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RESEARCH ARTICLE

bla$_{NDM-1}$-producing *Vibrio parahaemolyticus* and *V. vulnificus* isolated from recreational beaches in Lagos, Nigeria

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Abstract

Twenty-six strains of *Vibrio parahaemolyticus* and 14 strains of *V. vulnificus* isolated from selected beaches in Lagos State, Nigeria, were examined for virulence and antimicrobial resistance genes. The *V. parahaemolyticus* isolates were further serotyped and subjected to pulsed field gel electrophoresis (PFGE). Five strains of *V. vulnificus* and one of *V. parahaemolyticus* carried the New Delhi-metallo-beta-lactamase gene *bla*$_{NDM-1}$, seven strains carried *bla*$_{TEM}$, and four strains of *V. vulnificus* and one of *V. parahaemolyticus* carried *bla*$_{CMY}$. Real-time PCR assay for detection of virulence genes *tdh* and *trh* in the *V. parahaemolyticus* isolates showed that five isolates were positive for *tdh*, two for *trh*, and one isolate carried both genes. Ten *V. parahaemolyticus* serogroups and 23 pulsotypes were identified from 26 isolates based on O and K antigens typing and PFGE. Five of the isolates belong to the pandemic strains O1:Kut and O3:K6, and three belonged to the highly virulent O4:Kut serotype. Nineteen of the isolates showed distinct PFGE banding patterns. These results highlighted the importance of Nigerian recreational beaches as reservoirs of antimicrobial resistance genes of global public health interest, such as *bla*$_{NDM-1}$.

Keywords *V. parahaemolyticus* · *V. vulnificus* · Recreational beaches · *bla*$_{NDM-1}$ · *bla*$_{TEM}$ · *bla*$_{CMY}$ · *tdh* · *trh*

Introduction

Antimicrobial resistance in bacteria has become a serious public health problem worldwide (WHO 2015). Initially, concerns about the problem of resistance focused on hospitals, but recently, the presence of clinically relevant antimicrobial resistance genes in the natural environment has become a source of worry to the scientific community (Pruden et al. 2013; Berendonk et al. 2015). Routes of exposure to antimicrobial resistance outside the hospital setting include contact with human and animal carriers, and contaminated food and water (Valverde et al. 2008; Kennedy and Collignon 2010; Coleman et al. 2012). Exposure through the water route can occur by drinking contaminated water or by contact with recreational water at freshwater and marine beaches.

Beach tourism has evolved to be an important and rapidly growing activity in the world due to its suitability for recreation and sporting activities (Orams 2003) leading to a concomitant increase in the incidence of infections associated with recreational waters over the past decade (Halliday and Gast 2011). Thus, the current challenge posed by the presence of antimicrobial-resistant bacteria in the environment is an important public health issue for beach goers and those living in coastal areas. This problem is more acute in the developing world where the aquatic ecosystem serves as a sink for untreated wastewater from domestic, industrial, hospital, agricultural, and aquaculture sources. Through this practice, residual antimicrobial agents, antimicrobial-resistant bacteria, and antimicrobial resistance genes enter the aquatic environment (Taylor et al. 2011). In addition, the usual flow of human traffic to coastal areas in search of jobs and recreation with the attendant anthropogenic impact, and the corresponding lack of surface water quality monitoring in many developing countries, make recreational beaches in these countries an area of special interest for the development and spread of antimicrobial resistance (Overbey et al. 2015). Unfortunately, very

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little is known about the health status of beaches in Nigeria, especially as it relates to the presence of bacteria carrying clinically relevant antimicrobial resistance genes.

Lagos State, located in the south-western part of Nigeria on the narrow coastal floodplain of the Bight of Benin, consists of a long coastline of about 255 km long stretching from Epe in the east to Cotonu in the Republic of Benin to the west. This long coastline is home to a number of marine recreational beaches who by virtue of their location in the tropical ecosystem makes them potential breeding ground for the proliferation of pathogenic or potentially pathogenic bacteria such as *Vibrio* species whose presence in the aquatic ecosystem has been known to be positively correlated with mean water temperature (Blackwell and Oliver 2008; Rosec et al. 2009; Johnson et al. 2010; Urquhart et al. 2016).

Potentially, human pathogenic species of *Vibrio* such as *V. parahaemolyticus*, *V. vulnificus*, and non-O1/non-O39 *V. cholera* are common inhabitants of marine and brackish water environments worldwide (Fabbro et al. 2010; Johnson et al. 2012; Oliver et al. 2013; Ceccarelli et al. 2013; Wu et al. 2014). These organisms are important causal agent of septicemia, and gastrointestinal and wound infections, especially in immunocompromised patients (Daniels and Shafaie 2000; Drake et al. 2007). *V. parahaemolyticus* and *V. vulnificus* in particular are leading causes of human intestinal and wound infections after consumption of raw and undercooked seafood or contact with seawater during beach activities (Letchumanan et al. 2014). However, despite the growing importance of environmental *Vibrio* species as agents of opportunistic infections and reservoir of antimicrobial resistance genes (García-Aljaro et al. 2014), most studies of antimicrobial resistance in *Vibrio* species have focused on clinical and aquaculture isolates (Okoh and Igbinosa 2010). Only few studies have investigated the resistance of environmental isolates of *Vibrio* to antimicrobial agents (Baker-Austin et al. 2008; Okoh and Igbinosa 2010; Mudryk et al. 2013; Shaw et al. 2014; Baron et al. 2016; De Menezes et al. 2017; Ghenem and Elhadi 2018); fewer still among these studies focused on recreational beaches and none of these studies emanated from Nigeria, the most populous nation in Africa where little is known of antimicrobial resistance in environmental reservoirs.

In this study, we investigated the prevalence of resistance to selected antimicrobials, and the presence of virulence and antimicrobial resistance genes in *V. parahaemolyticus* and *V. vulnificus* isolated from public beaches along the shoreline of Lagos in southwestern Nigeria. We also used pulse field gel electrophoresis (PFGE) to detect clonal relationship among *V. parahaemolyticus* isolates. Our aim is to generate important baseline information on the health status of Nigerian recreational beaches and their role as reservoir of antimicrobial resistance that can pose a threat to the health of beach goers.

Material and methods

Strains of *V. parahaemolyticus* and *V. vulnificus* used in this study

Eighty-five strains of *V. parahaemolyticus* and *V. vulnificus* were isolated from Eleko (ELK), Lekki (LEK), Takwa Bay (TAK), and Badagry (BDG) beaches, Lagos, Nigeria, as previously described (Oyelade et al. 2018). Typical *V. parahaemolyticus* and *V. vulnificus* colonies were selected from CHROMagar™ Vibrio plates (CHROMagar, Paris, France) after enrichment in alkaline peptone water. Presumptive *V. vulnificus* colonies were further subcultured onto mCPC agar and TCBS agar before the identities of both *V. parahaemolyticus* and *V. vulnificus* were confirmed using API20E (API system, France) (Dumontet et al. 2000) and qPCR detection of *tlh* genes (*V. prahaemolyticus*) and *vvhA* genes (*V. vulnificus*) as previously described (Nordstrom et al. 2007; Campbell and Wright 2003). The isolated organisms were screened for resistance to ceftazidime (16 μg/ml) and meropenem (4 μg/ml) as indicators of extended spectrum beta-lactamase (ESBL) and carbapenemase production based on CLSI breakpoints listed in Table 20 of CLSI document M45-A3 (CLSI 2016). Forty isolates (26 *V. parahaemolyticus* and 14 *V. vulnificus*) that showed resistance to ceftazidime and meropenem were selected for further analysis. The origins, sources, and dates of isolation of the strains are listed in Table 1.

DNA extraction

Genomic DNA was extracted from the isolates by boiling lysis as described by Blackstone et al. (2003) and used as template in the qPCR detection of virulence and antimicrobial resistance genes as described below.

Real-Time qPCR amplification of *tlh*, *tdh*, *trh*, and *vvha*

Real-time qPCR was used for the multiplex detection and quantification of the *tlh*, *tdh*, *trh* (*V. parahaemolyticus*), and *vvha* (*V. vulnificus*) genes with primers (Table 2) and conditions previously described (Campbell and Wright 2003; Nordstrom et al. 2007) using an ABI 7500 instrument (Applied Biosystems Carlsbad, CA, USA). The reaction mixture (25 μl) contains the following: 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), 5.0 mM $MgCl_2$ (Invitrogen), 300 nM of each of the dNTPs (Roche, Indianapolis, USA), 300 nM each of the *trh* and *vvhA* primers, 200 nM *tlh* primers, 100 nM of *tdh* primers, 75 nM of *tlh* and *vvha* IAC primers, 25 nM of *tdh* and *trh* IAC primers, 150 nM *tlh* probe and IAC, 75 nM *tdh* and *trh* probes, 200 nM *vvha* probe, and 1.50 U, 1.12 U, 2.25 U platinum *Taq* polymerase (Invitrogen) for *tlh*, *vvha*, and *tdh/trh* respectively. All primers, IACs, *tlh*, and *vvha* probes were obtained from

Table 1 Origin and source of *V. parahaemolyticus* and *V. vulnificus* used in this study

Location	*V. parahaemolyticus*			*V. vulnificus*		
	Isolate ID Isolated	Source	Date	Isolate ID Isolated	Source	Date
Eleko Beach	ELK 121	Dry sand	10/31/2016	ELK 175	Water	10/31/2017
	ELK 124	Wet sand	10/31/2016	ELK 174	Water	10/31/2017
	ELK 126	Wet sand	10/31/2016	ELK 173	Water	10/31/2017
	ELK 169	Water	10/31/2017	ELK 125	Dry sand	10/31/2016
	ELK 170	Water	10/31/2017			
	ELK 127	Wet sand	10/31/2016			
Lekki Beach	LEK 100	Wet sand	10/31/2016	LEK 163	Water	10/31/2017
	LEK 101	Wet sand	10/31/2016	LEK 164	Water	10/31/2016
	LEK 103	Dry sand	10/31/2016	LEK 95B	Dry sand	10/31/2016
	LEK 108	Wet sand	10/31/2017	LEK 243	Dry sand	10/31/2017
	LEK 110	Wet sand	10/31/2017			
Takwa Bay	TAK 139	Dry sand	11/01/2017	TAK 145B	Wet sand	11/01/2016
	TAK 142	Dry sand	11/01/2017	TAK 181B	Water	11/01/2017
	TAK 145	Wet sand	11/01/2017	TAK 184	Water	11/01/2017
	TAK 132	Wet sand	11/01/2016	TAK 141	Dry sand	11/01/2016
	TAK 133	Wet sand	11/01/2016	TAK 178	Water	11/01/2017
	TAK 135	Dry sand	11/01/2016	TAK 196	Water	11/01/2017
	TAK 137	Dry sand	11/01/2017			
	TAK 138	Dry sand	11/01/2017			
Badagry Beach	BDG 151	Dry sand	11/01/2016			
	BDG 151B	Dry sand	11/01/2016			
	BDG 152	Dry sand	11/01/2016			
	BDG 154	Dry sand	11/01/2016			
	BDG 156	Dry sand	11/01/2017			
	BDG 157	Dry sand	11/01/2017			
	BDG 189	Water	11/01/2017			

Integrated DNA Technologies (Coralville, IA, USA) while the *tdh* and *trh* probes were obtained from Life Technologies (Carlsbad, CA, USA). The rest of the reaction mixture volume consisted of a previously quantified IAC DNA (2 μl), nuclease-free water and DNA templates (2 μl of boiled cell lysates). *V. parahaemolyticus* ATCC 17802 carrying *tlh*, *tdh*, and *trh*, and *V. vulnificus* ATCC 27562 possessing *vvha* gene were used as positive controls while nuclease-free water served as negative control.

Serotyping of *V. parahaemolyticus*

Serotyping of the *V. parahaemolyticus* isolates was performed using a commercially available *V. parahaemolyticus* antiserum test kit (Denka Seiken, Tokyo, Japan) according to the manufacturer's instructions. For O antigen typing, a heavy cell suspension was prepared in a normal saline solution and was then autoclaved at 121 °C for 2 h. A 1-μl loopful of the autoclaved suspension was added to one drop of each O antiserum which was observed for agglutination. For K antigen typing, a 1-μl loopful of isolate colony was mixed with one drop of each pool of K antisera and was observed for agglutination. If an isolate agglutinated in any K pool, the individual antisera of that pool were tested.

Antimicrobial resistance analysis

Antimicrobial resistance analysis was carried out using MicroScan Dried Gram Negative antibiotic panel (Beckman Coulter, Inc., Brea, CA, USA) containing 30 antimicrobial agents. The list of antimicrobial agents used, breakpoints, and references are listed in Table S3. The production of extended spectrum beta-lactamase (ESBL) in isolates showing phenotypic resistance to third-generation cephalosporins was confirmed using MicroScan ESBL *plus* panel. Inoculum preparation was performed in 2% NaCl solution according to the manufacturer's instruction based on CLSI document M07-A10 (CLSI 2015a). Test panels were incubated for 16–20 h at 35 °C and results interpreted using the CLSI breakpoints described for Enterobacteriaceae in Table 2A of document M100-S25 for the following antimicrobial agents: aztreonam, cefotetan,

Table 2 Serotype and detection of *tlh* and pathogenic determinants *tdh* and *trh* among the *V. parahaemolyticus* isolates

Location	Sample	Source	*tlh* gene	*tdh* gene	*trh* gene	Serotype
Eleko Beach	ELK 121	Dry sand	+	–	–	O4:K untypeabe
	ELK 124	Wet sand	+	+	–	O3:K6
	ELK 126	Wet sand	+	+	–	O1:K untypeabe
	ELK 169	Water	+	–	–	O11:K untypeabe
	ELK 170	Water	+	–	–	O11:K untypeabe
	ELK 127	Wet sand	+	+	–	O3:K6
Lekki Beach	LEK 100	Wet sand	+	–	–	O2:K3
	LEK 101	Wet sand	+	–	–	O5:K untypeabe
	LEK 103	Dry sand	+	–	–	O5:K untypeabe
	LEK 108	Wet sand	+	+	+	O10:K untypeabe
	LEK 110	Wet sand	+	–	–	O1:K untypeabe
Takwa Bay	TAK 139	Dry sand	+	–	–	O8:K70
	TAK 142	Dry sand	+	–	–	O8:K70
	TAK 145	Wet sand	+	–	–	O3:K31
	TAK 132	Wet sand	+	–	–	O2:K3
	TAK 133	Wet sand	+	–	–	O1:K untypeabe
	TAK 135	Dry sand	+	–	+	O4:K untypeabe
	TAK 137	Dry sand	+	–	–	O5:K untypeabe
	TAK 138	Dry sand	+	–	–	O1:K untypeabe
Badagry	BDG 151	Dry sand	+	–	–	O5:K30
	BDG 151B	Dry sand	+	–	–	O3:K31
	BDG 152	Dry sand	+	+	–	O3:K31
	BDG 154	Dry sand	+	–	–	O4:K untypeabe
	BDG 156	Dry sand	+	–	–	O5:K untypeabe
	BDG 157	Dry sand	+	–	–	O5:K untypeabe
	BDG 189	Water	+	–	–	O5:K untypeabe

tlh thermolabile hemolysin gene, *tdh* thermostable direct hemolysin, *trh* thermostable direct hemolysin-related hemolysin

ceftriaxone, cephalothin, ertapenem, nitrofurantoin, ticarcillin/K clavulanate, and tobramycin (CLSI 2015b). Breakpoints specific for *Vibrio* spp. in Table 20 of document M45-A3 was used for interpretation of the following antimicrobial agents: amikacin, ampicillin, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, cefoxitin, ceftazidime, cefuroxime, chloramphenicol, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, tetracycline, and trimethoprim/sulfamethoxazole (CLSI 2016). Cefotaxime/K clavulanate and ceftazidime/K clavulanate do not have interpretive breakpoint criteria, a ≥3-fold MIC decrease between CAZ and CAZ/CA; CTX and CTX/CA are considered positive for ESBL production according to the manufacturer's manual. For tigecycline, the manufacturer's breakpoint was used.

Detection of beta-lactams resistance genes

qPCR was carried out to detect β-lactams resistance genes from ESBL-producing *V. parahaemolyticus*) and *V. vulnificus* isolates with primers targeting the following beta-lactam resistance genes: bla_{KPC}, bla_{NDM-1}, bla_{TEM}, bla_{SHV}, $bla_{CTX-M-1}$ and $_{-M-9}$ (Cluster A), and bla_{CMY} (Table S1). Total genomic DNA for antimicrobial resistance gene detection was extracted from each isolate according to the Center for Disease Control and Prevention (CDC) protocol for Multiplex Real-Time PCR Detection of bla_{KPC} and bla_{NDM-1} (CDC 2011). The reaction mixture (25 μl) contains 1× PCR buffer (Invitrogen, Carlsbad, CA, USA), 5.0 mM $MgCl_2$ (Invitrogen), 300 nM of each of the dNTPs (Roche, Indianapolis, USA), 200 nM forward and reverse primers (Integrated DNA Technologies), 75 nM for IAC forward and reverse primers (Integrated DNA Technologies), 150 nM probes (Integrated DNA Technologies), and 1.50 U, platinum *Taq* polymerase (Invitrogen). The rest of the reaction mixture volume consisted of a previously quantified IAC DNA (2 μl), nuclease-free water, and a DNA template (2 μl cell lysates). The cycling parameters consisted of a 95 °C hold for 3 min for the initial denaturation and activation of the hot-start Taq polymerase, followed by 35 cycles of amplification, with each cycle consisting of denaturation at 95 °C for 3 s and a combined annealing/extension step at 60 °C for 30 s. *Klebsiella*

pneumoniae ATCC BAA-1705 strain possessing bla_{KPC} (as described by ATCC) and *K. pneumoniae* ATCC BAA-2146 carrying the bla_{NDM-1} (as described by ATCC), bla_{TEM}, bla_{SHV}, $bla_{CTX-M-15}$, and bla_{CMY} (Leski et al. 2012) were used as positive controls while the negative control was nuclease-free water added to the PCR mix as a template.

Pulse field gel electrophoresis analysis of *V. parahaemolyticus*

Pulse field gel electrophoresis (PFGE) was performed according to the CDC PulseNet protocol for *V. parahaemolyticus* (CDC 2013) using *Sfi* I (40 U/sample) (Roche Diagnostics, Indianapolis, IN, USA) for restriction digestion. The restricted fragments were resolved in 1% seakem gold agarose gel in 0.5% Tris-boric acid-EDTA buffer using CHEF Mapper system (Bio-Rad Laboratories, Richmond, CA, USA). The following electrophoresis conditions were used: Auto Algorithm of 78 kb and 396 kb for low and high molecular weight, initial and final switch time of 10 s and 35.03 s, and run time of 16.5 h at 14 °C. Voltage density and included angle were 6.0 V/cm and 120° respectively, while linear ramping and pump speed was 1 l/min. *Xba* I digested DNA from *Salmonella enterica serovar Braenderup* strain H9812 was used as a molecular size marker. Stained gels were visualized using Gel Doc XR$^+$ (Bio-Rad Laboratories, Richmond, CA, USA). The PFGE patterns were analyzed using BioNumerics software version 7.6 (Applied Maths, Austin, TX, USA). Clustering was performed using the unweighted pair group method and the Dice correlation coefficient with position tolerance of 1.5%. Clusters were defined on the basis of an 80% similarity cutoff (CDC 2013).

Results and discussion

In Nigeria, studies of *Vibrio* species are mostly focused on the detection of these organisms in seafoods (Oramadike and Ogunbanwo 2015; Adebayo-Tayo et al. 2011) with few studies focusing on environmental isolates (Eyisi et al. 2013). In this study, we isolated *V. parahaemolyticus* and *V. vulnificus* from recreational beaches along the shoreline of Lagos, Nigeria. *V. parahaemolyticus* was isolated from all the beaches sampled and occurred more frequently in beach sand compared to seawater samples (Table 1). *V. vulnificus* was, however, only detected from water samples of ELK, LEK, and TAK, and sand from ELK and LEK.

Detection of *tlh* and *tdh/trh* genes and serotyping of *V. parahaemolyticus*

The results of real-time PCR assay showed that all the tested *V. parahaemolyticus* were positive for thermolabile hemolysin (*tlh*) gene (Table 2) confirming the identity of these isolates as *V. parahaemolyticus* (Brasher et al. 1998; Bej et al. 1999; Rojas et al. 2011). Similarly, all *V. vulnificus* isolates were positive for the cytotoxin hemolysin protein encoded by *vvha*. Further, results of real-time PCR assay for detection of virulence genes *tdh* and *trh* showed that five isolates were positive for *tdh* and two isolates for *trh* while one isolate (LEK 108) carried both *tdh* and *trh* (Table 2). *tdh* and *trh* are the most predictive indicators of potential virulence in *V. parahaemolyticus* (Baker-Austin et al. 2008) and are responsible for hemolytic activity in many clinical strains (Ceccarelli et al. 2013). Similar to our results, previous studies (Cook et al. 2002; Hara-Kudo et al. 2003; Robert-Pillot et al. 2004; Baker-Austin et al. 2008; Li et al. 2015; He et al. 2016; Ghenem and Elhadi 2018) have reported a low prevalence of these virulence factors in food and environmental *V. parahaemolyticus* strains, confirming that non-clinical isolates of this bacteria rarely possess virulence factors. However, the prevalence of virulence factors reported in our study is higher than those reported in the studies cited above. Additionally, we also detected the co-occurrence of *tdh* and *trh* genes in an isolate from one of our study sites.

Serotyping revealed 10 different serogroups among the 26 isolates based on O and K antigens (Table 2). The most prevalent serogroup is the O5:Kut (K untypeable) with six isolates, followed by O1:Kut (4 isolates), O3:K31 and O4:Kut (3 isolates each), O2:K3, O3:K6, O8:K70, and O11:Kut with two isolates each, and O5:K30 and O10:Kut with one isolate each. Two serotypes of public health importance were isolated in three of the beaches included in this study. The first of such serotype, O3:K6, was detected in *V. parahaemolyticus* strain isolated from ELK. Serotype O3:K6 has been reported in large outbreaks of *V. parahaemolyticus* infections worldwide (Raszl et al. 2016) but are rarely detected in food and environmental isolates (Raghunath 2014). Secondly, serotype O4:Kut, known to be more virulent than other pathogenic strains of *V. parahaemolyticus* and has previously caused large outbreaks in the USA, Europe, and South America (Martinez-Urtaza et al. 2013; Newton et al. 2014; Haendiges et al. 2015), was isolated from ELK, TAK, and BDG. The detection of these serotypes in the Nigerian coastal environment, especially from recreational beaches, is of great public health concern for beach users.

Antimicrobial resistance analysis of *V. parahaemolyticus* and *V. vulnificus*

Results of susceptibility testing using 30 antibiotics revealed that all the *V. parahaemolyticus* and *V. vulnificus* isolates showed resistance to multiple antibiotics with the least being resistance to seven antibiotics in combination. There was 100% resistance to AMP, CFZ, CEF, and PIP among the isolates; and all *V. parahaemolyticus* isolates were in addition resistant to CAZ, TIM, AMK, and TOB (Fig. 1, Table S2). In contrast,

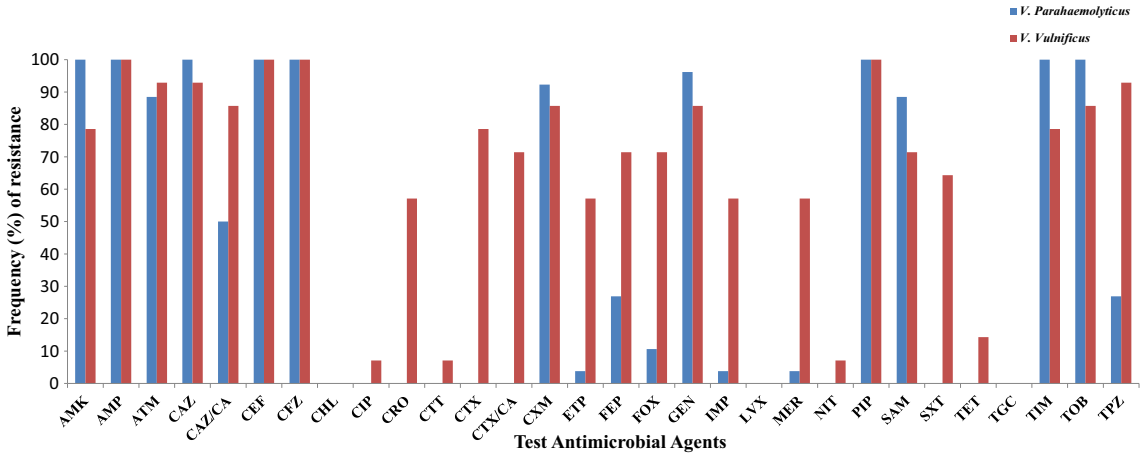

Fig. 1 Frequency of resistance to antimicrobial agents among *V. parahaemolyticus* and *V. vulnificus* isolates from the Nigerian Beaches. AMK amikacin, *AMP* ampicillin, *ATM* aztreonam, *CAZ* Ceftazidime, *CAZ/CA* ceftazidime/clavulanate, *CEF* cephalothin, *CFZ* cefazolin, *CHL* chloramphenicol, *CIP* ciprofloxacin, *CRO* ceftriaxone, *CTT* cefotetan, *CTX* cefotaxime, *CTX/CA* cefotaxime/clavulanate, *CXM* cefuroxime, *ETP* ertapenem, *FEP* cefepime, *FOX* cefoxitin, *GEN* gentamicin, *IMP* imipenem, *LVX* levofloxacin, *MER* meropenem, *NIT* nitrofuratoin, *PIP* piperacillin, *SAM* ampicillin/sulbactam, *SXT* trimethoprim/sulfamethoxazole, *TET* tetracycline, *TGC* tigecycline, *TIM* ticarcillin/clavulanate, *TOB* tobramycin, *TZP* piperacillin/taxobactam,

all isolates were sensitive to CHL, LVX, and TGC while in addition to these three antibiotics, all *V. parahaemolyticus* were sensitive to CTX, CTX/CA, CRO, CTT, SXT, CIP, NIT, and TET (Fig. 1, Table S2). One *V. parahaemolyticus* isolate from Takwa Bay (TAK 145) and ten *V. vulnificus* isolates from ELK (ELK 125, 173, 175), LEK (95B, 164), and TAK (TAK 141, 178, 181B, 184, 196) showed resistance to carbapenems (IMP, MER, and ETP) (Table S2).

The widespread resistance shown by the isolates of the present study to third (CAZ, CRO, CTX) and fourth-generation (FEP) cephalosporins, which remains rare among *Vibrio* species (Han et al. 2007; Baker-Austin et al. 2008; Wong et al. 2012; Li et al. 2015), is worrisome. Further, in contrast to previous observations by Baker-Austin et al. (2008), we found no significant difference in the resistance (12.6 antimicrobials per isolate) of *V. parahaemolyticus* isolates possessing the *tdh* and *trh* virulence determinants their counterparts lacking these genes (13.5 antimicrobials per isolate).

Detection of β-lactams resistance genes in ESBL-producing *Vibrio* isolates

The New Delhi metallo-β-lactamase gene $bla_{\text{NDM-1}}$ was found in five strains of *V. vulnificus* from ELK (ELK125, ELK175), LEK (LEK164), and TAK (TAK141, TAK196) and one *V. parahaemolyticus* isolated from TAK (TAK145) (Table 3). All the $bla_{\text{NDM-1}}$-positive isolates except one which carried bla_{TEM} (ELK175) were also positive for bla_{TEM} and bla_{CMY}. None of the tested genes was found in one isolate of *V. vulnificus* (TAK 178). All the $bla_{\text{NDM-1}}$ producers showed resistance to multiple antibiotics with the least being resistant to 17 antibiotics. One $bla_{\text{NDM-1}}$ producer (ELK175) was resistant to 24 of the 30 antibiotics tested. $bla_{\text{NDM-1}}$ producers are always resistant to several classes of antibiotics (Rolain et al. 2010; Nordmann and Poirel 2014).

There are increasing reports of the carriage of cephalosporinase-encoding genes among *Vibrio* species in other parts of the world. These include $bla_{\text{CMY-2}}$ located on IncA/C plasmids in *V. parahaemolyticus* (Li et al. 2015) and *V. alginolyticus* (Ye et al. 2016), $bla_{\text{VEB-2}}$ in *V. parahaemolyticus* (Li et al. 2016), and $bla_{\text{PER-1}}$ located on an IncN plasmid in *V. parahaemolyticus*, all isolated from seafood in China. Further complicating this disturbing observation is the increasing report of carbapenemases, which in some cases are novel (Bier et al. 2015; Mangat et al. 2016) in members of the genus *Vibrionaceae*. Previous studies have detected $bla_{\text{NDM-1}}$ in *V. parahaemolyticus* isolated from shrimps (Briet et al. 2018) and other *Vibrio* species such as environmental *V. cholera* in India (Walsh et al. 2011), clinical *V. cholera* O1 E1 Tor in india (Mandal et al. 2012), environmental non O1/non O39 *V. cholera* in Vietnam (Diep et al. 2015); clinical non O1/non O39 *V. cholera* in the UK (Darley et al. 2012), and

Table 3 Profile of antibiotic resistance genes detected among ESBL-producing isolates

Isolates	Identity	Resistance genes profile
ELK 175	*Vibrio vulnificus*	$bla_{\text{NDM-1}}$, bla_{TEM}
ELK 125	*V. vulnificus*	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{CMY}
LEK 163	*V. vulnificus*	bla_{TEM}
LEK 164	*V. vulnificus*	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{CMY}
TAK 145	*V. parahaemolyticus*	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{CMY}
TAK 141	*V. vulnificus*	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{CMY}
TAK 196	*V. vulnificus*	$bla_{\text{NDM-1}}$, bla_{TEM}, bla_{CMY}

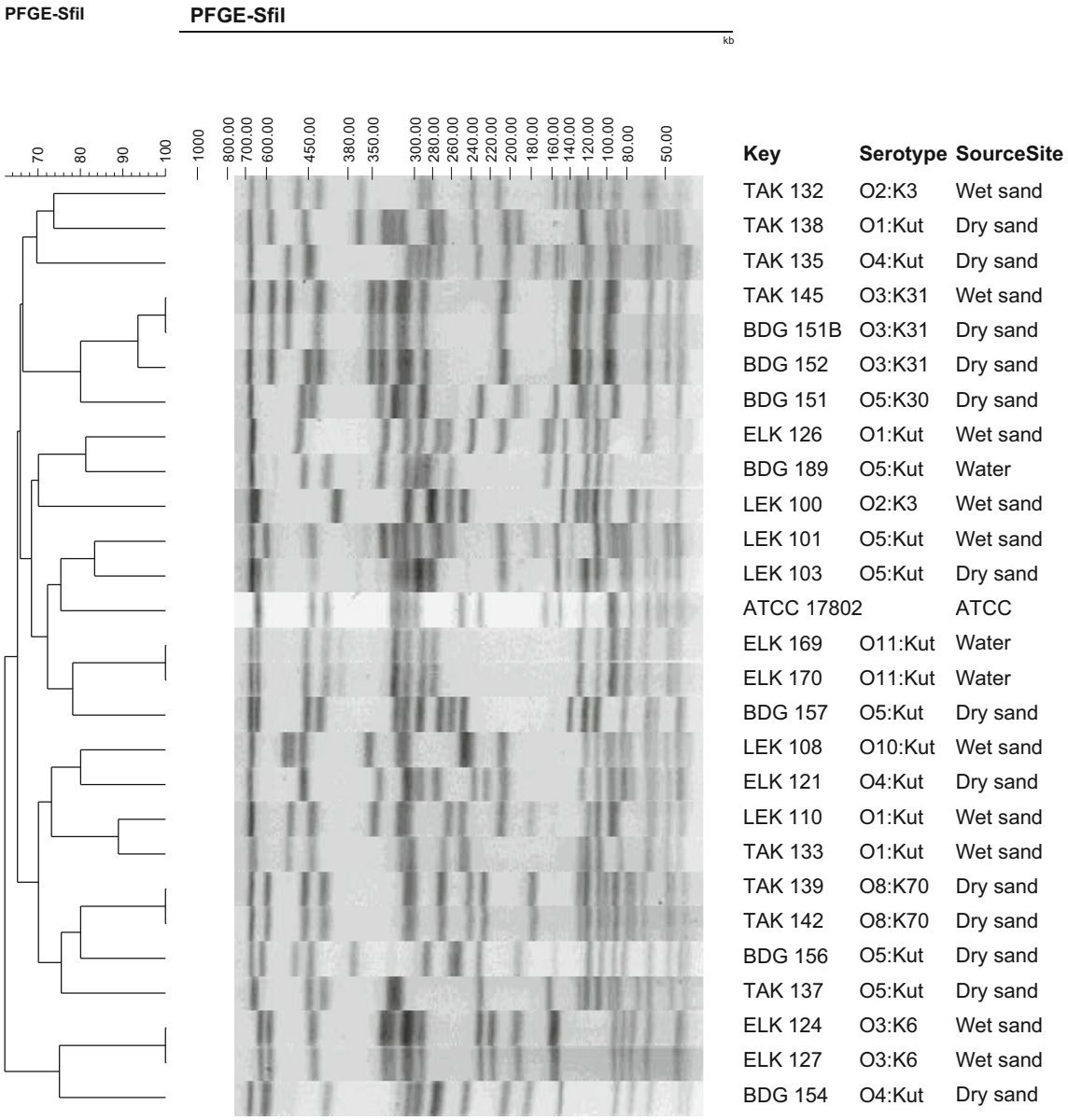

Fig. 2 PFGE profile of 26 strains of *V. parahaemolyticus* isolates after *Sfi*I enzyme restriction. *V. parahaemolyticus* ATCC 17802 was used as a control

V. fluvialis of clinical origin in India (Chowdhury et al. 2016). However, reports of bla_{NDM-1} in *V. parahaemolyticus* and *V. vulnificus* are not very common. The presence of these genes in bacteria of public health importance such as *V. parahaemolyticus* and *V. vulnificus* portend great danger for beach goers as they not only limit treatment options in case of infection, but they can also transmit their resistance elements to other pathogenic and potentially pathogenic bacteria in the beach ecosystem.

Pulse field gel electrophoresis analysis of *V. parahaemolyticus* strains

The PFGE profiles of the *Sfi*I-digested DNA of *V. parahaemolyticus* are presented in Fig. 2. A total of 23 pulsotypes were recognized among 26 *V. parahaemolyticus* strains with 19 isolates showing distinct banding patterns; the remaining four banding patterns included at least two isolates. This suggested a large degree of genomic variation among the isolates even within the same sampling sites. It also suggested that the isolates included in this study are not just clonal replicates. The isolates sharing the same banding patterns are TAK 145 and BDG 151B both belonging to serotype O3:K31, ELK 169 and ELK 170 both with serotype O11:Kut, TAK 139 and TAK 142 both with serotype O8:K70, and ELK 124 and ELK 127 with serotype O3:K6 respectively. It is interesting to note that the only clade found among this organism is between isolate TAK 145 from wet sand sample collected from Takwa Bay beach and BDG 151B from dry sand sample collected from Badagry beach.

In conclusion, we isolated *V. parahaemolyticus* and *V. vulnificus* showing resistance to multiple antimicrobial agents from four recreational beaches in Lagos, southwestern Nigeria. The virulence gene *tdh* and *trh* was detected in six isolates of *V. parahaemolyticus* with one isolate positive for both genes, confirming the presence of potentially pathogenic strains of *V. parahaemolyticus* in Nigerian recreational waters. Five *V. vulnificus* and one *V. parahaemolyticus* tested positive for the metallo-β-lactamase gene bla_{NDM-1}which occurred together with bla_{TEM} and bla_{CMY} in five isolates. The detection of these genes in public beaches visited by beach goers is of great public health concern and highlights the need for urgent mitigation efforts to protect beach goers and curbs further dissemination of these resistance genes to other bacterial population through horizontal gene transfer.

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