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VIRULENCE GENES OF ENTERIC ISOLATES OF VIBRIOAND VIBROID

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ABSTRACT

The vibrio species and the vibroid enteric isolates were found as Vibrio fluvialis andAeromonashydrophila though using API20E identification system. The to be total DNApreparations were separated from both of the organisms on agarose gel electrophoriesisand confirmed through the use of UV trsnsillumination. Such prepared and purifiedDNAwere amplified by PCR and allowed to pair with primers of four virulence genes as; Vhf,hupO ,tox and VhpfA for V .fluvialis and the primers of the genes Aer, hyl for A.hydrophila.Only Vhf and tox genes were positive in V.fluvialis and the genes Aer ,hyl were positive inA.hydrophila. Thus, PCR can be of use for the diagnosis for both of these organisms fromclinical samples,this will reduce labor and time needed for diagnosis.The authors were of theopinion holds that,four virulence genes from each of these organisms may served as abattery useful in molecular diagnosis of these disease agents

KEYWORDS:Haemolysin ,Aerolysin ,Virulence genes ,attenuation ,expression ,exotoxins.

INTRODUCTION

So far long is known that animal and human gut bacterial pathogens are mostly of gramnegative groups. Among which Vibrio and vibroids organisms are of rather commonoccurrence(1).Clinical and environmental resources isolates of such pathogens may expressputative virulence factor phenotypes.These phenotypes are encoded by genes within thepathogenicity islands.They may be located on the chromosome,plasmid,and/ortransposons (2).For the vibrio virulence genes encoding for virulence factorphenotypes,they were as; Swarming growth, biofilm, adherence, skin permeability factor, extracellularm etalloproteinases as well as haemolysins(3,4.5,6,7,).While, for thevirulence genes of vibroidsvriulence factor phenotypes,they were ;exotoxins,haemagglutinins ,adhesines and

hydrolytic enzymes(8). The objective of the present workwas to identify the vibrio and viboid isolates and to detect some of their virulence genesusing PCR technique.

MATERIALS AND METHODS

1-Organisms

Two laboratory cultures,thedescendents of an enteric clinical isolates preserved in thelaboratory local collection.They were assigened as Vibrio spp.andvibroid,and kept on longterm maintenance approach.

2-Identification

Each of the isolates were revived and identified using API20E miniaturized system.Theywere processed as in the manufacturer instructions.

3-Extrtaction of the total DNA

Both of the organisms were in shake broth culture method at 37C in a shaker incubatorfor 18 hrs.Dense washed cell suspension were re suspend in TES buffer with 600 ul 25%SDSwas added and incubated in 50C waterbath for five mins.Cell lysates were cooled to roomtemperature and mixed with 2ml.5N NaCl through frequent inversions.The resultant lysateswere mixed V:V with chloroform gently by several inversions within 30mins.Then mixtureswere separated by centrifugation at 10 000 rpm in 4C for 15 mins.The aqueous phase s weresaved into polyethylene tubes.Chloroform treatments were repeated till protein layer was disappeared. The purified DNA extract was concentrated by isopropanol 1:0.6 V:V and theDNA spirals on pasteurepipette,the pellet was dissolved in 1ml.TE buffer and stored at -5C.The to be DNA preparations were loaded on agarose gel electrophoriesis to assure thepresence of DNA(9),then transilluminated using the UV transilluminator(10).

4-Amplification of the total DNA

Specific primers and the amplification conditions were presented in tables 1 and 2.

Table(3-1) Primers and amplification conditions used in this study.

Primer Sequences (5'-3')	Target size (bp)	References
primers of *V.fluvialis*		
*tox*R-F GACCAGGGCTTTGAGGTGGACGAC	217	[15]
*tox*R-R AGGATACGGCACTTGAGTAAGACTC		
vfh-F GCGCGTCAGTGGTGGTGAAG	800	[15]
vfh-R TCGGTCGAACCGCTCTCGCTT		
hupO-F ATTACGCACAACGAGTCGAAC	600	[15]
hupO-R ATTGAGATGGT AAACAGCGCC		
vfpA –F TACAACGTCAAGTTAAAGGC	1790	[15]
vfpA –R GTAGGCGCTGTAGCCTTTCA		
primers of *A. hydrophila*		
aerA-F 5'-AGCGGCAGAGCCCGTCTATCCA-3'	416	[9]
aerA-R 5'-AGTTGGTGGCGGTGTCGTAGCG -3'		
hyl –F 5'-GGCCCGTGGCCCGAAGATGCAGG-3'	597	[9]
hyl –R 5- CAGTCCCACCCACTTC-3'		

Table (2) PCR Program That Apply In TheThermocyler

Gene	Initial denaturation	denaturation	Cycles annealing	elongatioin	Final elongation
toxR-F toxR-R	94ºC for 4 min	94ºC for 40 sec.	30 cycles 65ºC for 30 sec.	72ºC for 1 min	72ºC for 6 min
Vfh-F Vfh-R	94ºC for 4 min	94ºC for 40 sec.	30 cycles 61ºC for 30 sec.	72ºC for 1 min	72ºC for 6 min
hupO-F hupO-R	94ºC for 4 min	94ºC for 40 sec.	30 cycles 56ºC for 30 sec.	72ºC for 1 min	72ºC for 6 min
vfpA –F vfpA –R	94ºC for 4 min	94ºC for 40 sec.	30 cycles 55ºC for 30 sec.	72ºC for 1 min	72ºC for 6 min
aerA-F aerA-R	94ºC for 3 min	94ºC for 30 sec.	30 cycles 52ºC for 30 sec.	72ºC for 30 sec.	72ºC for 10 min
hyl –F hyl –R	95ºC for 5 min	95ºC for 30 min	30 cycles 55ºC for 1 min	72ºC for 1 min	72ºC for 7 min

RESULTS

1-Organismic Identity: The vibrio is being identified as Vibrio fluvialis,while the vibroid is characterized as Aeromonashydrophila Table3.

TABLE -3- Biochemical Characterization of the test Isolates

Tests	Vibrio	Vibroid
ONPG	+	
Arginine dihydrolase	+	-
Lysine decarboxylase	-	-
Ornithine decarboxylase	-	-
Citrate utilization	-	+
H2S production	-	+
Urease	-	
Gelatinase	+	+
Indole	+	+
Voges–Proskauer test	+	+
- Fermentation of		
Glucose	+	+
Mannitol	+	+
Inositol	-	
Sorbitol	-	
Rhamnose	-	
Sucrose	+	-
Melibiose	-	
Arabinose	+	V
Oxidase test	+	+
Growth in absence of NaCl	-	
Growth in 7% NaCl	+	
Growth in TCBS	+	+
Conclusion	Vibrio. fluvialis	Aeromonashydrophila

2-V.fluvialis Virulence genes

The prepared ,purified and amplified total DNA this V. fluvialis was screened for thepresence of the virulence genes Vfh ,hupO ,VfpA and tox ,using their specific primers by PCRtechnique.It was positive for both Vhf and tox genes Figure 1 and 2.

Detection of *vfh*gene by PCR technique is presented (Fig 1).

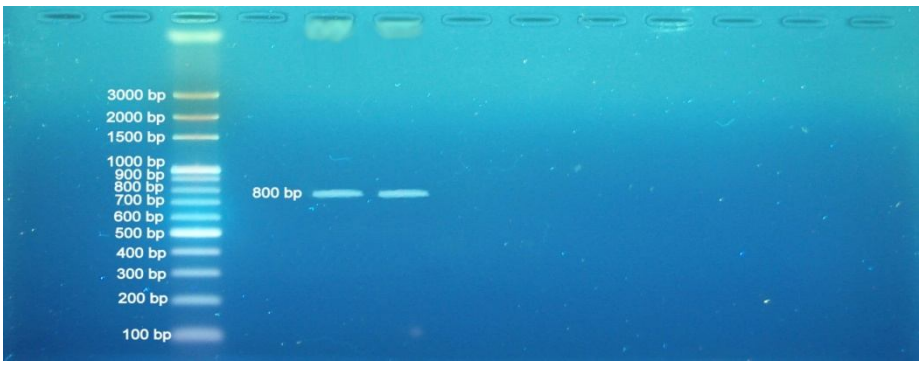

Fig. 1 Agarose Gel Electrophoresis 1.5% of PCR Amplify of *vfh*gene of *V.fluvialis*isolate for55 min at 100 Volt.

Detection of *tox*Gene :- Detection of*tox*gene by PCR technique is presented (Fig -2).

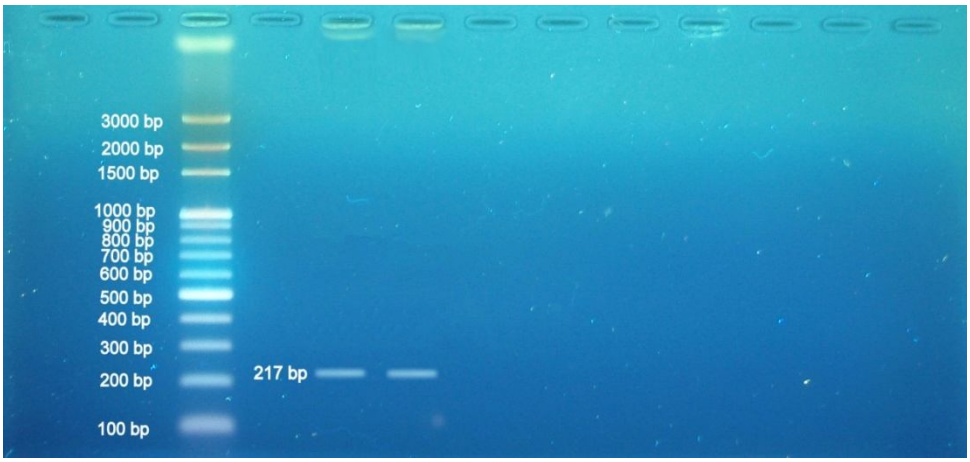

Fig. 2 Agarose Gel Electrophoresis 1.5% of PCR Amplify of *tox*gene of *V.fluvialis*isolate for55 min at 100 Volt.

3-Aeromonas hydrophila virulence genes:The recovered total DNA from this organism was screened for the genes Aerly .and hylIt was positive for the presence of these genes Figure 3 and 4.the presence of heamolysin toxin gene (Fig -3).

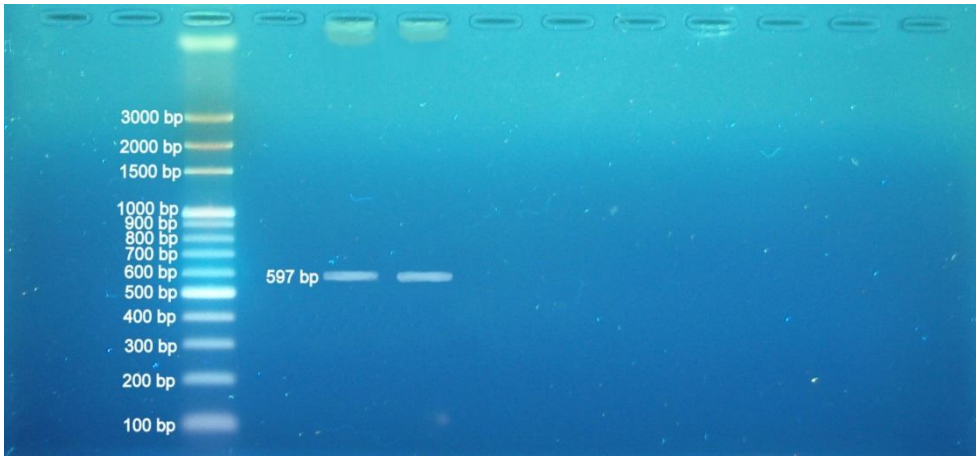

Fig. 3 Agarose Gel Electrophoresis 1.5% of PCR Amplified of (*hyl*) gene 597 bp of *A. hydrophila*isolates for 55 min at 100 Volt.

Detection of *Aerolysin*Gene (*aerA*)

Detection of *Aerolysin*gene (*aerA*) by PCR technique is presented (Fig-4).

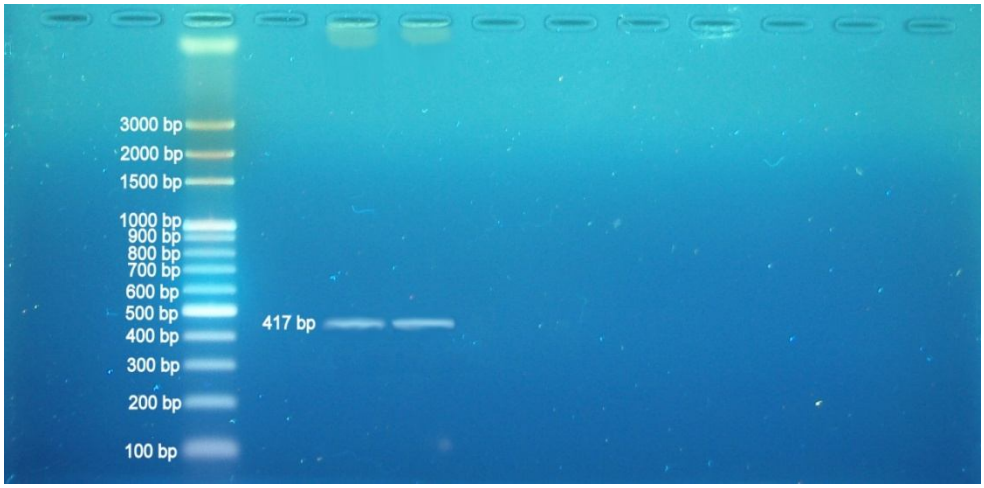

Fig. 4 - Agarose Gel Electrophoresis 1.5% of PCR Amplify of (*aerA*) gene of *A. hydrophila*isolates for 55 min at 100 Volt.

DSCUSSION

The identity the study organisms were consistent with; Vibrio fluvialis and Aeromonashydrophila(2,11).The obtained pure total DNA preparations were expected to contain DNA segments ofdifferent sizes that might or might not bear specific genes.PCR amplification increasesequally those having and not having genes. This study put forward two virulence genes ofeach of the study isolates .Figures 1,2,3,4.The Vhf genes encode for haemolysin production phenotype .Such haemolysinphenotypes were found to be of pore forming potentials to the erythrocyte membrane.TheV,fluvialis Vhf pores were larger than that formed by V. cholerae andV.parahaemolyticus(12,13).The Vhf genes were equally detected in patients and sea foodisolates(14).The tox gene is an anscestral gene for the family vibrionaceae which encods fortranscriptional activator domain(TAD),a transmembrane domain(TMD) and peripasmicdomain(PD)(15).The transmembrane regulatory protein ToxR is essential for expression ofvirulence factors in pathogenic vibrios. It plays a pivotal role in bile resistance which an initialphase of progression of V. fluvialis as potential gut pathogen(16).A.hydrophila pathogenic isolates are evidently aerolysin toxin secretors(17,18).Theirhaemolysins have shown to be of two immunotypes using immunologic approachs (19).Thenucliotid sequence homology between aerolysin and haemolysin were reaching 23.8%homology. Hence ,the evolutionary origin of the two genes might be different .The aerolysinand haemolysin gene attenuation have shown to be parallel with attenuation of virulence inmouse in mouse model.A finding that bears an indication for the possible control of A.hydrophila virulence by these genes(20)Finally,the utility of PCR assay to detect V. fluvialis and A .hydrophila clinical isolates of stoolsamples and help in the

reduction of labor and/or time needed for diagnosis of thesepathogen(15).Thse authors were of the opinion holds that a minimum of four virulencegenes from each of these pathogens may served as a battery of molecular diagnosis of thesedisease agents

CONCLUSIONS

The Vhf and tox genes were detected in a clinical isolate of V. fluvialis. Likewise ,Aerland hyl genes were identified in A,hydrophila

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