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Research Article
IGS-Fingerprinting and Virulence Properties of *Vibrio alginolyticus* Isolated from Farmed Fishes in Saudi Coast of Arabian Gulf

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Abstract

Background and Objective: *Vibrio* sp. are a crucial constituent of marine ecosystems worldwide. *Vibrio alginolyticus* is extremely significant and major pathogenic agent facing wild and farmed fishes. The main objective of this study was to record the molecular characterization (IGS-Typing pattern) by single PCR step and virulence genes in *V. alginolyticus* isolates. **Materials and Methods:** For this study 360 fishes were collected, including gilt head seabream (*Sparus auratus*), two bar seabream (*Acanthopagrus bifasciatus*), black bream (*Acanthopagrus latus*), red sea seabream (*Diplodus noct*), brown-spotted grouper (*Epinephilus coioides*) and rabbitfish (*Siganus canaliculatus*) between July, 2015-June, 2016. Primary identification of isolates was done conventionally using API 20NE systems (Biomerieux), while confirmation was done by sequencing of 16S rDNA and *V. alginolyticus* specific gene targets, construction of phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA) 6.0 software. IGS-Typing and antibiogram test screening were also carried out for isolates. **Results:** Two hundred and eighty isolates of *V. alginolyticus* were recovered from farmed fishes. Clinical signs expressed as general septicaemia, ulcers and corneal opacity. Conventional methods identified isolates as *V. alginolyticus* (ID% 96-99.9), sequencing and phylogenetic tree confirmed this identification. The PCR results for IGS-Typing were expressed as four typical band patterns ≈ 420, 580, 650 and 820 bp, while those for virulence genes were positive amplification for collagenase, toxR and ompK, while tlh and VPI virulence genes were not recognized. **Conclusion:** Isolated *V. alginolyticus* were highly pathogenic to farmed fishes as determined by virulence genes identified. Results of IGS-Typing facilitate further identification of isolates by single PCR step as it records the pattern for *V. alginolyticus*.

Key words: Virulence genes, *Vibrio alginolyticus*, antibiogram test, genetics analysis, intergenic spacer (IGS-Fingerprinting), farmed fishes, Arabian gulf

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish aquaculture is a growing industry in Saudi Arabia. However, vibriosis outbreak caused by various pathogenic *Vibrio* sp. is considered a significant problem to the development of farmed fish and shellfish with severe economic losses[1,2]. Moreover, Many *Vibrio* sp. are serious pathogen in human causing septicemia, gastroenteritis as well as wound infection[3,].

Vibrio alginolyticus is Gram-negative halophilic bacterium, ubiquitous in marine and estuarine ecosystems and has been associated with extensive body ulceration of gilthead sea bream (*Sparus aurata)*, silver sea bream (*Sparus sarba)*, sea bass (*Dicentrarchus labrax),* turbot (*Scophthalmus maximus)*, crimson snapper (*Lutjanus erythopterus)*, sea mullet (*Mugilcephalus)* and freshwater fish, Nile tilapia (*Oreochromis niloticus*)[2-10]. Recently, *V. alginolyticus* was recorded as a responsible pathogen for *Porites andrewsi* white syndrome in coral reefs and was associated with white spot disease in black tiger shrimp (*Penaeus monodon*)[11,12]. Hassan *et al.*[1] isolated *V. vulnificus* and *V. alginolyticus* from cage-cultured marine fish *Acanthopagrus bifasciatus* and *Sparus aurata* in Arabian Gulf.

More than 74 distinct *Vibrio* spp. has been identified till now (http://www.vibriobiology.net/) and continuously increases annually. This gave rise to the extreme importance for identification of *Vibrio* sp. based on well-known IGS-regions located between the 16S and 23S rRNA genes on the bacterial chromosomes[13]. This rapid, reliable and efficient IGS-Fingerprinting was able to identify *Vibrio* sp. not only at species level, but also, in some cases, at subspecies level using one primer for one PCR reaction especially if combined with 16S-rRNA gene sequence[13].

Previous studies reported *V. alginolyticus* as the dominant living species in water and sediments[14]. It can maintain virulence properties and pull out from several stressors including nutrient stress[8]. Presence of *V. cholerae pathogenicity* island (VPI) virulence gene and thermolabile haemolysin (tlh) virulence gene of *V. parahemolyticus* in *V. alginolyticus* reported by Zhang *et al.*[15] and Snoussi *et al.*[16] respectively. *OmpK* virulence gene of *V. alginolyticus* believed to play important role in infection and pathogenicity to host and the expression of this gene is controlled by *tox*R gene[17]. Collagenase has been widely utilized as a biomarker of *V. alginolyticus* as well as capable of causing severe pathology and distribution to blood stream[18]. Although *V. alginolyticus* has been reported in many cases from Arabian Gulf fish and shellfish[19-21,1]. No data available on the presence of virulence genes especially those derived from *V. cholera* as well as *V. parahaemolyticus*. Similarly, to the best of author's knowledge, no studies till now clarified the most important virulence genes for pathogenicity of *V. alginolyticus*.

The aims of the present study were to clarify the molecular identification of *V. alginolyticus* isolated from Saudi coast in Arabian gulf, by sequencing of targets in the 16S rDNA and *V. alginolyticus* specific gene regions, phylogenetic tree, recording 16S-23S rRNA Intergenic Spacer Regions pattern (IGS-Fingerprinting) and detect presence of *V. cholerae pathogenicity* island (VPI), *thermolabile haemolysin* (tlh), collagenase, *tox*R and *omp*K virulence genes by means of PCR for better understanding how to control or eradicate the disease in an aquaculture setting.

MATERIALS AND METHODS

Fish sampling: About 360 fishes were collected and examined as a part of ongoing monitoring program related to Fisheries Research Center, Ministry of Environment, Water and Agriculture, Saudi Arabia from cage cultured fish owned by private farm in the eastern province. About 6 species of fishes (60 fish/Species) were examined include, gilthead seabream (*Sparus auratus*), two bar seabream (*Acanthopagrus bifasciatus*), sobaity seabream (*Sparidentex hasta*), red sea seabream (*Diplodus noct*), brown-spotted grouper (*Epinephilus coioides*) and rabbitfish (*Siganus canaliculatus*) exhibits septicemic signs such as dermal ulcers and/or external hemorrhage, in addition to 96 water samples were also collected aseptically in sterile bottles and examined, during the period from June, 2015 to May, 2016.

Fishes were transferred alive in a special vessels supplied with oxygen to the Lab. of Fisheries Research Center in Al-Qatif (17025:2005 ISO Certified) where clinical and postmortem examination were carried out according to Noga[22]. Salinity, DO and water temperature were analyzed for water samples.

Bacterial strains and culturing conditions: Samples were taken from each fish separately from hematogenous organs of each fish (liver, kidney, spleen) in addition to water samples by using sterile bacteriological loop and inoculated in TSB and TSA (Oxoid) supplemented with 2% NaCl, incubated at 28°C for 24 h and further purificationin TSA with 2% NaCl according to Austin and Austin[23].

Phenotypic identification: Gram staining and oxidase test were done as a preliminary step of identification. Purification and further phenotypic identification were done with the

Table 1: Selected primer pairs sequence used in the study

Primers	Sequence	Product size (bp)	Targets	References
16S rDNA	27F AGAGTTTGATC(C/A)TGG CTCAG 1492R TACGG(C/T)TACCTTGTTACGACTT	1500	(Eu) bacterial 16S rDNA	Polz and Cavanaugh[24]
Species-specific primer	VA 1198230 F: 5' ACGGCATTGGAAATTGCGACTG VA 1198230 R: 5' TACCCGTCTCACGAGCCCAAG	199	Whole genome shotgun sequence	Kim *et al.*[25]
16S-23S rRNA (IGS)	16S.6 5'-ACTGGGGTGAAGTCGTAACA-3' 12S.1 5'-CTTCATCGCCTCTGACTGC-3'	-	Intergenic spacer regions	Hoffmann *et al.*[13]
tlh virulence gene	*Tlh*-F: AGCGGATTATGCAGAAGCAC *Tlh*-R: GCTACTTTCTAGCATTTTTGC	448	*tlh* virulence gene	Xie *et al.*[27]
VPI virulence gene	*VPI*-F: GCAATTTAGGGGCGCGACGT *VPI-R*: CCGCTCTTTCTTGATCTGGTAG	680	VPI virulence gene	Sechi *et al.*[28]
Collagenase virulence gene	VA-F: CGAGTACAGTCACTTGAAAGCC VA-R: CACAACAGAACTCGCGTTACC	737	Collagenase virulence gene	Di Pinto *et al.*[30]
*tox*R virulence gene	F: GATTAGGAAGCAACGAAAG R: GCAATCACTTCCACTGGTAAC	658	*tox*R virulence gene	Xie *et al.*[27]
*omp*K virulence gene	F: GGCGGTCGCTCTGGTATT R: TTGCCATCGTAAGTGCTGTA	319	*omp*K virulence gene	Cai *et al.*[10,29]

Table 2: Cycling conditions for different PCR reactions in the study

Target gene	Initial denaturation	Denaturation	Annealing	Extension	Cycles
16S rDNA	94°C for 3 min	94°C for 1 min	50°C for 1 min	72°C for 2 min	25
Species-specific primer	94°C for 5 min	94°C for 30 sec	60°C for 30 sec	72°C for 30 sec and final extension step 72°C for 10 min	25
16S-23S rRNA (IGS)	95°C for 15 min	95°C for 30 sec	73-64°C (decreasing 1°C/cycle) for 10 sec	72°C for 45 sec	10
	Complete amplification achieved as follow:				
		95°C for 30 sec	64°C for 10 sec	72°C for 45 min and final extension step 72°C for 1 min	34
	Finalization by single amplification cycle to resolve heteroduplex formation as follow:				
		95°C for 15 min	64°C for 1 min	72°C for 10 min	1
tlh virulence gene	94°C for 3 min	94°C for 1 min	54°C for 1 min	72°C for 1 min and final extension step 72°C for 10 min	35
VPI virulence gene	94°C for 3 min	94°C for 1 min	52°C for 1 min	72°C for 1 min and final extension step 72°C for 10 min	35
Collagenase virulence gene	95°C for 5 min	94°C for 30 sec	57°C for 30 sec	72°C for 50 sec	34
*tox*R virulence gene	94°C for 4 min	94°C for 1 min	54°C for 1 min	72°C for 1 min	35
*omp*K virulence gene	94°C for 4 min	94°C for 1 min	58.1°C for 1 min	72°C for 1 min	35

mean of Thiosulphate Citrate Bile Salt Sucrose agar (TCBS) as a selective media, growth in presence of vibriostatic agent 0/129 and API 20NE identification system (Biomérieux) using Apiweb™ identification software according to manufacturer's instructions. Pooling of strains were done and kept in TSB (15% glycerol) in -80°C.

DNA extraction: Pooled strains (phenotypically identified) were incubated at 28°C in TSB supplemented with 2% NaCl and DNA were extracted using a commercial kit (Qiagen, Germany), DNA quantification were done by mean of Nano Drop Technology (Thermo Scientific NanoDrop 2000 UV-Vis spectrophotometer) and stored in -20°C. Molecular identification, characterization and detection of virulence genes were carried out as follow:

- **Sequencing of 16S rDNA gene target:** Amplification of a specific target of the 16s rDNA using a pan-bacterial universal primer set (27F and 1492R, Table 1) following the method described by Polz and Cavanaugh[24]. The reaction was carried out in 25 µL reaction and the cyclic condition explained in (Table 2). PCR product directly sequenced and results were compared with those held in GenBank database (National Center for Biotechnology Information "NCBI", Bethesda, MD, USA) by using Blast program
- **Sequencing of *V. alginolyticus* specific gene target:** Using a Species-specific primer set (VA 1198230 F and VA 1198230 R, Table 1) following the method described by Kim *et al.*[25]. The PCR product directly sequenced and data BLASTed

Phylogenetic tree: A phylogenetic analysis was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) 6.0 software[26].

PCR of *V. alginolyticus* targeting 16S-23S rRNA intergenic spacer regions (IGS-fingerprinting): PCR of *V. alginolyticus* targeting 16S-23S rRNA Intergenic Spacer Regions (IGS-Fingerprinting) was performed in a 50 µL reaction following the method described by Hoffmann *et al.*[13]. The cyclic condition includes a second round amplification step to eliminate artifacts as possible as explained in Table 2.

PCR for Detection of *V. alginolyticus* virulence genes were performed in 50 µL reaction following the procedures described by Xie *et al.*[27] for tlh and *tox*R virulence genes, Sechi *et al.*[28] for VPI virulence gene, Cai *et al.*[29] for *Omp*K and Di Pinto *et al.*[30] for Collagenase virulence genes. Primer sequence for all reactions were described in Table 1 and detailed cyclic condition in Table 2.

Gel electrophoresis: The PCR products in all reactions were run on 1.5% agarose gel stained with Ethidium bromide (10 mg mL^{-1}) in Tris Acetate EDTA buffer (TAE) using Biorad PowerPac universal electrophoresis and visualized with UV Transilluminator using Molecular Imager Gel DOC™ with Image Lab™ software (USA).

RESULTS

Clinical signs of examined fishes: Symptoms of septicemic vibriosis are clear with Sluggish movement, nervous manifestation represented by listlessness, loss of body reflexes and Fin erosions (Fig. 1a). Generalized erythematic hemorrhage distributed all over the body surface especially anal fins and operculum, marked ulcers with different sizes especially on grouper fish (Fig. 1b and d), corneal opacity (bilateral Fig. 1c) or (unilateral Fig. 1e), hemorrhagic swollen vent and sometimes dark pigmentation were also observed.

Postmortem examination: Severe congestion in gills, liver, kidney and intestine were observed. Distended gall bladder,

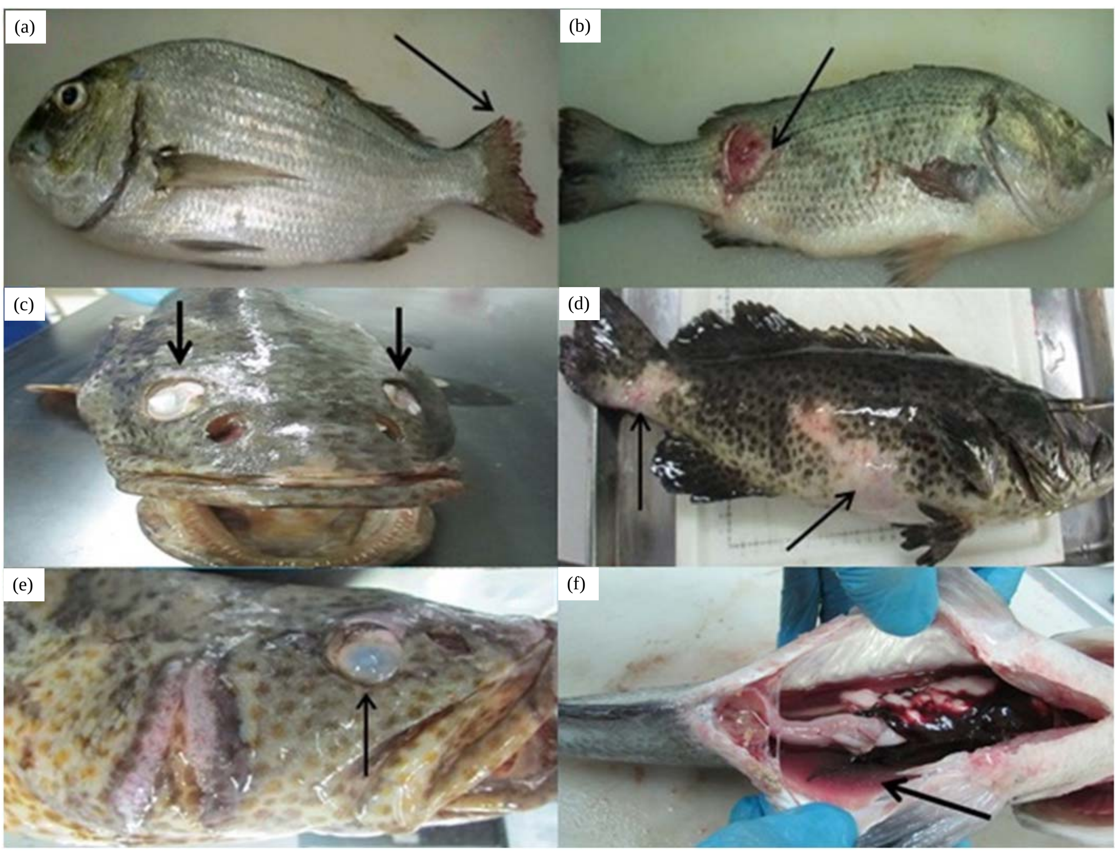

Fig. 1(a-f): (a) Gilthead seabream (*Sparus auratus*) showing fin erosion, (b) Black bream (*Acanthopagrus latus*) showing deep skin ulcer, (c) Brown-spotted grouper (*Epinephilus coioides*) showing bilateral corneal opacity, (d) Brown-Spotted Grouper (*Epinephilus coioides*) showing skin lesion and ulcers with different sizes. (E) Brown-spotted Grouper (*Epinephilus coioides*) showing unilateral corneal opacity and (f) Black bream (*Acanthopagrus latus*) showing bloody ascites

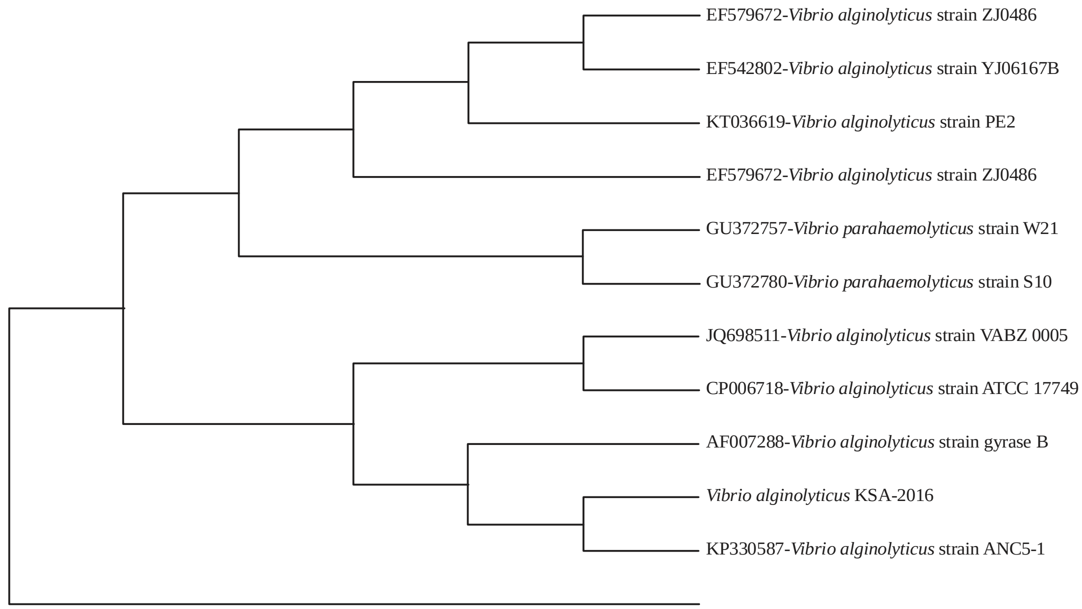

Fig. 2: Phylogenetic tree based on 16S r DNA gene sequence showing the relationships of *V. alginolyticus* with related species. The tree was created using MEGA 6.0 software

Table 3: Nucleotides sequence results for both 16S r DNA and *V. alginolyticus* specific gene targets

Target gene	Sequence results
16S rDNA (1500 bp)	CGAGCGGCAGGCCTACACATGCAAGTCGAGCGGAACGAGTTATCTGAACCTTCGGGGAACGATAACGGCGTCGAGCGGCGGACGGGTGAGTAATG CCTAGGAAATTGCCCTGATGTGGGGGATAACCATTGGAAACGATGGCTAATACCGCATGATGCCTACGGGCCAAAGAGGGGGACCTTCGGGCCTC TCGCGTCAGGATATGCCTAGGTGGGATTAGCTAGTTGGTGAGGTAAGGGCTCACCAAGGCGACTATC
V. alginolyticus specific gene (199 bp)	CACCTAAGTTACGCACTATCAGAGAAGTTGAGCTAACGATTCATCGTGGTGGCCATATCCATACGCAAACCTACCGCCATGGTGAGCCTCAAGCGCCACTA GCTGTTGTGGGTGATACGGATAAAACCGGTACACAAATTCGTTTCTGGCCAAGTGCCGAAACGTTCTCTAACACTGAGTTCCACTATGACATTCTGG CGAAACGCCTGCGTGAACTGTCATTCCTGAACTCTGGTGTGTCGATCAAATTGGTTGATGAACGTGAAGCGGACAAACATGATCACTTCATGTATGA AGGTGGTATTCAAGCGTTCGTTGATCACCTAAACACCAACAAAACGCCAATCATCGAAAAAATCTTCCACTTTAACTCTGAGCGTGAAGACGGCATT TCAGTTGAAGTGGCGATGCAATGGAACGATGGTTTCCAAGAGAACATCTTCTGCTTTACCAACAATATCCCACAGCGTGATGGTGGTACTCACCTTG CTGGTTTCCGTGCTGCGCTAACACGTACATTGAACAGCTTTATGGATAAAGAAGGTTTCTCGAAAAAAGCGAAAACAGCGACTTCAGGCGACGATG CGCGTGAAGGTCTAAC

the abdominal cavity filled with copious exudate (hemorrhagic or ascetic) (Fig. 1f). Inflammation and thickening of swim bladder wall were also recorded.

Phenotypic results: About 280 isolates recovered from examined fishes were Gram-negative, straight or slightly curved (comma shaped) rods, motile, oxidase positive, sensitive to vibriostatic agents 0/129. Small, circular creamy colored colonies on TSA supplemented with 2% NaCl. Large yellow colonies on TCBS agar plates were also recorded.

Biochemical results using API 20NE system: Indicated that all isolates were phenotypically identified as *V. alginolyticus* (ID % 96-99.9) with reference No. 7474644, 7464644, 7456744 and 7456744.

Sequencing results: BLASTing of sequence results from 16S rDNA (1500 bp) and from sequencing of *V. alginolyticus* specific target (199 bp) (Table 3) against those in GenBank "NCBI" resulted in accurate identification of isolates with 99% similarities to reference strains *V. alginolyticus* strain NBRC 15630-16S-rRNA gene (accession No. NR_121709) and *V. alginolyticus* strain ANC5-1 (accession No. KP330587) respectively.

Phylogenetic tree: Construction of phylogenetic tree using sequences output from both 16S rDNA and *V. alginolyticus* specific target accurately put isolates of the present study in *V. alginolyticus* group and indicated its closest proximity to *V. alginolyticus* strain NBRC 15630-16S-rRNA (Fig. 2) and *V. alginolyticus* strains ANC5-1 (Fig. 3) respectively.

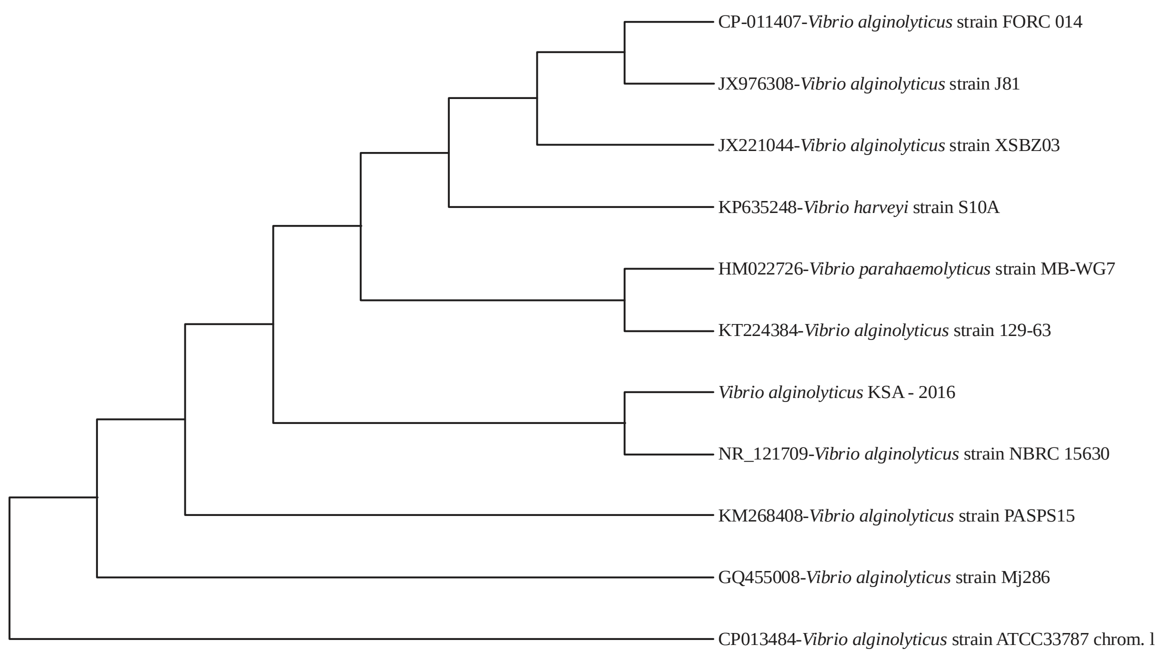

Fig. 3: Phylogenetic tree based on sequencing of targets of *V. alginolyticus* specific gene regions showing the relationships of *V. alginolyticus* with related species. The tree was created using MEGA 6.0 software

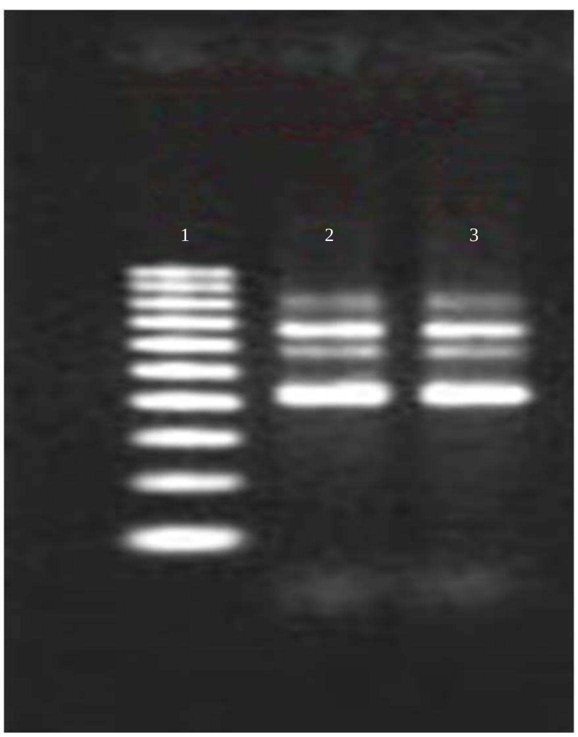

Fig. 4: About 1.5% agarose electrophoretic gel picture showing the 16S-23S intergenic spacer pattern (IGS-Fingerprinting) of *V. alginolyticus* where Lane 1: 100 bp DNA ladder, Lane 2 and 3: 4 bands of *V. alginolyticus* near 420, 580, 650 and 820 bp

Intergenic spacers (IGS) results: A polymerase chain reaction (PCR) targeting 16S-23S rRNA intergenic spacer (IGS) region was conducted with extracted DNA from pooled samples and resulted in amplicons. The latter were analyzed by gel electrophoresis and resulted in 4 typical bands ≈420, 580, 650 and 820 bp (Fig. 4).

Presence of virulence genes: The extracted DNA from pooled samples in the present study was subjected to virulence genes detection by mean of a conventional PCR and gave similar results. They were positive for the presence of collagenase, *tox*R and *omp*K genes and lacking *tlh* and VPI genes (Fig. 5).

The accurate molecular identification of *V. alginolyticus* isolates in present study revealed that the highest incidence of infection was in rabbitfish (*Siganus canaliculatus*) (29.64%) followed by brown-spotted grouper (*Epinephilus coioides*) (27.14%), two bar seabream (*Acanthopagrus bifasciatus*) (20.36%), red sea seabream (*Diplodus noct*) (8.93%), black bream (*Acanthopagrus latus*) (7.5%) and gilthead seabream (*Sparus auratus*) (6.43%) as shown in Table 4. As well as the highest incidence of infection was recorded from kidney tissues (46.79%) followed by liver (31.43%) and spleen (21.78%) as shown in Table 5.

The seasonal prevalence of *V. alginolyticus* infection among the total number of examined fishes revealed that, the

Table 4: Total no. of *V. alginolyticus* isolates/fish species

				Sum of isolates	
Fish sp.	Liver	Kidney	Spleen	No.	%
Gilthead seabream (*Sparus auratus*)	6	8	4	18	6.43
Twobar seabream (*Acanthopagrus bifasciatus*)	11	29	17	57	20.36
Black bream (*Acanthopagrus latus*)	7	8	6	21	7.50
Red sea seabream (*Diplodus noct*)	9	11	5	25	8.93
Brown-spotted grouper (*Epinephilus coioides*)	28	36	12	76	27.14
Rabbitfish (*Siganus canaliculatus*)	27	39	17	83	29.64
Total	280	100			

Table 5: Total No. of *V. alginolyticus* isolates/organs of various fish species

	Liver		Kidney		Spleen		Total	
Organ	No.	%	No.	%	No.	%	No.	%
No. of *V. alginolyticus* isolates	88	31.43	131	46.79	61	21.78	280	100

Table 6: Seasonal prevalence of *V. alginolyticus* in the examined fishes

	Winter		Autumn		Summer		Spring		Total	
Organ	No.	%	No.	%	No.	%	No.	%	No.	%
No. of fishes examined	90	25.00	90	25.00	90	25.00	90	25.00	360	100
No. of *V. alginolyticus* isolates	22	7.86	32	11.43	173	61.78	53	18.93	280	100

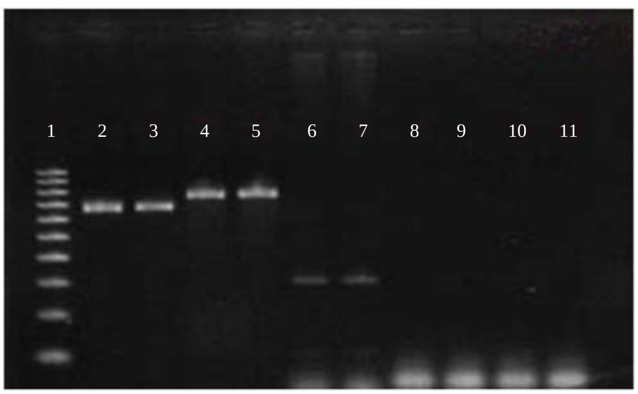

Fig. 5: About 1.5% agarose electrophoretic gel picture showing amplification of *V. alginolyticus* virulence genes under study where Lane 1: 100 bp DNA ladder, Lane 2 and 3: Positive *tox*R-gene amplification, Lane 4 and 5: Positive collagenase-gene amplification, Lane 6 and 7: Positive *omp*K-gene amplification, Lane 8 and 9: Negative *tlh*-gene amplification and Lane 10 and 11: Negative VPI-gene amplification

highest prevalence was in summer season (61.78%) followed by spring (18.93%), Autumn (11.43) and winter (7.86%) as shown in Table 6.

DISCUSSION

Arabian gulf area characterized by higher salt concentration and extreme higher temperature specially in summer season which favor *Vibrio* sp. Mainly *V. alginolyticus*[2]. Virulence of *V. alginolyticus* strain for fish epizootics outbreaks have been reported in gilthead seabream (*Sparus auratus*) by Paperna[31] and in two bar seabream (*Acanthopagrus bifasciatus*), black bream (*Acanthopagrus latus*), red sea seabream (*Diplodus noct*), brown-spotted grouper (*Epinephilus coioides*) and rabbitfish (*Siganus canaliculatus*) by Hassan *et al.*[1].

General septicemic picture were obvious on the examined fishes. In some cases, the symptoms were markedly prominent such as ulcers with different sizes and corneal opacity (uni-bilateral) especially in grouper fish. Internally, congestion of internal organs, distension of gall bladder and abdominal cavity with copious exudate (hemorrhagic or ascetic) were also recorded. Similar clinical signs and postmortem lesions stated by Labella *et al.*[32] and Moustafa *et al.*[33], which may came as a result of higher salinity, lack of dissolved oxygen by the rise of water temperature especially in summer season together with the presence of *V. alginolyticus* virulence factors. Gomathi *et al.*[34] stated that natural disease caused by *V. alginolyticus* in sea bream includes, septicemia, hemorrhage and skin ulcers in some cases. Internally, fish accumulate fluid in the peritoneal cavity and in some cases have hemorrhagic livers.

Due to plastic nature and higher number of identified vibrios (73 spp.) till now, Phenotypic and conventional methods including culture-based, API 20E and 20NE identification system were unreliable for identification of *Vibrio* sp.[13,35]. Hence, molecular biological DNA-based methods have been well established for rapid and accurate identification of *Vibrio* spp.[25]. It provide privileges than/or sequel to conventional biochemical and culture-based methods[36].

Sequencing of 16S rRNA and *V. alginolyticus* specific targets is of quiet importance for accurate identification and classification of isolates in the present study. The 16S rRNA gene sequence alone is less trust worthy in differentiation between species or even subspecies of G. *vibrio*[37] due to continuous increases in the number of known *Vibrio* sp., moreover several *Vibrio* sp. Contain almost similar sequence of 16S rRNA gene with fewer differences. Hence, in the present study, 16S rRNA sequence combined with *V. alginolyticus* specific gene sequence for accurate identification. BLASTing of sequence results against those held in GenBank (NCBI, Bethesda, USA) identified these isolates as *V. alginolyticus* with 99% similarity to *V. alginolyticus* strain NBRC 15630-16SrRNA gene and *V. alginolyticus* strain ANC5-1 respectively.

In bacterial chromosome, Intergenic Spacer (IGS) regions situated among 16S and 23S rRNA genes and considered as a fingerprinting to discriminate vibrios not only at species level but also at subspecies level as well[13]. The IGS-Typing pattern of *V. alginolyticus* on agarose gel was recorded in the present study as illustrated in Fig. 3 and represented by 4 typical bands ≈420, 580, 650 and 820 bp. The differences in the length and sequence of the 16S-23S intergenic spacer regions (IGSs) of rRNA persons were used to develop IGS-Typing system for *Vibrio* species, hence, *Vibrio* species can be distinguished from each other by one PCR reaction[13].

Concerning the results of virulence genes detection in the present study, *V. alginolyticus* possess positive results for collagenase, *tox*R and *omp*K genes and lacking tlh and VPI virulence genes. These results were supported by Effendy *et al.*[5], who stated that 67% of tested *V. alginolyticus* strains carry *omp*K, *tox*R and collagenase genes, while, 19% of them carrying both *omp*K and *tox*R genes and supported as well by Xie *et al.*[27], who detect the presence of tlh virulence gene which is homologous to those of *V. parahaemolyticus* and presence of VPI gene in 2 strains of *V. alginolyticus*. Hence, it proposed that *V. alginolyticus* act as substantial reservoir of several known virulence genes from other species of genus *Vibrio*.

The pathogenicity mechanism of *V. alginolyticus* might be different from other pathogenic *Vibrio* species or the virulence may be caused by multi virulent factors as reported by Kahla-Nakbi *et al.*[8] and Xie*et al.*[27] when they did not found any correlation between presence or absence or virulence genes in *V. alginolyticus* and its virulent strains.

In present study, the highest incidence of infection was in rabbitfish (*Siganus canaliculatus*) (29.64%) while, the lowest was in gilthead seabream (*Sparus auratus*) (6.43%). Similarly, Al-Sunaiher *et al.*[2] stated that *Vibrio* species detected in 16.8% of the total examined fish with higher incidence in rabbitfish (50%). On the contrary, Balebona *et al.*[38] reported that incidence of infection was (67.8%) intensive cultured gilt-head seabream (*Sparus aurata* L.). This variation in prevalence may be attributed to the differences salinity level, the sample sizes of studies, different climate.

The seasonal prevalence of *V. alginolyticus* infection among the total number of examined fishes revealed that, the highest prevalence was in summer season (61.78%) and these were supported by Giorgetti and Ceschia[39], who revealed that the main predisposing factors in all *Vibrios* outbreaks were increased in water temperature and epizootics were always associated with temperature higher than 15°C.

CONCLUSION

The present study suggested that the isolated *V. alginolyticus* was virulent to fish, shellfish, shrimp and crustacean present in Arabian gulf as it carry virulence genes. Subsequently, it should receive enough attention, may be by vaccination to avoid the resulting mortality and hence economic losses in commercial farms. Accurate identification and characterization of the dominant strain of local bacteria greatly support the epidemiological studies and tracing of diseases outbreaks. Moreover knowing the type of dominant bacteria is very helpful in developing of protection programs against it as well as to discover suitable methods to control the infection transmission to consumers.

SIGNIFICANCE STATEMENT

This study discovered the molecular characterization and virulence properties of *V. alginolyticus* isolated from Saudi coast of Arabian gulf and recorded its IGS-Typing pattern. This study will help the researcher to definitely identify *V. alginolyticus* using one PCR reaction in references to data of this study.

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