Virulence and Antibiotic Resistance Characteristics of Vibrio Isolates From Rustic Environmental Freshwaters

Oyama Gxalo1,2, Tennison O. Digban1,2, Bright E. Igere1,2, Ola A. Olapade3, Anthony I. Okoh1,2 and Uchechukwu U. Nwodo1,2*

1 South Africa Medical Research Center (SAMRC) Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice, South Africa, 2 Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa, 3 Biology Department, Albion College, Albion, MI, United States

The study investigated the occurrence of antimicrobial resistance genes and virulence determinants in Vibrio species recovered from different freshwater sheds in rustic milieu. A total of 118 Vibrio isolates comprising Vibrio fluvialis (n=41), Vibrio mimicus (n=40) and V. vulnificus (n=37) was identified by amplification of ToxR, vmh and hsp60 genes. The amplification of virulence genes indicated that V. mimicus (toxR, zot, ctx, VPI, and ompU) genes were detected in 12.5%, 32.5%, 45%, 37.5% and 10% respectively. V. fluvialis genes (stn, hupO and vfh) were harboured in 48.8%, 14.6% and 19.5% isolates congruently. The other virulence genes that include vcgC and vcgE were observed in 63.1% and 29% of isolates belonging to V. vulnificus. With the exceptions of imipenem, meropenem and ciprofloxacin, most isolates exhibited more than 50% resistance to antibiotics. The antimicrobial resistance was more prevalent for polymyxin B (100%), azithromycin (100%) and least in ciprofloxacin (16.1%). Multiple antibiotic resistance index range was 0.3 and 0.8 with most isolates showing MARI of 0.8. The blaTEM, AmpC, blaGES, blalMP, blaOXA-48 and blakPC genes were detected in 53.3%, 42%, 29.6%, 16.6%, 15%, 11.3% and 5.6% of the isolates. Non-beta lactamases such as streptomycin resistance (aadA and strA), gentamicin resistance (aphA1) and quinolone resistance gene (qnrVC) were found in 5.2%, 44.3%, 26% and 2.8%. Chloramphenicol resistance genes (cmlA1 and catI1) were found in 5.2% and 44.3% among the isolates. Our findings reveal the presence of antimicrobial resistance genes and virulent Vibrio species in aquatic environment which can have potential risk to human and animal’s health.

Keywords: antibiotic resistance, Vibrio isolates, virulence genes, MARI, South Africa

INTRODUCTION

Water containing infectious pathogens has been associated with ailments globally amongst human populations (Ramirez-Castillo et al., 2015). The importance and need for better water quality cannot be over-emphasized. Water scarcity is becoming more serious due to urbanization and population growth (Tzanakakis et al., 2020) hence there is an increase in demand for water. However, in most
countries a strategy to minimize water shortage has been put in place which involves treating and recycling wastewater (Voulvoulis, 2018). Many treatment plants for wastewater in South Africa discharge their final effluents openly into adjoining streams or rivers used by the nearby villages/suburb for their several domestic needs (Wall, 2018). However, reports suggest that some wastewater are not properly treated and therefore discharge effluent of poor quality (Edokpayi et al., 2015). Wastewater and sewage from several sources contribute to spreading antimicrobial resistance (AMR) to the environment and adversely impacting on the habituation of aquatic animals and their surrounding water (Fouz et al., 2020). Several studies have emphasized on the effect of sewage as a huge environmental pool of AMR, as it signifies a suitable milieu for AMR organisms and antimicrobial resistance genes to thrive (Rizzo et al., 2013; Singer et al., 2016). Numerous resistant pathogens have been identified in various environmental sewage, hospital effluents, surface water and wastewater in treatment plants (Lundborg and Tamhankar, 2017). The aquatic environment has become reservoir of many microbes including Vibrios that are potentially infectious to humans. Vibrio genus is grouped in the family Vibrionaceae, which comprises of opportunistic pathogenic microbes infecting both humans and animals (Takekura et al., 2014). Vibrio species are a group of Gram-negative, curved-rod shaped bacteria being regular constituent of estuarine, freshwater and marine environs (Baker-Austin et al., 2018). Their genomes are separated inside two chromosomes having been shaped by recombination and the acquiring of genes by horizontal gene transfer from other bacteria (Oliveira et al., 2017). Infections caused by pathogenic Vibrio remain severe public health significance. Cholera causing Vibrio (Vibrio cholerae) is implicated in serious diarrhoeal infection which can be rapidly fatal when not treated and is typically disseminated through person-to-person contact as well as infected water (Das et al., 2020). Non-cholera causing Vibrio species (including V. paraahaemolyticus, V. anguillarum, V. metschnikovii, Vibrio alginolyticus, V. harveyi, V. mimicus, V. fluvialis, and V. vulnificus) are known to cause vibriosis that is contracted via sea water or through ingesting raw or parboiled infected seafood causing necrotizing fasciitis, self-limiting gastroenteritis, severe septicaemia (Gennari et al., 2012). Vibrio species pathogenic to humans produce a variety of virulence genes which contribute to some ailments (Alvarez-Contreras et al., 2021). Some of the virulence trait includes the secretion of extracellular products (ECP), thermostable direct haemolysin (tdh), and tdh-related haemolysin expressed in V. paraahaemolyticus, expression of capsular polysaccharides, cytotoxicity, biofilm formation, cholera toxin and the toxin-coregulated pilus (tcp) in Vibrio cholerae (Binesse et al., 2008). The V. fluvialis protease gene (vfp), heme utilization protein gene (hupO) and extracellular haemolysin gene (vfh), are also among the Vibrio virulence genes (Ramamurthy et al., 2014). The emergence of Vibrio strains in aquatic environments have created more apprehension leading to upsurge in resistance among Vibrio isolates against most clinically prescribed antibiotics and this has initiated apprehension in the healthcare sector thwarting current therapeutic outcomes (Banerjee and Farber, 2018). Vibrio species are documented to be very sensitive to many antimicrobials that are clinically recommended for therapeutic purpose and comprises of tetracycline, cephalosporins quinolones, aminoglycosides, and folate pathway inhibitors (Elmahdi et al., 2016). The incessant abuse of antibiotics has over time contributed immensely to the rapid emergence of drug resistance microbial strains including several Vibrio species (Manyi-Loh et al., 2018). Antibiotics are broadly used to manage or treat bacterial ailments in humans as well as in veterinary medicine. The beginning of this millennium signifies the end of the golden era of antibiotics and the commencement of more alertness of the public health alertness on the distribution of antimicrobial resistance. Documented reports have emphasized the significant importance of various aquatic environments in the spread of AMR (Baquero et al., 2008) especially predominantly in wastewater effluents which are regarded as hot spots for horizontal genes transfer (Bouki et al., 2013). South Africa is situated in a semi-dry area and typically experience limited annual rainfalls giving rise to scarcity of portable water. Consequently, most inhabitants in the rural areas resort to the use of untreated water from vulnerable sources, like rivers, and dams for their daily supply (Fobosi, 2013). Over the years, many researchers have focused on the severity of diseases caused by Vibrio cholerae opting out relatively other Vibrio species of medical interest, some of which are emerging pathogens that are able to cause mild to severe human diseases (Osunla and Okoh, 2017). Therefore, the aim of this study was to assess the antibiogram, virulence and resistance genes of non-cholera causing Vibrio species recovered from aquatic sources.

MATERIALS AND METHODS

Study Site

The Eastern Cape is one of the nine provinces within South Africa; it is separated into two Buffalo City and Nelson Mandela Bay and six district municipalities for local government purposes. The district municipalities are in turn split into thirty-one local municipalities. The Eastern Cape mostly consists of rural settlements with minimal basic services. This study was carried out in two of the six district municipalities of this region, specifically at the Amathole District and Chris Hani District Municipalities. The Chris Hani District Municipality is located in the central of the Eastern Cape hinterland, between the Eastern Cape coastline and the Drakensberg mountains and spans an estimate of 37 294 km². The district municipality is located on the watershed of four river viz. Orange River, Great Fish River, Mbashe River and Great Kei River. Surface water sources supply water for most of the towns in the area while only a few depend on groundwater supplies. The Amathole District Municipality is located in the central part of the Eastern Cape and spans along the Sunshine Coast from the Fish River Mouth and along the Eastern Seaboard to the south of Hole in the Wall alongside the Wild Coast. It is also bordered to the north by the
Amathole Mountain Range. The rivers examined in this study were selected due to the fact that they serve as final receiving bodies of water for the release of treated domestic sewage operated in activated sludge systems. The targeted hospitals were also chosen because they serve as referral hospitals for the indigenous inhabitants with various medical conditions, they do not have independent wastewater treatment mechanism but are joined with that of the municipal waste treatment system.

Collection of Samples
A total of seventy-two (72) wastewater samples were collected aseptically fortnightly from the 9 sampling sites (shown in Table 1) over a period of 4 months (July 2018-October 2018) with sterile 500 mL Borosilicate Glass bottles. The samples were conveyed on ice in a cooler box from the sampling site to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare for analysis within few hours of collection.

Isolation and Identification of Presumptive Vibrio Species
Standard membrane procedure was employed in processing the wastewater samples (APHA, A. 1998). Each wastewater sample was serially diluted and a 100 ml volume was filtered through 0.45 µm mixed cellulose ester membrane filters (Millipore, Ireland) using a vacuum pump. The membrane filter paper was transferred onto alkaline peptone water as means of enrichment and incubated aerobically at 37°C for 18-24 h. A loopful from the alkaline peptone water was sub-cultured onto thiosulphate citrate bile salts sucrose (TCBS, Darmstadt, Germany) and incubated for another 24 h at 37°C. Four to six colonies were randomly chosen from each plate and ed in fresh TCBS agar plates. Puriﬁcation was achieved with PCR assay using speciﬁc primers targeting the 16SrRNA gene. The conﬁrmed isolates of the Vibrio isolates were also delineated into V. mimicus, V. fluvialis and V. Vulnificus. The PCR cocktail comprised of 12 µl of Master-mix (inqaba), 1µl of both forward and reverse oligonucleotide primers (Inqaba, SA), 6 µl of nuclease free water and 5µl of DNA template which summed up to 25 µl reaction mixture. The amplicons were differentiated by electrophoresis in 0.8% agarose gels containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, St. Louis, USA) run at 90 V for 50 min and visualised under a UV transilluminator (Alliance 4.7 Uvitec, Cambridge). The targeted genes, primer sequences, band sizes and cycling condition of the Vibrio species are shown on Appendix 1 (Supplementary Material).

Antimicrobial Sensitivity Test Performed on the Vibrio Isolates
Antibiotic sensitivity test was carried out using Mueller-Hinton agar (Basingshike, Hampshire, England) by the standard disk diffusion technique (Kirby-Bauer test) as endorsed by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2018). Few colonies of conﬁrmed isolates of the Vibrio species were reconstituted on sterile normal saline to give an approximately 0.5 McFarland standard. A lawn of the bacterial suspension was made on Mueller-Hinton agar and allowed to stand for 15 min. A panel of 12 antibiotics which are usually recommended for treating Vibrio infections were selected and impregnated unto the agar plates. The antibiotic disks include: ampicillin (10 µg), ampicillin-sulbactam (10/10 µg), imipenem (10 µg), meropenem (10 µg), kanamycin (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefuroxime (30 µg), polymyxin B (300 µg), azithromycin (15 µg), nitrofurantoin (300 µg) and Chloramphenicol (30 µg). The plates were incubated for 24 h at 37°C and results documented as sensitive, intermediate or resistant based on the elucidation of the diameter of the zone of inhibition.

Multiple Antibiotic Resistance Index
The Multiple antibiotic resistance index was estimated using the formula: MRI=a/b, where a = is the number of antibiotics to which the isolates exhibited resistance and b = is the total number of antibiotics which the resistant isolates were

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**TABLE 1 | Sampling sites and their coordinates.**

| District municipality | Sampling site     | Geographical coordinates |
|-----------------------|-------------------|--------------------------|
| Chris Hani district municipality | Komani River | 31°54’54''S 26°50’71’’E |
|                        | OT WWTP          | 31°53’38’’9’’S 26°53’26’’61’’E |
| Frontier Hospital      | Cofimvaba River  | 32°00’85’’5’’S 27°35’59’’E |
|                        | Cofimvaba Hospital | 32°00’43’’9’’S 27°34’59’’E |
| Amathole District Municipality | Victoria hospital | 32°46’37’’E 26°50’46’’E |
|                        | Thyme River      | 32°41’3’’S 26°54’18’’E |
|                        | Seymour upstream, downstream and final effluent | 32°34’8’’S 26°45’2’’E |
|                        | Fort Beaufort final effluent and downstream | 32°46’44’’S 26°38’7’’E |
subjected to. MARI value of more than 0.2 recommends that the isolates are from envirion where there are excessive or indiscriminate use of antibiotics (Blanco et al., 2008).

### Molecular Detection of Virulence Genes

The identification of virulence genes in the *Vibrio* species was done through polymerase chain reaction (PCR) analyses. The targeted virulence genes include toxin coregulated pilus (tcp), toxin regulon (toxR), outer membrane protein (ompU), zonula occludens toxin (zot), cholera toxin gene (ctx) and *Vibrio* pathogenicity island (VPI) in *V. mimicus*. Others include *V. vulnificus* virulence genes (*vcgC* and *vcgE*) in *V. vulnificus*, extracellular haemolysin gene (vflh), heme utilization protein gene (*hupO*), *V. fluvialis* protease gene (*sfpA*) and heat stable enterotoxin (*stn*) in *V. fluvialis*. Each reaction comprises a preliminary denaturation at 94°C for 4 min, accompanied by 35 cycles each consisting of an initial denaturation at 94°C for 40 sec followed by annealing (52°C and 62°C) and extension steps for 90 sec while final elongation was 72°C for 6 min. Primers and their annealing temperatures are presented in Appendix 2 (Supplementary Material).

### Detection of Antibiotic Resistance Genes Among the *Vibrio* Species

Also, PCR analysis was employed to identify various antibiotic resistant genes in the *Vibrio* species using pairs of specific primers and cycling conditions as follows: initial denaturation of 94°C for 5 min, accompanied by 35 cycles of 94°C for 1 min, annealing temperatures (60-62°C) for 45 s and 72°C for 91 s, final elongation step at 72°C for 5 mins. The selected targets genes were: aminoglycoside resistance genes (*strA*, *aadA*, *aacC2*, *apHAII* and *apHAII*), phenicols resistance genes (*catl*, *catII* and *cmIaI*), β-lactamases-encoding genes (*ampC*, *blaTEM* and *blaZ*) and Carbapenem resistance genes (*blaGES*, *blaOXA-48*, *blaIMP*, *blaVIM*, *blaKPC*, and *qnrVC*). The oligonucleotide sequences, amplicon sizes and annealing temperatures of the primers used in this study are presented in Appendix 3 (Supplementary Material).

### RESULTS

During the study period, a total of 695 presumptive *Vibrio* isolates were recovered from all the sampling sites. PCR analysis was used to confirm 690 isolates as *Vibrio* isolates. Of the confirmed *Vibrio* species: *V. mimicus*, *V. fluvialis* and *V. vulnificus* accounted for 40(6.7%), 41(6.8%) and 37(6.2%) respectively from seven of the nine sampling sites. Other isolates not confirmed could belong to *Vibrio* species not identified in this study. Confirmation and delineation of *Vibrio* species recovered from the different sampling sites is shown in Table 2. The frequency of *Vibrio* species recovered from the different site Figure 1, while the molecular gel electrophoresis delineating *Vibrio* into species is shown in Figure 2.

### Distribution of Antimicrobial Susceptibility Profile of the *Vibrio* Species

All 118 confirmed *Vibrio* species were subjected to seven antimicrobial classes comprising of twelve antibiotics. The antibiogram of the *Vibrio* isolates from all sampling sites showed varying degrees of resistance to most of the antibiotics used. A 100% resistance pattern was observed among all 118 isolates to azithromycin and polymyxin B. More than 70% of the isolates also showed resistance to ampicillin, ampicillin-sulbactam, cefuroxime and chloramphenicol. The highest susceptibility among the isolates was observed with imipenem.

![Figure 1](image-url)
and meropenem antibiotics. Meropenem susceptibility among *V. mimicus* and *V. fluvialis* was greater than 60%. A resistance pattern of more than 50% was observed among all the isolates to kanamycin, while fluoroquinolones had variations in the susceptibility pattern. High resistance level was observed with ofloxacin (62%) and ciprofloxacin (16%). The distribution of the antibiotic susceptibility pattern is shown in Table 3. The multiple antibiotic resistance patterns among the *Vibrio* species showed a range of 0.3 and 0.8. The multi-drug resistance profile among the *Vibrio* species is shown in Table 4.

### Frequency of Virulence Genes Among the *Vibrio* Species

A total of ten of the twelve virulence genes were detected among the *Vibrio* species recovered from all the sites. *Vibrio mimicus* harboured five virulence genes in the following proportion: toxR (12.5%), zot (32.5%), ctx (45%), VPI (37.5%), tcp (0%), and ompU (10%). Two virulence genes detected among the *V. vulnificus* isolates were vgcC (63.1%) and vgcE (29%). Three of the four virulence genes were likewise detected among the *V. fluvialis* isolates recovered from all the sampling sites in the following proportion: strA (48.8%), hupO (14.6%), vfpA (0) and vfh (19.5%). Figures 3A–C represent the frequency of virulence genes among the three *Vibrio* species (*V. mimicus, V. vulnificus* and *V. fluvialis*) recovered from all sampling sites. The gel representative pictures for the amplification of the virulence genes for the three *Vibrio* species are shown in Figure 4.

### Detection of Resistance Genes Among the *Vibrio* Species

Five resistant genes encoding for aminoglycoside were examined in this study and three were detected of which *aadA* (50%) was observed as the most prevalent while *strA* was the least with a prevalence of 4.3%. *V. mimicus* was devoid of any *strA* gene. However, 26% of *V. mimicus* solely harboured *aph(3)-Ia (aphA1)* a gene among all the *Vibrio* isolates. For the phenics,
# TABLE 4 | Multiple antibiotic resistance pattern and MAR1 of *Vibrio* isolates.

| Name of species | No. of isolates | Antibiotic resistance pattern | MAR1 |
|-----------------|-----------------|-------------------------------|------|
| *V. mimicus*    | 3               | AMP-CHL-PB-KAN-AZM-CXM-SAM    | 0.6  |
|                 | 7               | AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM| 0.7  |
|                 | 5               | IMP-AMP-CHL-NIN-PB-KAN-AZM-CXM-OFX-SAM | 0.8  |
|                 | 2               | AMP-CHL-NIT-PB-KAN-AZM-CXM    | 0.6  |
|                 | 2               | AMP-CHL-NIT-PB-KAN-MEM-AZM-CXM-OFX-SAM | 0.8  |
|                 | 3               | AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 3               | AMP-CHL-NIT-PB-AZM-CXM-OFX-SAM | 0.7  |
|                 | 1               | AMP-CHL-NIT-PB-KAN-AZM-PB     | 0.6  |
|                 | 1               | AMP-CHL-NIT-PB-AZM            | 0.4  |
|                 | 2               | AMP-CHL-NIT-PB-AZM-CXM        | 0.5  |
|                 | 2               | AMP-CHL-NIT-PB-KAN-MEM-AZM-SAM | 0.7  |
|                 | 2               | CHL-PB-KAN-AZM-CXM-OFX        | 0.6  |
|                 | 3               | CIP-AMP-CHL-NIT-PB-KAN-AZM-CXM-OFX-SAM | 0.8  |
|                 | 2               | AMP-CHL-NIT-PB-AZM-OFX-SAM    | 0.6  |
|                 | 1               | AMP-CHL-NIT-PB-MEM-AZM-CXM-OFX-SAM | 0.8  |
|                 | 1               | CIP-IMP-AMP-C-NIT-PB-AZM-CXM-OFX-SAM | 0.8  |
|                 | 5               | IMP-AMP-CHL-NIT-PB-AZM-CXM-SAM | 0.7  |
| *V. vulni*      | 1               | IMP-AMP-CHL-NIT-PB-MEM-AZM-CXM-SAM | 0.8  |
|                 | 5               | IMP-AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 5               | AMP-CHL-NIT-PB-AZM-CXM-OFX-SAM | 0.7  |
|                 | 1               | IMP-AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 1               | IMP-AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 1               | IMP-AMP-CHL-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 1               | IMP-AMP-CHL-NIT-PB-AZM-CXM-OFX-SAM | 0.8  |
|                 | 2               | CIP-AMP-CHL-NIT-PB-MEM-AZM-CXM-OFX-SAM | 0.8  |
|                 | 1               | CIP-IMP-AMP-C-NIT-PB-KAN-AZM-CXM-SAM | 0.8  |
|                 | 5               | IMP-AMP-CHL-NIT-PB-AZM-CXM-SAM | 0.7  |
| *V. fluvialis*  | 1               | AMP-CHL-NIT-PB-MEM-AZM-CXM    | 0.6  |
|                 | 1               | NIT-PB-MEM-AZM-CXM            | 0.4  |
|                 | 1               | NIT-PB-AZM-CXM                | 0.3  |
|                 | 3               | CIP-IMP-AMP-C-NIT-PB-AZM-CXM-SAM | 0.8  |
|                 | 12              | AMP-CHL-NIT-PB-KAN-AZM-CXM-OFX-SAM | 0.8  |
|                 | 8               | AMP-CHL-NIT-PB-AZM-OFX-SAM    | 0.7  |
|                 | 3               | IMP-AMP-CHL-NIT-PB-MEM-AZM-CXM-SAM | 0.8  |
|                 | 5               | AMP-CHL-NIT-PB-AZM-OFX-SAM    | 0.7  |

**FIGURE 3** | (A) = Frequency of *V. mimicus* virulence gene from all sampling sites, (B) = Frequency of *V. vulni* virulence genes from all sampling sites and (C) is Frequency of *V. fluvialis* virulence genes from all sampling sites.
three resistance genes were detected in 5.2% among the confirmed *Vibrio* species. 44.3% of the *Vibrio* species harboured the *catII* gene while *catI* gene was not detected among the three *Vibrio* species studied. The ampC gene was identified in 42% of the *Vibrio* isolates, blaTEM in 53.3% of the *Vibrio* isolates, and no isolate harboured the blaZ gene. The frequency of resistance genes among the carbapenems class of antibiotics detected in the studied isolates varied from 5.6% for and 29.6%. The frequency of resistance genes with carbapenem was *blaGES* (16.6%), *blaOXA*-48 (11.3%), *blaIMP* (15%), *blaVIM* (29.6%) and *blaKPC* (5.6%). Among the resistance genes to fluoroquinolones assayed, only two isolates representing 2.8% harboured the *qnrVC* gene in all the isolates studied. The distribution of the resistance genes among the *Vibrio* resistance isolates is shown in Table 5. The gel representative pictures for the amplification of the resistance genes for aminoglycoside, phenicols, beta-lactams, carbapenems and fluoroquinolones are shown in Figure 5 respectively.

**DISCUSSION**

Water is considered as one of the basic necessities for the existence of all living organisms. South Africa is a semi-arid region which obtains minimal amount of rain annually. In lieu of this and with the incessant shortages of portable and clean water in the country, most inhabitants, especially in the rural areas have resorted to different alternatives for water sources such as river and dams for their routine domestic activities (Meissner et al., 2018). Additionally, the upsurge in various informal settlements around the country as well as the present poor conditions of some wastewater treatment plants have significantly contributed to the diminished quality of water sources found in most of the rural areas (Govender et al., 2011). Most hospital settings in the rural areas do not treat their effluents and are channelled with municipal wastewater prior (Edokpayi et al., 2017). Often times, compromised sewage pipelines leak their contents into the environment which eventually end up in adjoining streams and rivers.

Our study detected three *Vibrio* species (*V. mimicus, V. fluvialis* and *V. vulnificus*) from all sampling sites through molecular analyses. While culture-based techniques are relied upon for detecting microbial presence in various environments, however current molecular methods are comparatively more specific and sensitive than culture-based methods (Franco-Duarte et al., 2019). Specifically, the confirmation of *Vibrio* isolates from targeted sites in this study was achieved by molecular amplification of 16SrRNA gene followed by target genes *hsp60, toxR* and *vmh* for *V. vulnificus, V. fluvialis* and *V. mimicus* isolates. Previous studies have also employed similar molecular methods in the confirmation and delineation of *Vibrio* isolates into species (Kim et al., 2015; Okoh et al., 2015; Guardioala-Avila et al., 2016). Overall, *Vibrio* species recovered during this study were absent in two sampling sites (FHE and...
CHE) and could be Vibrio isolates belonging to other species not identified in this study. The presence of the Vibrio isolates from seven sampling sites is shown in Table 5. The QTE site had the highest recovery of the Vibrio isolates and poor treatment plant performance could have contributed to the recovery of the Vibrio isolates in the final effluent sample. The occurrence of pathogens in waterbodies is often due to the aftereffect from faecal contamination involving humans and animals (Penakalapati et al., 2017). The uncontrolled discharge of sewage as well as nonchalant management of wastewater treatment plants have been incriminated in water pollution of microbial origin especially around the rural settlements in South Africa (Teklehaimanot et al., 2015). The antibiotic sensitivity patterns among the Vibrio isolates recorded showed varying degrees of resistance pattern. All Vibrio species in the study exhibited more than 95% resistance to polymyxin B, chloramphenicol and nitrofurantoin. Results from our findings on the resistance pattern among the Vibrio isolates were slightly higher to those

| Antimicrobial family | Total number of Vibrio species resistant to antibiotics | Resistant genes | V. mimicus | V. vulnificus | V. fluvialis | Total percentage of resistant genes |
|---------------------|-------------------------------------------------------|----------------|------------|--------------|--------------|----------------------------------|
| Aminoglycosides     | 70                                                    | strA           | –          | 2            | 1            | 4.3                              |
|                     |                                                       | aacA           | 6          | 12           | 17           | 50.0                             |
|                     |                                                       | aac(3)-IIa (aacC2) a | –           | –            | –            | –                               |
|                     |                                                       | aph(3)-la (aphA1) a | 18          | –            | –            | 26.0                             |
|                     |                                                       | aph(3)-IIa (aphA2) a | –           | –            | –            | –                               |
| Phenicols           | 115                                                   | cmA1           | 2          | 4            | –            | 5.2                              |
|                     |                                                       | catI           | –          | –            | –            | –                               |
|                     |                                                       | catII          | 18         | 16           | 17           | 44.3                             |
| Beta-lactams        | 102                                                   | ampC           | 26         | 16           | 5            | 42.0                             |
|                     |                                                       | blaTEM         | 38         | 15           | 35           | 53.3                             |
|                     |                                                       | blaZ           | –          | –            | –            | –                               |
| Carbapenems         | 71                                                    | GES            | 4          | 7            | 1            | 16.6                             |
|                     |                                                       | blaOXA-48      | 3          | 3            | 2            | 11.3                             |
|                     |                                                       | blaMP          | 2          | 4            | 5            | 15.5                             |
|                     |                                                       | blaVM          | 1          | 15           | 5            | 29.6                             |
|                     |                                                       | blaKPC         | –          | 4            | –            | 5.6                              |
| Fluoroquinolones    | 92                                                    | qnrVC          | –          | –            | 2            | 2.8                              |

FIGURE 5  (A) = Gel multiplex electrophoresis showing the aphA1, aadA and strA genes among the Vibrio species. Lane L: Molecular Marker (100 bp), lane 1: Negative control; Lane 2-7: positive isolates of aadA gene (525 bp), Lane 11: positive isolate of strA gene (348 bp) and lane 13: positive isolate of aphA1 gene (600 bp), (B) = Gel multiplex electrophoresis showing the cmA1 and catI genes among the Vibrio species. Lane L: Molecular Marker (100 bp), lane 1: Negative control; Lane 2-5, 7, 10 and 12: positive isolates of catI gene (543 bp), Lane 8 and 9: positive isolate of cmA1 gene (115 bp), (C) = Gel multiplex electrophoresis showing the blaTEM and ampC genes among the Vibrio species. Lane L: Molecular Marker (100 bp), lane 1: Negative control; Lane 2-9: positive isolates of blaTEM gene (690 bp), Lane 10-12: positive isolate of ampC gene (550 bp), (D) = Gel multiplex electrophoresis showing the blaKPC, blaGES, blaVM and blaOXA-48 genes among the Vibrio species. Lane L: Molecular Marker (100 bp), lane 1: Negative control; Lane 2, 3, 9 and 10: positive isolates of blaKPC gene (543 bp), Lane 4 and 5: positive isolate of blaGES gene (599 bp), Lane 2 and 3: positive blaVM isolates (390 bp), Lane 4 and 5: positive isolates of blaOXA-48 (281 bp), (E) = Gel electrophoresis showing the qnrVC genes among the Vibrio species. Lane L: Molecular DNA-marker (100 bp), lane 1: Negative control; Lane 3 and 4: positive isolates of qnrVC gene (621 bp).
reported by Beshiru et al. (2020). In general, all confirmed Vibrio species from our study showed more than 85% resistance pattern to ampicillin. The carbapenems have been proven to be broad spectrum antibiotic and active against a diverse range of Gram positive and Gram negative bacteria (Papp-Wallace et al., 2011). From our study, a high susceptibility pattern was observed with the carbapenems (imipenem and meropenem) against V. mimicus and V. fluvialis. However, low sensitivity pattern was noticed among V. vulnificus isolates. Kanamycin also exhibited a sensitivity pattern of more than 50% with the test isolates and in strong agreement with previous report by Maje et al. (2020). A third generation antibiotic (cefoxime) also showed a resistance pattern of more than 75% among the Vibrio isolates and is comparable with the findings by Manjusha and Sarita (2011). Ofloxacin also had a higher sensitivity pattern compared to ciprofloxacin but however, more isolates had an intermediate pattern of sensitivity with ciprofloxacin.

Multiple antibiotic resistance (MAR) analysis has been introduced to discern bacteria from sources using antimicrobial agents regularly approved for human therapy. In bacteria, MAR is most frequently associated with the presence of plasmids which harbours single or multiple resistance genes (Ruppé et al., 2015). Multiple antibiotic resistance patterns among the Vibrio species had a range of 0.5 to 0.8 while most of the isolates had a MARI of 0.8. This inferred that the Vibrio isolates originated from sources where antibiotic were greatly utilized.

Microbial virulence factors involve a wide range of molecules produced by pathogenic bacteria with increasing propensity to elude their host defence and cause infection. Also, some secretes microbial products which have the ability to gain entry into the host cell and utilize their mechanism assisting in the attainment of infection (Kitamoto et al., 2016). Virulence genes in Vibrio species have assisted in the initiation of infection ranging from diarrhoea, gastroenteritis, cholera, wound and blood infections (Schirmieister et al., 2014). It is commonly assumed that environmental bacterial strains are devoid of virulence traits that are present in clinical strains. Nonetheless, recent studies have reported that virulence genes, or their homologues could be found in strains from environmental sources and that acquiring such genes may have occurred in the aquatic environment (Persson et al., 2009). From this study, ten virulence genes were detected in varying frequencies among the Vibrio species isolated. The toxR virulence is made up of a set of genes encoding proteins that encourages internal colonization, toxin production and survival in the host cell coordinately expressed and survival in the host cell and utilizes their mechanism assisting in the attainment of infection ranging from diarrhoea, gastroenteritis, cholera, wound and blood infections (Kitamoto et al., 2016). Virulence genes in Vibrio species have assisted in the initiation of infection ranging from diarrhoea, gastroenteritis, cholera, wound and blood infections (Schirmieister et al., 2014). It is commonly assumed that environmental bacterial strains are devoid of virulence traits that are present in clinical strains. Nonetheless, recent studies have reported that virulence genes, or their homologues could be found in strains from environmental sources and that acquiring such genes may have occurred in the aquatic environment (Persson et al., 2009). From this study, ten virulence genes were detected in varying frequencies among the Vibrio species isolated. The toxR virulence is made up of a set of genes encoding proteins that encourages internal colonization, toxin production and survival in the host cell coordinately expressed and dependent upon transcriptional activator (toxR) (Bina et al., 2003). The toxR gene was detected among V. mimicus isolates from two sampling sites (QTE and THR). This study affirms with previous report (Chen et al., 2011) detecting toxR gene in V. mimicus from sea foods. Zot gene was initially detected in Vibrio cholerae and encoded by ctx prophage while the core part of ctx gene comprises of many structural genes including zot genes which has significance in phage morphogenesis and assembly (Pant et al., 2020). The detection of ctx and zot genes from our study is comparable to the reports by Menezes et al. (2014). The presence of a zot gene in ctx-positive V. mimicus indicates a possible significant role of zot in the toxigenicity of the species. Vibrio pathogenicity island (VPI) gene and ompU (encoding a porin in the outer membrane of the bacteria) were also detected among some V. mimicus isolates. VPI has been attributed to be an essential virulence gene cluster for colonization of the human intestine as well as receptor for infection in most toxigenic Vibrio strains (Labbate et al., 2016). Detection of VPI and ompU genes among some V. mimicus in this study is in agreement with previous study by Guardiola-Avila et al. (2020). The detection of virulence correlated gene in this study among the V. vulnificus isolates can be distinguished apparently as clinical (vcgE) and environmental (vcgC) (Bier et al., 2015). Our finding is in accord with the report by Warner and Oliver (2008) who detected vcgE (47%) and vcgC (53%) from oysters’ isolates and water surrounding oysters harvest in North Carolina. Among the V. fluvialis isolates, three genes detected were strA (48%), hupO (14.6%) and vhf (19.5%). These factors have been reported to assist in haemolysin production which intensifies the pathogenic prowess of V. fluvialis and increase diarrhoea in infected patients (Liang et al., 2013). A similar trend was documented by Fri et al. (2017) that also identified similar virulence genes from a dusky farm and estuary in South Africa.

Antibiotic resistance is one of the imminent challenges to global health and with the current trends in escalating environmental contaminants such as antibiotic resistance genes; natural environments likely to be potential repositories for the spread of antibiotic resistance (Hernando-Amado et al., 2020). Aminoglycosides are potent antibiotics that function via the inhibition of protein synthesis (Wasserman et al., 2015). We found in this study that there was a wide range from relatively moderate to low and eventually no detection of aminoglycoside resistance genes among the Vibrio isolates. The detection of streptomycin resistance genes (aadA, strA) and gentamicin resistance genes (aphA2) from our study varied among the Vibrio species which corresponds with a previous study by Adesfoye and Okoh (2017). Chloramphenicol resistance genes was analysed among all Vibrio species showing phenotypic resistance to the antibiotic. Chloramphenicol resistance is attributed to the enzymatic inhibition of the drug facilitated by chloramphenicol acetyl-transferases (CAT) (Alcala et al., 2020). The detection of cat resistance genes among some Vibrio isolates from our study is akin with other studies (Yoo et al., 2003; Zhang et al., 2019; Håkonsholm et al., 2020). Beta lactams are the most broadly used class of antimicrobial agents, characterized by minimal toxicity and employed in the treatment of various bacterial ailments including those attributed with different Vibrio species (Bush and Bradford, 2016). Apparently, resistance among the beta-lactam drugs continues to be on the surge. The broad spectrum TEM-beta lactamase has been reported to be widely distributed in plasmids (Tooke et al., 2019). AmpC genes are situated chromosomally but nonetheless, plasmid associated variants among some bacteria have become increasingly recognized and disseminated via horizontal transfer (Lan et al., 2017). Our study detected the presence of ampC and blaTEM genes among the Vibrio species. Carbapenem introduction as broader antimicrobial spectrum
are usually the last resort for most antimicrobial agents due to the proliferation of resistance among most antimicrobial classes. But resistance has been observed among the carbapenems. Our study detected a few beta lactamase resistance genes to carbapenems. Detection of resistance genes from this study correlates with recent reports (Elbadawi et al., 2021) identifying carbapenem resistance genes (blaOXA-48, blaIMP, blaVIM and blaKPC) among Gram negative isolates in Sudan. In addition, blaGES was identified among the three Vibrio species in our study. Documented reports have shown limited Vibrio carrying blaGES genes (Dahanayake et al., 2020; Hossain et al., 2020). These beta-lactam resistance genes are frequently identified in Enterobacteriaceae which portends concern on the impact of sewage in aquatic environment, giving rise to drug resistance bacteria and fortified selective pressure increasing the advent and dissemination of antimicrobial resistance (Fresia et al., 2019). The acquisition of these genes in Vibrio isolates in our study may have been through plasmid mediation in the aquatic environment from other bacteria strains. The addition of fluoride to quinolone creates fluoroquinolones with enhanced bactericidal activity. However, the plasmid-mediated quinolone resistance gene (qnrVC) was identified in two V. fluvialis isolates from our study and similar trend have been reported by Vinothkumar et al. (2016).

**CONCLUSION**

Our present study is a broad assessment of the antimicrobial susceptibility of various Vibrio species that were successfully recovered from aquatic and hospital environments. Detection of multiple drug resistance Vibrio species, isolates harbouring virulence genes as well as the detection of antimicrobial resistance genes suggests risk for public health concern. The maintenance of dysfunctional wastewater treatment plants as well as burst sewage pipes in rural settlements should be rectified to further prevent the leakages of sewage into the surrounding environments. More importantly, the promotion of proper antibiotic stewardships should be upheld in the society to forestall the continuous propagation and sustenance of antimicrobial resistance among bacteria population in nature.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**AUTHOR CONTRIBUTIONS**

UN, Conceptualization. OG and BI, methodology. OG and TD, formal analyses. NU, resources. OG and TD, writing—original draft preparation. OG, TD, BI, OO, AO, and UN, writing—review and editing. UN, supervision. All authors contributed to the article and approved the submitted version.

**ACKNOWLEDGMENTS**

We are grateful to the South Africa Medical Research Council and the University of Fort Hare for financial support.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.732001/full#supplementary-material

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