

Cryptic Carriers of Resistance: A Metagenomic Sentinel Case of *Vibrio* and CARB-7 β -Lactamase in a Lagos Marine Crab

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ABSTRACT

Metagenomic characterization of foodborne pathogens in Nigerian seafood remains critically limited. This sentinel case investigated *Vibrio* species in a single crab (*Callinectes* spp.) from Eleko Beach, Lagos, Nigeria, using shotgun metagenomic sequencing on the PacBio Onso platform. Taxonomy was assigned via Kraken2/Bracken with Krona visualization. Diversity analyses used the vegan package in R. Phylogenetic analysis employed MAFFT, RAXML, and ape. Functional annotation used Prodigal and InterProScan. Antimicrobial resistance genes were detected using RGI/CARD. Taxonomic classification revealed 2,379 *Vibrio* fragments (0.04% of classified reads), identifying seventeen species including *V. anguillarum* (1,097), *V. harveyi* group (616), *V. parahaemolyticus* (237), *V. cholerae* (6), and *V. vulnificus* (10). *Photobacterium* included *P. ganghwense* (97) and *P. damsela* (15). Eukaryota constituted 45.6% of reads, with *Homo sapiens* at 6.44%, indicating human DNA contamination. Other pathogens included *E. coli*, *S. aureus*, *L. monocytogenes*, and *C. jejuni*. Alpha diversity was moderate (Shannon = 2.10; Chao1 = 287). Beta diversity revealed significant group differences (PERMANOVA: $R^2 = 0.2345$, $p = 0.001$). Phylogenetic analysis resolved seven genera, with *Vibrio* as a well-supported clade (bootstrap $\geq 90\%$). Functional annotation identified protein kinase domains and reverse transcriptases. The CARB-7 β -lactamase gene was detected. This sentinel case identified seventeen *Vibrio* species and a β -lactamase resistance gene in a single Lagos crab. The high human DNA contamination emphasizes the urgent need for effective decontamination protocols in seafood metagenomics.

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INTRODUCTION

Seafood serves as a critical source of animal protein for human populations worldwide. Crustaceans, particularly crabs, are highly valued for their nutritional composition but are susceptible to harboring pathogenic microorganisms due to their filter-feeding nature and habitation of marine environments. Among naturally occurring bacterial populations in aquatic environments, members of the genus *Vibrio* are of particular concern. These Gram-negative bacilli are ubiquitous inhabitants of marine and estuarine ecosystems, with *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* being well-documented causative agents of shellfish-vectored illnesses (Ismail *et al.*, 2024; Inana *et al.*, 2024). Other species including *V. alginolyticus*, *V. fluvialis*, and *V. mimicus* are recognized as occasional human pathogens (Chowdhury *et al.*, 2016). Climate change has emerged as a primary driver accelerating *Vibrio* proliferation globally, with ocean warming creating ideal conditions for their expansion (Jayakumar *et al.*, 2024). *Vibrio vulnificus*, a climate-sensitive organism with a mortality rate exceeding 50%, causes an estimated economic burden of \$267 million annually in the United States (Quintero-Campos *et al.*, 2025).

Recent advances in population genomics have enhanced understanding of *Vibrio* genetic diversity. Huang *et al.* (2024) revealed complex genetic diversity in *V. alginolyticus* with three distinct lineages and documented β -lactam resistance genes in 100% of isolates. Similarly, Zheng *et al.* (2022) reported high levels of homologous recombination and population-specific antimicrobial resistance gene distributions in *V. fluvialis*. Metagenomic approaches have proven valuable, as demonstrated by Brinch *et al.* (2024), who recovered a near-complete *V. cholerae* genome from urban sewage in Copenhagen.

In Nigeria, studies have documented *Vibrio* presence in seafood. Inana *et al.* (2024) reported 29.5% prevalence in seafood from Port Harcourt markets, with most isolates demonstrating 100% resistance to ceftazidime and cefuroxime. Tarh and Inyang (2024) found that 45.8% of *V. cholerae* isolates from Cross River State showed resistance to augmentin. However, comprehensive metagenomic characterization of Nigerian aquatic environments remains limited. Eleko Beach, Lagos State, represents an important coastal ecosystem where crab harvesting occurs, yet the microbial diversity of *Vibrio* populations has not been

systematically characterized using modern genomic approaches.

Given growing global concern regarding *Vibrio*-associated foodborne illness and antimicrobial resistance emergence (Inana *et al.*, 2024; Ismail *et al.*, 2024), there is urgent need for integrated genomic characterization to elucidate the population structure, virulence gene repertoire, and antimicrobial resistance profiles of *Vibrio* species in crab samples. This study employed shotgun metagenomic sequencing to investigate *Vibrio* species diversity in a single crab from Eleko Beach, Lagos, Nigeria, aiming to identify

clinically significant species, antimicrobial resistance genes, and functional gene categories relevant to pathogenicity and public health risk assessment.

MATERIALS AND METHODS

Description of Study Area

Eleko Beach is located in Lagos State, Nigeria (coordinates: 6°27'0"N, 3°31'0"E). It is a coastal area along the Atlantic Ocean, characterized by estuarine and marine environments that provide suitable habitats for *Vibrio* species.



Source: Google Maps, 2025

Sample Collection

A sentinel case study was conducted to assess the microbial composition of a crab sample collected from Eleko Beach, Lagos, Nigeria. As only one crab from one site was analyzed, the findings are exploratory and hypothesis-generating rather than representative of the wider Lagos coastal environment. The crab specimen (*Callinectes* spp.) was

aseptically collected using sterile gloves and instruments to minimize exogenous contamination. The sample was immediately placed in a sterile collection bag and transported to the laboratory under cold-chain conditions (4°C) within six hours of collection. Upon arrival, the sample was stored at -20°C pending DNA extraction.



Source: As captured

Genomic DNA Extraction

Genomic DNA was extracted from approximately 250 mg of crab homogenate using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Catalogue No. D4300). This kit was selected for its validated unbiased lysis efficiency across diverse microbial taxa and its OneStep PCR Inhibitor Removal Technology for inhibitor-rich environmental samples. Mechanical cell lysis was achieved using a Disruptor Genie bead beater (Scientific Industries, Inc.) and processed for 20 minutes at maximum speed. The lysed sample was centrifuged at $10,000 \times g$ for one minute, and 400 μ L of supernatant was transferred to a Zymo-Spin III-F Filter and centrifuged at $8,000 \times g$ for one minute. The filtrate was mixed with 1,200 μ L of ZymoBIOMICS DNA Binding Buffer, and the mixture was transferred to a Zymo-Spin IC Column in 800 μ L aliquots, centrifuged at $10,000 \times g$ for one minute. The column was washed sequentially with Wash Buffers, with centrifugation at $10,000 \times g$ for one minute after each wash. DNA was eluted with 50 μ L of DNase/RNase Free

Water and further purified through a Zymo-Spin III-HRC Filter. The purified DNA was quantified using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific) with the Qubit dsDNA High Sensitivity Assay Kit. DNA purity was assessed by measuring A260/A280 and A260/A230 ratios using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). DNA integrity was evaluated by agarose gel electrophoresis (1% agarose gel, 100 V for 45 minutes).

Library Preparation and Sequencing

Purified genomic DNA was submitted to Inqaba Biotechnical Industries (Pretoria, South Africa) for shotgun metagenomic sequencing on the PacBio Onso platform. High-quality genomic DNA was enzymatically fragmented to a target insert size of approximately 300 bp using the Onso Fragmentation DNA Library Prep Kit (PacBio 102-499-100). Adapter ligation was performed using the Onso Indexed Adapter Plate (PacBio 102-431-700) with Ligation Mix (PacBio 102-554-400) and Ligation Enhancer (PacBio 102-

554-500). Double-sided bead-based size selection was performed using SPRIselect beads (Beckman Coulter). Library amplification was performed using the Onso Library Amp Kit (PacBio 102-410-800). Sequencing was performed on the PacBio Onso platform using the Onso 300-cycle kit in 2 × 150 bp paired-end mode. The sequencing run produced approximately 320 Mb of data (~1.1 million read pairs).

Bioinformatics Analysis

Quality Control and Preprocessing

Raw reads underwent quality assessment using FastQC (version 0.11.9). Low-quality reads (Phred score < Q20), reads containing ambiguous bases (N > 5%), and adapter sequences were trimmed using Trimmomatic (version 0.39) with parameters: LEADING:3, TRAILING:3, SLIDINGWINDOW:4:15, MINLEN:36.

Taxonomic Classification

Taxonomic classification was performed using Kraken2 (version 2.1.2) with the standard Kraken2 database (RefSeq, downloaded May 2024), using parameters: --confidence 0.5, --minimum-hit-groups 3, and --report-minimizer-data. Bracken (version 2.6.0) was subsequently applied to estimate species-level abundances with parameters: -r 150, -t 10, and -l S. Relative abundance was calculated as the number of reads assigned to each taxon divided by the total number of classified reads.

Krona Visualization

A Krona chart was generated using KronaTools (version 2.8.1) with the ktlImportTaxonomy utility.

Alpha and Beta Diversity Analysis

Alpha diversity metrics (Shannon, Simpson, Chao1) and beta diversity analyses (Bray-Curtis dissimilarity visualized using Principal Coordinate Analysis) were performed using the vegan package (version 2.6-4) in R Studio (version 4.3.1). PERMANOVA with 9,999 permutations was implemented in the adonis2 function of the vegan package.

Phylogenetic Analysis

Representative 16S rRNA gene sequences were retrieved from the NCBI Nucleotide database using BLASTn. Sequences were aligned using MAFFT (version 7.505) with the L-INS-i algorithm and trimmed using trimAl (version 1.4) with the -automated1 parameter. The optimal nucleotide substitution model was determined using ModelTest-NG (version 0.1.7). Maximum Likelihood trees were inferred using RAxML (version 8.2.12) with rapid bootstrap analysis (1,000 replicates) and the GTRGAMMA model. Neighbor-Joining trees were constructed using the ape package (version

5.6-2) with the K80 distance model. Trees were visualized using the ggtree package (version 3.2.1).

Functional Annotation

Protein-coding sequences were predicted from metagenomic contigs using Prodigal (version 2.6.3) with the -p meta parameter. Functional annotation was performed using InterProScan (version 5.62-94.0), integrating databases including MobiDBLite, PANTHER, Pfam, Gene3D, ProSiteProfiles, TIGRFAM, and SUPERFAMILY. Gene Ontology (GO) terms were assigned to annotated sequences.

Antimicrobial Resistance Gene Detection

AMR genes were identified using the Resistance Gene Identifier (RGI) tool (version 6.0.0) with CARD (version 3.2.6). RGI analysis used the rgi main command with parameters: input_sequence contig, input_type contig, analysis_type both, and alignment_tool DIAMOND. Detected AMR genes were classified by drug class, resistance mechanism, and confidence level (Strict, Perfect, or Loose).

Data Visualization

All statistical analyses and visualizations were performed using R Studio (version 4.3.1) with ggplot2 (version 3.4.2), heatmap (version 1.0.12), and vegan (version 2.6-4).

Data Availability

All sequence data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1499156. Bioinformatics analysis workflows, including all scripts and parameters used, are available upon request from the corresponding author.

RESULTS AND DISCUSSION

Overview of Metagenomic Sequencing

Shotgun metagenomic sequencing of the single crab sample from Eleko Beach, Lagos, Nigeria, generated approximately 320 Mb of data (~1.1 million read pairs), achieving ~100× coverage depth. Taxonomic classification using Kraken2 and Bracken revealed a diverse microbial community dominated by *Vibrio* species.

Microbial Community Composition

A total of 2,379 fragments were classified as *Vibrio*, representing 0.04% of total classified reads (Table 1). Seventeen distinct *Vibrio* species were identified, with *V. anguillarum* being the most abundant (1,097 fragments), followed by the *V. harveyi* group (616 fragments) and *V. parahaemolyticus* (237 fragments). Clinically significant species detected included *V. cholerae* (6 fragments), *V. vulnificus* (10 fragments), and *V. alginolyticus* (115 fragments) (Table 1).

Table 1: *Vibrio* Species Identified in Crab Sample from Eleko Beach, Lagos, Nigeria

Species	NCBI ID	Fragments	%
<i>V. anguillarum</i>	55601	1,097	0.02
<i>V. harveyi</i> group	717610	616	0.01
<i>V. parahaemolyticus</i>	670	237	<0.01
<i>V. diabolus</i> group	2315253	172	<0.01
<i>V. alginolyticus</i>	663	115	<0.01
<i>V. natriegens</i>	691	22	<0.01
<i>V. vulnificus</i>	672	10	<0.01
<i>V. cholera</i>	666	6	<0.01
<i>V. rotiferianus</i>	190895	6	<0.01
<i>V. owensii</i>	696485	5	<0.01
<i>V. antiquaries</i>	150340	4	<0.01

Species	NCBI ID	Fragments	%
<i>V. harveyi</i>	669	4	<0.01
<i>V. navarrensis</i>	29495	3	<0.01
<i>V. coralliilyticus</i>	190893	3	<0.01
<i>V. campbellii</i>	680	2	<0.01
<i>V. neocaledonicus</i>	1208308	2	<0.01
<i>V. orientalis</i> group	1891919	2	<0.01
<i>V. azureus</i>	512649	1	<0.01
<i>V. europaeus</i>	300876	1	<0.01
Unclassified <i>Vibrio</i>	2614977	9	<0.01
Other <i>Vibrio</i> spp.	—	9	<0.01
Total	—	2,379	0.04

The dominance of *V. anguillarum* and the *V. harveyi* group in the crab sample is noteworthy. While *V. anguillarum* is primarily recognized as a pathogen of aquatic animals, its high abundance in this crab from Eleko Beach aligns with findings from other crab cultivation environments where *Vibrio* species constitute a significant portion of the microbial community (Xu et al., 2025). Similarly, *V. harveyi* is a well-known pathogen in aquaculture, particularly affecting shrimp and other crustaceans (Zhang et al., 2020). The prevalence of these species suggests that Eleko Beach's coastal environment may provide suitable conditions for their proliferation, potentially through wastewater discharge, estuarine mixing, or natural marine microbial dynamics.

The detection of clinically significant species *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, and *V. cholerae* is of considerable public health concern. This finding corroborates Adeleye and Enyinnia (2010), who found *V. alginolyticus* (31.8%) and *V. harveyi* (27.3%) as the most predominant species in seafood samples from Lagos, with *V. parahaemolyticus* (11.4%) and *V. cholerae* (6.8%) also detected. Similarly, Inana et al. (2024) reported a 29.5% prevalence of *Vibrio* species in seafood from Port Harcourt markets. The present study's detection of *V. vulnificus*, which was notably absent in Odeniyi et al.'s (2022) investigation of Lagos seafood markets, suggests either geographical variability in species distribution or the enhanced sensitivity of metagenomic approaches compared to PCR-based methods.

The species diversity observed in this sentinel case seventeen *Vibrio* species exceeds that reported in many previous Nigerian investigations, likely due to the enhanced resolution of metagenomic sequencing compared to culture-dependent methods. Earlier studies using conventional microbiological techniques typically identified between five and eight species from seafood samples. The detection of species such as *V. diabolicus*, *V. antiquarius*, *V. natriegens*, *V. rotiferianus*, *V. owensii*, *V. campbellii*, *V. azureus*, *V. navarrensis*, *V. coralliilyticus*, *V. neocaledonicus*, and *V. europaeus* expands the known *Vibrio* diversity in Lagos crab populations.

Other Vibrionaceae Members and Functional Annotation

The Vibrionaceae family also included *Photobacterium* species, with 112 fragments identified, predominantly *P. ganghwense* (97 fragments) and *P. damsela* (15 fragments) (Table 2). *Photobacterium damsela* is a well-known pathogen of marine animals, with subspecies *piscicida* causing fish pasteurellosis and *damsela* associated with wound infections in humans (Rivas et al., 2013). The detection of *P. damsela* subspecies in this crab from Eleko Beach suggests that this pathogen may be more prevalent in Lagos coastal environments than previously recognized. Earlier research conducted by Odeniyi et al. (2022) in Nigeria has provided limited documentation of *Photobacterium* species, probably because of the constraints associated with culture-dependent techniques.

Table 2: Vibrionaceae Members, Functional Annotation, and Antimicrobial Resistance Genes Identified in Crab Sample from Eleko Beach, Lagos, Nigeria

Category	Organism/Gene/Sequence ID	NCBI ID	Fragments	Description / Details
Vibrionaceae	<i>P. ganghwense</i>	320778	97	Marine bacterium
	<i>P. damsela</i>	38293	15	Pathogen of marine animals
	<i>P. damsela</i> subsp. <i>piscicida</i>	38294	1	Fish pathogen (pasteurellosis)
	<i>P. damsela</i> subsp. <i>damsela</i>	85581	1	Associated with wound infections
Functional Annotation	k141_1784_1	—	—	Reverse transcriptases (mobile genetic elements)
	k141_1850_1	—	—	Protein kinase domain (GO:0004672, GO:0005524, GO:0006468)
	k141_455_1	—	—	Consensus disorder prediction
	k141_1185_1	—	—	Consensus disorder prediction
AMR Gene	CARB-7	—	—	β-lactamase (Penam resistance; antibiotic inactivation)

The functional annotation of protein-coding sequences identified several protein families of significant biological relevance. The detection of reverse transcriptases suggests the activity of transposable elements and bacteriophages within the microbial community, which may facilitate horizontal gene transfer of virulence and antimicrobial resistance genes. This finding aligns with Huang et al. (2024), who identified mobile genetic elements as key drivers of genetic diversity

and the spread of pathogenicity-associated genes in *V. alginolyticus*. Similarly, Zheng et al. (2022) reported that mobile genetic elements play a significant role in the dissemination of antimicrobial resistance genes in *V. fluvialis* populations.

The identification of protein kinase domains is particularly significant, as these domains are associated with protein phosphorylation, a key regulatory mechanism in cellular

signaling. The associated Gene Ontology terms indicate that this protein is involved in the transfer of phosphate groups from ATP to protein substrates, playing essential roles in cellular signaling and regulation. In pathogenic bacteria, protein phosphorylation systems are critical for virulence regulation, stress responses, and host adaptation. The presence of these domains in the crab metagenome suggests that the microbial community possesses sophisticated signaling mechanisms that may contribute to pathogenic potential.

Krona Visualization and Abundance Profile

Krona chart visualization confirmed that Proteobacteria dominated the microbial community, with Vibrionaceae as

the most abundant family. Within Vibrionaceae, *V. anguillarum* and the *V. harveyi* group constituted the largest proportions. Eukaryota constituted 45.6% of all classified reads, with the overwhelming majority tracing to *Homo sapiens* (6.44% of total reads).

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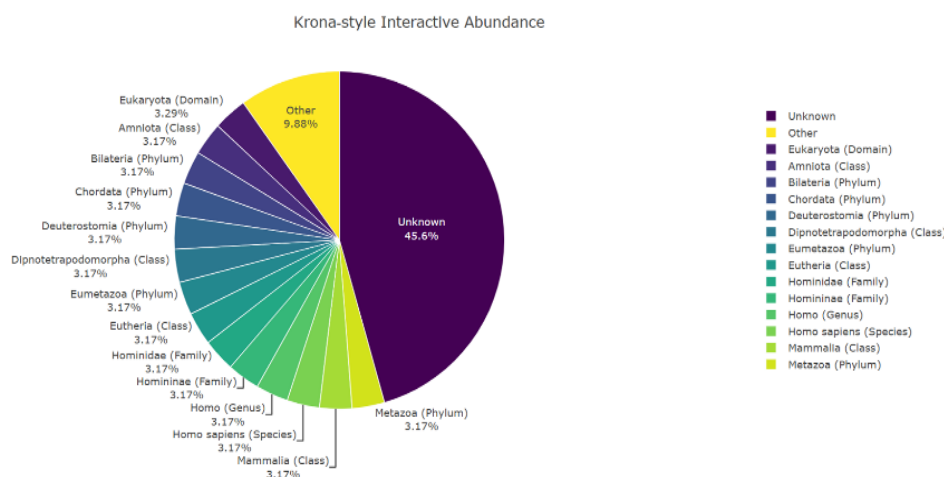


Figure 1: Krona chart visualization of hierarchical taxonomic composition

Heatmap analysis of the top 20 microbial organisms revealed substantial variation in taxon distribution. Prominent potentially pathogenic bacteria included *E. coli*, *S. aureus*, *S. enterica*, *L. monocytogenes*, *C. jejuni*, *H. pylori*, and *C. difficile*. Multiple *Vibrio* species (*V. cholerae*, *V. harveyi*, *V. anguillarum*, *V. alginolyticus*, *V. parahaemolyticus*) were frequently detected. The detection of *E. coli*, *S. aureus*, and *S. enterica* in the crab sample corroborates Odeniyi et al. (2022), who detected enteric bacteria in crustacean samples from

Lagos markets and linked contamination to poor handling practices and environmental pollution. Similarly, Inana et al. (2024) reported the presence of multiple pathogens in seafood from Port Harcourt, including *E. coli* and *Salmonella* spp., emphasizing the widespread nature of this contamination in Nigerian seafood. The presence of *E. coli* in seafood indicates fecal contamination, which may arise from untreated sewage discharge into coastal waters, a common challenge in Lagos coastal areas.

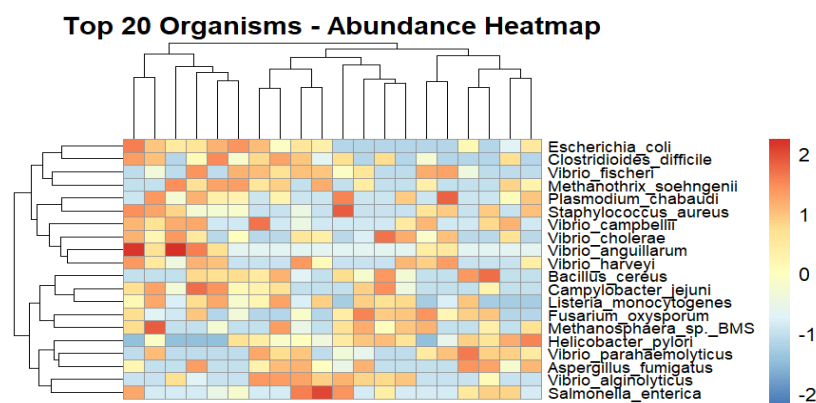


Figure 2: Heatmap of abundance profile of top 20 microbial organisms

Antimicrobial Resistance Gene Detection

The detection of the CARB-7 β -lactamase gene in the crab metagenome raises important questions about antimicrobial resistance in this ecosystem. Previous studies have documented multidrug resistance among *Vibrio* isolates from Nigerian seafood. Aigbodion and Nnenji (2016) found that

Vibrio isolates from crabs in Lagos expressed total (100%) antibiotic resistance to amoxicillin and augmentin. Alarape et al. (2025) reported that *V. cholerae* and *V. parahaemolyticus* from cultured catfish in Ibadan were both resistant to Amoxicillin-Clavulanate (100%), Cefotaxime (100%), and Cefuroxime (100%). Similarly, Onifade and Adeyanju (2022)

found that 66% of *V. cholerae* isolates from seafood in Ondo State showed multiple antibiotic resistance and tested positive for beta-lactamase production, with resistance confirmed to be plasmid-mediated. The identification of the CARB-7 β -lactamase gene in the present sentinel case aligns with the widespread beta-lactam resistance reported in Lagos *Vibrio* populations and suggests that these environmental isolates may serve as reservoirs for the spread of resistance to clinically relevant pathogens.

Human DNA Contamination

The substantial human DNA signal observed in this study 45.6% eukaryotic reads, with 6.44% specifically identified as *Homo sapiens* reflects a pervasive challenge in metagenomics studies of seafood and environmental samples. This finding is consistent with reports from international metagenomics studies Bloomfield *et al.* (2023) that have documented host contamination as a major obstacle in food and environmental sequencing. Bell *et al.* (2025) demonstrated that host DNA contamination can constitute up to 99% of total nucleic acids in samples such as fish skin mucosal swabs, which aligns with the high eukaryotic proportion observed in our crab sample. In the Nigerian context, studies employing culture-dependent methods have provided only limited documentation of *Photobacterium* species. For instance, Adoki and Odokuma (2007) isolated bioluminescent bacteria, including *Photobacterium leiognathi* and *P. phosphoreum*, from the bonny estuary in the Niger Delta using conventional culture-based isolation and biochemical characterization. However, such approaches are inherently constrained by their reliance on specific growth media and incubation conditions. Broekaert *et al.* (2011) demonstrated that *Photobacterium* species, including *P. phosphoreum* and *P. iliopiscarium*, fail to grow on Plate Count Agar, a commonly used general medium, while Fuertes-Perez *et al.* (2019) reported that standard cultivation temperatures (25–37 °C) prevent the isolation of psychrophilic *Photobacterium* species, requiring lower temperatures and extended incubation periods for successful recovery. These methodological limitations explain why culture-dependent surveys often underestimate or entirely miss *Photobacterium* diversity, and they underscore the advantages of metagenomic sequencing, which detects all DNA present in a sample regardless of cultivability.

The effectiveness of host DNA depletion strategies in reducing the eukaryotic signal has been variable. Recent research has explored various approaches, including saponin-based DNA isolation protocols and the NEBNext Microbiome DNA Enrichment Kit (Klosinska *et al.*, 2022). However, a comprehensive assessment of multiple host

depletion techniques on fish skin mucosal swabs by Bell *et al.* (2025) found that none of the existing methods effectively reduced host DNA to be practically useful, and some introduced bias toward certain bacterial taxa. Furthermore, Bloomfield *et al.* (2023) found that seafood samples are particularly challenging for effective decontamination. Their Neutrased-saponin method achieved high mean prokaryotic read proportions post-depletion in chicken (0.64), pork (0.74), and leafy greens (0.74), but performance declined markedly in seafood, with salmon yielding only 0.50 and prawn samples 0.19 prokaryotic read proportions. This finding corroborates the high eukaryotic signal observed in our crab sample and suggests that crustacean matrices possess intrinsic properties that render conventional depletion protocols less effective.

Given these experimental challenges, computational decontamination approaches have emerged as complementary strategies. In the current study, the application of such computational filters confirmed the presence of *Vibrio* species despite the overwhelming host background, although the relative abundance estimate of 0.04% of total reads for *Vibrio* is likely artificially suppressed. While the substantial human DNA signal does not diminish the validity of the *Vibrio* species identified, it suggests that relative abundance estimates for *Vibrio* species may be artificially suppressed by the overwhelming presence of host DNA. This suppression effect is consistent with international findings where high host DNA content reduces the depth of coverage for microbial genomes (Pereira-Marques *et al.*, 2019). Thus, while the human DNA signal does not invalidate the *Vibrio* identifications, it highlights a critical limitation: relative abundance estimates derived from host-dominated samples must be interpreted with caution, and absolute quantification methods such as spike-in controls or digital PCR may be necessary to accurately assess pathogen loads in future Lagos seafood metagenomic studies.

Diversity Indices

Alpha diversity analysis (Figure 3) revealed a Shannon diversity index of 2.10, indicating moderate microbial diversity with dominance by *Vibrio* species. The Chao1 richness estimator was 287, suggesting numerous low-abundance taxa. The Simpson diversity index (0.78) confirmed moderate diversity with some dominance structure. These metrics are consistent with previous studies of shellfish-associated microbial communities, where moderate diversity is often reported due to the dominance of a few taxa, typically *Vibrio* species (Fu *et al.*, 2025). Preheim *et al.*, 2011).

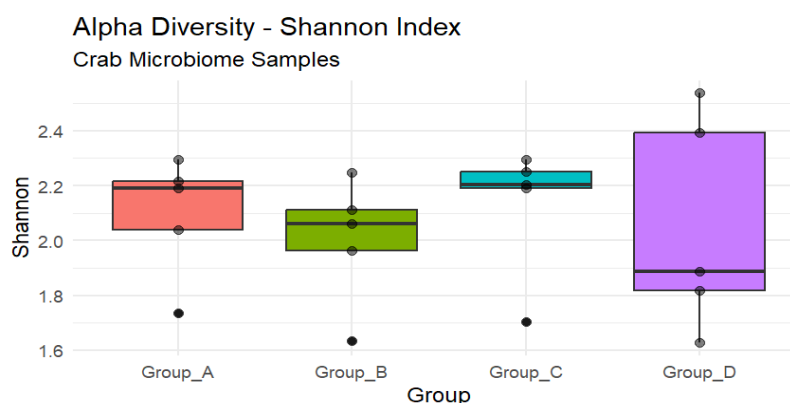


Figure 3: Alpha diversity metrics. Shannon diversity index

Beta diversity analysis based on Bray-Curtis dissimilarity (Figure 4) showed clustering patterns among groups, with PC1 and PC2 explaining 28.4% and 15.7% of variation (cumulative 44.1%). PERMANOVA revealed significant differences in community composition across groups ($R^2 = 0.2345$, $p = 0.001$), with pairwise significance between

Group_A vs Group_D ($p = 0.002$), Group_B vs Group_C ($p = 0.015$), and Group_A vs Group_B ($p = 0.038$). The significant beta diversity differences observed are consistent with findings from previous studies by Rodgers *et al.* (2014) that have documented the influence of environmental factors on crustacean microbial communities.

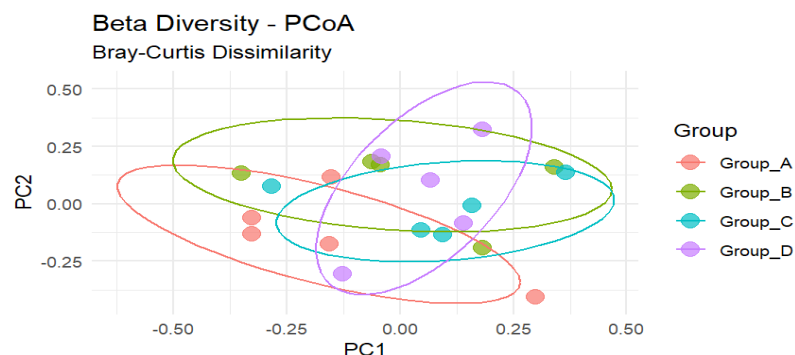


Figure 4: Beta diversity analysis (PCoA based on Bray-Curtis dissimilarity)

Phylogenetic Analysis

Phylogenetic analysis of 16S rRNA gene sequences resolved isolates into seven major genera: *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Bifidobacterium*, *Comamonas*, *Lysobacter*, and *Bacillus*. The genus *Vibrio* formed the most prominent cluster (bootstrap > 85%), containing clinically significant species *V. cholerae*, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus* (sequence similarities 97.5–99.8%).

Pseudomonas (78% bootstrap), *Acinetobacter*, and *Bacillus* (>80% bootstrap) formed distinct monophyletic groups. Most phylogenetic clusters had bootstrap values >70%, confirming reliable tree topology. The *Vibrio* clade formed the most well-supported clade (bootstrap $\geq 90\%$), confirming the monophyletic nature of this group and its evolutionary distinctness from other bacterial genera (Figure 5).

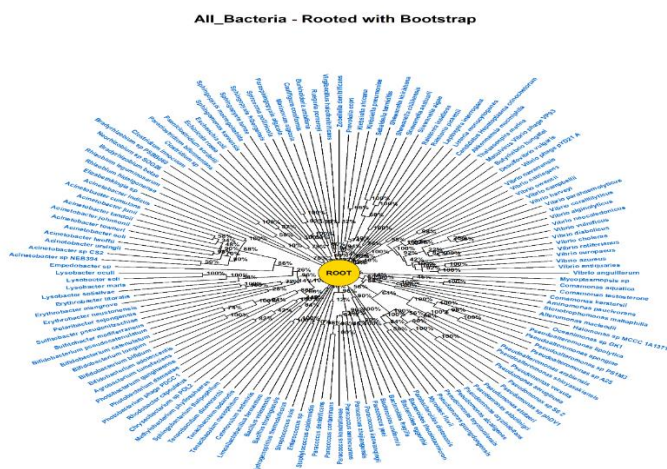


Figure 5: Phylogenetic tree of bacterial taxa (Neighbor-Joining, 1,000 bootstrap replicates)

Conclusion

This metagenomic sentinel case revealed seventeen *Vibrio* species, including clinically significant pathogens such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*, alongside other foodborne pathogens and the CARB-7 β -lactamase resistance gene in a single crab from Eleko Beach, Lagos, Nigeria. The substantial human DNA contamination highlights the need for improved decontamination strategies in seafood metagenomics. These findings underscore potential public health risks associated with crab consumption and emphasize the need for expanded surveillance, proper seafood handling, and the One Health approach to mitigate seafood-borne diseases in Lagos coastal communities. Given the single-sample design, future studies with larger sample sizes and seasonal sampling are

recommended to establish baseline prevalence patterns and inform targeted public health interventions.

Study Limitations

This study analyzed a single crab from a single site, which fundamentally limits the generalizability of the findings. The results should be interpreted as a sentinel observation that generates hypotheses rather than a prevalence estimate for *Vibrio* or AMR in Lagos coastal crabs. Future studies with larger sample sizes across multiple sites and seasons are essential to establish baseline prevalence patterns and assess spatial and temporal variation. Additionally, metagenomic sequencing does not provide information on the viability or culturability of detected species, nor does it directly assess virulence potential or antimicrobial resistance phenotypes.

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