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Characterization of *Vibrio parahaemolyticus* isolated from coastal water in Eastern Province of Saudi Arabia

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ABSTRACT

Aims: *Vibrio parahaemolyticus* is a marine and estuarine bacterium that has been documented as the causative agent of food-borne outbreak worldwide. The aim of this study was to confirm the identification of presumptive *V. parahaemolyticus* isolates to the species level by using PCR targeted to the outer membrane protein regulation operon gene (*tox*R) and to investigate antibiotic resistance, plasmid profile, and the main core virulence genes of thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin (*trh*).
Methodology and results: A total of 56 presumptive isolates of *V. parahaemolyticus* were isolated from seawater collected during year a 2010 sampling pilot study performed along the Arabian Gulf coast of the Eastern Province of Saudi Arabia. The purpose of this study was to confirm the identification of presumptive *V. parahaemolyticus* isolates to the species level by using PCR targeted to the *toxR* gene and to investigate antibiotic resistance, plasmid profile, and the main core virulence genes of *tdh* and *trh*. The *toxR*-specific PCR assay revealed that a total of 30 out of 56 isolates tested positive for *V. parahaemolyticus*. None of the 30 strains of the *tox*R gene were tested positive for *tdh* and *trh* genes. All (100%) of isolates were highly resistant to amikacin, cefuroxime, ampicillin, ticarcillin, cefaclor (80%), and tetracycline (70%). The multiple antibiotic resistance (MAR) index was measured for all 16 antimicrobial agents, and the high ranged from 0.25 to 0.56. Among the isolated *V. parahaemolyticus*, 22 out of 30 strains contained plasmid DNA bands ranging in size from 1.5 to 55 kb and no correlation was observed between the plasmid profiles and antibiotic resistance patterns.
Conclusion, significance and impact of study: The results obtained in this study indicate that *V. parahaemolyticus* is present in the coastal environment of the Eastern Province of Saudi Arabia.

Keywords: Vibrio parahaemolyticus, coastal water, *tox*R gene, virulence genes, antibiotic resistance

INTRODUCTION

The genus *Vibrio* contains aquatic microorganisms that live in coastal and estuarine waters. *Vibrio parahaemoylticus* is a Gram-negative halophilic bacterium, is one of the pathogenic species of the genus *Vibrio*, and is commonly isolated from coastal and estuarine waters worldwide (Zimmerman, 2007). Since its discovery in 1950 in Japan (Fujino *et al.*, 1953), *V. parahaemolyticus* has been recognized as a leading cause of seafood food poisoning throughout the world (Alam *et al.*, 2002; Su and Liu, 2007; Newton *et al.*, 2012; Yu *et al.*, 2013). This bacterium is responsible for causing three major syndromes of clinical manifestations in humans with the most common syndrome being gastroenteritis by the ingestion of *V. parahaemolyticus* in seafood, followed by wound infections when an opened wound is exposed to warm seawater; also, wound infections and septicemia have been reported in cases following bacterial exposure to open wounds, mainly in immunocompromised patients and aged people (Nishibuchi *et al*., 1989; Honda and Iida, 1993; Daniels *et al.*, 2000). Infections with *V. parahaemolyticus* are frequently reported in coastal areas due to high consumption of seafood and direct contact with estuarine waters (Marano *et al.*, 2000). The abundance, presence, and distribution of *V. parahaemolyticus* in the marine and estuarine environments depends on the water temperature, and it has been suggested that *V. parahaemolyticus* might survive in sediments during the winter and be released into the water surface in late

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spring or early summer when the temperature rises to 15 °C or higher (Kaneko and Colwell, 1973).

Among the medically significant *Vibrio* species, *V. parahaemolyticus* produce virulence factors, which encode the thermostable direct hemolysin (*tdh*) and/or the *tdh*-related hemolysin (*trh*); they are considered to be pathogenic (Nishibuchi and Kaper, 1995) and can cause acute gastroenteritis. Both *tdh* and *trh* genes are reported in very low frequencies (1 to 2%) in environmental strains of *V. parahaemolyticus* strains (Nishibuchi and Kaper, 1995; Baker-Austin *et al.*, 2008; Vieira *et al.*, 2011; Gutierrez West *et al.*, 2013). The abundance presence of *V. parahaemolyticus* and other vibrios in the estuarine-marine environment are of particular concern for human health (Baker-Austin *et al.*, 2010). In the United States, for instance, reported cases of *V. parahaemolyticus* is increasing and is one of the three most commonly reported sources of *Vibrio* infection incidence (Newton *et al.*, 2012). This bacterium is implicated as the primary source of vibriosis infections, and the highly pathogenic serotypes of this species are emerging on a global scale, including the Atlantic coasts of the United States and Spain (Newton *et al.*, 2012; Martinez-Urtaza *et al.*, 2013).

Despite the importance of the global emergence of *V. parahaemolyticus* infections worldwide in the coastal areas, the aim of this study was to confirm the identification of *V. parahaemolyticus* isolates to the species level by using PCR targeted to the *tox*R gene, determine the virulence factors by using PCR method directed to *tdh* and *trh* genes, determine antibiotic resistance and plasmid profile. To our knowledge, this is the first report on the molecular characterization and antibiotic resistance of *V. parahaemolyticus* in Saudi Arabia.

MATERIALS AND METHODS

Bacterial strains

A total of 56 presumptive isolates of *V. parahaemolyticus* were isolated from seawater collected during a 2010 sampling pilot study performed along the Arabian Gulf coast of the Eastern Province of Saudi Arabia. These isolates were isolated on CHROM agar Vibrio, and identification were confirmed by biochemical and phenotypical tests described in the Food and Drug Administration Bacteriological Analytical Manual (FDA, 2004) and another published study (DePaola *et al.*, 2003) was used for isolation.

Control strains

Vibrio parahaemolyticus ATCC 17802 and AQ3815 were used in PCR amplification as positive control strains for confirmation of *tox*R, *tdh*, and *trh* genes, and *V. alginolyticus* ATCC 17749 was used as a negative control in PCR amplification. The reference strain of *E. coli* ATCC 25922 was used as a control while performing antibiotic susceptibility testing.

Genomic DNA extraction

Vibrio parahaemolyticus isolates were cultured in Luria-Bertani agar supplemented with 1% NaCl and incubated at 37 °C for 24 h. The genomic DNA from the presumptive isolates of *V. parahaemolyticus* were extracted by using a boiled cell lysate method (Elhadi, 2016). Three to five colonies were selected, re-suspended in 500 µL of sterile distilled water, and boiled for 20 min to liberate the DNA (Elhadi, 2016). The obtained crude DNA were used as a template for PCR analysis of *tox*R, *tdh*, and *trh* genes.

*tox*R gene detection

The 56 presumptive isolates of *V. parahaemolyticus* identification was confirmed with presence of the *toxR* gene by using the primer sequence described by Kim *et al.* (1999), which produced a 368-bp amplicon, as shown in Table 1. The *toxR* gene fragment was amplified in all the isolates to confirm that they were in fact *V. parahaemolyticus*, as this gene can be used to identify the species (Kim *et al*., 1999). Reaction for *toxR* analysis was performed in a total volume of 20 µL reaction containing 1.2 µL of DNA sample, 11.9 µL of deionized water, 2 µL of 10× buffer ($MgCl_2$ free), 1.6 µL of 25 mM concentration of $MgCl_2$, 1.6 µL of 2.5 mM concentration of dNTP solution, 0.8 µL (10 µM) of ToxR4 and ToxR7 primer (Table 1), and 0.1 µL of 5 U/µL of *Taq* DNA polymerase (Promega, USA). The PCR assay was performed at 94 °C for 5 min followed by 20 cycles of 94 °C for 1 min, 63 °C for 1.5 min, and 72 °C for 1.5 min with a final extension at 72 °C for 7 min in a DNA thermal cycler (Swift MaxPro thermocycler, Esco, Singapore). The PCR fragments were subjected to electrophoresis on a 1.5% agarose gel in Tris-borate EDTA buffer solution (Promega, USA) at 120 V for 30 min, visualized by ethidium bromide staining and photographed using a Gel Doc XR apparatus (Biorad, USA).

Detection of virulence gene

The screening of *V. parahaemolyticus* virulence genes (*tdh* and trh) genes were amplified with the specific primers adapted from Tada *et al.* (1992) (Table 1), which produced 251 and 250 bp amplicons, respectively. The reactions for (*tdh* and trh) examination were performed in a total final volume of 20 µL, containing1.2 µL of DNA template, 11.9 µL of deionized water, 2 µL of 10x buffer ($MgCl_2$ free), 1.6 µL of 25 mM concentration of $MgCl_2$, 1.6 µL of 2.5 mM concentration of dNTP solution, 0.8 µL (10 µM) of *tdh* and *trh* primer (Table 1), and 0.1 µL of 5 U μL^{-1} of *Taq* DNA polymerase (Promega, USA). The PCR amplifications for the two genes were performed using a Swift MaxPro thermocycler (Esco, Singapore) with the following cycling conditions: initial denaturation at 96°C for 5 min, 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final elongation at 72°C for 7 min. Positive and negative controls were included in all

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Table 1: Primer sequence and amplicon size used in this study.

Target gene	Primer sequence 5'-3'	Product size (bp)	Reference
*tox*R	F-GTCTTCTGACGCAATCGTTG R-ATACGAGTGGTTGCTGTCATG	368	Kim *et al.*, 1999
tdh	F-GGTACTAAATGGCTGACATC R- CCACTACCACTCTCATATGC	251	Tada *et al.*, 1992
trh	F- GGCTCAAAATGGTTAAGCG R- CATTTCCGCTCTCATATGC	250	Tada *et al.*, 1992

Note: *tox*R, Regulatory protein; *tdh*, thermostable direct hemolysin; *trh*, thermostable related hemolysin

reaction mixtures. Amplified products were separated by electrophoresis in ethidium bromide-stained 1.5% agarose gel in Tris-borate-EDTA buffer solution at 120 volts for 30 min. The gels were visualized and photographed using a Gel Doc XR apparatus (Biorad, USA).

Plasmid DNA extraction

Plasmid DNA was extracted from each strains of *V. parahaemolyticus* using the Pure Yield^{TM} Plasmid Mini-prep System kit (Promega, USA) following the instructions of the manufacturer. The Pure Yield^{TM} Plasmid Mini-prep System kit system provides a rapid method to purify plasmid DNA using a silica-membrane column and was achieved without isopropanol precipitation.

Antibiotic susceptibility testing

Some of the 16 antimicrobials chosen in this study were in accordance with the M100-S23 guidelines and represent antimicrobials of clinical importance to non-cholera *Vibrio* species, particularly tetracycline, cefotaxime, and fluoroquinolones. *Vibrio parahaemolyticus* isolates were examined for antibiotic susceptibility testing according to the published CLSI M100-S23 document, which presented the most current information for drug selection, interpretation, and quality control for MIC testing of infrequently isolated or fastidious bacteria, including non-cholera *Vibrio* species (Jorgensen and Hindler, 2007; CLSI, 2013). Antibiotic susceptibility testing of the *V. parahaemolyticus* was performed using a disc diffusion method (Bauer *et al.*, 1966; CLSI, 2013) on Muller-Hinton Agar. The following antibiotics were tested: amikacin (AK: 30 µg), gentamicin (CN: 10 µg), imipenem (IMP: 10 µg), cefaclor (CEC: 30 µg), cefuroxime (CXM: 30 µg), cefotaxime (CTX: 30 µg), ceftizoxime (ZOX: 30 µg), ceftriaxone (CRO: 30 µg), nitrofurantion (F : 17 µg), ampicillin (AMP: 10 µg), ticarcillin (TIC: 75 µg), amoxicillin/clavulanate (AMC: 30 µg), ciprofloxacin (CIP: 5 µg), norfloxacin (NOR: 10 µg), trimethoprim sulfamethoxazole (SXT: 25 µg), and tetracycline (TE: 30 µg). Antibiotic susceptibility testing results were interpreted using recommendations from the Clinical and Laboratory Standards Institute (CLSI, 2013).

Statistical analyses

The chi-square goodness-of-fit test (Hammer *et al*., 2001) was used to test the uniformity of the distribution of antimicrobial resistance of *V. parahaemolyticus* isolated from coastal sea water of the Arabian Gulf. The sign test was used to test significance of the difference between the values of the MAR index and the median value of 0.2.

RESULTS

Bacterial strains

A total of 56 presumptive isolates of *Vibrio parahaemolyticus* were isolated from seawater collected during a 2010 sampling pilot study performed along the Arabian Gulf coast of the Eastern Province of Saudi Arabia.

PCR analysis

The *tox*R-specific PCR assay revealed that a total of 30 out of 56 isolates tested positive for *V. parahaemolyticus* (Figure 1 and Table 2). None of the 30 strains of the *tox*R gene tested positive for DNA sequences of the *tdh* and *trh* genes (Table 2).

Antibiotic resistance profiles

All 30 isolates of *V. parahaemolyticus* that were confirmed *tox*R gene positive were tested for a total of nine classes of antibiotics (aminoglycosides, carbapenems, second generation of cephalosporins, third generation of cephalosporins, nitrofurans, penicillins, quinolones, sulfonamides, and tetracyclines), which include sixteen antibiotic disks (Oxoid, UK). Table 3 presents the rate of resistance to the 16 antibiotics tested. A very high incidence of resistance was observed against the majority of the antibiotics. 100% resistance were found for the studied isolates against amikacin, cefuroxime, ampicillin and ticarcillin. Almost 80% of the isolates were resistant to cefaclor, closely followed by that against tetracycline (70%). The resistance recorded against amoxicillin/clavulanate (56.6%) were also very high, whereas very low resistance was observed with gentamicin, nitrofurantion, and norfloxacin, contributing 3.3% resistance individually. Based on the chi-square goodness-of-fit test, with the exception of one case (amoxicilin/ clavulanate), all the antibiotics showed

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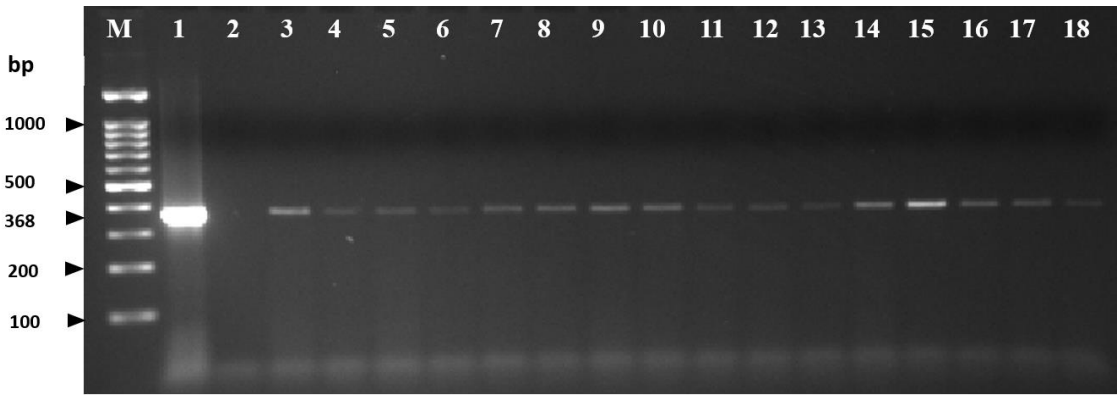

Figure 1: Detection of the *V. parahaemolyticus tox*R gene by PCR. Lane M, molecular size marker (100 bp DNA ladder; Promega); 1, *V. alginolyticus* ATCC 17749 (negative control); 2, *V. parahaemolyticus* ATCC 17802 (positive control); 3 to 18, *V. parahaemolyticus* strains isolated in this study.

Table 2: PCR results obtained with *V. parahaemolyticus* using specific primers.

Strains	PCR results for the target genes *tox*R gene	*tdh* gene	*trh* gene
Control strains			
V. parahaemolyticus ATCC 17802	+	-	+
V. parahaemolyticus AQ3815	+	+	-
V. alginolyticus ATCC 17749	-	-	-
Seawater isolates			
AGVP1	+	-	-
AGVP2	+	-	-
AGVP3	+	-	-
AGVP4	+	-	-
AGVP5	+	-	-
AGVP6	+	-	-
AGVP7	+	-	-
AGVP8	+	-	-
AGVP9	+	-	-
AGVP10	+	-	-
AGVP11	+	-	-
AGVP12	+	-	-
AGVP13	+	-	-
AGVP14	+	-	-
AGVP15	+	-	-
AGVP16	+	-	-
AGVP17	+	-	-
AGVP18	+	-	-
AGVP19	+	-	-
AGVP20	+	-	-
AGVP21	+	-	-
AGVP22	+	-	-
AGVP23	+	-	-
AGVP24	+	-	-
AGVP25	+	-	-
AGVP26	+	-	-
AGVP27	+	-	-
AGVP28	+	-	-
AGVP29	+	-	-
AGVP30	+	-	-

Table 3: Distribution of antimicrobial resistance of *V. parahaemolyticus* isolated from coastal sea water of the Arabian Gulf (n= 30).

Antibiotics	No. (%) of *V. parahaemolyticus* resistant to selected antibiotics	*p*-value based on the chi-square goodness of fit test
Aminoglycosides		
Amikacin (AK: 30 µg)	30 (100)	$p < 0.001$
Gentamicin (CN: 10 µg)	1 (3.3)	$p < 0.001$
Carbapenems		
Imipenem (IMP: 10 µg)	0	$p < 0.001$
Cephalosporins (Second generation)		
Cefaclor (CEC: 30 µg)	24 (80)	$p = 0.001$
Cefuroxime (CXM: 30 µg)	30 (100)	$p < 0.001$
Cephalosporins (Third generation)		
Cefotaxime (CTX: 30 µg)	4 (13.3)	$p < 0.001$
Ceftizoxime (ZOX: 30 µg)	6 (20)	$p = 0.001$
Ceftriaxone (CRO: 30 µg)	4 (13.3)	$p < 0.001$
Nitrofurans		
Nitrofurantion (F : 17 µg)	1 (3.3)	$p < 0.001$
Penicillins		
Ampicillin (AMP: 10 µg)	30 (100)	$p < 0.001$
Ticarcillin (TIC: 75 µg)	30 (100)	$p < 0.001$
Amoxicillin/clavulanate (AMC: 30 µg)	17 (56.6)	$p = 0.465$
Quinolones		
Ciprofloxacin (CIP: 5 µg)	0	$p < 0.001$
Norfloxacin (NOR: 10 µg)	1 (3.3)	$p < 0.001$
Sulfonamides		
Trimethoprim sulfamethoxazole (SXT: 25 µg)	5 (16.6)	$p < 0.001$
Tetracyclines		
Tetracycline (TE: 30 µg)	21 (70)	$p = 0.028$

showed significant results. The number that were resistant were significantly different from those that were not resistant, and the *p*-values were mostly less than 0.001 (Table 3).

Various antibiotic resistance patterns expressed by *V. parahaemolyticus* are shown in Table 4. 96.3 % of isolates exhibited multiple antibiotic resistance pattern (I to X). Among these multiple antibiotic resistance patterns, the majority of the patterns consisted of isolates showing resistance to amikacin, ampicillin, ticarcillin, cefaclor, and cefuroxime in combination with each other and along with other antibiotics. The resistance pattern VI, which showed resistance to AK-AMC-TIC-CEC-SXT-TE-CXM-AMP, was found to be the most widely expressed pattern. As many as 20% of *V. parahaemolyticus* shared this resistance pattern (VI). The second most widely expressed pattern was VIII, which was resistant to AK-AMC-TIC-CEC-TE-CXM-AMP with 16.6% of isolates.

The multiple antibiotic resistance (MAR) index

The multiple antibiotic resistance (MAR) index was measured for all 16 antimicrobial agents, and the high ranged from 0.25 to 0.56. All the resistance patterns observed in this study were found to be above the significant MAR value of 0.2. Based on the sign test, the values of the MAR index were significantly different from the median value of 0.2 (Table 4). The resistance patterns I, V, VII, and X shared similar MAR values near 0.37. Similarly, resistance patterns II, III, and VI were found to have MAR values of 0.5. All the test isolates from all the phenotypic groups were subjected to a plasmid analysis (Table 4).

Plasmid DNA analysis

Among the isolated *V. parahaemolyticus*, 22 out of 30 strains contained plasmid DNA bands ranging in size from 1.5 to 55 kb and no correlation was observed between the plasmid profiles and antibiotic resistance patterns.

DISCUSSION

V. parahaemolyticus is a bacterium that is distributed in marine water environments and is usually associated with gastroenteritis, wound infections, and septicemia. Diseases caused by this pathogen are frequently reported in coastal areas and are caused by seafood consumption and seawater exposure (Marano *et al.*, 2000). Most of the *V. parahaemolyticus* isolates tested in this study were revealed to be 100 % resistant to amikacin, cefuroxime, ampicillin, ticarcillin, cefaclor (80%), tetracycline (70%), amoxicillin/clavulanate (56.6%), trimethoprim sulfamethoxazole (16.6%), ceftizoxime

Table 4: The antibiotic resistance profile patterns and multiple antibiotic resistance (MAR) index of *V. parahaemolyticus* isolated from coastal sea water of the Arabian Gulf.

Patterns	Strain No.	*Antibiotic Profiles	MAR	(% of occurrence)
I	AGVP1, AGVP16	AK, AMC, TIC, CEC, CXM, AMP	0.37	6.6
II	AGVP2, AGVP7, AGVP8	AK, CTX, TIC, CEC, TE, CXM, AMP, ZOX	0.5	10
III	AGVP3, AGVP4, AGVP13	AK, TIC, CEC, TE, CXM, AMP, ZOX, CRO	0.5	6.6
IV	AGVP5, AGVP6, AGVP21, AGVP22	AK, TIC, CXM, AMP	0.25	13.3
V	AGVP9, AGVP10, AGVP11	AK,TIC, CEC, TE, CXM, AMP	0.37	10
VI	AGVP12, AGVP15, AGVP17, AGVP19, AGVP27, AGVP30	AK, AMC, TIC, CEC, SXT, TE, CXM, AMP	0.5	20
VII	AGVP14	AK, TIC, F, CEC, CXM, AMP	0.37	3.3
VIII	AGVP18, AGVP20, AGVP26, AGVP28, AGVP29	AK, AMC, TIC, CEC, TE, CXM, AMP	0.43	16.6
IX	AGVP23	AK, CTX, AMC, TIC, CEC, TE, CXM, AMP, CRO	0.56	3.3
X	AGVP24, AGVP25	AK, AMC, TIC, CEC, CXM, AMP	0.37	6.6

Note: AK, Amikacin; CN, Gentamicin; CEC, Cefaclor; CXM, Cefuroxime; CTX, Cefotaxime; ZOX, Ceftizoxime; CRO, Ceftriaxone; F, Nitrofurantion; AMP, Ampicillin; TIC, Ticarcillin; AMC, Amoxicillin/clavulanate; NOR, Norfloxacin; SXT, Trimethoprim sulfamethoxazole; TE, Tetracycline.

(20%), cefotaxime (13.3%), ceftriaxone (13.3%), and Gentamicin (3.3%), as shown in Table 3. The clinical treatment recommended by CDC for *Vibrio* infections include tetracyclines (doxycycline, tetracycline), flouroquinolones (ciprofloxacin, levofloxacin), third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone), aminoglycosides (amikacin, apramycin, gentamicin, streptomycin), and folate pathway inhibitors (trimethoprim-sulfamethoxazole) (CDC, 2016). However, the majority of tested *V. parahaemolyticus* isolates in this study were susceptible to imipenem and ciprofloxacin, and although one isolate of *V. parahaemolyticus* demonstrated resistance to norfloxacin, gentamicin and nitrofurantoin.

A recent study of antimicrobial susceptibility of *V. parahaemolyticus* recovered from recreational and commercial areas of the Chesapeake Bay and Maryland Coastal Bays (Shaw *et al.*, 2014) produced interesting comparisons to our findings. In a Chesapeake Bay study of *V. parahaemolyticus* from environmental samples, no resistance were found to Amikacin, amoxicillin clavulanic acid, tetracycline, cefotaxime, ceftriaxone, whereas our study found high levels of resistance to these antibiotics. The Chesapeake Bay and Maryland Coastal Bays study found *V. parahaemolyticus* isolates to be 53% (n = 77) resistant to ampicillin, while our study detected 100% (n = 30) resistance. Resistance to amikacin and tetracycline were found in 0% of the Chesapeake Bay and Maryland Coastal Bays study, compared to 100% and 70% resistance in this study, respectively. Our study is in agreement with the study from the Chesapeake Bay and Maryland Coastal Bays, which detected full susceptibility to imipenem and ciprofloxacin. Our study also contrasts the results of one study conducted in Malaysia (Letchumanan *et al*., 2015) in *V. parahaemolyticus* isolates from retail shrimps, which found 98% of isolates were highly susceptible to imipenem, ampicillin (96%), trimethoprim-sulfamethoxazole (93%), gentamicin (85%), and tetracycline (82%). The results in this study indicated all *V. parahaemolyticus* isolates were highly susceptible to imipenem, ciprofloxacin, gentamicin (96.7%), norfloxacin (96.7%), nitrofurantoin (96.7%), cefotaxime (86.7%), ceftriaxone (86.7%), and trimethoprim-sulfamethoxazole (86.7%). Also, reduced susceptibility to ampicillin from 81% of *V. parahaemolyticus* isolated from Louisiana Gulf and retail raw oysters was found (Han et al., 2007).

The antibiotic result of *V. parahemolyticus* in this study confirms the multiple drug resistance among the isolates from the coastal water of the Eastern Province of Saudi Arabia. Tetracycline has been recommended as the antimicrobial of choice for treatment of severe *Vibrio* infections (Morris and Tenney, 1985) and alternative treatments are combinations of expanded-spectrum cephalosporins (e.g., ceftazidime) and doxycycline or a fluoroquinolone alone (Tang *et al*., 2002). Trimethoprim-sulfamethoxazole plus an aminoglycoside is used to treat children in whom doxycycline and fluoroquinolones are contraindicated (CDC, 2016). Traditionally, *Vibrio* is considered highly susceptible to virtually all antimicrobials (Oliver, 2006). During the past few decades, however, antimicrobial resistance has emerged and evolved in many bacterial genera due to the excessive use of antimicrobials in human, agriculture, and aquaculture systems (Mazel and Davies, 1999; Cabello, 2006). In

contrast, the awareness of antimicrobial resistant bacteria in the aquatic environment is less documented (Cabello 2006; Gordon *et al*., 2007). Recently, few studies have examined the antimicrobial susceptibilities of *Vibrio* spp. isolated from the aquatic environment (Ottaviani *et al*., 2001; Zanetti *et al.*, 2001; Maluping *et al*., 2005). In this study, 100% of *V. parahaemolyticus* isolates were resistant to ampicillin and should not be used empirically to treat *V. parahaemolyticus* infection. Several studies worldwide reported that *V. parahaemolyticus* were resistant to ampicillin and exhibited β-lactamase production (Joseph *et al.*, 1978; Zanetti *et al*., 2001; Maluping *et al*., 2005; Shaw *et al*., 2014).

None of the isolates in this study were *tdh* or *trh* positive; this is due to the relationship between *V. parahaemolyticus* strains isolated from the aquatic environment and those isolated from cases of infection in humans, which is poorly understood due to the low prevalence of *tdh* and/or *trh* positive strains in the environment (Cabrera-García *et al.*, 2004; Ottaviani *et al.*, 2013; Haley *et al*., 2014). Most strains of *V. parahaemolyticus* isolated from the environment or seafood, in contrast to clinical strains, do not produce *tdh* and *trh* (Alam *et al*., 2002; Ceccarelli *et al*., 2013; Shaw *et al*., 2014). In this study, the plasmid profiles did not correlate with antibiotic resistance patterns and our observation in agreement with similar findings reported by Silvester *et al*. (2015).

CONCLUSION

Our findings present the first report of *tox*R gene positive and antimicrobial susceptibility of *V. parahaemolyticus* recovered from the coastal water of the Eastern Province of Saudi Arabia and provide a baseline against which future studies can be conducted to cover more sample locations along the coastal areas of the Eastern Province of Saudi Arabia. *V. parahaemolyticus* isolates tested in this study displayed high antibiotic resistance when compared to similar studies conducted worldwide. These data will be helpful in short and long-term predictions of human health risks associated with exposures to *V. parahaemolyticus* and other *Vibrio* populations in the Arabian Gulf coastal area. Also, we recommend an extensive investigation into the prevalence of toxigenic *V. parahaemolyticus* and other *Vibrio* species and antimicrobial susceptibility in the Eastern Province of Saudi Arabia marine coastal waters.

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