



# MICROBIOLOGICAL ASSESSMENT AND ANTIBACTERIAL SUSCEPTIBILITY PROFILE OF *VIBRIO* SPECIES FROM SELECTED SURFACE WATER IN ABALALI KI LOCAL GOVERNMENT AREA, EBONYI STATE, SOUTH-EAST, NIGERIA

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## ABSTRACT

*Vibrio species has been associated with infection of epidemic proportion. This is further compounded with reports of multiple antibiotics resistance in Vibrio species. This study aims at isolating and identifying the pathogenic Vibrio species from selected surface water in Abakaliki. Three water samples were collected from three selected surface water sources. The temperature and pH were measured insitu. Twenty-four putative vibrio isolates were obtained from Thiosulfate Citrate Bile Salt Agar after a 24hrs growth in alkaline peptone water and subsequent tenfold dilution and identified using some specified biochemical test and further characterized based on susceptibility to twelve antibiotics. Isolates presumptive to be Vibrio parahaemolyticus was higher (8 (33.3%)) compared to V. mimicus (7 (29.17%)), V. alginolyticus (3 (25%)), V. vulnificus (2 (8.33%)) and V. cholerae (1 (4.17%)). Most Vibrio species isolated from all surface water samples had highest susceptibility to Gentamycin 21 (87.5%) with the least occurring in Ciprofloxacin, 3 (12.5%) while high resistance occurred in Amoxicillin Clavulanic acid, Cefepime, Cotrimixazole, Meropenem and Tetracycline all having a 24 (100%) while the least was observed in Gentamycin with a frequency of 3 (12.5%). Findings of the Multiple Antibiotic resistance index which ranged from 0.60 to 0.90 indicates that the vibrio isolates originate from an environment with high contamination or antibiotics usage. This study shows the increase in multi-antibiotics resistance of Vibrio isolates in the wastewater sample rendering it polluted and unsafe for use.*

**Keywords:** *Vibrio species; Antibiotic resistance; Surface water; Multidrug resistance; Water pollution*

## 1.0 Introduction

Water is essential for life; however, pollution of surface-water bodies can reduce aquatic biodiversity and pose serious risks to human, animal, and environmental health (Igwe et al., 2017). Anthropogenic activities, including agriculture, mining, domestic waste disposal, urbanization, and the discharge of untreated wastewater, alter the physicochemical properties and microbial quality of water (Onyegeme & Ogunka, 2015). Population growth also increases pressure on water resources because rivers are often used simultaneously for domestic

purposes, waste disposal, farming, and other activities. Runoff containing poultry manure, fertilizers, sewage, and other contaminants may therefore introduce pathogenic microorganisms into surface-water bodies. In addition, drainage channels and the natural slope of urban landscapes can facilitate the movement of wastewater and pollutants into rivers (Igwe et al., 2017; Olorode et al., 2015).

The availability of safe and accessible drinking water is closely associated with human health. Water contaminated with human or animal faeces may contain microorganisms capable of causing outbreaks of waterborne diseases. Among these organisms are pathogenic *Vibrio* species, which are commonly associated with aquatic environments. *Vibrio cholerae* is a Gram-negative bacterium belonging to the family *Vibrionaceae* and is the principal causative agent of cholera. Although more than 200 serogroups of *V. cholerae* have been identified, serogroups O1 and O139 are mainly associated with epidemic cholera, whereas non-O1/non-O139 strains may cause sporadic or localized cholera-like disease (Bulus et al., 2015). Because water serves as an important reservoir and transmission route, monitoring *Vibrio* species in water sources is essential.

Pathogenic *vibrio* species may be transmitted through the consumption of contaminated water or undercooked seafood and, in some cases, through direct contact. Infections may range from mild, self-limiting gastroenteritis to severe conditions such as septicaemia, wound infection, and necrotizing fasciitis. Although *V. cholerae* and *V. parahaemolyticus* are among the most important human pathogens, other species, including *V. alginolyticus*, *V. mimicus*, *V. vulnificus*, *V. fluvialis*, *V. harveyi*, *V. anguillarum*, and *V. metschnikovii*, have also been reported as emerging pathogens, particularly in aquatic and marine environments (Gennari et al., 2012; Cazorla, 2011; Newton, 2012).

Antimicrobial agents remain important in the management of bacterial infections. Drugs such as doxycycline and azithromycin have been used in the treatment of selected *Vibrio* infections. However, the increasing occurrence of antimicrobial resistance among bacterial pathogens threatens effective therapy and may result in treatment failure, prolonged illness, increased morbidity, and mortality (Mpho et al., 2020; Sack et al., 2004). Monitoring the antimicrobial susceptibility of *Vibrio* isolates is therefore necessary for guiding treatment and detecting resistant strains. Therefore, this study focuses on isolating, identifying, and characterizing pathogenic *Vibrio* species from selected surface waters in Abakaliki, South-Eastern Nigeria, and determining their antibiotic susceptibility patterns. The findings will help assess the microbiological quality of the water, identify potential public-health risks, and support measures for preventing *Vibrio*-associated infections in this area.

## 2. Materials and Method

The materials and equipment used in the study included thiosulfate-citrate-bile salts-sucrose (TCBS) agar, nutrient agar, triple sugar iron (TSI) agar, alkaline peptone water, sodium deoxycholate, bile salts, sterile swab sticks, Petri dishes, McCartney bottles, microscope slides, moist filter paper, and antibiotic discs. The reagents used for microbiological characterization included Gram-staining reagents, Kovac's reagent, indole spot reagents, oxidase reagent, and 3% hydrogen peroxide. Absolute ethanol was used for laboratory disinfection and other routine procedures. Analytical equipment comprised a multiparameter ion-specific meter and a turbidity meter, while appropriate data-analysis software was used for statistical analysis of the experimental data.

## 3. Description of Study Site

Abakaliki is the capital city of Ebonyi State in South-Eastern Nigeria, located 64km (40mi) south-east of Enugu. The wet season is warm, and the dry season is hot, muggy and partly cloudy. Over the course of the year, the temperature typically varies from 65°F to 89°F and is rarely below 58°F or above 92°F.

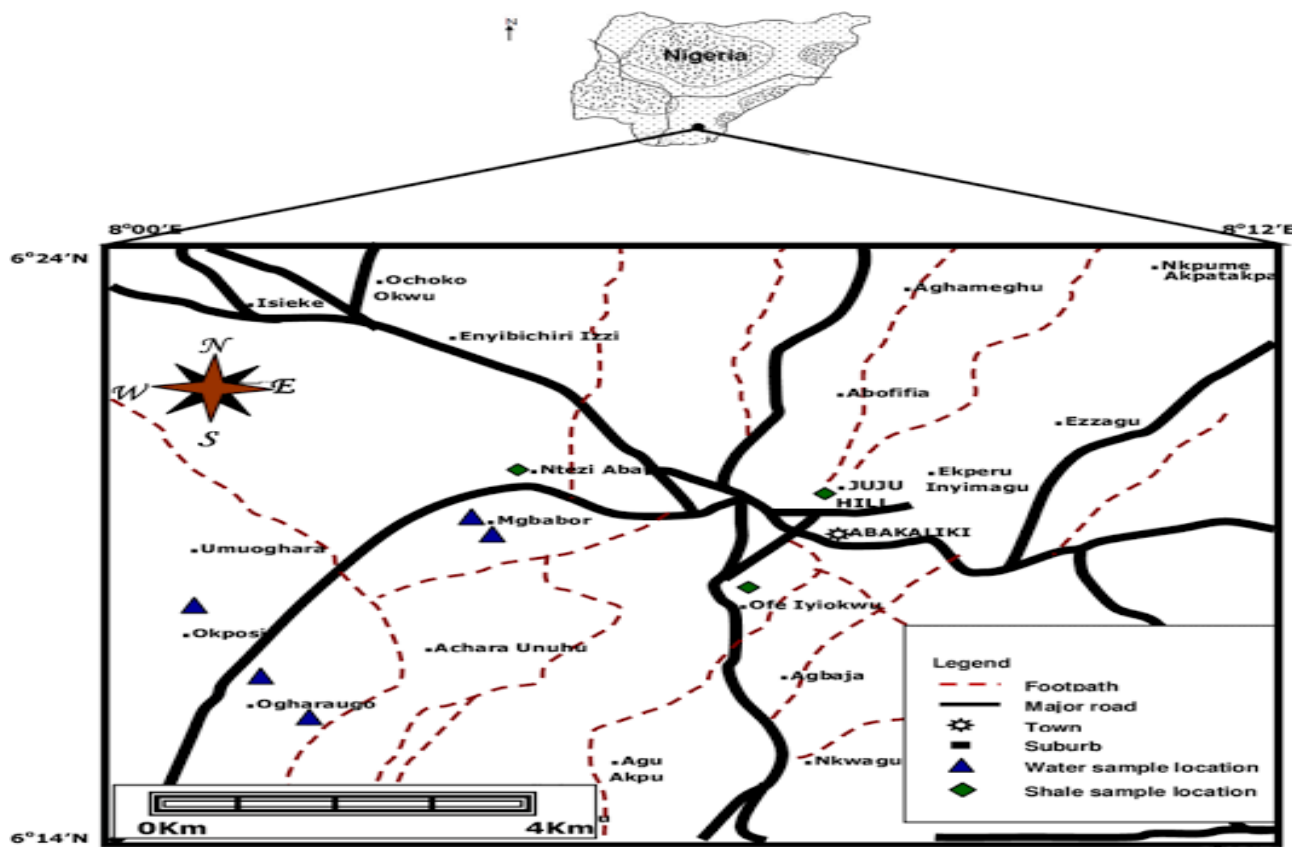


Figure 1: Map of Abakaliki Local Government Area

### Sample Collection

Four water samples from three selected rivers in Abakaliki local government area, state (two samples from Azuiyioku, one sample from Onuebonyi). The water samples was collected aseptically using a sterile 500ml container 15m below the surface of the selected water sources in the direction of flow. The lid from the sample container will be removed, and without touching the inside of the bottle or lid, and closed tightly after which the sample collection to prevent leakage before transporting in an ice box to the microbiological laboratory of Alex Ekwueme Federal University Ndufu-Alike Ikwo, for immediate analysis within 3-5 hours of collection.

### Physicochemical Analysis

Two physicochemical parameters, temperature and pH, were measured at the respective sampling sites immediately after water sample collection. All meters and other measuring equipment were inspected and calibrated in accordance with the manufacturers' specifications before use to minimize measurement errors and ensure the reliability of the results.

### Sample Preparation and Enumeration of *Vibrio* Species

Water samples were examined for the presence and enumeration of presumptive pathogenic *Vibrio* species. 1 mL of each water sample was aseptically inoculated into 9 mL of alkaline peptone water (APW) enrichment broth. The inoculated broths were incubated at 37°C for the specified enrichment period to facilitate the recovery and growth of *Vibrio* species. Following enrichment, serial tenfold dilutions of each culture were prepared, and 0.2 mL from a dilution factor of  $10^{-5}$  of the appropriate dilution was inoculated onto Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar using the spread-plate technique in triplicate. The inoculated plates were incubated at 37°C for 24 hours. After incubation, colonies exhibiting morphological characteristics were examined and enumerated. The presumptive *Vibrio* colonies were subsequently subcultured onto sterile nutrient agar (Oxoid) to obtain pure

cultures. A representative pure colony from each sample was selected and maintained on nutrient agar slants for subsequent identification and characterization. Colony counts were expressed as colony-forming units per millilitre (CFU/mL).

### **Identification of Bacterial Isolates**

The pure isolates which are the representative of each plate were cultured on nutrient agar slants and stored at 4°C. From the stock culture, each representative isolate was examined biochemically by screening for String test, Triple sugar iron test, Oxidase reaction, Gram staining, H<sub>2</sub>S, Hemolysis, Indole, Catalase reaction and salt tolerance using appropriate processes (AL-Fatlawy, 2013a; AL-Fatlawy, 2017b).

### **Gram Staining**

Gram reaction was performed on the overnight growth of each bacterial isolate on nutrient agar. A loop-full of culture was picked up using a sterile inoculating loop and applied on clean slides to form a thin layer, forming a smear. The slide will be left to air dry before being heat-fixed by passing it over a Bunsen burner flame. After being drenched with crystal violet for 60 seconds, the slide was rinsed with running water and then flooded with Lugol's iodine for 60 seconds to fix it with mordant. The slide was decolorized with a 50 percent acetone and 50 percent ethanol solution for 15 seconds, and then it was rinsed with water. The slide is then counter-stained with safranin, left for 60 seconds, and then rinsed off with running water. The slides will be left to air dry before being seen under a light microscope equipped with a ×100 objective lens (oil immersion).

### **Catalase Test**

A loop full of the microbe was emulsified with a loop full of a drop of 3% v/v hydrogen peroxide solution on a sterilized glass slide. The presence of effervescent gas bubbles indicates a positive result, whereas the lack of bubbles indicates a negative result (Hafezi & Khamar, 2024).

### **Coagulase Test**

The coagulase test was performed using previously prepared plasma to determine the ability of the bacterial isolates to produce coagulase. A drop of plasma was placed on a clean glass slide using a sterile dropper. A portion of a 24 hour bacterial culture was aseptically transferred to the plasma with a sterile wire loop and emulsified thoroughly. The mixture was observed for approximately 15 seconds for visible agglutination or clumping. The formation of distinct clumps was interpreted as a positive coagulase reaction, whereas the absence of clumping indicated a negative reaction.

### **Oxidase Test**

The reagent is made by dissolving 0.1g in 10ml of distilled water. The test organisms were picked up with a sterile stick onto filter paper soaked in 1% oxidase reagent (diamine hydrochlorine). After then, the organisms were observed for the development of a bright blue hue within ten seconds, which signifies a favorable response (Hafezi & Khamar, 2024).

### **Indole Test**

After pipetting five milliliters of peptone water broth into test tubes and properly corking them, the broth was autoclaved for 15 minutes at 15 psi to sterilize it. It was allowed to cool to 40°C before the test organism was added and allowed to grow for 72 hours. Kovac's reagent was then added in three drops to the culture, and it was left to stand for 30 minutes. Red development at the surface layer is a sign of a positive response (Hafezi & Khamar, 2024).

### Citrate Utilization Test

The test, which measures an organism's ability to use citrate as its only carbon source, was conducted by inoculating the test organism on Simon citrate agar slopes for 48 hours at 37 °C. Citrate is greenish, thus when it turns blue, it indicates a positive result (Hafezi & Khamar, 2024).

### Urease Test

The urease test was performed to determine the ability of the bacterial isolates to hydrolyse urea. A heavy inoculum obtained from a 24-h-old pure culture was aseptically used to streak the entire surface of the urea agar slant. The inoculated tubes were incubated at 37°C for 24 h. Following incubation, the slants were examined for a visible colour change. A change in the medium from its original colour to pink or red was interpreted as a positive urease reaction, whereas the absence of colour change indicated a negative reaction.

### Salt Tolerance Test

Salt tolerance was assessed to determine the ability of the bacterial isolates to grow under elevated sodium chloride (NaCl) concentrations. A single colony from a 24 hours old pure culture was aseptically inoculated into 6% NaCl peptone water and incubated at 37°C. The cultures were examined for visible turbidity after 24 hour and, where no growth was observed, re-examined at 48 hours and 72 hours. The development of turbidity was interpreted as evidence of bacterial growth and, consequently, tolerance to the tested NaCl concentration, whereas the absence of turbidity indicated inability to grow under the test conditions.

### Antibiotic Susceptibility Testing

The antimicrobial susceptibility profiles of the confirmed *Vibrio* isolates were determined using the Kirby-Bauer disc diffusion method in accordance with the applicable Clinical and Laboratory Standards Institute (CLSI) guidelines (Kirby et al., 1996; CLSI, 2014). A fresh bacterial suspension was prepared for each pure isolate in sterile 0.8% (w/v) normal saline and homogenized using a vortex mixer. The turbidity of each suspension was adjusted to a 0.5 McFarland standard to obtain a standardized inoculum. An aliquot (100 µL) of the standardized suspension was evenly inoculated onto the surface of Mueller–Hinton agar using the spread-plate technique. Commercially prepared antimicrobial discs were aseptically placed on the inoculated agar surface at appropriate distances to prevent overlapping inhibition zones. The antimicrobial agents tested were gentamicin (CN, 10 µg), amoxicillin-clavulanic acid (AMC, 30 µg), meropenem (MEM, 10 µg), ceftriaxone (CRO, 30 µg), aztreonam (ATM, 30 µg), and cefepime (FEP, 30 µg). The plates were incubated at 37°C for 24 h, after which the diameters of the zones of inhibition were measured in millimetres. The results were interpreted according to the applicable CLSI breakpoints and reported as susceptible, intermediate, or resistant, where interpretive criteria were available.

### Antibiotic Resistance Index, Multiple Antibiotic Resistance Index and Multiple Antibiotic Resistance Phenotype

The Antibiotic Resistance Index (ARI) was determined as the proportion of antimicrobial agents to which each bacterial isolate exhibited resistance relative to the total number of antimicrobial agents tested. The ARI was calculated using the following formula:

$$ARI = a/b$$

Where **a** represents the number of antimicrobial agents to which the isolate was resistant and **b** represents the total number of antimicrobial agents tested.

The Multiple Antibiotic Resistance Index (MARI) was calculated using the method described by Krumperman (1983):

$$MARI = x/y$$

Where **x** represents the number of antimicrobial agents to which an isolate was resistant and **y** represents the total number of antimicrobial agents tested.

The Multiple Antibiotic Resistance Phenotype (MARP) was expressed as the specific combination of antimicrobial agents to which an isolate exhibited resistance.

## 4. Results

### 4.1: Physicochemical parameters of selected surface water samples

The physicochemical characteristics of the selected surface water samples are presented in Table 1. Sampling was conducted between 11:06 am and 1:15 pm. Water temperature ranged from 27.3°C to 38.7°C, with the highest value recorded at Azuiyokwu upstream (38.7°C) and the lowest at Onuebonyi (27.3°C). The pH values were relatively similar across the sampling locations, ranging from 6.29 to 6.34, indicating slightly acidic to near-neutral conditions. Azuiyokwu upstream recorded a pH of 6.29, while Onuebonyi and Azuiyokwu downstream recorded 6.34 and 6.33, respectively.

**Table1: Physicochemical parameters of selected surface water samples**

| Location                | Time of collection | Temperature | pH   |
|-------------------------|--------------------|-------------|------|
| Onuebonyi               | 11:06am            | 27.3°C      | 6.34 |
| Azuiyokwu<br>upstream   | 12:57pm            | 38.7°C      | 6.29 |
| Azuiyokwu<br>downstream | 01:15pm            | 38.5°C      | 6.33 |

### 4.2: Total *Vibrio* Counts in Surface Water Samples from Selected Sampling Locations in Abakaliki, Ebonyi State

The total *Vibrio* counts varied across the three sampling locations, ranging from  $1.06 \times 10^8$  to  $1.52 \times 10^8$  CFU/mL. The highest count was recorded at Azuiyokwu downstream ( $1.52 \times 10^8 \pm 8.78 \times 10^6$  CFU/mL), followed by Azuiyokwu upstream ( $1.36 \times 10^8 \pm 1.89 \times 10^7$  CFU/mL), while Onuebonyi recorded the lowest count ( $1.06 \times 10^8 \pm 2.91 \times 10^7$  CFU/mL). However, the superscript notation indicates that the differences among the sampling locations were not statistically significant ( $p > 0.05$ ).

**Table 2: counts of *Vibrio* isolates from Abakaliki surface water samples.**

| Sampling location    | Total <i>Vibrio</i> species count in (CFU/mL).       |
|----------------------|------------------------------------------------------|
| Azuiyokwu upstream   | $1.36 \times 10^8 \pm 1.89 \times 10^7$ <sup>a</sup> |
| Azuiyokwu downstream | $1.52 \times 10^8 \pm 8.78 \times 10^6$ <sup>a</sup> |
| Onuebonyi            | $1.06 \times 10^8 \pm 2.91 \times 10^7$ <sup>a</sup> |

Values are mean  $\pm$  SD (CFU/mL). Means sharing the same superscript letter are not significantly different at  $p > 0.05$ .

### 4.3: Biochemical Identification

**Table 3: Morphological color and biochemical characteristics of vibrio isolates from Azuiyokwu upstream**

| Isolate | Colony colour | Gram reaction | Citrate | Indole | Oxidase | Catalase | Salt tolerance | Presumptive organism       |
|---------|---------------|---------------|---------|--------|---------|----------|----------------|----------------------------|
| AZU1    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZU2    | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>          |
| AZU3    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZU4    | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>          |
| AZU5    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZU6    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZU7    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZU8    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |

**Legend: AZU:** Azuiyokwu upstream, +: Positive, -: Negative

**Table 4: Morphological color and biochemical characteristics of *Vibrio* isolates from Azuiyokwu downstream**

| Isolate | Colony colour | Gram reaction | Citrate | Indole | Oxidase | Catalase | Salt tolerance | Presumptive organism       |
|---------|---------------|---------------|---------|--------|---------|----------|----------------|----------------------------|
| AZD1    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZD2    | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i>    |
| AZD3    | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i>    |
| AZD4    | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>          |
| AZD5    | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>          |
| AZD6    | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>          |
| AZD7    | Green         | –             | +       | +      | +       | +        | +              | <i>V. parahaemolyticus</i> |
| AZD8    | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i>    |

**Legend: AZD:** Azuiyokwu downstream, +: Positive, -: Negative

**Table 5: Morphological color and biochemical characteristics of *Vibrio* isolates from Onuebonyi**

| Isolate | Colony colour | Gram reaction | Citrate | Indole | Oxidase | Catalase | Salt tolerance | Presumptive organism    |
|---------|---------------|---------------|---------|--------|---------|----------|----------------|-------------------------|
| OE1     | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i> |
| OE2     | Green         | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>       |
| OE3     | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. mimicus</i>       |
| OE4     | Yellow        | –             | +       | +      | +       | +        | +              | <i>V. cholerae</i>      |
| OE5     | Yellow        | –             | +       | +      | –       | +        | –              | <i>V. vulnificus</i>    |
| OE6     | Yellow        | –             | +       | +      | –       | +        | –              | <i>V. vulnificus</i>    |
| OE7     | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i> |
| OE8     | Yellow        | –             | +       | +      | –       | +        | +              | <i>V. alginolyticus</i> |

**Legend:** OE: Onuebonyi, +: Positive, -: Negative

#### 4.4: Antibiotic Susceptibility Profiles of *Vibrio* Isolates from Surface Water Samples

The antibiotic susceptibility profiles of *Vibrio* isolates from the three sampling locations are presented in Tables 6-8. The isolates exhibited high levels of resistance to several of the tested antibiotics. At Azuiyokwu upstream, all isolates were susceptible to aztreonam and gentamicin but resistant to amoxicillin–clavulanic acid, cefepime, meropenem, co-trimoxazole, and tetracycline. At Azuiyokwu downstream, all isolates were resistant to amoxicillin–clavulanic acid, cefepime, meropenem, ciprofloxacin, erythromycin, co-trimoxazole, and tetracycline, while gentamicin retained activity against five of the eight isolates. At Onuebonyi, all isolates were resistant to amoxicillin–clavulanic acid, ceftriaxone, cefepime, meropenem, ciprofloxacin, co-trimoxazole, and tetracycline, whereas all eight isolates remained susceptible to gentamicin.

**Table 6: Antibiotic susceptibility testing of *Vibrio* spp from Azuiyokwu upstream**

| Isolate | ATM | AMC | CN | CRO | FEP | MEM | CIP | E | COT | TE |
|---------|-----|-----|----|-----|-----|-----|-----|---|-----|----|
| AZU1    | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZU2    | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZU3    | S   | R   | S  | I   | R   | R   | S   | I | R   | R  |
| AZU4    | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZU5    | S   | R   | S  | I   | R   | R   | R   | R | R   | R  |
| AZU6    | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZU7    | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZU8    | S   | R   | S  | I   | R   | R   | R   | R | R   | R  |

**Legend:** AZU = Azuiyokwu upstream; S = susceptible; I = intermediate; R = resistant. ATM = aztreonam; AMC = amoxicillin–clavulanic acid; CN = gentamicin; CRO = ceftriaxone; FEP = cefepime; MEM = meropenem; CIP = ciprofloxacin; E = erythromycin; COT = co-trimoxazole; TE = tetracycline.

**Table 7: Antibiotic susceptibility testing of *Vibrio* spp from Azuiokwu downstream**

| Isolate | ATM | AMC | CN | CRO | FEP | MEM | CIP | E | COT | TE |
|---------|-----|-----|----|-----|-----|-----|-----|---|-----|----|
| AZD1    | I   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZD2    | I   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZD3    | S   | R   | S  | I   | R   | R   | R   | R | R   | R  |
| AZD4    | I   | R   | R  | R   | R   | R   | R   | R | R   | R  |
| AZD5    | I   | R   | R  | R   | R   | R   | R   | R | R   | R  |
| AZD6    | I   | R   | R  | R   | R   | R   | R   | R | R   | R  |
| AZD7    | I   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| AZD8    | S   | R   | S  | I   | R   | R   | R   | R | R   | R  |

**Legend:** AZU = Azuiyokwu upstream; S = susceptible; I = intermediate; R = resistant. ATM = aztreonam; AMC = amoxicillin–clavulanic acid; CN = gentamicin; CRO = ceftriaxone; FEP = cefepime; MEM = meropenem; CIP = ciprofloxacin; E = erythromycin; COT = co-trimoxazole; TE = tetracycline.

**Table 8: Antibiotic susceptibility testing of *Vibrio* spp from Onuebonyi**

| Isolate | ATM | AMC | CN | CRO | FEP | MEM | CIP | E | COT | TE |
|---------|-----|-----|----|-----|-----|-----|-----|---|-----|----|
| OE1     | R   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| OE2     | I   | R   | S  | R   | R   | R   | R   | S | R   | R  |
| OE3     | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| OE4     | I   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| OE5     | S   | R   | S  | R   | R   | R   | R   | S | R   | R  |
| OE6     | R   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| OE7     | R   | R   | S  | R   | R   | R   | R   | R | R   | R  |
| OE8     | S   | R   | S  | R   | R   | R   | R   | R | R   | R  |

**Legend:** AZU = Azuiyokwu upstream; S = susceptible; I = intermediate; R = resistant. ATM = aztreonam; AMC = amoxicillin–clavulanic acid; CN = gentamicin; CRO = ceftriaxone; FEP = cefepime; MEM = meropenem; CIP = ciprofloxacin; E = erythromycin; COT = co-trimoxazole; TE = tetracycline.

#### 4.5: Antibiotic Susceptibility Profile of *Vibrio* Isolates Recovered From Surface Water Samples.

The 24 *Vibrio* isolates demonstrated marked resistance to several of the antibiotics tested. Complete resistance (100%) was observed for amoxicillin–clavulanic acid, cefepime, meropenem, co-trimoxazole, and tetracycline. High resistance was also observed to erythromycin (95.8%), ciprofloxacin (87.5%), and ceftriaxone (79.2%). In contrast, gentamicin exhibited the highest susceptibility rate (87.5%), followed by aztreonam (54.2%). Ceftriaxone had no susceptible isolates, with 79.2% classified as resistant and 20.8% as intermediate.

**Table 9: Antibiotic Susceptibility Profile of *Vibrio* Isolates Recovered From Surface Water Samples.**

| Antibiotic                  | Disc code | Potency (µg) | Resistant, n (%) | Intermediate, n (%) | Susceptible, n (%) |
|-----------------------------|-----------|--------------|------------------|---------------------|--------------------|
| Aztreonam                   | ATM       | 30           | 3 (12.5)         | 8 (33.3)            | 13 (54.2)          |
| Amoxicillin–clavulanic acid | AMC       | 30           | 24 (100)         | 0 (0)               | 0 (0)              |
| Gentamicin                  | CN        | 10           | 3 (12.5)         | 0 (0)               | 21 (87.5)          |
| Ceftriaxone                 | CRO       | 30           | 19 (79.2)        | 5 (20.8)            | 0 (0)              |

|                       |     |    |           |         |          |
|-----------------------|-----|----|-----------|---------|----------|
| <b>Cefepime</b>       | FEP | 30 | 24 (100)  | 0 (0)   | 0 (0)    |
| <b>Meropenem</b>      | MEM | 30 | 24 (100)  | 0 (0)   | 0 (0)    |
| <b>Ciprofloxacin</b>  | CIP | 10 | 21 (87.5) | 0 (0)   | 3 (12.5) |
| <b>Erythromycin</b>   | E   | 15 | 23 (95.8) | 1 (4.2) | 0 (0)    |
| <b>Co-trimoxazole</b> | COT | 30 | 24 (100)  | 0 (0)   | 0 (0)    |
| <b>Tetracycline</b>   | TE  | 30 | 24 (100)  | 0 (0)   | 0 (0)    |

**Legend:** **ATM** = aztreonam; **AMC** = amoxicillin–clavulanic acid; **CN** = gentamicin; **CRO** = ceftriaxone; **FEP** = cefepime; **MEM** = meropenem; **CIP** = ciprofloxacin; **E** = erythromycin; **COT** = co-trimoxazole; **TE** = tetracycline.

#### 4.6: Antibiotics Resistance Index and Multiple Antibiotic Resistance Index

The Antibiotic Resistance Index (ARI) and Multiple Antibiotic Resistance Index (MARI) showed substantial multidrug resistance among the *Vibrio* isolates. ARI values ranged from 0.07-0.10 in Azuiyokwu upstream, 0.09-0.11 in Azuiyokwu downstream, and 0.09-0.11 in Onuebonyi. MARI values ranged from 0.6-0.8, 0.7-0.9, and 0.7-0.9, respectively. The highest MARI (0.9) was recorded in AZD4, AZD5, AZD6, OE1, OE6, and OE7, whereas the lowest (0.6) occurred in AZU3. The findings indicate widespread multiple antibiotic resistance among the *Vibrio* isolates across the three sampling locations.

**Table 10: Antibiotics Resistance Index and Multiple Antibiotic Resistance Index**

| <b>Sample A:</b>    |            |             | <b>Sample B:</b>    |            |             | <b>Sample C:</b>    |            |             |
|---------------------|------------|-------------|---------------------|------------|-------------|---------------------|------------|-------------|
| <b>Azuiyokwu</b>    |            |             | <b>Azuiyokwu</b>    |            |             | <b>Onuebonyi</b>    |            |             |
| <b>upstream</b>     |            |             | <b>downstream</b>   |            |             |                     |            |             |
| <b>Isolate code</b> | <b>ARI</b> | <b>MARI</b> | <b>Isolate code</b> | <b>ARI</b> | <b>MARI</b> | <b>Isolate code</b> | <b>ARI</b> | <b>MARI</b> |
| <b>AZU1</b>         | 0.10       | 0.8         | <b>AZD1</b>         | 0.10       | 0.8         | <b>OE1</b>          | 0.11       | 0.9         |
| <b>AZU2</b>         | 0.10       | 0.8         | <b>AZD2</b>         | 0.10       | 0.8         | <b>OE2</b>          | 0.09       | 0.7         |
| <b>AZU3</b>         | 0.07       | 0.6         | <b>AZD3</b>         | 0.09       | 0.7         | <b>OE3</b>          | 0.10       | 0.8         |
| <b>AZU4</b>         | 0.10       | 0.8         | <b>AZD4</b>         | 0.11       | 0.9         | <b>OE4</b>          | 0.10       | 0.8         |
| <b>AZU5</b>         | 0.09       | 0.7         | <b>AZD5</b>         | 0.11       | 0.9         | <b>OE5</b>          | 0.09       | 0.7         |
| <b>AZU6</b>         | 0.09       | 0.7         | <b>AZD6</b>         | 0.11       | 0.9         | <b>OE6</b>          | 0.11       | 0.9         |
| <b>AZU7</b>         | 0.10       | 0.8         | <b>AZD7</b>         | 0.10       | 0.8         | <b>OE7</b>          | 0.11       | 0.9         |
| <b>AZU8</b>         | 0.09       | 0.7         | <b>AZD8</b>         | 0.09       | 0.7         | <b>OE8</b>          | 0.10       | 0.8         |

#### 4.7: Multiple Antibiotic Resistance Phenotypes (MARP)

The *Vibrio* isolates exhibited multiple antibiotic resistance phenotypes involving resistance to 6–9 antibiotics. The most extensive resistance patterns involved nine antibiotics, including AMC/CN/CRO/FEP/MEM/CIP/E/COT/TE and ATM/AMC/CRO/FEP/MEM/CIP/E/COT/TE. These extensive resistance phenotypes were observed among isolates from both Azuiyokwu downstream and Onuebonyi, indicating widespread multidrug resistance among the isolated *Vibrio* isolates.

**Table 11. Multiple antibiotic resistance patterns of *Vibrio* isolates from selected surface-water sampling locations**

| Sampling location           | Isolate code | Resistance pattern               |
|-----------------------------|--------------|----------------------------------|
| <b>Azuiyokwu upstream</b>   | AZU1         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZU2         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZU3         | AMC/FEP/MEM/CIP/COT/TE           |
|                             | AZU4         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZU5         | AMC/FEP/MEM/CIP/E/COT/TE         |
|                             | AZU6         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZU7         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZU8         | AMC/FEP/MEM/CIP/E/COT/TE         |
| <b>Azuiyokwu downstream</b> | AZD1         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZD2         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZD3         | AMC/FEP/MEM/CIP/COT/TE           |
|                             | AZD4         | AMC/CN/CRO/FEP/MEM/CIP/E/COT/TE  |
|                             | AZD5         | AMC/CN/CRO/FEP/MEM/CIP/E/COT/TE  |
|                             | AZD6         | AMC/CN/CRO/FEP/MEM/E/COT/TE      |
|                             | AZD7         | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | AZD8         | AMC/FEP/MEM/CIP/E/COT/TE         |
| <b>Onuebonyi</b>            | OE1          | ATM/AMC/CRO/FEP/MEM/CIP/E/COT/TE |
|                             | OE2          | AMC/CRO/FEP/MEM/E/COT/TE         |
|                             | OE3          | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | OE4          | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |
|                             | OE5          | AMC/CRO/FEP/MEM/E/COT/TE         |
|                             | OE6          | ATM/AMC/CRO/FEP/MEM/CIP/E/COT/TE |
|                             | OE7          | ATM/AMC/CRO/FEP/MEM/CIP/E/COT/TE |
|                             | OE8          | AMC/CRO/FEP/MEM/CIP/E/COT/TE     |

**Legend:** **AZU** = Azuiyokwu upstream; **AZD** = Azuiyokwu downstream; **OE** = Onuebonyi; **ATM** = aztreonam; **AMC** = amoxicillin–clavulanic acid; **CN** = gentamicin; **CRO** = ceftriaxone; **FEP** = cefepime; **MEM** = meropenem; **CIP** = ciprofloxacin; **E** = erythromycin; **COT** = co-trimoxazole; **TE** = tetracycline.

## 5. Discussion

Water is an essential resource for life, and access to safe drinking water is a fundamental human need and a basic right of every individual [50]. Safe drinking water should be void of high concentrations of chemicals and minerals, as well as pathogenic microorganisms. South Africa is located in a semiarid region and receives very less rainfall, resulting in shortage of water, especially potable water. Besides, increased industrialisation and frequent establishment of informal settlements also affect the quality of water, especially in rural areas. Due to scarcity of potable water, most individuals resort to water from other unprotected sources, such as boreholes, rivers, and dams for daily activities. Unprotected sources of water could be contaminated with faecal matter from human and animal origin.

All 24 isolates were identified as *Vibrio* species by the morphological translucent green and yellow forming colonies on Thiosulphate Citrate Bile Salt Agar. Isolates presumptive to be *Vibrio parahaemolyticus* was higher (8 (33.3%)) compared to *V. mimicus* (7 (29.17%)), *V. alginolyticus* (3 (25%)), *V. vulnificus* (2 (8.33%)) and *V. cholerae* (1 (4.17%)). A similar observation with a slightly different prevalence of *harveyi* (10 (45.45%)), *V. parahaemolyticus* (5 (22.73%)), *V. mimicus* (3 (13.64%)), *V. vulnificus* (2 (9.09%)), and *V. cholerae* (2 (2.09%)) water sources as

reported by Mpho *et al.*, (2020) in water sources including taps, boreholes and dams in North West province, South Africa. A P-value of 0.433 shows no significant difference in the counts of all *Vibrio species* isolated from all selected water sources.

The finding on susceptibility had susceptibilities observed in the order: Gentamycin (87.5%) and Azetronam (54.2%). Similar observations reported by Mpho *et al.*, (2020). Conversely, all the isolates showed high resistance to Amoxicillin Clavulanic Acid (100%) Cefepime (100%), Meropenem (100%), Cotrimoxazole (100%), Tetracycline (100%), Erythromycin (95.83%), Ciprofloxacin (87.5%), followed by Ceftriazone (79.2%) and Gentamycin (12.5%). Statistical analysis revealed, no significant difference between the susceptibility (0.508) and resistance ( $P=0.141$ ) of *Vibrio spp* obtained across the sampling period ( $P < 0.05$ ).

All isolates tested showed multi-resistant to the antibiotics used, Highest MARP obtained was (ATM/AMC/CRO/FEP/MEM/CIP/E/COT/TE) with a dual occurrence and the least was a dual occurrence of (AMC/FEP/MEM/CIP/COT/TE) (Table 7). The MARI ranged from 0.7 to 0.9, surpassing the 0.2 threshold limit, showing that the isolates are of high antimicrobial usage. MARI has been used to estimates the health risks associated with the spread of drug resistance in an environment. A MARI value of 0.2 is used to differentiate between low and high risks and MARI greater than 0.2 suggests that the isolates originate from an environment with high contamination or antibiotics usage. This study shows the increase in multi-antibiotics resistance of *Vibrio* isolates in the wastewater sample rendering it polluted and unsafe for use.

Drug resistance is currently an issue of severe public health concern worldwide, mainly due to the alarming rate of dissemination of resistant determinants in environmental bacterial pathogens. These resistant have a negative impact on antibiotic therapy, resulting in difficulties in controlling and managing diseases Mpho *et al.*, (2020). Improper discharge of municipal and industrial wastewater, as well as water from aquaculture systems, has been reported to play significant roles in the dissemination of resistant genes within aquatic ecosystems. It is worthy to note that several clinically used antibiotics are released in an active biological form through faeces and urine to the environment (Verlicchi & Zambello, 2016; Li & Zhang, 2010) improperly treated wastewater containing such residues.

## 6. Conclusion

These findings of this study revealed the presence of antibiotic-resistant virulent *Vibrio* species from selected surface water, making it unsafe for domestic use. Cluster analysis of bacterial growth inhibition zone diameter data revealed very close similarities among isolates from different sources and locations, indicating similar histories of exposure to antibiotics, which is a public health concern.

In order to avert the menace that may arise from the outbreak of infections of *Vibrio species* from the use of this contaminated water bodies, there is need for the initiation of government regulation of the sewage disposal into this sources and routine check of the microbial level of the water sources as well as more research to enable ascertain more more effective drug to treat already infected individual and provision of safer pipe borne water sources as a social amenity to reduce the use of these contaminated water sources.

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