(15.95)	439 (0.45)
<i>Bacillales</i>	-	8551 (3.23)	-	563 (0.21)	-	1840 (1.87)
<i>Rhizobiales</i>	6923 (3.68)	7911 (2.99)	3545 (2.30)	2544 (0.97)	14713 (6.91)	-
<i>Burkholderiales</i>	4674 (2.49)	4112 (1.55)	-	3366 (1.28)	1097 (0.52)	65 (0.07)
<i>Clostridiales</i>	-	-	-	-	-	12649 (12.88)
<i>Planctomycetales</i>	2130 (1.13)	639 (0.24)	534 (0.35)	2285 (0.87)	690 (0.32)	-
<i>Acidobacteriales</i>	1925 (1.02)	1194 (0.45)	1746 (1.13)	1866 (0.71)	-	-
<i>Spathiida</i>	1794 (0.95)	733 (0.28)	207 (0.13)	-	-	-
<i>Xanthomonadales</i>	1102 (0.59)	-	237 (0.15)	-	541 (0.25)	-
<i>Chloroflexales</i>	805 (0.43)	4019 (1.52)	707 (0.46)	1121 (0.43)	834 (0.39)	-
<i>Sphingobacteriales</i>	520 (0.28)	-	-	9353 (3.55)	-	-
<i>Chlamydiales</i>	-	585 (0.22)	-	-	-	-
<i>Rubrobacterales</i>	-	-	404 (0.26)	-	-	-
<i>Caudovirales</i>	-	-	384 (0.25)	-	-	-
<i>Enterobacteriales</i>	-	-	-	3801 (1.44)	-	3562 (3.63)
<i>Pseudomonadales</i>	-	-	-	-	5580 (2.62)	566 (0.58)
<i>Flavobacteriales</i>	-	-	-	-	429 (0.20)	-
<i>Verrucomicrobiales</i>	-	-	-	-	402 (0.19)	-
<i>Pasteurellales</i>	-	-	-	-	-	49 (0.05)
<i>Neisseriales</i>	-	-	-	-	-	41 (0.04)
<i>Lactobacillales</i>	-	-	-	-	-	172 (0.18)
Unknown	150849(80.22)	189996(71.77)	137203(89.03)	198547(75.34)	153564(72.10)	78605(80.04)

Table 1e. Top Family Members of Microorganisms from Studied Coastal Waters

Family	CW23 Reads (%)	CW25 Reads (%)	CW24 Reads (%)	CW26 Reads (%)	CW27 Reads (%)	CW28 Reads (%)
<i>Nocardioidaceae</i>	1656 (0.88)	3211 (1.21)	6938 (4.50)	-	4303 (2.02)	-
<i>Intrasporangiaceae</i>	-	-	-	34740 (13.18)	-	-
<i>Acidobacteriaceae</i>	1925 (1.02)	1194 (0.45)	1746 (1.13)	1866 (0.71)	-	-
<i>Mycobacteriaceae</i>	-	22840 (8.63)	-	-	13107 (6.15)	-
<i>Streptomyetaceae</i>	-	9220 (3.84)	-	-	-	-
<i>Micrococcaceae</i>	14337 (7.62)	9034 (3.41)	1135 (0.74)	-	-	-
<i>Planctomycetaceae</i>	2130 (1.13)	-	534 (0.35)	2285 (0.87)	690 (0.32)	-
<i>Methylobacteriaceae</i>	-	-	452 (0.29)	-	-	-
<i>Burkholderiaceae</i>	-	2226 (0.84)	-	3145 (1.19)	967 (0.45)	-
<i>Oxalobacteraceae</i>	-	1430 (0.54)	-	-	-	-
<i>Sphingomonadaceae</i>	-	-	-	9353 (3.55)	-	-
<i>Enterobacteriaceae</i>	-	-	-	3801 (1.44)	-	3562 (3.63)

<i>Rubrobacteraceae</i>	-	-	404 (0.26)	-	-	
<i>Podoviridae</i>	-	-	384 (0.25)	-	-	
<i>Xanthomonadaceae</i>	1102 (0.59)	-	237 (0.15)	-	-	
<i>Actinosynnemataceae</i>	-	-	224 (0.15)	-	-	
<i>Rhizobiaceae</i>	1806 (0.96)	-	-	-	-	
<i>Thermomonosporaceae</i>	-	-	-	920 (0.35)	-	
<i>Pseudonocardiaceae</i>	-	-	-	691 (0.26)	-	
<i>Spathidiidae</i>	1794 (0.95)	-	-	-	-	
<i>Hyphomicrobiaceae</i>	3680 (1.96)	-	-	-	-	
<i>Paenibacillaceae</i>	-	8540 (3.23)	-	-	-	
<i>Bradyrhizobiaceae</i>	-	7446 (2.81)	-	1461 (0.55)	14576 (6.84)	
<i>Williamsiaceae</i>	-	-	-	-	13147 (6.17)	
<i>Pseudomonadaceae</i>	-	-	-	-	5519 (2.59)	
<i>Cellulomonadaceae</i>	-	-	-	-	1797 (0.84)	
<i>Gordoniaceae</i>	-	-	-	-	1305 (0.61)	
<i>Clostridiaceae</i>	-	-	-	-	-	12565 (12.82)
<i>Bacillaceae</i>	-	-	-	-	-	1723 (1.76)
<i>Moraxellaceae</i>	-	-	-	-	-	543 (0.55)
<i>Propionibacteriaceae</i>	-	-	-	-	-	170 (0.17)
<i>Streptococcaceae</i>	-	-	-	-	-	142 (0.14)
<i>Corynebacteriaceae</i>	-	-	-	-	-	106 (0.11)
<i>Listeriaceae</i>	-	-	-	-	-	54 (0.06)
<i>Neisseriaceae</i>	-	-	-	-	-	41 (0.04)
Not assigned	4669 (2.48)	-	-	-	-	-
Unknown	152953(81.34)	194019(73.29)	141094(91.55)	200670(76.15)	154532(72.55)	78751(80.32)

Table 2a. Summary of sequences prior to filtering and clustering into amplicon sequence variants (ASVs)

sample ID	forward sequence count	reverse sequence count
TM-CW25	271549	271549
TM-CW26	271436	271436
TM-CW27	217816	217816
TM-CW23	192276	192276
TM-CW24	157621	157621
TM-CW28	118664	118664
Total	1229362	1229362

Table 2b. Total ASVs and Frequency per-sample

Sample ID	Frequency of ASVs	Number of ASVs
TM-CW25	5883	136
TM-CW23	4483	102
TM-CW26	4477	111
TM-CW28	4177	61
TM-CW27	3884	88
TM-CW24	3845	81
Total ASVs		536

Key:

CW25 – Kaa River; CW23 – Andoni River; CW26 – Okrika; CW28 – Bonny River; CW27 – Abua River; CW24 – Ogu/Bolo River; ASV – Amplicon Sequence Variant

Sequence summary

A total number of both forward and reverse sequence counts was obtained before filtering and clustering into amplicon sequence for each of the water samples with sample CW 25 which has a total number of 271549 sequences each exhibiting the highest number of counts and CW 28 with sequence count of 118664 being the lowest count. After clustering the sequences into different categories based on phylogenetic relationships, the number of amplicon sequence variants (ASV) was obtained for each sample. This ASV is 100% similar for each cluster. The highest number was recorded in sample CW 25 and the lowest was seen in sample CW 28. More so, the frequency of ASV was measured for each sample

and sample CW 24 showed the lowest frequency of 3845 ASVs. Rarefaction of the sequences was carried out at the depth of 3845 prior to diversity analysis to remove all bias in the total number of species per sample.

- 34.53% of the ASVs were present in all three regions
- 15.83% were unique to Rivers West
- 19.42% were unique to Rivers South-east
- 13.67% were unique to Rivers East
- 7.19% of the ASVs were present in both Rivers West and Rivers South-east
- 6.47% of ASVs were present in both Rivers South-east and Rivers East
- Only 2.88% of the ASVs were shared between Rivers West and Rivers East

4. Alpha Diversity Analysis

Alpha diversity of bacterial species across the three regions investigated showed no significant difference

while that of individual samples was significantly different at $P \leq 0.05$ using observed features, Shannon diversity index, Faith's phylogenetic diversity and Pielou's evenness tools.

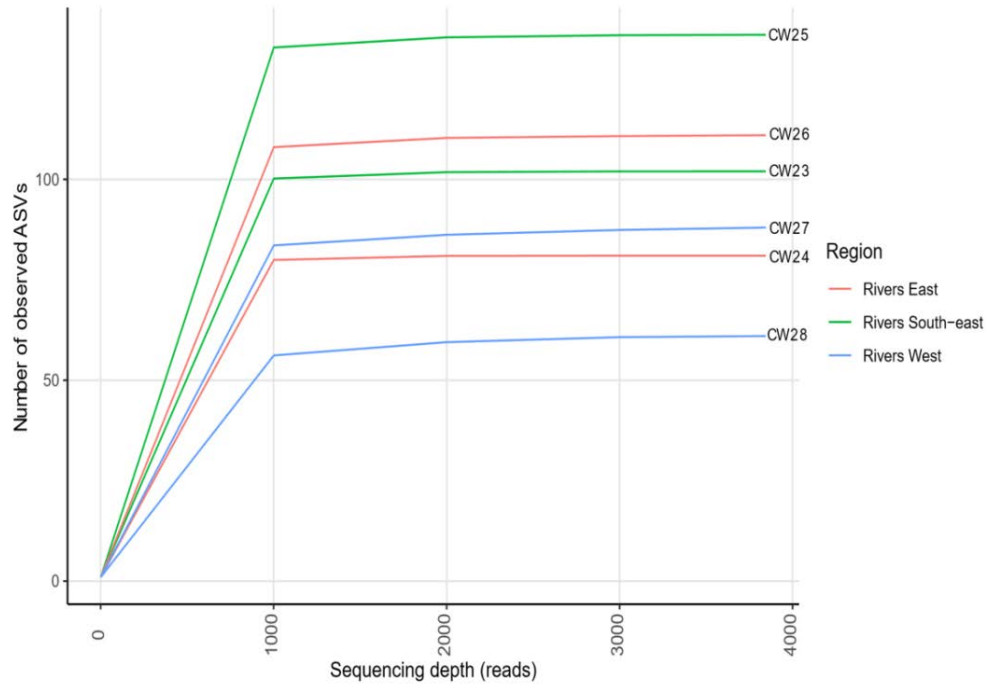


Figure 1. Rarefaction of sequences at a depth of 3845 prior to diversity analysis

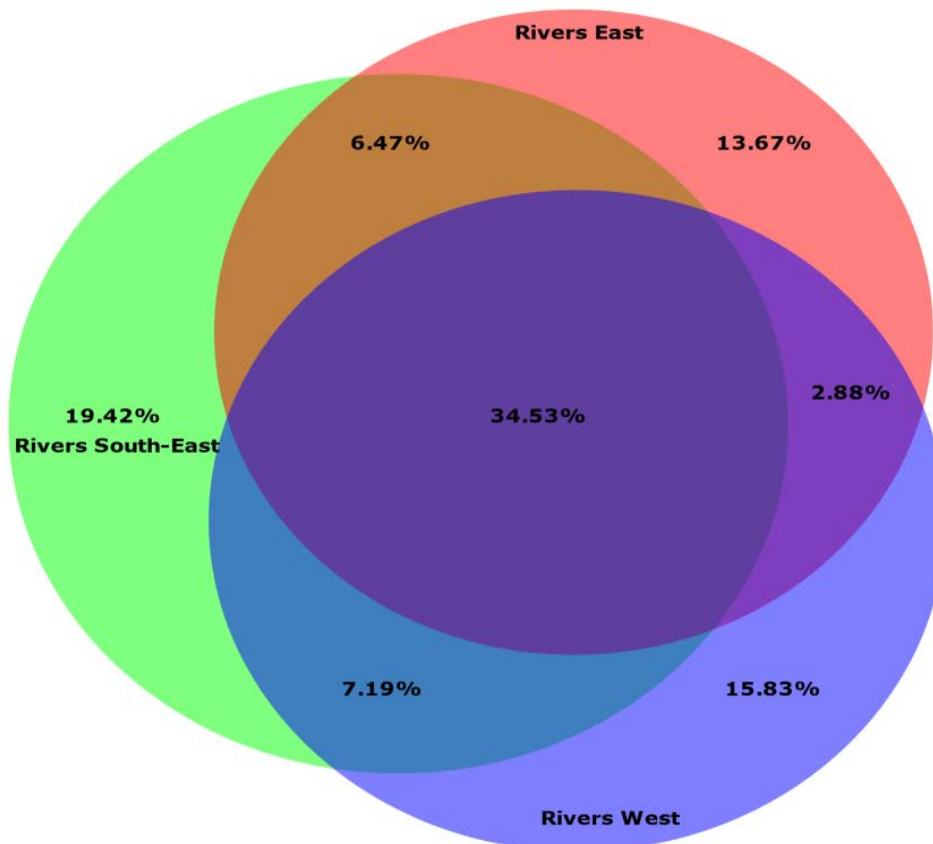
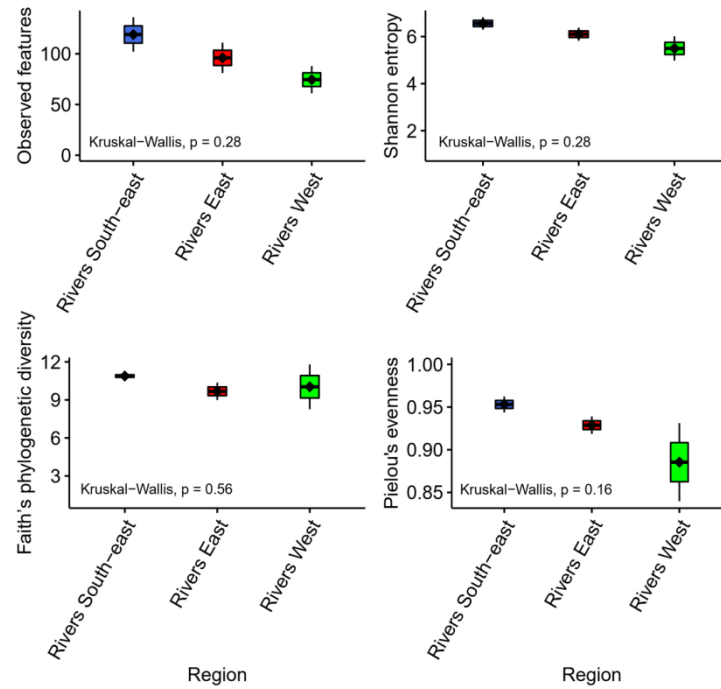


Figure 2. Venn diagram showing the percentage of shared and unique ASVs across the different geopolitical regions



*No significant differences ($p \leq 0.05$) observed in alpha diversity across the three regions

Figure 3. Alpha diversity of bacterial species across the three regions investigated

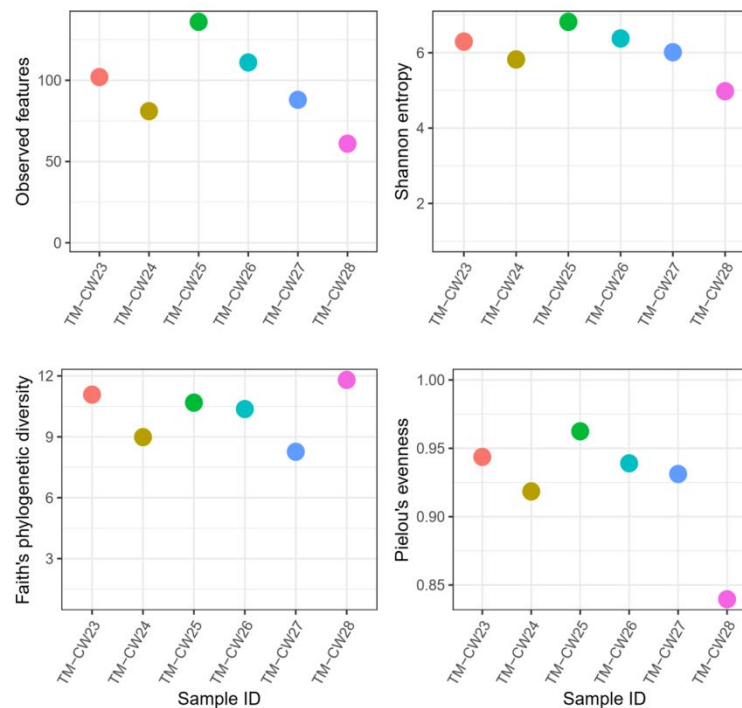


Figure 4. Alpha diversity of individual samples

Table 3. Correlation Analysis between Alpha Diversity Metrics and Physicochemical Parameters

Parameters	Temperature		pH		DO		EC		TDS		Redox	
	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value	R	p-value
Observed	0.0857	0.8717	0.6571	0.1562	-0.0580	0.9131	0.7143	0.1108	0.5429	0.2657	0.2571	0.6228
Faith	-0.4286	0.3965	0.2000	0.7040	-0.6088	0.1997	0.0857	0.8717	-0.771	0.0724	0.3143	0.5441
Evenness	0.6228	0.6228	0.8286	0.0416*	0.0870	0.8699	0.6000	0.2080	0.4286	0.3965	0.5429	0.2657
Shannon	0.0857	0.8717	0.6571	0.1562	-0.0580	0.9131	0.7143	0.1108	0.5429	0.2657	0.2571	0.6228

Alpha FDR-adjusted: $\leq 0.05^*$ (Significance value)

The table above showed that pH affected the proportional distribution of species in the coastal waters.

5. Beta-Diversity Analysis

Bray-Curtis dissimilarity between the bacterial communities structures in the three zones investigated did not reveal distinct differentiation in multivariate space (Figure 5). The observed clustering pattern of samples within the PCoA (Principal coordinate analysis) plot was found to be non-significantly (FDR-adjusted $p > 0.05$) different (Table 4a and 4b) among the zones in Rivers State (PERMANOVA $R^2 = 43.3\%$, $p = 0.07$).

The table above showed that the water samples were impacted by the same factor, the non-significant difference on bacterial communities across the regions.

The redundancy analysis (RDA) model for the association between bacterial species and chemical properties (environmental cues) in all three zones of Rivers state was not significant ($p > 0.05$) (Table 4c). Permutational test of the significance of the environmental parameters in the RDA model revealed that only Redox significantly influenced the assembly of bacterial communities (Table 4c). Notably, most individual bacterial phylotypes (genus level) were largely uninfluenced by the measured chemical parameters.

The ASVs detected in all zones of Rivers state spanned 15 phyla (Rivers East = 13; Rivers South-east = 13; Rivers West = 10). Presented in Figure 4.8 are the phyla with at least 1% relative abundance in any of the zones. *Actinobacteriota* was the most relatively abundant phyla in both Rivers East (43.9%) and Rivers South-east (35.5%)

while *Firmicutes* (36.2%) was the most abundant phyla in Rivers West. Other relatively abundant ($>5\%$) phyla in at least one of the zones include *Proteobacteria*, *Chloroflexi*, *Planctomycetota*, and *Acidobacteriota*. Notably, *Chloroflexi*, *Acidobacteriota*, *Patescibacteria*, and *Gemmatimonadota* were not detected in Rivers West unlike in Rivers East and River South-east where the relative abundance of these phyla was relatively high.

At the genus taxonomic rank, *Noviherbaspirillum*, KD4-96 (*Chloroflexi*), *Nocardioides*, *Mycobacterium*, *Rhodococcus*, *Sphingaurantiacus*, and *Sphingomonas* were the relatively most abundant ($\geq 3\%$) genera in Rivers East. In Rivers South-east, KD4-96 (*Chloroflexi*), *Mycobacterium*, *Sphingomonas*, *Streptomyces*, *Rhodococcus*, *Bacillus*, and *CandidatusUdaeobacter* were the relatively abundant ($\geq 3\%$) genera whereas, in Rivers West, the most abundant ($\geq 3\%$) genera were in these order: *Mycobacterium* > *Escherichia-Shigella* > *Bacillus* > *Pseudoxanthomonas* > *Clostridium_sensu_stricto_7* > *Rhodococcus*.

Differentially (False Discovery Rate adjusted $p < 0.05$) abundant genera, also referred to as biomarkers for the different zones are presented in Figure 7 and has shown that *Acidobacteriota* and *Chloroflexi* were biomarkers in Rivers East and Rivers South-east respectively whereas *Firmicutes* was biomarker phylum for Rivers West. At the genus taxonomic rank *Clostridium*, *Escherichia-Shigella*, and *Pseudoxanthomonas* were biomarkers in Rivers West while KD4-96 (*Chloroflexi*) was differentially abundant in Rivers South-east.

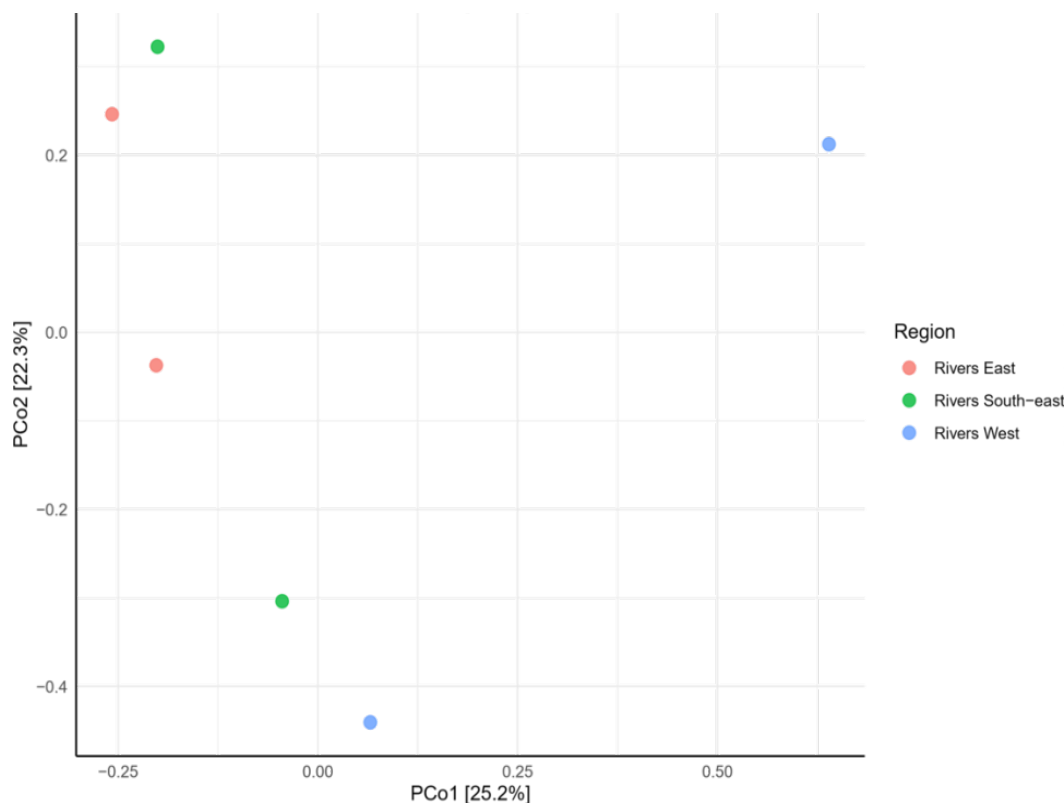


Figure 5. Multivariate differentiation of bacterial communities in the different regions of rivers state based on Bray-Curtis dissimilarities

Relationship between bacterial communities and environmental parameters

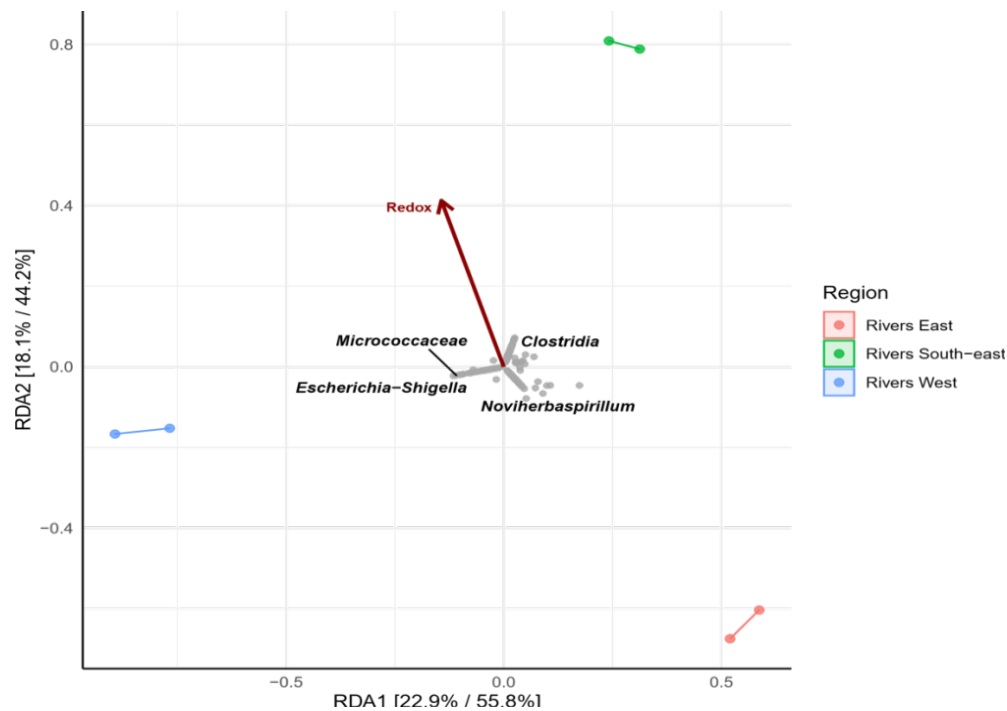


Figure 6. Redundancy analysis (RDA) of bacteria – environment interaction across regions in Rivers State

Table 4a. Permutational multivariate analysis of variance (PERMANOVA) of the bacterial communities based on regions in Rivers State

	Df	Sum of Sqs	R ²	F	Pr(>F)
Region	2	0.96457	0.43286	1.1449	0.06667.
Residual	3	1.26377	0.56714		
Total	5	2.22834	1.00000		

Significancecodes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4b. Pair-wise PERMANOVA on bacterial communities across regions

	Pairs	Df	Sums of Sqs	F.Model	R ²	p-value	p-adjusted
1	Rivers South-east vs Rivers East	1	0.4407894	1.154246	0.3659340	0.3333333	1
2	Rivers South-east vs Rivers West	1	0.5284889	1.227086	0.3802458	0.3333333	1
3	Rivers East vs Rivers West	1	0.4775692	1.058445	0.3460729	0.3333333	1

Significancecodes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4c. RDA ANOVA analysis

	Df	Variance	F	Pr(>F)
Model	2	0.37719	1.0436	0.3333
Residual	3	0.54216		

Significancecodes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4d. Goodness of fit of environmental variables that affects the assembly bacterial communities

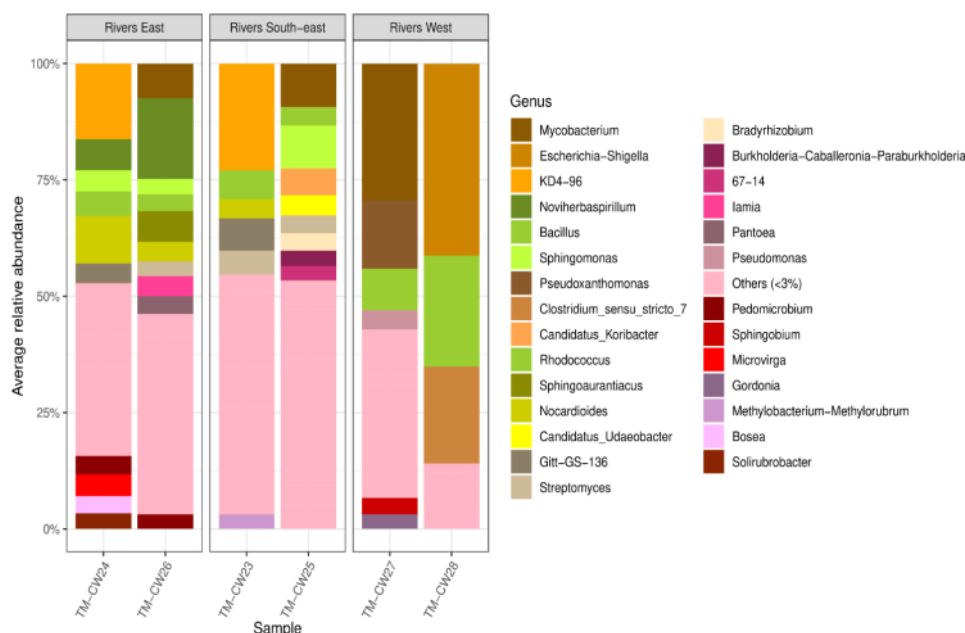
	RDA1	RDA2	r2	Pr(>r)
Temperature	0.45446	0.89077	0.1570	0.72222
pH	0.07023	0.99753	0.5461	0.27500
DO	0.96228	-0.27205	0.2499	0.55833
EC	0.95739	0.28880	0.7259	0.19861
TDS	-0.98635	-0.16468	0.2759	0.98194
Redox	-0.32829	0.94458	0.7640	0.02222 *

Table 4e. Statistical table for differentially abundant phyla

Row	Base Mean	log2 Fold Change	lfcSE	stat	pvalue	P-value FDR-adj
<i>Chloroflexi</i>	159.8289	10.06424	2.344365	4.29295	1.76E-05	2.64E-04
<i>Firmicutes</i>	1347.827	-7.58949	2.328109	-3.25994	0.001114	0.008358
<i>Acidobacteriota</i>	103.5795	7.033172	2.479587	2.836429	0.004562	0.022811

Table 4f. Statistical table for differentially abundant bacterial genera

Row	Base Mean	log2 Fold Change	lfcSE	Stat	pvalue	p-value FDR-adj
<i>Escherichia-Shigella</i>	146.6122	-29.9603	4.785047	-6.26124	3.82E-10	1.03E-08
<i>Pseudoxanthomonas</i>	62.74091	-27.3381	4.785346	-5.71287	1.11E-08	1.50E-07
<i>Clostridium_sensu_stricto_7</i>	73.87816	-26.5143	4.785267	-5.54081	3.01E-08	2.71E-07
<i>KD4-96 (Chloroflexi)</i>	215.6035	11.9274	4.530458	2.632713	0.008471	0.057176

**Figure 7.** Relative abundance of bacteria at the genus taxonomic rank across different zones in Rivers State

6. Discussion

This study has shown that the microbial communities in coastal waters were highly diversified. The abundance of *Actinobacteria* and *Proteobacteria* as the most common phyla in all the coastal water studied is in agreement with the findings of previous works [11,12]. The observations were also made in some coastal water possessing unique phyla such as *Cyanobacteria* and *Fusobacteria* in Bonny, *Gemmatimonadetes* in Andoni, and *Nitrospira* in Kaa.

Actinobacteria have been reported to play a critical role in the bioremediation of water and soil sites that have been polluted with recalcitrant toxic compounds. It also produces secondary metabolites useful in antibiotic production. Despite these useful functions of *Actinobacteria*, they pose a serious Public Health challenge as they cause human diseases such as tuberculosis, allergic pneumonia, paratuberculosis, mycetomas, actinomycosis, and various types of abscesses [13]. The study also agrees with a similar study which reported that *Actinobacteria* constitute the largest bacteria phyla ubiquitously distributed in both aquatic and soil environments [14]. The highest percentage abundance of *Actinobacteria* was detected in the Abua River (26.85%). This value is very high compared with that recorded in Bonny which is in the same senatorial district. The difference could be explained by the difference in the Global Positioning System between these two communities. The percentage abundance of *Actinobacteria* in Ogu/Bolo and Okrika is similar indicating that the two coastal communities have similar activities and environmental factors taking place.

Proteobacteria have three major classes all detected in all the water samples examined in this study. The *Alphaproteobacteria* which include the *Chlamydia*s and *Rickettsia*; the *Betaproteobacteria* which include the *Neisseria*spp and the *Bordetella pertussis* that causes whooping cough; the *Gammaproteobacteria* which include the *Pseudomonas*spp, the *Pasteurella*spp, the *Haemophilus ducreyi* that causes chancroid, the *Vibrio* species and intestinal *Enterobacteriaceae*. All these pathogenic bacteria pose a very serious threat to Public Health [15].

Fusobacteria causes a wide variety of infections such as Vincent angina, pelvic and postsurgical infection, soft tissue infection, inflammatory bowel disease, Lemierre's syndrome, and abscess of bone, lungs, and brain. They possess adhesion, slime, and fimbriae as virulence factors. *Cyanobacteria* are widely present in all aquatic environments because of their ability to source carbon from different environments, possessing neurotoxins, hepatotoxins, and enterotoxins. When contracted through contaminated water cause gastroenteritis. *Cyanobacteria* also deplete dissolved oxygen when in abundance [16]. This explained why this study reported a low value of dissolved oxygen in Bonny which has the highest abundance of *Cyanobacteria*.

According to molecular data, *Nitrospira* are the most diverse nitrifiers that are widely distributed in biological wastewater, and natural ecosystems and yet, are barely studied and uncultured nitrite-oxidizing bacteria [17]. *Nitrospira* are distributed worldwide in oceans, freshwater habitats, hot spring, and, wastewater treatment plants and is notoriously recalcitrant to isolation via cultural methods [18]. In all the coastal water examined, there was a higher percentage abundance of unknown and unassigned

microbial communities. This suggested that the investigated coastal waters are potentially rich hubs for establishing novel microbial strains, and the findings are in tandem with a similar study done in the mangroves region [19].

In this study, the diversity of microbial communities in the coastal waters located in three senatorial districts in Rivers State was also determined using IlluminaMiSeq sequencing technique. The amplicon sequence variants, the sampling depth, location, and some physicochemical parameters used in shaping microbial communities were also determined. The amplicon sequence variants (ASVs) in this study were 100% similar for each sample, which agrees with Muwawaet *al.* [19]. The number of ASVs detected in each sample ranged from 61-136. This is far less than the findings of Houet *al.* (2017) which recorded ASVs range of 659-1835. The difference could be due to different methods used and differences in sampling locations. It could also indicate that the coastal waters studied were disturbed more than that of Hou and co-researchers [20]. This study also showed that environmental factors such as redox and pH strongly influence the microbial community structure and distribution of functional groups by shaping the metabolic niches in coastal waters. A similar study recorded temperature as the most environmental contributor to the shaping of microbial community which is in contrast to the findings of this work [21].

7. Conclusion

This study used the metagenomics technique to determine the presence of culturable and non-culturable bacteria in surface waters in the coastal area of Rivers State. The microbial communities present in coastal waters are highly diversified. The amplicon sequence variants were obtained following the removal of erroneous sequences generated during PCR amplification and sequencing. The amplicon units obtained in this study shared 100% DNA sequence which was used to classify groups of species, and determine biological and environmental variation as well as ecological patterns.

A lot of unknown and unassigned microorganisms were detected from all the coastal water examined, showing that these waters are hubs for highly potential microorganisms that could be inimical to human health. The relative abundance of *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Verracomicrobia*, and *Firmicutes* in this study is also a critical pointer showing that the environment is highly polluted with both environmental wastes and human wastes. This study also revealed that the metagenomics method and generated sequences detected the presence of bacteria that are difficult to culture and the existence of highly abundant unknown and unassigned bacteria in water, proving that metagenomics analysis is the better option for detection and identification of microorganisms in a given sample as microbes other than bacteria were also detected.

The microbial diversity also differs from one geographical location to another. This study showed that *Acidobacteriota* and *Chloroflexi* were biomarkers of microbial contamination in Rivers East and Rivers South-east whereas *Firmicutes* was a biomarker phylum for

Rivers West. At the genus taxonomic rank, *Clostridium*, *Escherichia-shigella*, and *Pseudoxanthomonas* were biomarkers of contamination in Rivers West; KD4-96 (*Chloroflexi*) was differentially abundant in Rivers South-east while *Nocardiodes*, *Mycobacterium*, *Rhodococcus*, *Sphingourantiacus* and *Sphingomonas* were relatively abundant in Rivers East. It was easy to compare the bacteria structure and diversity in each coastal community and to group them by the use of metagenomic tools.

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