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Antibiotic Resistance Profiles and Hydrocarbon-Utilisation Potential of Indigenous Bacteria Isolated from Oil-Impacted Surface Waters of the Niger Delta, Nigeria

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Abstract

Oil contamination can alter microbial communities in aquatic environments, potentially favouring the coexistence of hydrocarbon-utilising bacteria and antibiotic-resistant organisms. This study investigated the antibiotic resistance profiles and hydrocarbon-utilisation potential of indigenous bacteria isolated from oil-impacted surface waters at five locations in the Niger Delta, Nigeria. Surface-water samples were analysed for selected physicochemical parameters and total petroleum hydrocarbons (TPH). Bacterial isolates were recovered and characterised using conventional microbiological methods, screened for hydrocarbon utilisation, and tested for susceptibility to eight antibiotics using the disc-diffusion method. The TPH-removal efficiencies of selected isolates were also evaluated, and correlations among TPH concentrations, hydrocarbon-utilising bacteria and multidrug-resistant (MDR) isolates were examined. TPH concentrations ranged from 3.94 ± 0.27 mg/L at Bodo Creek to 8.21 ± 0.52 mg/L at Ataba Creek. Of the 87 bacterial isolates recovered, 38 (43.7%) demonstrated hydrocarbon-utilisation potential. The highest resistance rates were recorded against amoxicillin (81.6%) and ampicillin (76.3%), whereas resistance was lower against ciprofloxacin (13.2%) and gentamicin (18.4%). Among the selected isolates, Pseudomonas aeruginosa achieved the highest TPH removal (70.8%), followed by Rhodococcus sp. (68.6%) and Acinetobacter sp. (65.8%), compared with 5.6% in the uninoculated control. Positive correlations were observed between TPH concentration and hydrocarbon-utilising bacteria (r = 0.81), TPH concentration and MDR isolates (r = 0.74), and hydrocarbon-utilising bacteria and MDR isolates (r = 0.69). These findings highlight the coexistence of hydrocarbon-utilising and antibiotic-resistant bacteria in oil-impacted waters, underscoring their potential value in bioremediation and the need for environmental monitoring to address associated public health concerns.

Keywords: *Antibiotic resistance; hydrocarbon-utilising bacteria; multidrug resistance; Niger Delta; oil-impacted surface water; total petroleum hydrocarbons.*

1.0 Introduction

Petroleum exploration and production remain important components of the Nigerian economy, but the associated activities have generated persistent environmental concerns, particularly within the Niger Delta. The region contains an extensive network of rivers, creeks, estuaries, wetlands and intertidal ecosystems that receive contaminants from oil exploration, transportation, refining, gas flaring, spills and other anthropogenic activities [15,24,31].

Petroleum-derived contaminants in these environments include total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds and other associated pollutants [34,35,39].

The presence of petroleum hydrocarbons in surface waters is of environmental concern because these compounds can alter physicochemical conditions and impose selective pressure on indigenous microbial communities. TPH is commonly used as an aggregate indicator of petroleum contamination because it provides an estimate of the overall hydrocarbon burden in an environmental sample.

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Previous studies have reported measurable TPH concentrations in Niger Delta aquatic environments, including the Cross River Estuary and Ogboinbiri Creek [15,24]. Similarly, hydrocarbon contamination has been documented in the Bonny Estuary and other petroleum-producing environments of Rivers State [31,34].

Microorganisms are important components of the natural attenuation of petroleum-contaminated environments. Certain indigenous bacteria possess metabolic systems capable of utilising hydrocarbons as carbon and energy sources, thereby contributing to the transformation and removal of petroleum compounds. The ability of bacteria to degrade hydrocarbons is associated with specialised enzymes and metabolic pathways, including oxygenase and dioxygenase systems involved in the oxidation of hydrocarbon substrates [11,13]. Studies conducted in the Niger Delta have demonstrated the occurrence of indigenous hydrocarbon-utilising bacteria in crude-oil-impacted environments. Chikere and Ekwuabu [10], for example, characterised hydrocarbon-utilising bacteria from crude-oil-impacted sites in Bodo, Ogoniland, while Chikere and Omadudu [11] demonstrated dioxygenase activity among indigenous bacteria recovered from oil-polluted sites.

The microbial response to petroleum contamination is not restricted to changes in hydrocarbon utilisation. Contaminated environments may also exert selective pressures that influence the distribution of antimicrobial resistance among environmental bacteria. The natural environment is increasingly recognised as an important reservoir for antibiotic-resistant bacteria and antimicrobial resistance genes, with resistance determinants capable of persisting and circulating among environmental microbial populations [27]. Antibiotics and antibiotic resistance genes may enter aquatic environments through wastewater, agricultural activities, pharmaceutical discharges, domestic sewage and other anthropogenic pathways. Environmental matrices containing high microbial densities can consequently provide opportunities for the persistence and exchange of resistance determinants [8,28].

The relationship between petroleum contamination and antimicrobial resistance is particularly relevant in environments affected by multiple anthropogenic pressures. Oil-producing communities may experience simultaneous inputs from petroleum activities, domestic wastewater, industrial discharges and other sources of environmental contamination. In Rivers State, domestic wastewater discharge has been associated with deterioration of water quality and potential public-health concerns in receiving rivers [30]. Similarly, studies of surface and groundwater sources in Port Harcourt and Obio/Akpor have documented antibiotic-resistant bacterial isolates, demonstrating that resistance is already an important environmental concern within the region [14]. Evidence of antibiotic resistance genes in hospital wastewater further indicates that contaminated aquatic systems can serve as reservoirs and dissemination points for resistance determinants [36].

The coexistence of hydrocarbon contamination and antimicrobial resistance raises an important environmental question. Bacteria that persist in petroleum-impacted environments may possess physiological characteristics that enable them to survive under several environmental stresses. Experimental and environmental studies have demonstrated that bacteria recovered from petroleum-contaminated sites can exhibit both hydrocarbon-degrading capacity and antibiotic resistance.

Ataikiru et al. [1], for example, reported hydrocarbon biodegradation and variable antibiotic sensitivity among microorganisms isolated from an oil-polluted site in Kokori, Delta State. This provides an important Nigerian precedent for investigating the simultaneous occurrence of these two functional characteristics.

Petroleum-contaminated environments may also contain microbial communities that change in response to the availability of hydrocarbon substrates. Chikere [1] demonstrated changes in indigenous bacterial communities during crude-oil degradation and nutrient-enhanced bioremediation, while Ibiene [2]reported changes in microbial populations associated with crude-oil-polluted soils in the Niger Delta. Such microbial responses are important because selection for hydrocarbon utilisation may alter the relative abundance of particular bacterial groups. The presence of genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Enterobacter* and related organisms in petroleum-impacted environments has also been reported in previous studies [10,11].

The environmental significance of antimicrobial resistance extends beyond clinical settings. Resistant bacteria released into aquatic systems may interact with other microorganisms and contribute to the environmental circulation of resistance determinants. Horizontal transfer of resistance genes can occur among bacterial populations, providing a mechanism through which resistance traits may be disseminated within microbial communities [21]. Consequently, environmental monitoring of antimicrobial resistance is increasingly considered an important component of a broader One Health approach. The detection of antibiotic-resistant bacteria in drinking-water sources and other aquatic environments further illustrates the potential public-health relevance of environmental reservoirs of resistance [3,17].

The Niger Delta therefore presents a particularly important setting for integrated investigations of petroleum contamination, bacterial ecology, hydrocarbon utilisation and antimicrobial resistance. Existing studies have extensively documented petroleum contamination of surface waters and sediments across the region [24,31,34,38,39], while other investigations have independently demonstrated antimicrobial resistance in aquatic environments [14,17,36]. However, comparatively fewer studies have examined the relationship between hydrocarbon-utilising bacteria and antibiotic resistance phenotypes across different oil-impacted surface-water systems within the Niger Delta. The present study was therefore designed to investigate the antibiotic resistance profiles and hydrocarbon-utilisation potential of indigenous bacteria isolated from oil-impacted surface waters of selected creeks in the Niger Delta, Nigeria.

2.0 Materials and Methods

2.1 Study Area

The study was conducted in five oil-impacted surface-water systems within the Niger Delta region of Nigeria, comprising Nembe Creek and Kolo Creek in Bayelsa State, Bodo Creek and Ataba Creek in Rivers State, and Iko Creek in Akwa Ibom State. The waterways were selected to represent surface-water environments subject to varying degrees of petroleum-related anthropogenic influence. Nembe Creek and Kolo Creek are located within oil-producing areas of Bayelsa State, whereas Bodo Creek and Ataba Creek are situated within petroleum-impacted environments of Rivers State. Iko Creek is located in Akwa Ibom State.

The geographical coordinates of all sampling stations were recorded using a handheld Global Positioning System (GPS) receiver in decimal degrees. The coordinates were subsequently used to prepare the spatial distribution map of the study area.

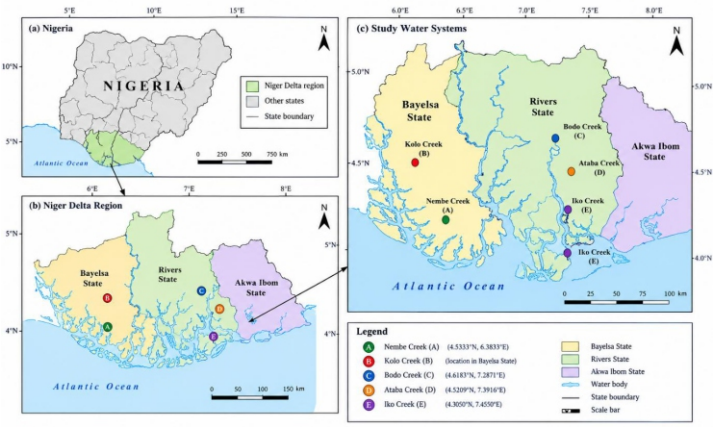


Figure 1. Map of the study area showing Nigeria, the Niger Delta region and the five study water systems

2.2 Study Design and Sampling Strategy

A comparative cross-sectional environmental study design was employed. Five oil-impacted surface-water systems were investigated and designated Sites A–E as follows: Site A, Nembe Creek; Site B, Kolo Creek; Site C, Bodo Creek; Site D, Ataba Creek; and Site E, Iko Creek.

Sampling stations were selected based on their proximity to petroleum-related activities, evidence of hydrocarbon contamination, accessibility and relevant environmental characteristics. The exact number of stations sampled within each waterway and the number of independent samples collected per station should be stated explicitly from the field sampling records.

Where multiple stations were established within a waterway, they were positioned to provide spatial coverage of the upstream, central and downstream sections where the physical configuration of the waterway permitted. Sampling was undertaken during the specified study period under comparable field conditions to minimise temporal variation.

2.3 Collection and Preservation of Surface-Water Samples

Surface-water samples intended for microbiological examination were collected aseptically into sterile, appropriately labelled sample bottles. Separate pre-cleaned amber glass containers were used for hydrocarbon analysis to minimise contamination and potential photochemical alteration of petroleum hydrocarbons.

At each sampling station, relevant field observations, including water colour, visible oil films, odour, surrounding land use and proximity to petroleum-related activities, were documented. Samples for microbiological analysis were transported to the laboratory in insulated ice-packed containers and processed within the appropriate holding period. Samples intended for TPH analysis were preserved and transported according to the requirements of the analytical method employed.

2.4 Determination of Physicochemical Parameters

Water temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO) were determined using calibrated field meters. Calibration of the instruments was performed before measurements were undertaken according to the manufacturer's instructions and appropriate quality-control procedures.

Chemical oxygen demand (COD) was determined using the dichromate oxidation method. Results were expressed in appropriate SI-compatible units.

2.5 Determination of Total Petroleum Hydrocarbons

Total petroleum hydrocarbons (TPH) in the surface-water samples were determined following solvent extraction and chromatographic analysis using gas chromatography coupled with a flame-ionisation detector (GC-FID).

A measured volume of each water sample was transferred into a clean extraction vessel and extracted using the analytical-grade solvent specified in the laboratory analytical protocol. The extraction procedure was repeated, and the extracts were combined, dried over anhydrous sodium sulphate and concentrated to the required final volume prior to chromatographic analysis.

Quantification was performed against appropriate petroleum-hydrocarbon calibration standards. Analytical quality control included laboratory blanks, calibration standards, replicate analyses and calibration-verification samples. TPH concentrations were reported as mg/L.

The final manuscript should provide the specific solvent, extraction volume, extraction ratio, GC-FID model, chromatographic column, carrier gas, temperature programme, calibration range, detection limit, quantification limit and analytical recovery obtained during the actual analysis.

2.6 Isolation and Enumeration of Bacteria

Surface-water samples were serially diluted using sterile physiological saline. Aliquots from appropriate dilution levels were inoculated onto the bacteriological medium used for recovery of heterotrophic bacteria and incubated under the conditions specified in the laboratory protocol.

Following incubation, morphologically distinct colonies were enumerated and expressed as colony-forming units per millilitre (CFU/mL), where applicable. Representative colonies differing in colony size, morphology, pigmentation, elevation, margin and texture were selected and repeatedly subcultured until pure cultures were obtained.

Pure cultures were maintained under appropriate preservation conditions pending hydrocarbon-utilisation screening, biochemical characterisation and antibiotic susceptibility testing.

2.7 Screening for Hydrocarbon-Utilising Bacteria

Purified bacterial isolates were screened for hydrocarbon-utilisation potential using a mineral-salt medium in which petroleum hydrocarbon served as the principal carbon source. Each isolate was inoculated into the hydrocarbon-containing medium and incubated under controlled laboratory conditions.

An uninoculated medium containing the hydrocarbon substrate served as the abiotic control. Bacterial growth was assessed using the predefined criterion adopted for the study, based on visible biomass formation and/or turbidity relative to the uninoculated control. Isolates demonstrating growth under the hydrocarbon-screening conditions were classified as hydrocarbon-utilising candidates.

The exact hydrocarbon substrate, medium composition, inoculum concentration, incubation temperature, incubation duration and criterion for positive utilisation should be reported from the laboratory records to ensure reproducibility.

2.8 Characterisation of Bacterial Isolates

Hydrocarbon-utilising isolates were characterised using colony morphology, Gram staining and conventional biochemical tests. Identification was assigned only to the lowest taxonomic level supported by the analytical evidence.

Where molecular identification was not performed, species-level identification should be interpreted cautiously. Isolates identified solely through conventional microbiological and biochemical procedures should preferably be reported at genus level where species-level confirmation cannot be established.

2.9 Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the standard disc-diffusion technique. Fresh cultures were suspended in sterile physiological saline and adjusted to approximately 0.5 McFarland turbidity standard. The standardised bacterial suspensions were uniformly inoculated onto the susceptibility-testing agar.

The antimicrobial agents examined were amoxicillin, ampicillin, tetracycline, gentamicin, ciprofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole and cefotaxime. Antibiotic discs were aseptically applied to the inoculated plates and incubated under the conditions prescribed by the interpretive guideline adopted for the study.

Following incubation, inhibition-zone diameters were measured in millimetres and interpreted as susceptible, intermediate or resistant according to the applicable Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

The exact guideline edition, antibiotic disc concentrations, agar medium, incubation conditions and quality-control strains used must be stated in the final manuscript.

2.10 Determination of Multidrug Resistance and Multiple Antibiotic Resistance Index

Multidrug resistance (MDR) was defined as non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes. The antimicrobial classes represented by the eight tested agents were identified before calculation to prevent double-counting antibiotics belonging to the same pharmacological class.

The multiple antibiotic resistance (MAR) index was calculated as:

MAR index = a/b

where a is the number of antimicrobial agents to which an isolate demonstrated resistance and b is the total number of antimicrobial agents tested.

Because amoxicillin and ampicillin belong to the same broad β-lactam group, MDR classification was based on antimicrobial classes rather than simply the number of resistant discs.

2.11 Quantitative Assessment of Hydrocarbon-Utilisation Potential

Selected hydrocarbon-utilising isolates were subjected to quantitative laboratory evaluation using a defined initial TPH concentration. An uninoculated medium containing the same initial TPH concentration served as the abiotic control.

Following the predetermined incubation period, the residual TPH concentration was determined using the same analytical procedure applied to the initial substrate concentration.

Percentage TPH removal was calculated as:

$$\text{TPH removal (\%)} = [(C_0 - C_t) / C_0] \times 100$$

where C_0 represents the initial TPH concentration, and C_t represents the residual TPH concentration following incubation.

The percentage reduction observed in the uninoculated control was considered when interpreting biological removal because losses may arise from abiotic processes such as volatilisation or adsorption.

The final manuscript should report the number of biological replicates, incubation period, inoculum density, incubation temperature, medium composition and statistical procedure used to compare isolates.

2.12 Quality Assurance and Quality Control

Quality assurance and quality-control procedures were incorporated throughout field sampling, microbiological analysis and hydrocarbon determination. Sampling containers were appropriately cleaned or sterilised according to their intended application, while analytical instruments were calibrated before use.

Laboratory blanks, media sterility controls, analytical replicates and calibration-verification standards were incorporated where applicable. Hydrocarbon analyses were conducted using appropriate analytical standards and quality-control samples.

All samples and bacterial isolates were assigned unique identification codes to ensure traceability from field collection through laboratory processing and analysis.

2.13 Statistical Analysis

Data were subjected to descriptive and inferential statistical analyses using appropriate statistical software. Continuous environmental variables were summarised using mean and standard deviation where replicate observations were available.

Prior to inferential analysis, the assumptions of normality and homogeneity of variance were assessed. Where the assumptions were satisfied, one-way analysis of variance (ANOVA) was used to evaluate differences among sampling locations, followed by an appropriate post-hoc multiple-comparison procedure. Where assumptions were violated, an appropriate non-parametric alternative was considered.

Antibiotic-resistance frequencies were expressed as numbers and percentages. The MAR index was calculated for individual isolates, while MDR was determined according to antimicrobial-class criteria.

Associations between TPH concentration, abundance of hydrocarbon-utilising bacteria and resistance characteristics were evaluated using an appropriate correlation procedure based on the actual number and structure of independent observations. Pearson correlation was used only where the assumptions of linearity, normality and independence were satisfied; otherwise, Spearman rank correlation was considered. Statistical significance was set at $p < 0.05$.

3.0 Results

3.1 Physicochemical Characteristics and Total Petroleum Hydrocarbon Concentrations of the Surface Waters

The physicochemical characteristics and total petroleum hydrocarbon (TPH) concentrations of the surface-water samples from the five investigated locations are presented in Table 1. The measured water temperature ranged from 28.6 ± 0.4 °C at Site A to 29.4 ± 0.4 °C at Site D.

The pH values ranged from 6.18 ± 0.10 at Site B to 6.61 ± 0.13 at Site E, indicating slightly acidic to near-neutral conditions across the investigated surface-water systems.

Electrical conductivity (EC) varied from 592 ± 26 µS/cm at Site C to 913 ± 41 µS/cm at Site D. Dissolved oxygen (DO) concentrations ranged from 3.28 ± 0.17 mg/L at Site D to 4.51 ± 0.24 mg/L at Site C. Chemical oxygen demand (COD) ranged from 38.7 ± 2.4 mg/L at Site C to 64.2 ± 3.7 mg/L at Site D.

The TPH concentration showed considerable spatial variation among the sampling sites. The highest TPH concentration was recorded at Site D (8.21 ± 0.52 mg/L), followed by Site B (7.36 ± 0.48 mg/L), while Site C recorded the lowest concentration (3.94 ± 0.27 mg/L). Thus, Site D had approximately twice the TPH concentration observed at Site C.

The relatively high TPH and COD concentrations recorded at Site D indicate a greater petroleum-hydrocarbon and organic pollution burden at this location compared with the other investigated sites.

Table 1: Physicochemical characteristics and total petroleum hydrocarbon concentrations of the investigated surface waters

Parameter	Site A	Site B	Site C	Site D	Site E
Temperature (°C)	28.6 ± 0.4	29.1 ± 0.3	28.8 ± 0.5	29.4 ± 0.4	28.9 ± 0.3
pH	6.42 ± 0.12	6.18 ± 0.10	6.55 ± 0.14	6.27 ± 0.11	6.61 ± 0.13
EC (µS/cm)	684 ± 28	821 ± 34	592 ± 26	913 ± 41	647 ± 29
DO (mg/L)	4.18 ± 0.21	3.62 ± 0.18	4.51 ± 0.24	3.28 ± 0.17	4.06 ± 0.20
TPH (mg/L)	4.82 ± 0.31	7.36 ± 0.48	3.94 ± 0.27	8.21 ± 0.52	5.17 ± 0.35
COD (mg/L)	42.6 ± 2.8	58.4 ± 3.1	38.7 ± 2.4	64.2 ± 3.7	46.9 ± 2.6

3.2 Distribution and Enumeration of Bacterial Isolates

A total of 87 bacterial isolates were recovered from the five investigated surface-water systems. Of these, 38 isolates demonstrated growth under the hydrocarbon-utilisation screening conditions, representing 43.7% of the total isolates recovered.

Site D recorded the highest number of bacterial isolates, with 23 isolates, of which 11 demonstrated hydrocarbon-utilisation potential. Site B yielded 21 bacterial isolates, including 10 hydrocarbon-utilising isolates, whereas Site A yielded 17 isolates, including eight hydrocarbon-utilising isolates. Sites C and E each yielded 13 isolates, of which five and four, respectively, demonstrated hydrocarbon-utilisation potential.

The proportion of hydrocarbon-utilising isolates ranged from 30.8% at Site E to 47.8% at Site D. Sites A, B and D showed relatively comparable proportions of hydrocarbon-utilising isolates, whereas Site E recorded the lowest proportion.

Table 2: Distribution of recovered bacterial isolates and hydrocarbon-utilising isolates across the sampling sites

Sampling site	Total bacterial isolates (n)	Hydrocarbon-utilising isolates (n)	Hydrocarbon-utilising isolates (%)
Site A	17	8	47.1
Site B	21	10	47.6
Site C	13	5	38.5
Site D	23	11	47.8
Site E	13	4	30.8
Total	**87**	**38**	**43.7**

3.3 Identification and Qualitative Hydrocarbon-Utilisation Characteristics of Bacterial Isolates

Ten representative bacterial groups were identified based on cultural, morphological and biochemical characteristics. The identified groups comprised *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Acinetobacter* sp., *Bacillus cereus*, *Staphylococcus* sp., *Enterobacter* sp., *Micrococcus* sp., *Alcaligenes* sp., *Rhodococcus* sp. and *Klebsiella* sp.

Pseudomonas aeruginosa had the highest frequency among the representative isolates, with six isolates, followed by *Bacillus subtilis* with five isolates. *Acinetobacter* sp., *Bacillus cereus* and *Enterobacter* sp. each had four isolates. *Staphylococcus* sp., *Micrococcus* sp., *Alcaligenes* sp., *Rhodococcus* sp. and *Klebsiella* sp. each occurred at frequencies of three isolates.

Strong hydrocarbon-utilisation growth was observed among *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Alcaligenes* sp. and *Rhodococcus* sp. Moderate growth was observed for *Bacillus subtilis*, *Bacillus cereus*, *Enterobacter* sp. and *Klebsiella* sp., while *Staphylococcus* sp. and *Micrococcus* sp. demonstrated comparatively weak growth.

Table 3: Bacterial isolates and qualitative hydrocarbon-utilisation characteristics

Isolate code	Bacterial identification	Frequency (n)	Hydrocarbon-utilisation growth
A1	*Pseudomonas aeruginosa*	6	Strong
A2	*Bacillus subtilis*	5	Moderate
A3	*Acinetobacter* sp.	4	Strong
A4	*Bacillus cereus*	4	Moderate
A5	*Staphylococcus* sp.	3	Weak
A6	*Enterobacter* sp.	4	Moderate
A7	*Micrococcus* sp.	3	Weak
A8	*Alcaligenes* sp.	3	Strong
A9	*Rhodococcus* sp.	3	Strong
A10	*Klebsiella* sp.	3	Moderate
Total		**38**	

3.4 Antibiotic Susceptibility Profiles of Hydrocarbon-Utilising Bacteria

The antibiotic susceptibility profiles of the representative hydrocarbon-utilising isolates are presented in Table 4. Considerable variation in antimicrobial susceptibility was observed among the isolates.

Resistance to amoxicillin and ampicillin was common across all ten representative isolates. Resistance to tetracycline was observed particularly among *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Staphylococcus* sp., *Enterobacter* sp., *Alcaligenes* sp. and *Klebsiella* sp.

Ciprofloxacin demonstrated the lowest apparent resistance among the representative isolates, with all ten isolates classified as susceptible. Gentamicin also showed comparatively favourable activity, although intermediate susceptibility was observed in some Gram-negative isolates.

Multiple resistance phenotypes were particularly evident among *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp., *Alcaligenes* sp. and *Klebsiella* sp.

Table 4: Antibiotic susceptibility profiles of representative hydrocarbon-utilising bacterial isolates

Bacterial isolate	AMX	AMP	TET	GEN	CIP	CHL	SXT	CTX
P. aeruginosa	R	R	R	S	S	R	R	R
B. subtilis	R	R	S	S	S	S	S	S
Acinetobacter sp.	R	R	R	I	S	R	R	R
B. cereus	R	R	S	S	S	S	I	S
Staphylococcus sp.	R	R	R	S	S	S	R	I
Enterobacter sp.	R	R	R	I	S	R	R	R
Micrococcus sp.	R	R	S	S	S	S	S	S
Alcaligenes sp.	R	R	R	S	S	R	R	R
Rhodococcus sp.	R	R	S	S	S	S	I	S
Klebsiella sp.	R	R	R	I	S	R	R	R

Key: *AMX = amoxicillin; AMP = ampicillin; TET = tetracycline; GEN = gentamicin; CIP = ciprofloxacin; CHL = chloramphenicol; SXT = trimethoprim-sulfamethoxazole; CTX = cefotaxime; R = resistant; I = intermediate; S = susceptible.*

3.5 Frequency of Antibiotic Resistance

The frequency of antibiotic resistance among the 38 hydrocarbon-utilising isolates is presented in Table 5. Amoxicillin recorded the highest resistance frequency, with 31 of the 38 isolates resistant, corresponding to 81.6%.

Ampicillin followed closely, with 29 isolates (76.3%) exhibiting resistance.

Tetracycline resistance occurred in 19 isolates (50.0%). Chloramphenicol and trimethoprim-sulfamethoxazole recorded resistance frequencies of 44.7% and 42.1%, respectively. Cefotaxime resistance was recorded in 14 isolates (36.8%).

The lowest resistance frequencies were observed for gentamicin and ciprofloxacin, with seven isolates (18.4%) and five isolates (13.2%), respectively.

The results demonstrate a pronounced resistance pattern towards amoxicillin and ampicillin, whereas ciprofloxacin and gentamicin showed comparatively lower resistance frequencies.

Table 5: Frequency and percentage of antibiotic resistance among hydrocarbon-utilising bacterial isolates (n = 38)

Antibiotic	Resistant isolates (n)	Resistance (%)
Amoxicillin	31	81.6
Ampicillin	29	76.3
Tetracycline	19	50.0
Chloramphenicol	17	44.7
Trimethoprim-sulfamethoxazole	16	42.1
Cefotaxime	14	36.8
Gentamicin	7	18.4
Ciprofloxacin	5	13.2

3.6 Quantitative Hydrocarbon-Utilisation Potential of Selected Bacterial Isolates

The quantitative TPH-utilisation experiment demonstrated marked differences in the ability of the selected bacterial isolates to reduce TPH concentration under the experimental conditions (Table 6).

Pseudomonas aeruginosa demonstrated the highest TPH removal, reducing the initial concentration from 500 mg/L to 146 mg/L. This represented a removal of 354 mg/L or 70.8%.

Rhodococcus sp. recorded the second-highest removal efficiency, reducing TPH concentration to 157 mg/L, corresponding to 343 mg/L removal and 68.6% reduction. *Acinetobacter* sp. reduced the TPH concentration to 171 mg/L, representing 329 mg/L removal and 65.8% reduction.

Alcaligenes sp. recorded 62.4% TPH removal, while *Bacillus subtilis* and *Bacillus cereus* recorded 54.2% and 50.6%, respectively. *Enterobacter* sp. demonstrated the lowest removal among the inoculated isolates, with a reduction of 43.8%.

The uninoculated control decreased from 500 mg/L to 472 mg/L, representing only 28 mg/L or 5.6% reduction. Thus, the substantially greater reductions observed in the inoculated treatments indicate that biological activity contributed considerably to TPH removal under the experimental conditions.

Table 6: Quantitative TPH-utilisation potential of selected bacterial isolates

Isolate	Initial TPH (mg/L)	Final TPH (mg/L)	TPH removed (mg/L)	Removal (%)
P. aeruginosa	500	146	354	70.8
Acinetobacter sp.	500	171	329	65.8
Alcaligenes sp.	500	188	312	62.4
Rhodococcus sp.	500	157	343	68.6
B. subtilis	500	229	271	54.2
B. cereus	500	247	253	50.6
Enterobacter sp.	500	281	219	43.8
Uninoculated control	500	472	28	5.6

3.7 Relationship between TPH Concentration, Hydrocarbon-Utilising Bacteria and Multidrug Resistance

The relationships among environmental TPH concentration, hydrocarbon-utilising bacterial abundance and multidrug-resistant bacterial characteristics are presented in Table 7.

TPH concentration showed a strong positive correlation with hydrocarbon-utilising bacterial abundance (r = 0.81). A positive correlation was also observed between TPH concentration and multidrug-resistant bacterial abundance (r = 0.74). Hydrocarbon-utilising bacterial abundance was similarly positively correlated with multidrug-resistant bacterial abundance (r = 0.69).

The strongest relationship was therefore observed between TPH concentration and hydrocarbon-utilising bacterial abundance, followed by the relationship between TPH concentration and multidrug-resistant bacterial abundance.

These findings indicate that locations with higher measured TPH concentrations tended to exhibit greater hydrocarbon-utilising bacterial abundance and a greater occurrence of multidrug-resistant phenotypes. Nevertheless, the correlation coefficients describe association rather than causation and should not be interpreted as evidence that petroleum hydrocarbons directly caused antibiotic resistance.

Table 7: Correlation matrix for TPH concentration, hydrocarbon-utilising bacterial abundance and multidrug-resistant bacterial abundance

Variable	TPH	Hydrocarbon-utilising bacteria	Multidrug-resistant bacteria
TPH	1.00	0.81	0.74
Hydrocarbon-utilising bacteria	0.81	1.00	0.69
Multidrug-resistant bacteria	0.74	0.69	1.00

3.8 Multiple Antibiotic Resistance Patterns

The multiple antibiotic resistance profiles of the selected isolates are presented in Table 8. The highest resistance burden was observed among *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp. and *Klebsiella* sp., each of which demonstrated resistance to six of the eight antibiotics tested.

Alcaligenes sp. demonstrated resistance to five antibiotics, while *Staphylococcus* sp. demonstrated resistance to four. *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus* sp. and *Rhodococcus* sp. demonstrated resistance to two antibiotics each.

The corresponding MAR indices ranged from 0.25 among isolates resistant to two antibiotics to 0.75 among isolates resistant to six antibiotics. The highest MAR index was therefore recorded for *P. aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp. and *Klebsiella* sp.

Table 8: Multiple antibiotic resistance patterns and MAR indices of selected bacterial isolates

Isolate	Antibiotics tested	Number resistant	Resistance pattern	MAR index
P. aeruginosa	8	6	AMX–AMP–TET–CHL–SXT–CTX	0.75
B. subtilis	8	2	AMX–AMP	0.25
Acinetobacter sp.	8	6	AMX–AMP–TET–CHL–SXT–CTX	0.75
B. cereus	8	2	AMX–AMP	0.25
Staphylococcus sp.	8	4	AMX–AMP–TET–SXT	0.50
Enterobacter sp.	8	6	AMX–AMP–TET–CHL–SXT–CTX	0.75
Micrococcus sp.	8	2	AMX–AMP	0.25
Alcaligenes sp.	8	5	AMX–AMP–TET–CHL–SXT	0.625
Rhodococcus sp.	8	2	AMX–AMP	0.25
Klebsiella sp.	8	6	AMX–AMP–TET–CHL–SXT–CTX	0.75

4.0 Discussion

4.1 Physicochemical Characteristics and Petroleum Hydrocarbon Contamination

The physicochemical characteristics recorded across the five sampling locations demonstrate differences in the environmental conditions of the investigated surface waters.

Temperature varied only moderately among the locations, whereas greater differences were observed in electrical conductivity, dissolved oxygen, chemical oxygen demand and TPH concentrations. The relatively narrow temperature range is consistent with the tropical climatic conditions of the Niger Delta, where surface-water temperatures generally remain relatively stable. In contrast, the differences in conductivity and oxygen-related parameters may reflect variations in ionic loading, organic matter input, biological activity, tidal influence and anthropogenic discharge.

The pH values were slightly acidic to near neutral across the sampling locations. Such conditions are important for microbial activity because pH can influence bacterial growth, enzyme activity and the bioavailability of hydrocarbon substrates. Petroleum contamination can also interact with other environmental factors to modify microbial community structure. Studies of crude-oil-contaminated environments have demonstrated that physicochemical conditions influence the composition and abundance of indigenous bacterial populations involved in petroleum degradation [12,23].

The TPH concentrations ranged from 3.94 ± 0.27 mg/L at Site C to 8.21 ± 0.52 mg/L at Site D. The higher concentrations observed at Sites B and D indicate greater petroleum hydrocarbon burdens at these locations. Comparable petroleum contamination has been reported in other Niger Delta surface-water systems. Ekwere and Nna [15] documented TPH in surface waters of the Cross River Estuary, while Isueken et al. [24] reported petroleum hydrocarbon contamination in surface water from Ogboinbiri Creek. Recent investigations have likewise demonstrated hydrocarbon contamination of the Bonny Estuary and other petroleum-bearing aquatic environments in Rivers State [31,34].

The elevated TPH concentrations at some locations reflect differences in proximity to petroleum-related activities, surface runoff, accidental releases, boat traffic, domestic discharge and other local sources which is consistent with the complex contamination patterns previously reported in Niger Delta aquatic systems, where petroleum hydrocarbons frequently occur alongside other contaminants [33,35,37]. Chemical oxygen demand was highest at Site D, where the highest TPH concentration was also recorded. The concurrent elevation of COD and TPH may indicate a greater overall organic contaminant burden at this location.

4.2 Occurrence of Hydrocarbon-Utilising Bacteria

A total of 87 bacterial isolates were recovered from the investigated surface waters, of which 38 (43.7%) demonstrated hydrocarbon-utilisation capability. The proportion of hydrocarbon-utilising bacteria varied among the sampling locations, ranging from 30.8% at Site E to 47.8% at Site D. The recovery of hydrocarbon-utilising bacteria from all five locations suggests that the investigated aquatic systems support bacterial populations capable of utilising petroleum hydrocarbons.

This observation agrees with earlier investigations of petroleum-impacted environments in the Niger Delta. Chikere and Ekwuabu [10] reported hydrocarbon-utilising bacteria in crude-oil-impacted sites in Bodo, Ogoniland, while Chikere and Omadudu [11] demonstrated dioxygenase activity among indigenous hydrocarbon-utilising bacteria from oil-polluted Niger Delta environments. These findings supported the evidence that petroleum exposure is associated with the enrichment or persistence of microorganisms possessing hydrocarbon-degrading capabilities (Okpoji et al., 2025).

The relatively high proportion of hydrocarbon-utilising bacteria at Site D, which also had the highest TPH concentration, is noteworthy. Petroleum hydrocarbons serve as selective substrates for microorganisms possessing appropriate metabolic pathways. Continuous or repeated hydrocarbon exposure may therefore favour organisms capable of tolerating and metabolising petroleum compounds.

The occurrence of hydrocarbon-utilising bacteria is environmentally significant because indigenous microbial communities constitute an important component of natural attenuation. Petroleum biodegradation involves sequential microbial transformations of hydrocarbon molecules through enzymatic pathways, with the extent of degradation influenced by the chemical structure of the compounds and environmental conditions [13]. Previous research has also demonstrated that bacterial community composition changes during crude-oil degradation, indicating that hydrocarbon contamination can influence microbial ecology [12].

4.3 Bacterial Diversity and Hydrocarbon-Utilisation Characteristics

The selected hydrocarbon-utilising isolates comprised *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Acinetobacter* sp., *Bacillus cereus*, *Staphylococcus* sp., *Enterobacter* sp., *Micrococcus* sp., *Alcaligenes* sp., *Rhodococcus* sp. and *Klebsiella* sp. The diversity observed is consistent with previous reports that petroleum-contaminated environments harbour metabolically diverse bacterial communities. In particular, *Pseudomonas*, *Bacillus* and other hydrocarbon-associated bacteria have frequently been recovered from crude-oil-impacted environments [10,11].

The strong hydrocarbon-utilisation responses recorded for *P. aeruginosa*, *Acinetobacter* sp., *Alcaligenes* sp. and *Rhodococcus* sp. suggest that these organisms possessed comparatively effective mechanisms for using the petroleum substrate provided in the screening medium. The ability of bacteria to utilise hydrocarbons depends on the presence and expression of enzymes capable of initiating oxidation of hydrocarbon molecules. Oxygenases and dioxygenases are particularly important in the initial transformation of many petroleum hydrocarbons [11,13].

The occurrence of *Rhodococcus* sp. among the stronger hydrocarbon utilisers is also biologically plausible because members of this genus are recognised for their metabolic versatility and capacity to transform structurally diverse organic compounds. Similarly, *Pseudomonas* species are widely associated with petroleum hydrocarbon degradation [9,22].

4.4 Antibiotic Resistance Profiles

The antibiotic susceptibility results demonstrated substantial resistance among the 38 hydrocarbon-utilising isolates. Resistance was highest to amoxicillin (81.6%) and ampicillin (76.3%), whereas ciprofloxacin and gentamicin showed comparatively lower resistance frequencies of 13.2% and 18.4%, respectively. This pattern indicates considerable variation in the effectiveness of the tested antimicrobial agents against the environmental isolates.

The high resistance to amoxicillin and ampicillin is consistent with the widespread occurrence of resistance to commonly used β-lactam antibiotics among environmental bacteria. Bacterial populations in aquatic environments can be exposed to antibiotics and resistance determinants introduced through sewage, domestic wastewater, healthcare facilities and other anthropogenic sources [28].

In southern Nigeria, antibiotic-resistant bacteria have been reported in surface and groundwater sources, further demonstrating that antimicrobial resistance is present in aquatic environments within the region [14].

The relatively low resistance observed for ciprofloxacin and gentamicin suggests that these agents retained greater activity against the tested isolates than amoxicillin and ampicillin. The environmental importance of antibiotic resistance is further strengthened by evidence that wastewater can contain both resistant bacteria and resistance genes [8,36].

The resistance profiles also differed among bacterial groups. *P. aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp. and *Klebsiella* sp. demonstrated broad resistance patterns, whereas *Bacillus subtilis*, *B. cereus*, *Micrococcus* sp. and *Rhodococcus* sp. displayed narrower profiles. Such differences may reflect intrinsic physiological characteristics, acquired resistance determinants and differences in exposure history. Antibiotic resistance is a dynamic bacterial trait and can be influenced by both chromosomal mechanisms and horizontally acquired genetic determinants [21,27].

4.5 Multiple Antibiotic Resistance and MAR Indices

The MAR indices in Table 8 ranged from 0.25 to 0.75. The highest MAR index (0.75) was recorded for *P. aeruginosa*, *Acinetobacter* sp., *Enterobacter* sp. and *Klebsiella* sp., each resistant to six of the eight antibiotics tested. *Alcaligenes* sp. had a MAR index of 0.625, while *Staphylococcus* sp. recorded 0.50. The lowest value of 0.25 occurred among *B. subtilis*, *B. cereus*, *Micrococcus* sp. and *Rhodococcus* sp.

The high MAR values are consistent with the observation that environmental bacteria can acquire and maintain resistance to multiple antimicrobial agents when exposed to environments containing diverse selection pressures. Antibiotic resistance can persist outside clinical settings, and environmental microbial communities may provide opportunities for the maintenance and exchange of resistance determinants [27,28]. Horizontal transfer of antibiotic resistance genes is particularly important because resistance determinants may move between bacterial populations under suitable environmental conditions [21].

The finding is also supported by Nigerian evidence. Ataikiru et al. [1] reported both hydrocarbon biodegradation and antibiotic resistance among microorganisms isolated from an oil-polluted site in Delta State. Similarly, the recovery of hydrocarbon-utilising bacteria from oil-impacted sites in Bodo and other Niger Delta environments provides evidence that petroleum-contaminated ecosystems can support bacterial populations with diverse ecological and functional characteristics [10,11,14,30,36].

4.6 Quantitative TPH-Removal Potential

The quantitative degradation experiment demonstrated substantial differences in TPH removal among the selected isolates. *P. aeruginosa* achieved the highest removal efficiency (70.8%), followed by *Rhodococcus* sp. (68.6%), *Acinetobacter* sp. (65.8%) and *Alcaligenes* sp. (62.4%). *B. subtilis*, *B. cereus* and *Enterobacter* sp. recorded lower removal efficiencies of 54.2%, 50.6% and 43.8%, respectively. The uninoculated control showed only 5.6% reduction, indicating that most of the TPH reduction observed in the inoculated treatments was associated with biological activity, although abiotic losses cannot be completely excluded.

The superior performance of *P. aeruginosa* is consistent with the recognised capacity of members of this genus to utilise petroleum hydrocarbons. Indigenous bacterial communities can become enriched with hydrocarbon degraders following petroleum exposure, and these organisms may contribute to natural attenuation [11,12]. The results also support previous observations that microbial degradation can be an important component of petroleum-contaminated soil and water remediation [13,25].

The removal efficiency observed for *Rhodococcus* sp. is also noteworthy. The comparatively high removal by this isolate suggests that it possesses metabolic capabilities suitable for transformation of components of the petroleum substrate. The reduction recorded in the uninoculated control also highlights the importance of appropriate experimental controls. Loss of hydrocarbons may occur through volatilisation, adsorption to experimental materials, abiotic transformation or other processes. Therefore, the difference between inoculated treatments and the control provides stronger evidence of biological contribution than the final TPH concentration alone.

4.7 Relationship Between TPH, Hydrocarbon-Utilising Bacteria and MDR

Positive relationships were observed between TPH and hydrocarbon-utilising bacteria ($r = 0.81$), TPH and MDR bacteria ($r = 0.74$), and hydrocarbon-utilising bacteria and MDR bacteria ($r = 0.69$). These relationships suggest that the variables tended to increase together across the investigated sites. The strongest association was between TPH concentration and the abundance of hydrocarbon-utilising bacteria, which is biologically plausible because petroleum hydrocarbons can provide substrates for bacteria possessing appropriate metabolic pathways.

The positive association between TPH and MDR bacteria is potentially important because it suggests that petroleum-impacted sites may also contain substantial populations of antibiotic-resistant bacteria. However, correlation does not establish causation [8,27,28].

The positive relationship between hydrocarbon-utilising bacteria and MDR bacteria may also indicate that some bacterial populations were capable of simultaneously tolerating petroleum contamination and multiple antimicrobial agents. Ataikiru et al. [1] reported a similar coexistence of hydrocarbon biodegradation and antibiotic sensitivity/resistance characteristics among bacteria isolated from an oil-polluted site.

Conclusion

This study established the occurrence of indigenous hydrocarbon-utilising bacteria with varying antibiotic resistance profiles in oil-impacted surface waters across five locations in the Niger Delta, Nigeria. The measured total petroleum hydrocarbon (TPH) concentrations ranged from 3.94 ± 0.27 to 8.21 ± 0.52 mg/L, indicating differences in petroleum hydrocarbon contamination among the investigated water systems. Of the 87 bacterial isolates recovered, 38 (43.7%) demonstrated the ability to utilise hydrocarbons under the conditions of the screening assay. This indicates that a substantial proportion of the recovered bacterial isolates possessed hydrocarbon-utilisation potential.

The antibiotic susceptibility results revealed considerable resistance to amoxicillin (81.6%) and ampicillin (76.3%), while resistance to ciprofloxacin (13.2%) and gentamicin (18.4%) was comparatively lower.

The occurrence of multidrug-resistant phenotypes among the isolates is environmentally relevant because aquatic environments can serve as reservoirs of antibiotic-resistant bacteria. The findings therefore underscore the importance of including antibiotic resistance surveillance in the microbiological assessment of petroleum-impacted aquatic ecosystems.

The quantitative hydrocarbon-utilisation assessment further demonstrated differences in TPH-removal potential among the selected isolates. *Pseudomonas aeruginosa* recorded the highest TPH removal (70.8%), followed by *Rhodococcus* sp. (68.6%) and *Acinetobacter* sp. (65.8%). These values were substantially higher than the 5.6% removal observed in the uninoculated control, suggesting that the selected isolates have potential for further investigation as biological agents for hydrocarbon remediation. Positive correlations were observed between TPH concentration and the abundance of hydrocarbon-utilising bacteria (r = 0.81), TPH concentration and MDR isolates (r = 0.74), and hydrocarbon-utilising bacteria and MDR isolates (r = 0.69). These relationships suggest that petroleum contamination, hydrocarbon-utilisation potential and antibiotic resistance may co-occur in the investigated environments.

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