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Original Article

Molecular Assessment of Resistance and Virulence Potential of *Vibrio* Species Isolated From Dumpsites in Port Harcourt, Nigeria

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Abstract

Background: Dumpsites have the potential to serve as reservoirs for various medically important bacteria and their virulence and resistance gene markers. For *Vibrio* spp., numerous genes associated with virulence have been identified in environmental strains. Due to the specific growth requirements of *Vibrio* spp., such strains can often be overlooked. This study aimed to assess the potential of *Vibrio* spp. isolated from two dumpsites in Port Harcourt, Nigeria, to serve as reservoirs of virulence and resistance genes.

Methods: The soil samples were evaluated for the presence of *Vibrio* spp. following enrichment, using standard microbiological and biochemical test methods. DNA from Vibrio spp. was extracted using the boiling method, and isolates were tested for the presence of four resistance (*sxt, strB, BlaTEM*, and *dfrA1*) and four virulence (*ctxA, hlyA, tcpA*, and *toxR*) genes.

Results: The study found a 40% occurrence of resistance genes and a 10% occurrence of virulence genes, with the *strB* streptomycin resistance gene being the most commonly detected (42%). Two of the virulence genes (*ctxA* and *tcpA*) were not detected. Seven of the test isolates exhibited multiple gene markers, with four gene markers present in each of two isolates.

Conclusion: Overall, the study revealed a generally low potential for *Vibrio* sp. isolated from the dumpsites in Port Harcourt, Nigeria, to act as reservoirs of virulence and resistance genes. Additionally, the study reported an absence of major virulence markers associated with *V. cholerae*. A concerning finding was the high occurrence (42%) of the *strB* gene among these environmental isolates.

Keywords: Virulence, *Vibrio*, Waste sites, Resistance genes, Nigeria, Reservoir

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Introduction

Open solid waste disposal is a cost-effective waste management technique used in resource-limited settings, such as Nigeria.[1] Dumpsites have long been recognized as potential reservoirs of various medically significant bacteria, primarily due to their role in receiving waste from diverse sources. These dumpsites often contain animal or human fecal matter.[2] The presence of human feces at dumpsites can be attributed to unsafe fecal disposal practices, such as improper disposal of infant diapers or adult feces by households without adequate toilet facilities.

Several studies have investigated the presence of various medically significant bacteria at dumpsites.[3-5] While most pathogenic bacterial strains are distinct due to the presence of specific genetic markers, environmental strains of *Vibrio* sp have been reported to carry several genes associated with virulence.[6] In addition to environmental *Vibrio* strains, pathogenic *Vibrio* species are of particular importance, as the environment serves as a reservoir for these organisms. These pathogenic *Vibrio* species can be broadly categorized into two groups: cholera-causing *Vibrio* and non-cholera-causing pathogenic *Vibrio* spp., which are responsible for a range of diseases collectively known as vibriosis. Cholera, a disease associated with poor hygiene and lower socioeconomic classes, causes significant morbidity and mortality in children under 5 years of age. The unique growth requirements of *Vibrio* sp strains often make them overlooked and underestimated as potential reservoirs of both pathogenic strains and virulence genes, especially in the context of dumpsites' characteristics.

However, the specific potential of dumpsites in Rivers State, Nigeria, has not been extensively studied. Therefore, this study aimed to investigate the resistance and virulence potential of *Vibrio* sp strains isolated from two dumpsites in Port Harcourt, Rivers State, Nigeria.

Materials and Methods

Study Area

This study was conducted at two open dumpsites within the Port Harcourt metropolis, Rivers State, between November and December 2021. The sites included the Rivers State Waste Management Authority (RIWAMA) dumpsite, located along the Port Harcourt International Airport road (GPS Location: 4° 54'12''N 6° 57'52''E), and the Alakahia dumpsite along the East/West road (GPS Location: 4° 53'14.3''N 6° 55'19.9''E).

Sample Collection

A hundred soil samples were collected, each taken one meter apart from the vicinity of each dumpsite. Surface debris was carefully removed, and the topsoil was collected using a sterile trowel to a depth of 5 cm. The soil samples were promptly placed in sterile polythene bags and transported immediately to the laboratory for processing.[7]

Bacterial Isolation and Identification

The samples were processed following the CDC's guidelines.[8] In summary, 10 g of the homogenized soil sample was initially mixed with 90 mL of alkaline peptone water (APW) to create a homogeneous suspension. Subsequently, a ten-fold dilution was prepared by mixing 1 mL of this stock with 9 mL of APW. Pre-enrichment was conducted for 6 hours at room temperature (28 °C), followed by inoculation of a loopful of the topmost part of the broth culture onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar.

The plates were then observed after 24 hours of incubation at 37 °C for characteristic *Vibrio* colonies. *Vibrio* spp typically appear as either yellow or green colonies on TCBS, with *V. cholerae* specifically exhibiting yellow colonies. These colonies were purified, and isolates were identified using Gram stain and standard biochemical tests as previously described.[9,10] Biochemical tests included the citrate test, motility test, TSIA test, indole test, urease test, catalase test, sugar fermentation tests, methyl red/Voges Proskauer test, oxidase test, and sodium chloride tolerance test.

Detection of Resistance and Virulence Genes

Following bacterial identification, DNA from select identified *Vibrio* species was extracted using the boiling method, as previously described.[11] The purity and concentration of the extracted DNA were determined using a spectrophotometer. A total of eight genes were then assayed, four of which were resistance genes and four virulence genes (Table 1).

The resistance genes assayed for convey resistance to trimethoprim (*dfrA1*), beta-lactam antibiotics (*BlaTEM*), streptomycin (*strB*), and a group of antibiotics comprised of sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin (*sxt*). The virulence genes, on the other hand, encoded haemolysin (*hlyA*), cholera toxin gene (*ctxA*), a pilus essential for colonization (*tcpA*), and a transmembrane transcription regulator (*toxR*).

A standard amplification protocol was used, which involved an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 secs, annealing (with temperatures varying from primer to primer) (Table 1), elongation at 72 °C for 45 seconds, and final extension at 72 °C for 6 minutes. All targets were amplified in separate reactions in a final volume of 20 µL. Amplification products were visualized by running on a

Table 1. *Vibrio* Virulence Genes and Primer Sequences for Polymerase Chain Reaction

Gene	Primer Name	Primer Sequence (5' to 3')	Annealing Temp (°C)	Product Size (bp)	References
		Virulence Genes			
hlyA	hlyA F	AGCGTTCATTGCAATACGG	57	509	[12]
	hlyA R	GGTCGAACGATTGAGTAGCG			
toxR	toxR F	CCTTCGATCCCCTAAGCAATAC	60	779	[13]
	toxR R	AGGGTTAGCAACGATGCGTAAG			
ctxA	*ctxA F*	CTCAGACGGGATTTGTTAGGCACG	58	301	[14]
	ctxA R	TCTATCTCTGTAGCCCCTATTACG			
tcpA	*tcpA F*	CGACACCTTGTTGGTATTTT	60	878	[14]
	tcpA R	TGACTTTGTGTGGTTAAATGT			
		Resistance Genes			
sxt	*sxt F*	ATGGCGTTATCAGTTAGCTGGC	61	1035	[15]
	sxt R	GCGAAGATCATGCATAGACC			
BlaTEM	*BlaTEM F*	TTTCGTGTCGCCCTTATTCC	59	692	[16]
	BlaTEM R	CCGGCTCCAGATTTATCAGC			
dfrA1	*dfrA1 F*	CAATGGCTGTTGGTTGGAC	57	253	[17]
	dfrA1 R	CCGGCTCGATGTCTATTGT			
strB	*strB F*	GGCACCCATAAGCGTACGCC	67	470	[15]
	strB R	TGCCGAGCACGGCGACTACC			

1% agarose gel and viewed using a UV transilluminator.

Statistical Analysis

Descriptive statistics was employed on the data obtained using Microsoft Excel and the results have been shown as percentages and frequencies.

Results and Discussion

Out of a total of 97 isolates showing characteristic colonies on the TCBS media, 61% (59) were identified as *Vibrio* species (Figure 1), with only 9% (4) of the total confirmed as *Vibrio cholerae*. *Vibrio* sp. members are recognized for their link to different gastrointestinal tract infections, with cholera standing out as the most infamous. These organisms primarily reside in the aquatic environment, spreading among individuals through diverse channels. Dumpsites, due to their characteristics, have been extensively associated with potential infection sources, facilitated either by scavengers or by seepage into adjacent water sources.[5-7,18,19]

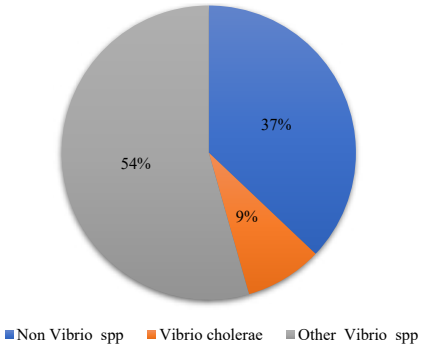

Figure 1. Percentage of Biochemically Confirmed *Vibrio* sp. and *Vibrio cholerae*

An assessment of resistance and virulence gene markers in the test isolates revealed a higher occurrence of resistance genes than virulence genes (Figure 2). A representative gel picture showing amplification of the *BlaTEM* gene is presented (Figure 3). The gene encoding resistance to streptomycin (*strB*) was the most commonly occurring (42%, 21/50), with 0% detection observed for the *ctxA* and *tcpA* virulence genes (Figure 4). A total of 32 isolates had at least one of the gene markers (Table 2).

While environmental and clinical strains of *Vibrio* species have been shown in different locales to carry various virulence markers, cholera-causing toxigenic strains of *Vibrio cholerae* often possess a unique set of markers.[6] This study reported a low potential of environmental strains of *Vibrio* species, in general, to serve as reservoirs of virulence, with a 10% occurrence of virulence genes. This finding aligns with a more recent study conducted in Nigeria on *Vibrio* isolated from dumpsites, which showed an occurrence of four virulence genes (*fliCH7, uidA, lf, stx*) at about 22.2%.[7] However, this was slightly lower than the 16% reported by a 2012 study exploring virulence genes in environmental *Vibrio* from a marine environment in Italy.[20] In contrast, a more recent study on *Vibrio* in aquatic habitats in China reported a 98.7% occurrence of virulence genes.[21] Much higher levels of virulence markers were reported by several other studies. Gxalo and colleagues reported rates of virulence markers ranging from 10% to 65.1%, while

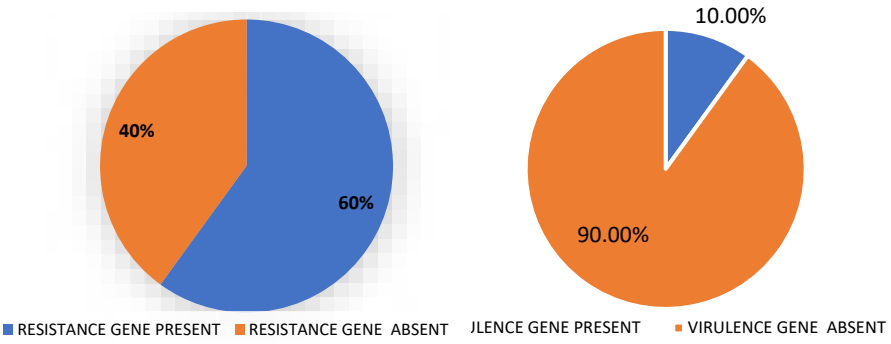

Figure 2. Occurrence of Resistance and Virulence Genes in *Vibrio* Species Isolated From Dumpsites in Port Harcourt, Nigeria

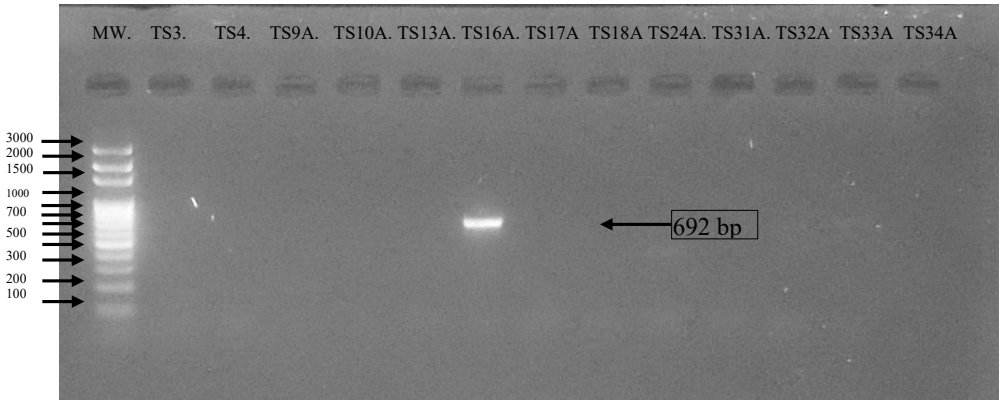

Figure 3. Representative gel Electrogram Capturing the Amplification of *BlaTEM* Gene in one of the 13 Products Assessed

Beshiru and colleagues had rates ranging from 28.6% to 95.7%.[22,23] These differences could reflect varying isolate sources, as the Beshiru and colleagues' study analyzed ready-to-eat shrimps.

Furthermore, there was a complete absence of the major *Vibrio* virulence markers of high concern in this study. Two such markers are the *ctxA* and *tcpA* genes, which encode the cholera toxin and the toxin co-regulated pilus, respectively. These two genes confer pathogenicity on *V. cholerae*, and strains lacking them are unable to cause cholera. The absence of these markers from *Vibrio* species isolated from dumpsites in this study strongly indicates the absence of cholera-causing strains of *V. cholerae* at these dumpsites. These findings contrast with those of the Omoruyi and Ojubiaja study, which reported a 5.6% occurrence of the *tcpA* gene and a 4/18 occurrence of the *ctxA* gene,[7] but are consistent with several other reports where both of these determinants were lacking in environmental strains.[24-26]

The *V. cholerae* haemolysin, encoded by the *hlyA* gene, is one of the virulence factors postulated to contribute to the pathogenicity of non-cholera *Vibrio*.[27] The occurrence of this gene, along with the regulatory *toxR* gene, in the environmental *Vibrio* isolates in this study suggests a potential, albeit low, role of these environmental strains as reservoirs of virulence genes. The *toxR* gene has been identified in *V. parahaemolyticus, V. fluvialis*, and *V. cholerae*, and is associated with the establishment of non-cholera vibriosis.[28]

A particularly concerning finding of this study was the high occurrence (42%) of the *strB* streptomycin resistance gene among these environmental isolates. Streptomycin resistance is mediated by several determinants, some of which are chromosomally borne while others are carried by mobile genetic elements. The *strB* gene is typically found linked to the *strA* gene and is associated with the Tn transposon.[29] The presence of this gene in environmental *Vibrio* species has been previously reported, with occurrence rates ranging from 25% to 78.3%.[15,22,30,31] One study with significantly different results was the recent study by Xiao conducted in China, which reported a widespread lack of antibiotic-resistant genes in the test isolates, with only 3 out of 78 isolates possessing the genes tested for.[21] The results of the present study could suggest a misuse of streptomycin in this locale and underscore the potential role of environmental *Vibrio* as reservoirs of antibiotic resistance genes.

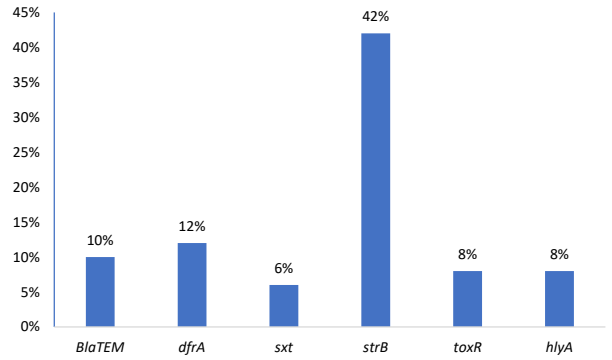

Figure 4. Percentage Occurrence of Resistance and Virulence Genes Among *Vibrio* Species Isolated From Dumpsites in Port Harcourt, Nigeria

Table 2. Distribution of Test Genes Among the *Vibrio* spp

Test Gene	Isolates With Gene
BlaTEM	TS16A, TS38C, SS11C, SS31B, SS35C,
dfrA	TS9A, TS17A, TS18A, TS32A, TS34B, SS8,
sxt	TS16A, SS9C, SS32A,
strB	TS4, TS10A, TS10D, TS29B, TS31B, TS33C, SS3, SS4, SS8, SS9B, SS9C, SS10C, SS29B, SS31A, SS31B, SS32A, SS33A, SS33B, SS34C, SS35B, SS38A
toxR	TS31A, TS31B, SS31B, SS32A
hlyA	TS31A, TS32B, SS31B, SS32A
ctxA	-
tcpA	-

Isolates With Multiple Gene Markers

Of the 32 test isolates associated with the gene markers, seven exhibited multiple gene markers (Table 3). Particularly notable were isolates SS31B and SS32A, each carrying four of the gene markers. An assessment of the antibiotic susceptibility profile of these isolates, determined using the Kirby Bauer disc diffusion method (Table 4), revealed that two of these isolates (TS31B and SS9C) were multidrug-resistant, demonstrating resistance to three or more drug classes.

Table 3. Co-occurrence of Gene Markers in Test Isolates

Isolate ID	Markers Present
TS16A	*BlaTEM-sxt*
TS31A	*hlyA-toxR*
TS31B	*strB-toxR*
SS8	*dfrA-strB*
SS9C	*strB-sxt*
SS31B	*BlaTEM-hlyA-strB-toxR*
SS32A	*hlyA-strB-sxt-toxR*

Table 4. Antibiogram of *Vibrio* sp. With Multiple Gene Makers Isolated From Dumpsites in Rivers State

Isolate ID	Antibiogram
TS16A	CXM
TS31A	AUG-CAZ-CRX-CXM
TS31B	AUG-CAZ-CRX-CXM-NIT
SS8	AUG-CAZ-CRX-CXM
SS9C	ACX-AUG-CRO-CTX-CXM-GEN-IMP-LBC-NA-NIT
SS31B	AUG
SS32A	-

Study Limitations

The study's scope was restricted regarding the range of virulence and resistance determinants examined, as only a specific subset of these determinants was investigated. Furthermore, the data collected here is derived solely from two out of the many dumpsites in the city.

Conclusion

Despite the detection of four resistance and two virulence genes from *Vibrio* sp. isolated from two dumpsites in Port Harcourt, Nigeria, this study reported a generally low potential of these specific isolates to act as reservoirs of virulence and resistance genes. Additionally, the study noted an absence of major *Vibrio* virulence markers associated with *V. cholerae*. However, a particularly concerning finding of this study was the high occurrence (42%) of the *strB* streptomycin resistance gene among these environmental isolates. Given its association with a mobile genetic element, this suggests that these dumpsites could serve as locations for the acquisition of this resistant gene.

Authors' Contribution

Conceptualization: Kome Otokunefor, Obakpororo Ejiro Agbagwa.
Data curation: Precious Chijindu Nwankwo, Kemuel Chinonye Nyema.
Formal analysis: Precious Chijindu Nwankwo, Kemuel Chinonye Nyema.
Funding acquisition: Kome Otokunefor, Precious Chijindu Nwankwo, Kemuel Chinonye Nyema, Obakpororo Ejiro Agbagwa.
Investigation: Precious Chijindu Nwankwo, Kemuel Chinonye Nyema.
Methodology: Kome Otokunefor.
Project administration: Kome Otokunefor, Obakpororo Ejiro Agbagwa.
Resources: Kome Otokunefor, Precious Chijindu Nwankwo, Kemuel Chinonye Nyema.
Software: Kome Otokunefor, Precious Chijindu Nwankwo.
Supervision: Kome Otokunefor.
Validation: Obakpororo Ejiro Agbagwa.
Visualization: Kome Otokunefor.
Writing–original draft: Kome Otokunefor, Precious Chijindu Nwankwo, Kemuel Chinonye Nyema, Obakpororo Ejiro Agbagwa.
Writing–review & editing: Kome Otokunefor.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

Ethical approval was not needed as no human subjects or samples were used in this study.

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