

## Characterization of *Vibrio vulnificus* Isolated from the Coastal Areas in the Eastern Province of Saudi Arabia

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*Vibrio vulnificus* is a Gram-negative, halophilic bacterium that mainly inhabits marine environments. It is responsible for causing gastroenteritis upon consuming contaminated seafood or exposure of an open wound to seawater. In addition, it has the ability to cause wound infection and septicaemia. It is also known to be an opportunistic organism that targets immunocompromised patients and those with liver disease. In the present study, 362 seawater samples were collected from 17 different locations along the coastal areas of the Eastern Province of Saudi Arabia and were analyzed for the presence of *V. vulnificus*. There were 65 (17.95%) positive samples and 234 isolates of *V. vulnificus*. All positive isolates were tested for pathogenicity using PCR to detect the hemolysin-cytolysin (*vvhA*) gene, which was found in 52 (22%) of the isolates. The antibiotic susceptibility test indicated high resistance to ampicillin (96%), cephalothin (73%), rifampicin (63%), and amoxicillin-clavulanic acid (56%). The MAR index was calculated for all antibiotics and revealed significant values (>0.2) for 34.6% of *V. vulnificus* isolates. Isolates positive for the *vvhA* gene were genotyped by using Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) DNA fingerprinting. ERIC-PCR fingerprints of 52 isolates of *V. vulnificus* generated high similarity scores ranging from 85 to 100%, indicating significant genetic relatedness between the isolates. This study is the first to report the isolation of *V. vulnificus* positive for the *vvhA* gene from the coastal water in the Eastern Province of Saudi Arabia.

**Keywords:** *Vibrio vulnificus*; hemolysin-cytolysin gene (*vvhA*); antibiotic resistance; ERIC-PCR.

Humans have a permanent relationship with marine systems, including different activities such as fishing for food, transportation, and entertainment. This has had an impact on the development of human life. However, it has a poor effect on coastal and estuarine water environments. The quality of water in developing countries has been declining throughout the years. Economic

development, industrial growth, and social progress are changing the balance of the ecosystem<sup>1</sup>. Pollution is the first factor that results from contaminating water by different waste, including oil, agricultural chemicals, sewage, and factory waste. All these environmental modifications lead to increased incidence of emerging diseases<sup>2</sup>.

Emerging pathogens that are causing new infections and antimicrobial resistance have had increasing incidence in the human population in the past two decades and are expected to increase in the future.<sup>2</sup> One of the emerging organisms is bacteria in the genus *Vibrio*, which belongs to the *Vibrionaceae* family. According to the Centers for

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Disease Control and prevention (CDC), *Vibrio* infections are classified as cholera and vibriosis<sup>3</sup>. The CDC has established a national system to monitor infections by *V. cholerae* and other vibriosis cases, which is known as Cholera and Other *Vibrio* Illness Surveillance (COVIS)<sup>3</sup>. The CDC initiated COVIS in collaboration with the Food and Drug Administration (FDA), and this system is used for reporting human infections with pathogenic *Vibrio* species. The program is also interested in testing data on antibiotic susceptibility and the route of transmission of these pathogens. In the United States, COVIS reported an increase of *Vibrio* infection between 1996 and 2010, and the analysis determined that the most cases of death were from *Vibrio vulnificus*<sup>3,4</sup>.

*V. vulnificus* is a halophilic bacterium that is naturally found in brackish and fresh water. It is an opportunistic organism that mainly targets immunosuppressed patients and those with liver disease<sup>5</sup>. The most common disease caused by *V. vulnificus* is gastroenteritis, which results from the consumption of contaminated seafood. *V. vulnificus* has the ability to cross the intestinal wall and enter the blood stream, which results in aggressive infection and primary septicemia. Direct contact between an open wound and sea water may lead to infection with a high mortality rate<sup>6,7</sup>. *V. vulnificus* is classified into different biotypes depending on genetics, serology, chemical reactions, and type of host. The essential virulence genes are well defined and affect motility, polysaccharides, the potential for neutralization by acid, the iron acquisition system, and hemolysin<sup>8</sup>.

Laboratory identification with the conventional culture method is done on proper media, followed by chemical and serology identification, as recommended by the FDA<sup>9</sup>. Molecular methods for detection are considered to be the most sensitive and specific. Infection by *V. vulnificus* is rare with approximately 100 cases occurring per year in the USA and Europe. It is responsible for 95% of deaths related to foodborne pathogens in the USA<sup>10</sup>. Increased infections by *V. vulnificus* are expected in the future<sup>11</sup>. The abundance of *V. vulnificus* has seasonal variability because of the effects of temperature and salinity on proliferation<sup>12</sup>. We conducted a study on the occurrence of *V. vulnificus* in seawater samples by testing the isolates for the hemolysin-cytolysin

(*vvhA*) gene and antimicrobial susceptibility. In addition, all the positive isolates for the *vvhA* gene were genotyped by ERIC-PCR.

## MATERIALS AND METHODS

### Study site

The Arabian Gulf is a subtropical marine system that is very shallow with a maximum depth of around 60 m. Many studies report that the gulf environment is under different stresses that lead to alteration of the surroundings. The most significant natural alterations are the warming of the climate and salinity<sup>13</sup>. Due to the impacts of development in Arabian Gulf countries, the water is polluted by industrial wastes such as heavy metals, oil, and gases, as well as sewage discharge. The Arabian Gulf consider has high pollution due to the semi-enclosed ecosystem with low water exchange<sup>1</sup>.

In this study, seawater samples were collected from 17 different locations that were divided into two or three sites, which were coordinated using GPS. The locations are along the coastline of the Arabian Gulf, and recreational areas were targeted, as shown in Fig. 1. The locations are Alaziziyah Beach (AZB), Corniche Tiba Jubail (CTJ), Dammam Corniche (DMC), Dammam Marina Front (DMF), Fanateer Corniche (FNC), Half-Moon Beach (HMF), Alkobar Corniche (KBC), Alkobar Marina Front (KBF), Albuhaireh Beach (LAK), Almorjan Island (MOI), Palm Beach Jubail (PBJ), Qatif Corniche (QTC), Ras Tanura Corniche (RTC), Saihat Corniche (SEC), Alshibaly (SHB), Tarout Corniche (TRC), and Imam Abdulrahman Bin Faisal University (IAU).

### Water sample collection and transportation

A total of 362 seawater samples were collected from the surfaces of the different locations between February 2015 and February 2016. The samples were collected using sterile 500-ml screw-cap bottles (Fischer, UK) and kept in insulated coolers. All samples were immediately transported to the microbiology research laboratory at Imam Abdulrahman Bin Faisal University and analyzed for the isolation of *V. vulnificus*.

### Sample treatment and cultivation

All seawater samples were enriched with alkaline peptone water (APW) containing 1% NaCl, as described in the Bacteriological Analytical Manual (BAM) of the FDA<sup>9</sup>. Briefly, 25 ml of the

samples were added to 225 ml of prepared APW and then incubated for 18-24 hours at 37°C. After the enrichment step, full loops of enriched seawater samples were streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Oxoid, UK) and Vibrio chromogenic agar (CHROM Agar, France) and incubated at 37°C for 18-24 hours. Since *V. vulnificus* varies in fermenting sucrose, the colonies appeared on TCBS in yellow and green colors. On the Vibrio chromogenic agar, the colonies appeared as turquoise. Around 3 to 5 typical colonies were selected and sub-cultured on tryptic soy agar (TSA) enriched with 3% NaCl and incubated at 37°C.

#### Biochemical identification

After the culture and isolation of pure colonies, presumptive *V. vulnificus* was confirmed by a series of biochemical tests. The confirmation includes Gram staining, oxidase tests, and Kligler's iron agar (KIA). For typical *V. vulnificus*, the results must be Gram negative rods and produce a purple color on strips that indicate a positive oxidase test. When the selected colonies are emulsified in bile salt on a clean slide, they appear viscous. Pulling the loop gently produces a mucoid string mass, which indicates a positive string test, and the colonies have a K/A pattern with no gas or H<sub>2</sub>S production. All the isolates of typical *V. vulnificus* according to biochemical tests were reconfirmed by API 20E strip tests (Bio Mérieux, France). The API suspension was prepared using 2% saline, and *V. vulnificus* ATCC 27562 strain was used as a positive control. The pure identified colonies of *V. vulnificus* were preserved in tryptic soya broth supplemented with 3% NaCl and glycerol and stored at -80°C.

#### Genomic DNA Extraction

For genomic DNA extraction, one loop full of the preserved presumptive *V. vulnificus* was sub-cultured on Luria-Bertani (LB) broth supplemented with 3% NaCl and then incubated at 37°C over night. The DNA was extracted according to Silvester et al. (2015) with little modification. Briefly, 1.5 ml of the incubated LB broth was centrifuged at 10,621 x g for 2 minutes, and the supernatant was decanted. The pellets were then suspended in sterile distilled water, followed by vortexing for 2 minutes. Finally, all sample suspensions were boiled in a water bath at 100°C for 15 minutes to lyse the bacterial cells and free the DNA. After boiling, all samples were kept at

-20°C to be used for detection of the *vvhA* gene and ERIC-PCR genotyping.

#### Detection of *vvhA* gene in *Vibrio vulnificus*

PCR amplification was performed to detect the *vvhA* gene as described elsewhere<sup>14</sup>. The total volume of the reaction mixture was 25 µl, which was composed of 1 µl of diluted DNA (1:10) template, 5 µl of 10X buffer (Promega, USA), 4 µl of 25 mM MgCl<sub>2</sub> solution (Promega, USA), 0.5 µl of 2.5 mM deoxynucleotide triphosphate (dNTP) solution (Promega, USA), 0.25 µl of Taq DNA polymerase (Promega, USA), 1 µl of forward primer 52 TTCCAACCTCAAACCGAACTATGA-32, 1 µl of reverse primer 52 ATTCCAGTCGATG CG AATACGTTG-32, and 12.25 µl of deionized water (Promega, USA). *V. vulnificus* ATCC 27562 was used as a positive control, while *V. alginolyticus* ATCC 17749 was used as a negative control.

The reaction program began at 94°C for initial denaturation for 3 minutes, followed by 30 cycles of amplification including 1 minute at 94°C for denaturation, 1 minute at 65°C for annealing, and 1 minute at 72°C for extension. The 30 amplification cycles were followed by a final extension step at 72°C for 5 minutes. After that, 10 µl of the PCR reaction mixture was loaded on 1.5% agarose electrophoresis gel with 1 µl of ethidium bromide dye (Promega, USA) added to make it visible under UV light. The buffer used was 1X Tris Borate EDTA. The positive *vvhA* gene was determined after comparison with the positive control, which has a 205-bp amplicon.

#### Enterobacterial repetitive intergenic consensus (ERIC) PCR

ERIC-PCR was performed according to a previously described method<sup>15</sup>. The total volume of the reaction mixture was 25 µl, which was composed of 1 µl of diluted DNA (1:10) template, 5 µl of 10X buffer (Promega, USA), 4 µl of 25 mM MgCl<sub>2</sub> solution (Promega, USA), 0.5 µl of 2.5 mM dNTPs solution (Promega, USA), 0.25 µl of Taq DNA polymerase (Promega, USA), 2 µl of ERIC primer, and 12.25 µl of distilled water. The amplification reaction was 4 minutes at 94°C for pre-denaturation, followed by 35 cycles of 45 seconds at 94°C for denaturation, 1 minute at 52°C for annealing, an extension step at 65°C for 8 minutes, and a final extension at 65°C for 10 minutes. After that, 10 µl of the PCR reaction

mixture was loaded on 1% agarose electrophoresis gel with 1 µl of ethidium bromide dye added (Promega, USA) to make it visible under UV light. The buffer used was 1X Tris Borate EDTA.

#### Fingerprinting analysis

All gels were scanned for DNA bands, and the images were captured using a gel documentation system (Syngene G: Box). The gel images were analyzed using GelJ software to analyze the DNA fingerprint<sup>16</sup>. The dendrogram tree was constructed based on the unweighted average pair group method (UPGMA) using a Dice coefficient. The numerical index of discrimination (D) was calculated empirically using a previously described formula<sup>17</sup>:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j - 1)$$

where D is the discriminatory index, N is the total number of strains in the sample population, s is the total number of types described, and  $n_j$  is the number of strains belonging to the  $j^{\text{th}}$  type.

#### Antibiotic susceptibility testing

The isolates of *V. vulnificus* recovered from sea water samples that were positive for the *vvhA* gene were tested against 24 antibiotics

(Oxoid, England). The disc diffusion method was performed according to the CLSI protocols<sup>18</sup>. The following antimicrobial agents were tested: ampicillin (AMP: 25 µg), amoxicillin-clavulanic acid (AMC: 30 µg), piperacillin (PRL: 100 µg), piperacillin-tazobactam (TZP: 110 µg), aztreonam (ATM: 30 µg), cephalothin (KF: 30 µg), cefoxitin (FOX: 30 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), ceftriaxone (CRO: 30 µg), cefepime (FEP: 30 µg), imipenem (IPM: 10 µg), meropenem (MEM: 10 µg), amikin (AK: 30 µg), gentamicin (GN: 10 µg), streptomycin (S: 10 µg), tetracycline (TE: 30 µg), doxycycline (DO: 30 µg), ciprofloxacin (CIP: 5 µg), levofloxacin (LEV: 5 µg), chloramphenicol (C: 30 µg), trimethoprim-sulfate (SXT: 25 mcg), nalidixic acid (NA: 30 µg), and rifampicin (RD: 5 µg). The reference strain *Escherichia coli* ATCC 1175 was used as a control while performing the antibiotic susceptibility testing.

#### Multiple antibiotic resistance index

The Multiple Antibiotic Resistance (MAR) index was calculated as  $a/b$ , where  $a$  is the number of multiple antibiotics to which the isolates are resistant, and  $b$  is the total number of multiple antibiotics to which particular isolates have been exposed<sup>19</sup>.

**Table 1.** Total number of seawater samples and *V. vulnificus* isolates recovered from different locations

Location	No. of samples	No. of positives samples	No. of isolates	No. of isolates positive for <i>vvhA</i> gene by using PCR
Alaziziyah Beach (AZB)	29	6	25	6
Corniche Tiba Jubail (CTJ)	11	3	9	2
Dammam Corniche (DMC)	25	5	23	6
Dammam marina Front (DMF)	14	2	6	2
Fanateer Corniche (FNC)	16	3	8	1
Half-Moon Beach (HMF)	36	2	5	1
Alkobar Corniche (KBC)	13	5	11	3
Alkobar Marina Front (KBF)	5	1	3	2
Albuhairah Beach (LAK)	101	17	82	27
Almorjan Island (MOI)	15	2	5	0
Palm Beach Jubail (PBJ)	15	4	13	2
Qatif Corniche (QTC)	12	2	4	0
Ras Tanura Corniche (RTC)	19	3	9	0
Saihat Corniche (SEC)	3	1	2	0
Alshibaly (SHB)	8	2	5	0
Tarout Corniche (TRC)	30	5	18	0
Imam Abdulrahman Bin Faisal University (IAU)	10	2	6	0
Total	362	65 (17.95 %)	234	52 (21.8%)

### Multidrug resistance

The categories of antibiotics used in this study were penicillins, cephalosporin, carbapenem, aminoglycosides, tetracycline, fluoroquinolone, amphenicol, folate inhibitor, quinolone, and rifampin. The strains with resistance to at least one antibiotic from three or more different classes of antimicrobial were considered as having multidrug resistance (MDR)<sup>20</sup>.

## RESULTS AND DISCUSSION

This study aimed to determine the occurrence and characterization of *V. vulnificus* isolated from seawater samples collected from the eastern coastal environment of Saudi Arabia (Fig. 1). *V. vulnificus* is a marine bacterium that naturally inhabits estuarine and coastal waters worldwide. Infection by *V. vulnificus* includes self-limited gastroenteritis, primary septicemia,

and wound infection with a 50% mortality rate<sup>6</sup>. Several studies report that approximately 95% of cases occur in the subtropical Western Pacific and Atlantic coastal regions, including Korea, Japan, Taiwan, and the United States from the Gulf of Mexico<sup>21</sup>. In this study, *V. vulnificus* was isolated from 65 (17.95%) of the 362 samples examined and yielded 234 isolates (Table 1).

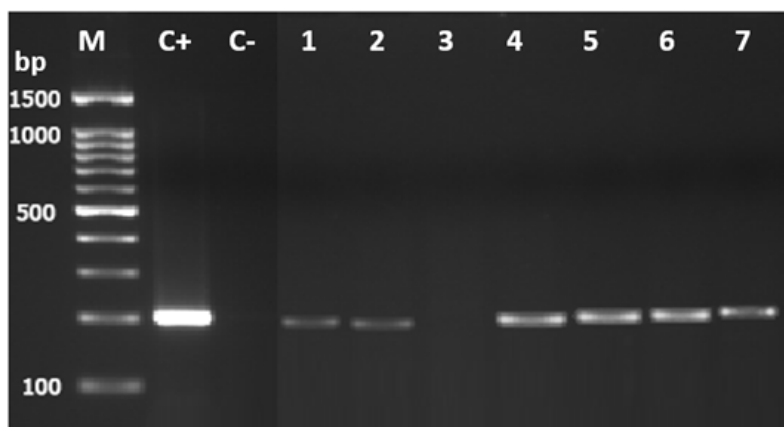
*V. vulnificus* was isolated and identified according to procedures recommended by BAM from the FDA<sup>9</sup>. The presumptively identified colonies on CHROMagar Vibrio (Paris, France) were phenotypically confirmed using API20E strips (BioMérieux, France). All the isolates of *V. vulnificus* (n=234) were tested for the presence of the *vvhA* virulence gene using PCR, and 52 (21.8%) of the isolates tested positive for this gene (Fig. 2). As shown in Table 2, the *vvhA* gene was detected in 10 out of 17 sample locations, and the highest number of positive isolates were from LAK.

Although it has high abundance in nature, infection by *V. vulnificus* is rare. This can be explained by most of the strains being non-pathogenic to humans. The identification of pathogenic markers and classifying the strains as pathogenic and non-pathogenic is very hard for environmental bacteria due to horizontal gene transfer<sup>22</sup>. The reported cases of *V. vulnificus* have been increasing in past four decades with rising mortality rates<sup>21</sup>. The cases of *V. vulnificus* infection had the highest hospitalization rate of 79% among all cases of vibrio according to COVIS, and they had the highest mortality rate of 18%<sup>3</sup>.

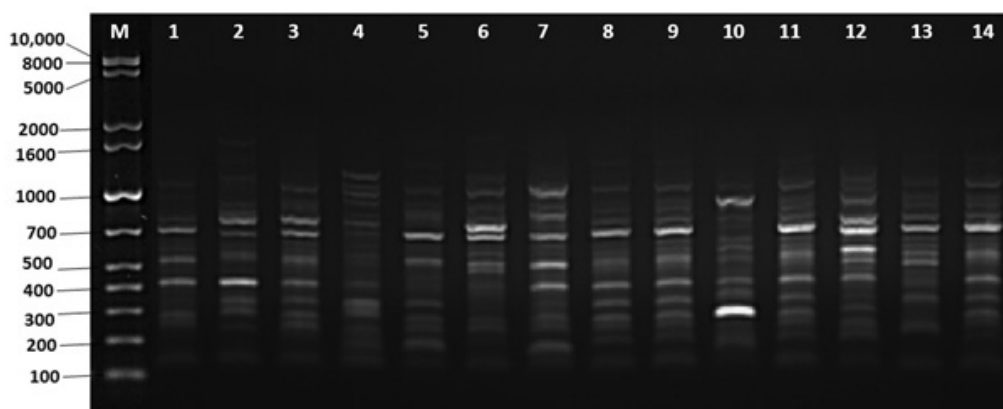
Out of 52 isolates of *V. vulnificus* that were positive for the *vvhA* gene, only 51 isolates were typed using ERIC-PCR to find out the genetic relationship among the isolates. All isolates generated 6 to 16 fingerprint bands ranging in size from 180 to 1100 bp by using the ERIC primer. The fingerprints of the isolates were grouped based on the similarities generated using the UPGMA algorithm, as shown in Fig. 4. The genetic similarities were calculated by using 1% optimization for the Dice coefficient and 1% for the position tolerance. As shown in Fig. 4, the dendrogram generated by the ERIC primer clustered the 51 isolates with 90% identical genetic similarity. However, the dendrogram fingerprint result revealed that there was no correlation between the locations and months of samples, and



**Fig. 1.** Sampling sites and locations in Eastern Province of Saudi Arabia



**Fig. 2.** PCR agarose gel electrophoresis analysis showing the *vvhA* gene in *V. vulnificus* by using PCR. Lane M: molecular weight marker (100 bp DNA ladder; Promega); C+, *V. vulnificus* (positive control); C-, negative control; 1 to 7, representative isolates of *V. vulnificus* isolated in this study.



**Fig. 3.** Amplified DNA fingerprints generated by ERIC-PCR using ERIC1R primer. Lane M: 1kb plus ladder; lane 1 to 14: representative isolates of *V. vulnificus* isolated in this study.

all the *V. vulnificus* isolates were identical and clustered together (Fig. 4). We used ERIC-PCR for genetic typing because it has multiple advantages, such as speed, simplicity, no need for advanced devices, ease, not requiring an expensive setup, and needing a small amount of DNA template<sup>23</sup>.

*V. vulnificus* showed high degrees of resistance to ampicillin (100%), cephalothin (73%), rifampicin (65.4%), amoxicillin with clavulanic acid (61.5%), and streptomycin (44%) (Table 2). The lowest resistance occurred for amikacin (23%), cefoxitin (15%), piperacillin (8%), aztreonam (4%), and piperacillin with tazobactam (2%). As presented in Table 2, nine antibiotics were effective (100%) for all *V. vulnificus* isolates: ceftazidime, cefepime, Imipenem, meropenem, tetracycline,

doxycycline, chloramphenicol, trimethoprim-sulfate, and nalidixic acid.

The results of antibiotic susceptibility testing showed that 100% of the *V. vulnificus* isolates were resistant to ampicillin, which has been reported previously<sup>24-26</sup>. As shown in Table 2, high resistance of *V. vulnificus* isolates was also found for cephalothin (73%), rifampicin (65%), amoxicillin-clavulanic acid (61.5%), and streptomycin (44%). Similar resistance to these antibiotics was reported by other researchers<sup>10, 25-27</sup>.

Interestingly, there no *V. vulnificus* isolates have been reported to be resistant to antibiotics recommended by the CDC in the USA for treating *V. vulnificus* infection, such as doxycycline and third-generation cephalosporin (ceftazidime)<sup>3</sup>. The MAR

**Table 2.** Results of antibiotic susceptibility testing performed on 52 *V. vulnificus* isolates

Antibiotics class	Antimicrobial agents	Antibiogram pattern of <i>V. vulnificus</i>		
		Resistant (%)	Intermediate (%)	Susceptible (%)
Penicillins	Ampicillin	52 (100%)	0	0
	Amoxicillin- clavulanic acid	32 (61.5%)	12 (23%)	8 (15.4%)
	Piperacillin	4 (8%)	14 (27%)	34 (65%)
	Piperacillin-Tazobactam	1 (2%)	2 (4%)	49 (94%)
Monobactams	Aztreonam	2 (4%)	7 (13%)	43 (83%)
Cephalosporins	Cephalothin	38 (73%)	12 (23%)	2 (4%)
	Cefoxitin	8 (15%)	37 (71%)	7 (14%)
	Cefotaxime	0	2 (4%)	50 (96%)
	Ceftazidime	0	0	52 (100%)
	Ceftriaxone	0	4 (8%)	48 (92%)
	Cefepime	0	0	52 (100%)
	Imipenem	0	0	52 (100%)
	Meropenem	0	0	52 (100%)
Aminoglycosides	Amikacin	12 (23%)	24 (46%)	16 (31%)
	Gentamicin	0 (0%)	9 (17%)	43 (83%)
	Streptomycin	23 (44%)	27 (52%)	2 (4%)
Tetracycline	Tetracycline	0	0	52 (100%)
	Doxycycline	0	0	52 (100%)
Quinolones	Ciprofloxacin	0	21 (40%)	31 (60%)
	Levofloxacin	0	4 (8%)	48 (92%)
Amphenicol	Chloramphenicol	0	0	52 (100%)
Folate inhibitor	Trimethoprim- sulfate	0	0	52 (100%)
Quinolone	Nalidixic acid	0	0	52 (100%)
Rifampin	Rifampicin	34 (65%)	17 (32.7%)	1 (4%)

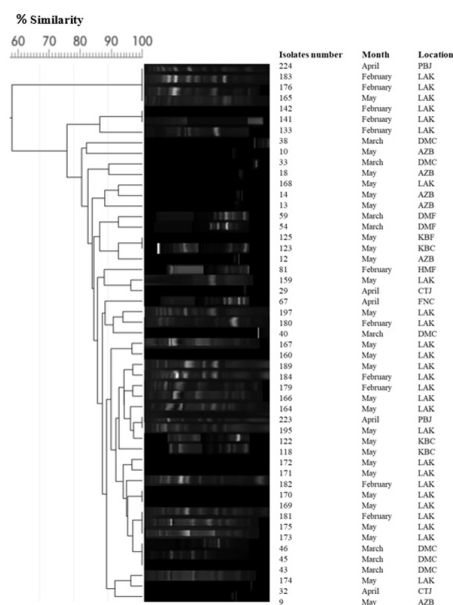
index ranged from 0.08 to 0.33, and nine different antibiotic resistance patterns had a significant MAR index value  $>0.2$ . (Table 3). The majority of them were isolated from LAK (Figure 1 and Table 1). A MAR index that exceeds 0.2 suggests there are high-risk sources of contamination where the use of antimicrobial agents is common<sup>19,25</sup>. Accordingly, the present study found that 41 out of 45 (86.5%) isolates of *V. vulnificus* had MDR, and most of them were isolated from LAK as well (Table 3). MDR has previously been reported in *V. vulnificus* isolates<sup>28</sup>. All 52 isolates of *V. vulnificus* that tested positive for the *vvhA* gene were multi-drug resistant (Table 3).

Our findings are also in agreement with other published studies that reported the spread of MDR in *V. vulnificus* isolated from coastal water environments<sup>29-31</sup>. In the present study, *V. vulnificus* was mostly isolated from samples collected from LAK (Fig. 1). The LAK location is an artificial lake that is linked with the Arabian Gulf by a canal, which feeds it with seawater. A potential reason for

the LAK site being the most contaminated location is that it is a closed area with poor water exchange and possible contamination by sewage. Some hypothesize that the deterioration of water quality and pollution are the causes of the increase in pathogenic strains in the environment<sup>7,32</sup>. Reasons for the spread of the resistant bacteria are selective pressure from antibiotics and excessive use of antimicrobials in agriculture, which are mostly discharged into marine water. Other reasons are the ability of bacteria to be reservoirs of resistance by transferring plasmids through conjugation, transformation, and integrons<sup>33</sup>. The multidrug resistant strains of bacteria are a major threat to human life and becoming an international health crisis<sup>34</sup>. The Food and Agriculture Organization set up an action plan to minimize the spread of antibiotic resistance by increasing the awareness of resistance to antibiotics, developing programs to monitor the resistance, and improving the systems of using antimicrobials in agriculture<sup>35</sup>.

**Table 3.** Antibiotic resistance profile and multiple antibiotic resistance (MAR) index of *V. vulnificus*.

Antibiotic Resistance Profile	No. of isolates	Multiple antibiotic resistance index (MAR)
AMP, S	1	0.08
AMP, KF	1	0.08
AMP, AMC	5	0.08
AMP, S, RD	1	0.13
AMP, KF, RD	6	0.13
AMP, AMC, S	1	0.13
AMP, AMC, KF	4	0.13
AMP, AMC, RD	2	0.13
AMP, KF, S, RD	1	0.17
AMP, AMC, KF, S	1	0.17
AMP, AMC, S, RD	1	0.17
AMP, AMC, KF, RD	8	0.17
AMP, AMC, KF, FOX	1	0.17
AMP, AMC, PRL, FOX	1	0.17
AMP, KF, AK, S, RD	6	0.21
AMP, TZP, KF, AK, S	1	0.21
AMP, AMC, KF, S, RD	4	0.21
AMP, AMC, KF, FOX, S	1	0.21
AMP, AMC, PRL, AK, RD	1	0.21
AMP, KF, FOX, AK, S, RD	2	0.25
AMP, ATM, KF, FOX, AK, S, RD	1	0.29
AMP, AMC, PRL, ATM, KF, FOX, S	1	0.29
AMP, AMC, PRL, KF, FOX, AK, S, RD	1	0.33

**Fig. 4.** Cluster generated by ERIC-PCR fingerprints of 51 isolates of *V. vulnificus* grouped by the ERIC1R primer according to their genetic similarities using the UPGMA algorithm. The fingerprints genetic similarities between the isolates were calculated using the Dice coefficient.



## CONCLUSION

The results of this study confirmed the presence of *V. vulnificus* in the Arabian Gulf coast of the Eastern Province of Saudi Arabia. In addition, this study is the first report of the presence of the *vvhA* gene in isolates of *V. vulnificus*, which may have a potential to cause human infection, especially in populations with a high percentage of diabetes mellitus and among hematological patients with high serum iron. The antibiogram results showed a high percentage of MDR of *V. vulnificus* to clinically important antibiotics. This constitutes a possible risk for humans from the consumption or handling of contaminated seafood or from the exposure of open wounds to seawater.

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