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Bacterial Diversity of Gastrointestinal Tract from Individuals in Hydrocarbon Impacted Ogoni Communities in Rivers States, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors designed the study. Author CBG performed the experiment, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CKW, SDA and EGN managed the analyses of the study. Author CBG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The Niger Delta region of Nigeria is popularly known for its huge deposit of crude oil which during explorative activities has led to the pollution of soil and water bodies of nearby coastal regions such as Ogoni.
Aim: This study aimed to assess the bacterial diversity of the GIT of induvial living in hydrocarbon-impacted Ogoni communities.
Methods: Eighty (80) stool samples were collected as case subjects (people living in hydrocarbon-impacted Ogoni communities). Twenty (20) subjects were collected as control samples (people living in Ikenga Ogidi in Idemili North LGAs of Anambra State Nigeria which is a non-hydrocarbon-impacted region). These samples were cultured and analyzed for genomic studies using standard procedures, antimicrobial profiling was done using standard antibiotic disc, the resistant and virulent genes were also assessed using the thermal cycler PCR techniques.
Results: The phylogenic analyses of the 16S rRNA for case and control subjects review the isolates within the *Escherichia coli, Klebsiella pneumoniae, Providencia, Morganella, Proteus, and*

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Lysinibacibacillus mecroides, Commamonas thiooxydans, Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae, and Enterobacter asburiae respectively. Antibiotic resistance profiling reviewed resistance to the following antibiotic AZN (43%), CIP (11%), EPT (20%), CT (47%), IPM (11%), CN (40%), NOR (11%), CTX (94%), and AZN (0%), CIP (40%), EPT (20%), CT (80%), IPM10%, CN20%, NOR (70%,) CTX (70%) for case and control subjects respectively. The prevalence of resistance genes were CTX (56%), SHV (28%), TEM (28%) for case subjects. The virulence genes, Stx1 had (16%), Adhesin (40%), Stx (10%) and Adhesin (20%) for case and control respectively. No resistance genes were found in the control while more virulence genes were found in the case than the control subjects.

Conclusion: These finding revealed that individual living in hydrocarbon impacted Ogoni communities harbor resistance genes that could pose great risk to their health.

Keywords: Genomic studies; bacterial; hydrocarbon impacted Ogoni communities.

1. INTRODUCTION

Crude oil exploration in the Ogoni which commenced in 1958 has led to the pollution of the coastal areas of Ogoni in communities. This oil pollution has affected both arable land and water bodies [1]. Epidemiological studies of the population living in the vicinity of hydrocarbon pollution had reviewed a close link between some non-communicable diseases and exposure to hydrocarbon [2]. Researchers have reviewed that exposure the chemical substance can affect the interaction with man and both microbiota of the GIT. Other research opined those environmental pollutants increase the virulence of some bacteria, therefore, making them more resistant to antimicrobial agents which possess serious health concerns. Exposure a chemical substance such as heavy metals like zinc and lead causes multidrug- resistance bacteria, this chemical can also affect pathogens found in the GIT and alter their composition thereby making them dangerous [3]. The health implication of hydrocarbon pollution is devastating to human health because of its carcinogenic and recalcitrant properties [4]. Interestingly, Microbial communities play an essential role in maintaining and preserving human life, and the human body houses the vast majority of these microbes. They have inherited features resulting from evolution and the development of the normal physiology of the body.

The gastrointestinal tract of human housed a diverse and dynamic microbial community which directly have a tremendous impact on health. This complex ecosystem harbors numerous organisms such as archaea, fungi, viruses, and bacteria, it is dominated by bacteria [5]. The gut bacterial are more than 200 different species abundances in the gut. The bacterial microbiota plays an essential role in the host which includes, competition with the pathogenic organisms for ecological dominance [6]. Immune system modulation, Substrate secretion [7], and it is also used as a nutrient by microbes, therefore it is the preferred site for colonization [8]. The GIT microflora can be influenced to some great extent by environmental factors, and dietary intake. Bacteria are known to occupy the majority of the gastrointestinal tract. However, due to the presence of hydrochloric acid, the stomach and small intestine have fewer bacteria [9]. Numerous bacteria, including *Lactobacillus sp., Escherichia coli, Klebsiella sp., and Proteus sp.,* live in the large intestine and aid in the production and 4r breakdown of food [10,11].

The intestinal microbiota is a living community of microorganisms that fluctuates along the gastrointestinal tract (GIT) and between the mucosa and the intestinal lumen. The microbiota evolves all the time as a result of the interplay of genetic variables, contact with the environment, nutrition, and disease, which explains why each individual has a distinct microbiota [9]. The molecular genomes of these microbes are essential to understand the pathogenesis, diagnosis, prevention and management of diseases. The amplicon sequence targeting the 16s ribosomal RNA (rRNA) genes enables the taxonomic characterization of bacteria. There is evidence that many individuals harbor multiple strains of bacterial species in the GIT microbiota, which is of public health conscience. Therefore, there is urgent need to improve accuracy and precision of cultural analysis in other to validate and develop microbiome-based therapy [12,13]. This research seeks to assess if individual living in some hydrocarbon impacted Ogoni communities harbor bacteria that could be a potential pathogen.

2. MATERIALS AND METHODS

2.1 Study Area

Eighty (80) stool samples were collected from case subjects from hydrocarbon impacted Ogoni communities while Twenty (20) stool samples were collected from control subjects from Anambra States Nigeria (non-hydrocarbon impacted Ogoni communities). The study was performed in four Local Government Areas in Ogoni. Ogoni area covers an area of 1,000 km^2 and is located in the eastern part of Rivers State Nigeria. It is located at a latitude 4° 40′ 5″ N and 4° 43′ 19.5″ N and longitude 7° 22′ 53.7″ E and 7° 27′ 9.8″ E. 11-13. It extends across four Local Government Areas (LGAs) namely Gokana, Khana, Tai and Eleme [1].

2.2 Sample Collection and Processing

Sampling point was geo-referenced using geographical positioning system (GPS). Stool sample was collected in sterile universal container and inoculated into peptone water for four (4) hours at 37°C, samples were then subculture from Selenite F medium unto MacConkey agar (MAC) and Deoxycholate citrate agar (DCA) and incubated at 37°C for 24 hours respectively. Isolates were also sub-cultured onto nutrient agar and incubated at 37°C for 24 hours to obtain a pure culture. Organisms were further inoculated onto alkaline peptone water and then aseptically poured on LB plate; it was further subjected to broad spectrum antibiotic for antibiotic susceptibility testing. Resistance isolate were processed for genomic studies [14].

2.3 DNA Extraction

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) were dispensed in a sterile 1.5 ml microcentrifuge tube and 500 µl of normal saline was added to each of the tube to makeup to 1.5 ml. It was spun at 12000 rpm for 4 minutes. The supernatant was discarded and 1 ml of normal saline was added vortexed, and centrifuge twice. Also, 500 µl of DNA eluting buffer was added in each of the tube and then placed on heated block at 95°C for 20 minutes. The heated bacterial suspension was allowed to cool on ice and spun for 4 minutes at 14000 rpm. Two hundred (200) µl of the supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tube and stored at -20°C for other downstream reactions.

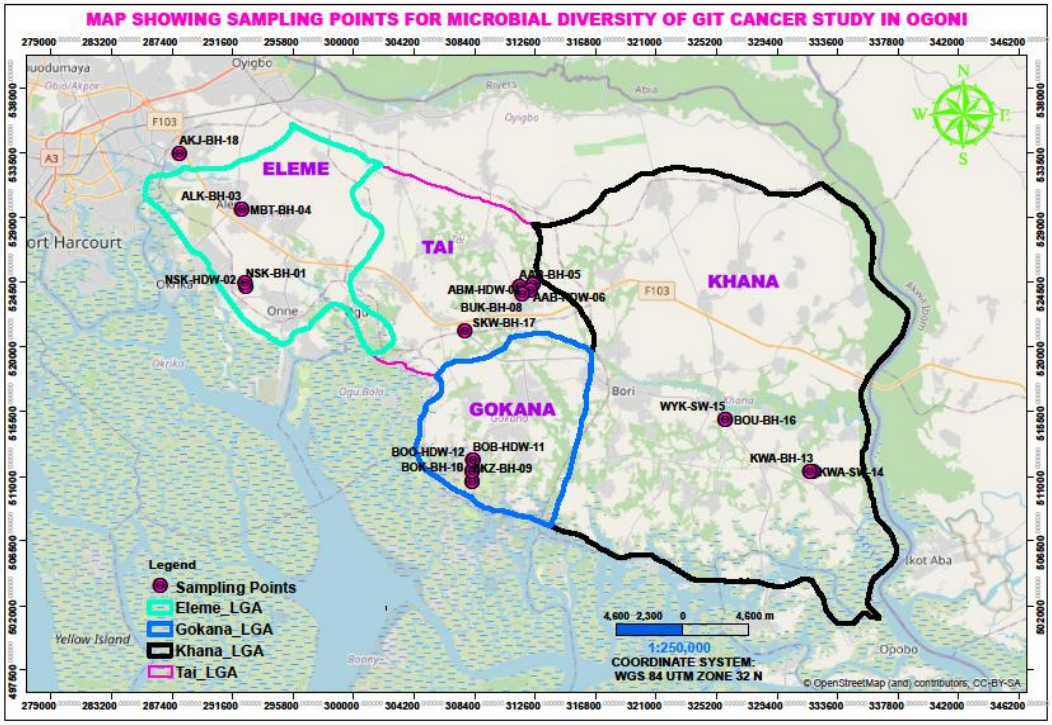

Fig. 1. Map of Ogoni showing sample location

2.4 DNA Quantification

The extracted genomic DNA were quantified using the Nanodrop 1000 spectrophotometer. The upper and lower pedestal were cleaned prior to usage. The equipment was initialized with 2 *µ*l of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration were measured by clicking on the "measure" button.

2.5 16S rRNA Amplification

Amplification of the 16s rRNA region of the rRNA gene were isolated using the 27F:5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'- CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystem thermal cycler at a final volume of 30 microliters for 35 cycles. The PCR mix used was supplied by Inqaba, South Africa it includes x2 Dream taq Master, (taq polymerase, DNTPs, MRgCL), the extracted DNA was used as a template and the primers at a concentration of 0.5 µM. The PCR concentrations were as follows: Initial denaturation, 95ºC for 5 minutes, final denaturation, 95ºC for 35 seconds and annealing temperature at 52ºC for 35 seconds, initial extension, 72ºC for 35 seconds and final extension at 72ºC for 7 minutes.

2.6 Amplification of Resistance Genes

The following resistance SHV genes were amplifiedfrom the bacterial isolates using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers at a final volume of 30 µl, annealing at 52^{o}C for 35 cycles. TEM genes isolated from the bacterial were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGAG-3' primers at final volume of 30 µl, annealing at 55^{o}C for 35 cycles. CTX-M genes from the bacterial isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGTTGGT-3' primers at a final volume of 30 µl, annealing at 52^{o}C for 35 cycles. All three genes were amplified on a ABI 9700 Applied Biosystems thermal cycler.

2.7 Amplification of Virulence Genes

Stx1 genes from the isolates were amplified using the Stx1F: 5´-TTGCGATGCTCTATGAGTGGCTA-3´ and StxR: 5´-CTCGAATGCCTGGCGTGTTT-3´ primers at a final volume of 30 µl, annealing at 58^{o}C for 35 cycles, using ABI 9700 Applied Biosystems thermal cycler. Adhesin (Adh) genes were amplified using AdhF: 5'-CCCGAATTTCAAATGATTGAAAA-3' and AdhrR: 5'-CGCCATCCTCCTGCAAAA-3' primers at a final volume of 30 µl, annealing at 58^{o}C for 35 cycles, using ABI 9700 Applied Biosystems thermal cycler.

2.8 Phylogenetic Analysis

The sequences obtained was edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbourhood-Joining method in MEGA 6.0 [15]. The bootstrap consensus tree inferred from 500 replicates [16] is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method [17].

2.9 Statistical Analyses

Statistical tools were used are mean ± SD, T-test, and Spearman's correlation and ANOVA. Statistical significance was defined as a *p*-valve of less than 0.05 at 95% confidence interval.

3. RESULTS

3.1 Prevalence of Bacteria Isolated from Individual in Hydrocarbon and Non-hydrocarbon Impacted Communities Based on LGAs

Table 1 shows the percentages of resistant bacteria isolated from the different hydrocarbon-impacted Local Government Areas in Ogoni based on antibiotic susceptibility testing. Eleme showed greatest resistance to CTX treatment (80%) and least in CIP and NOR (10%). Tai showed greatest resistance in CTX treatment (70%) and least in IPM (10%). Gokana showed greatest resistance in CTX treatment (80%) and least in NOR (10%). Khana showed greatest resistance (60%) and least in AZM, CIP and NOR (0%). The control showed highest resistance in CT (30%) and least in AZM and CTX (0%).

3.2 Detection of Resistance and Virulence Genes

The amplicons of resistance and virulence genes are represented in Figs. 2-5. While resistance and virulence genes were seen in the hydrocarbon-impacted community, none was found in the control communities. The CTX-M, SHV and TEM amplicons correspond with their sizes which are 500 bp (Figs. 4-9), 300 bp (Figs. 4-9) and 400 bp (Fig. 7) respectively. The Stx genes amplified corresponds to 950 bp while the Adhesin amplicons is 600 bp as seen in Figs. 6 and 7 respectively.

Table 2 shows the comparative analyses of both the resistance and virulence genes between the bacterial isolates of hydrocarbon-impacted and non-hydrocarbon-impacted communities. The resistance genes were significantly higher in all the isolated obtained from the hydrocarbon-impacted communities than the non-hydrocarbon communities; CTX-M (exposed 56%, control 0%, p=<0.0001), SHV and TEM (exposed 28%, control 0%, p=0.0069). Again, the virulence genes also showed that the isolates obtained from the hydrocarbon-impacted communities were all higher than the non-hydrocarbon communities however, not significant; Stx (exposed 16%, control 10%, p=0.7130) and Adhesin (exposed 40%, control 20%, p=0.1641).

3.3 Phylogeny of Bacteria from Hydrocarbon and Non-hydrocarbon-impacted Regions

The 16S rRNA of the isolate showed a percentage similarity to other species at 99-100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Escherichia coli, Klebsiella pneumoniae, Providencia, Morganella, Proteus, Alcaligenes, Enterobacter, Pseudomonas* and *aeromonas* and revealed a closely relatedness to *Escherichia, Klebsiella, Providencia rettgeri, Morganella morganii, Proteus mirabilis, Alcaligenes feacalis, Enterobacter hormaechii, Enterobacter asburiae Pseudomonas sp* and *aeromonas hydrophilia.*

Table 1. Resistant Bacteria Antibiogram from Individuals in Hydrocarbon-Impacted Communities based on LGAs

Community	AZM (%)	CIP(%)	ETP(%)	CT(%)	CTX(%)	IPM(%)	GEN(%)	NOR(%)
Eleme (N=20)	12(60)	2(10)	4(20)	10(50)	16(80)	6(30)	12(60)	2(10)
Tai (N=20)	8(40)	4(20)	4(20)	8(40)	14(70)	2(10)	6(30)	4(20)
Gokana (N=20)	6(30)	2(10)	6(30)	12(60)	16(80)	4(20)	4(20)	2(10)
Khana (N=20)	2(10)	2(10)	2(10)	12(60)	10(50)	2(10)	4(20)	2(10)
Ikenga Ogidi (Control) (N=20)	2(10)	6(30)	4(20)	16(80)	0(0)	2(10)	2(10)	12(60)

Results were analysed in percentages. AZN: Azithromycin, CIP: Ciprofloxacin, ETP: Ertapenem, CT: Colistin, CTX: Cefotaxime, IPM: Imipenem, CN: Gentamycin, NOR: Norfloxacin. Exposed: n=35, Control: n= 10

Table 2. Comparison of the Prevalence of Resistance and Virulence Genes in the Bacterial Isolates

	Resistance Gene		
	CTX-M	**SHV**	**TEM**
Exposed (n=50)	28 (56)	14 (28)	14 (28)
Control (n=20)	0 (0)	0 (0)	0 (0)
p-value	**<0.0001**	**0.0069**	**0.0069**
	Virulence Genes		
	Stx	**Adhesin**	
Exposed (n=50)	8 (16)	20 (40)	
Control (n=20)	2 (10)	4 (20)	
p-value	0.7130	0.1641	

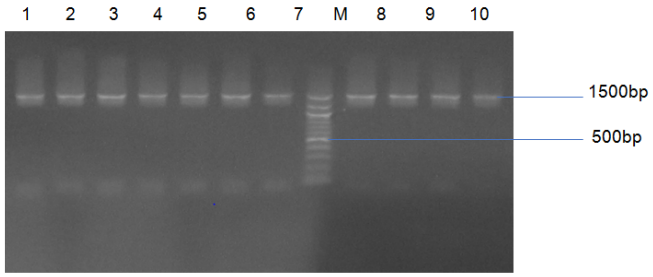

Fig. 2. Amplification of the 16S rRNA selected bacterial isolates
Lanes 1 – 11 represent 16S rRNA gene bands (1500 bp). Lane M represents the 100 bp Molecular ladder

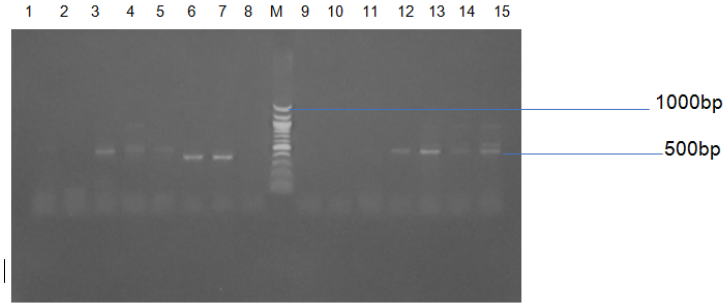

Fig. 3. Agarose gel electrophoresis of CTX-M gene of some selected bacterial isolates
Lanes 3-5 and 12-15 represent the CTX-M gene bands (500 bp). Lane M represents the 100 bp Molecular ladder

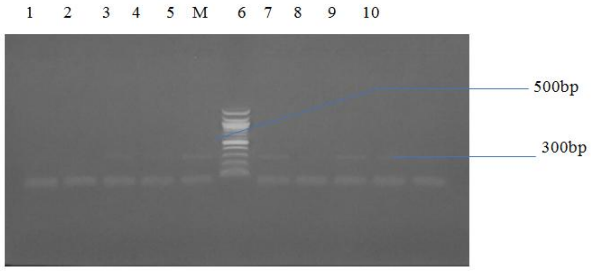

Fig. 4. Agarose Gel Electrophoresis of SHV Gene of Some Selected Bacterial Isolates
Lanes 3 – 6, 8 - 10 represent the amplified SHV gene bands (300 bp). Lane M represents the 100 bp molecular ladder

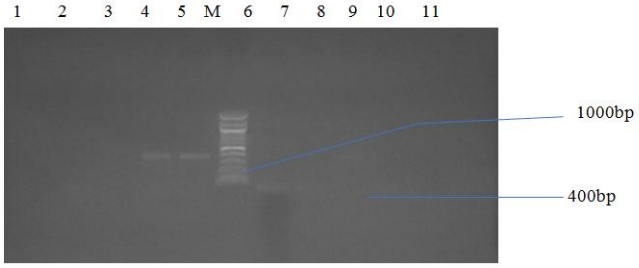

Fig. 5. Agarose Gel Electrophoresis of TEM Gene of Some Selected Bacterial Isolates
Lanes 4, 5 and 8 represent the TEM gene bands (500 bp). Lane M represents the 100 bp Molecular ladder

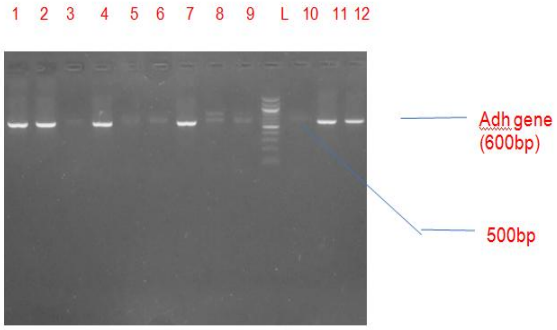

Fig. 6. Agarose gel electrophoresis showing the amplified Adh

Lane 1, 2, 4, 7, 11 and 12 showing the Adh at 600 bp gene while lane L represents the 100 bp molecular ladder

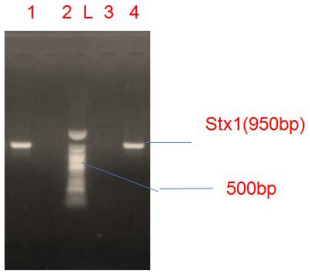

Fig. 7. Agarose gel electrophoresis showing the amplified stx1 gene of the various plants

Lane 1 and 4 represent the stx1 bands at 950 bp while lane L represents the 50 bp molecular ladder

Fig. 8. 16s rRNA phylogeny of bacterial isolates from hydrocarbon impacted region

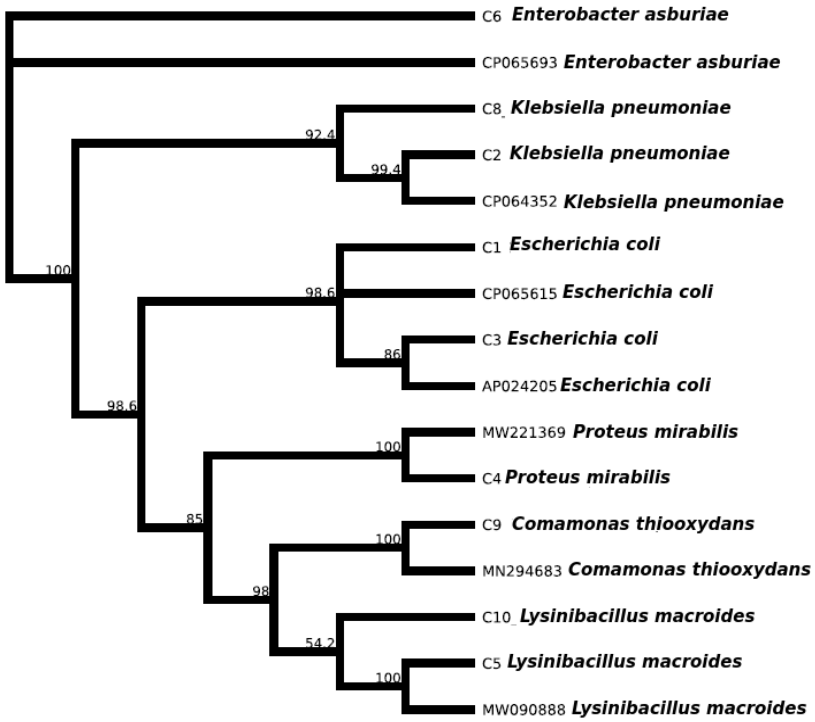

Fig. 9. 16S rRNA phylogeny of bacterial isolates from hydrocarbon impacted region

4. DISCUSSION

In our study, the phenotypic analysis of antimicrobial agents of the gut isolates from hydrocarbon-impacted (exposed population) and non-hydrocarbon impacted (control population) communities were studied [18]. The reported an increase in the tread of antimicrobial resistance in gut microbiota which is of a public health conscience, the reason behind these changes have not been properly discussed, some studies have established the concept that microorganism tended to develop resistance when exposed to antimicrobial agents [19]. Other researchers have confounded spectrum activity and ecological effects; however, considerations were not given to several other factors such as sub-inhibitory concentration, impact on the anaerobic flora [19]. To reduce the impact of antimicrobial agents on gut microbiota, consideration of antimicrobial spectrum of activities and the route of elimination must be integrated into the decision. Various strategies to prevent antimicrobial stewardship action on a residual antibiotic at the colonic level have been currently being developed. In our study, we observed resistance to β-Lactamase producing bacteria, seen in (Table 1) Cefotaxime had the highest resistance trend in Eleme, Tai, and Gokana LGAs 80%, 70%, and, 80% respectively, while Colistin had the highest resistant pattern in Khana LGAs in the exposed population. These results seem to agree with [20] that the β-Lactamase class of antibiotic had different effects on the fecal flora of volunteers. This means that failure to modify the fecal flora from exposed subjects resulted in the breakdown of antibiotics by β-Lactamase producing-bacteria. This seems also to agree with the work done by Monsi *et al.* [21] that observed resistance of prevalence of 64.2% in *K. pneumoniae* isolates from beta-lactamase producing *Enterobacteriaceae.* This is because diseases caused by beta-lactamase-producing *Enterobacteriaceae* are classified as a high-risk infection because of their resistance to antibiotic treatment [22]. The blaTEM-1, blaCTX, and blaSHV usually mediate the resistance to beta-lactamase antibiotic, the isolates were screened and it was confirmed to possess these enzymes from the several conventional identified isolates, this beta-lactamase screening was performed with beta-lactamase antibiotic which includes Azithromycin, ciprofloxacin ertapenem, colistin, Imipenem, Gentamycin and Norfloxacin using the disc diffusion technique [23]. This class of antibiotic had been previously used to determine both antimicrobial sensitivities of bacteria isolate and also in the production of

beta-lactamase in Enterobacteriaceae, in their report only three isolates were resistant to beta-lactamase- producing Enterobacteriaceae. In our study, a higher prevalence of resistance was observed in beta-lactamase- producing enterobacteria, the prevalence is higher than 42.9%. This result shows a possible higher multidrug resistance than non-beta-lactamase producers. Ubede and his colleagues, [4] reported an increase in the trend of antimicrobial resistance in gut microbiota within the last decade, several researchers have confounded spectrum activity and ecological effects, however, considerations were not given to other important factors such as sub-inhibitory concentration, impact on the anaerobic flora [8, 24]: Gut microbiota is vital for the development of intestinal tract and for the maturation of the nervous and immune system. Furthermore, alteration of the gut microbiota composition caused by multiple factors including antibiotic therapy can lead to modification of the gut microbiota [24]. Hence, this study was aimed at determining the Bacterial diversity of gastrointestinal and resistance genes in stool samples from people living in hydrocarbon impacted Ogoni communities.

The first goal of this study was to identify the gut microflora. The result from the conventional analysis and phylogenic placement of 16s rRNA shows most of the organisms isolated were normal gut microbiota. This agrees with [24], where they identified similar isolates from the gut of volunteers. The development of culture-dependent molecular techniques had made it easy to identify unknown species, thereby promoting novel insight into the composition and diversity of fecal microbiota [8]. The breaking of the resistance barrier to colonization is due to the direct or indirect mechanism of antimicrobial agents' various strategies to prevent antimicrobial stewardship action on a residual antibiotic at the colonic level have been currently developed in other studies.

In our study, PCR amplification of 16S rRNA was determined for specific bacteria isolates. The amplification (Fig. 2) of the 16S rRNA genes from bacterial isolates using a primer set that corresponds to a highly conserved region which is highly specific for a specific bacterial isolate. The band of DNA amplified is 1500 bp which corresponds to the gene of the 16S rRNA of a specific bacterium. The isolates are in agreement with the observation made [25]. However, on the contrary [26] and [27] observed that 16S amplification is a good method for detecting bacteria isolates, he also noted with despair that, this technique is not sensitive enough to allow for clear differentiation of bacteria isolated that are closely related.

To confirm the true identity of the organism, the 16s rRNA was sequenced and analyzed using bioinformatics software to generate a phylogenetic tree (Figs. 8-9). The phylogenic tree shows that the 16s rRNA of the isolated shows a percentage similarity to other species at 99 – 100%. The evolutionary distance computed using the Juke-cancer method were in agreement with the phylogenetic placement of the 1bs rRNA of the isolate within *Escherichia coli, Klebsiella, Pneumoniae, Providencia, Morganella, Proteus, Alcaligenes, Enterobacter, Pseudomonas and Aeromonas* and revealed a close relatedness to *Escherieria, Klebsiella, Pneumoniae, Providencia, Morganella, Proteus, Akaligenes, Enterobacteria Pseudomonas*,*and Aeromonas* are revealed a close relatedness to *Escherichia, Klebsiella, Providencia, rettgeri, Morganella morganii, Proteus mirabilis, Alcaligenes faecalis, Enterobacter hormaecchii, Enterobacteria, asburiae, Pseudomonas sp*, and *Aeromonas hydrophilia*, this is similar to the technique used by [18] to different gut bacteria isolate. Gut microbiota play a vital role in removing invading exogenous bacteria and inhibiting the indigenous bacteria according to [25] described the major the role of gut bacteria against an enteric pathogen, the bacteria isolates are generally belonging to the genus enterobacteria which other studies observed an increase in the proportion of potentially harmful proteobacteria. Mostly, the Enterobacteriaceae family can trigger imbalances in the gut microbiota which can lead to the persistence of inflammatory bowel disease (IBD). Recent studies have revealed pathology alteration in the gastrointestinal tract (GI) microbiota, this usually activates the immune response [9].

In addition, it was observed that genus *Escherichia coli* has the highest isolates and this is in agreement with the work done by [9] that observed about 70.5% with *Escherichia* and the most common isolated genes include: TEM, SHV, CTMX-1 (Figs. 3-5). Enterobacteriaceae in the gut of case subjects point toward the need for active screening and preventive management.

The majority of the isolates are *Klebsiella pneumoniae*, the colonization of the GI tract by opportunistic *K. pneumoniae* generally occur due

to the development of nosocomial infection, this is in agreement with the work by [28] that observed nosocomial infection of the GI tract due to *K. pneumoniae* infection. *K. pneumoniae* usually causes multidrug-resistance of extended-spectrum β-lactamase, this is usually becoming problematic in choosing antibiotics for treatment [4]. *Pseudomonas spp* in the GI tract is also of medical importance, most species of *Pseudomonas* are usually resistant to beta-lactam antibiotics, *Pseudomonas aeruginosa* has been recognized as an emerging opportunistic pathogen of clinical significance. This is due to its concerted action of multidrug effluent pumps, with its encoded antibiotics resistance gene. The gut microbiota is composed of several different bacteria, the microbial activities can be affected by environmental stimuli, this could alter and influence the metabolome and human health in the centrally, the bacterial isolated that were specific for control subjects were, *comamonas thiooxydans* and *lysinibacillus macrorides*. *Comamonas* species are mostly common environmental bacteria that occasionally cause human disease [29]. It is reported as being non-fermentative gram-negative bacilli that could be further identified. *Lysinibacillus macrolides* is an aerobe, spire-forming bacterium, it was first isolated from the colon, and however, this bacterium had lost standing in bacteriological nomenclature [30,31].

5. CONCLUSION

The phylogenic analysis showed that beta-lactamase genes share an evolutionary relationship with a virulent strains of bacteria such as *E. coli, Klebsiella pneumonia, Enterobacter asburiae, Proteus mirabilis*, and *Pseudomonas* species, resistance to beta-lactamase genes were observed in case of subjects and non in control subjects. More virulent genes were seen in case subjects more than in control subjects. This is a pointer that there are more resistant genes in the subjects from some hydrocarbon impacted Ogoni communities which could be of public health concern.

ETHICAL APPROVAL AND CONSENT

Prior to the commencement of this study, an ethical approval was obtained from Rivers State Ministry of Health with the number MH/PRS/391/VOL.2/624 on 10th February 2020. Oral informed and written consent were obtained before samples were collected.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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