



## Fish Bacterial Pathogen in Gills, Skin, Kidney, Intestines, and its Water at Elmahmoudia and Edfina

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### ABSTRACT

#### Key words:

bacterial pathogens, vibrio spp, ERIC-PCR

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#### Article History

Received: Aug 18, 2022

Accepted: Sep 19, 2022

Bacterial pathogens are major etiology of infectious diseases of fish. In this study we were isolated and identified the most important Gram negative bacteria as Aeromonas spp, Coliforms., Salmonella spp., Pseudomonas spp., Vibrio spp. from gills, skin, intestine, kidneys of diseased fish mainly in Tilapia niloticus and environmental water at Mahmoudia canal in Elmahmoudia city, Edfina city and spans as abrakish water, and assessment of genetic and biochemically relations of the prominent bacterium. Bacteria as Aeromonas spp, Coliforms., Salmonella spp., Pseudomonas spp., were identified phenotypically, except V. parahaemolytica spp which was the prominent bacterial species isolated was confirmed molecularly by PCR, typed its strains from fish and water by ERIC\_PCR. in order to answer the question whether Vibrio spp infection considered water borne disease or not. Unfortunately ERIC\_PCR nor answered as the bacterium have diverse genetic heterogenous where the isolated were similar but genetically differed. However, in most cases, the origin of the infection is suspected to be environmental due to phenotype similarity of isolates. The aim of this study was to investigate similarity of certain bacteria in clinically diseased fish and environmental water.

## 1. INTRODUCTION

Fish and fish products are the most important source of protein, and it is estimated that more than 30 % of fish for human consumption comes from aquaculture (Hastein et al. 2006). Consumption of infected fish and shell fish may cause diseases due to infection or intoxication. Some of these diseases are caused by bacteria present on the external surfaces including slime, gills and the gut of the fish (El-Sayed et al., 2019).

The presence of bacterial pathogens may lead to high fish mortalities and huge economic losses (Shaheen et al., 2013; and (Mapenzi & Mmochi., 2016). Among the Gram negative bacterial pathogens in fresh water fish Pseudomonas spp, Salmonella spp, Aeromonas spp, Vibrio spp. are the most common bacteria affect various fish species

, including Tilapia, as the most prominent infection of young fish (Akayh, T. and

Timur, 2002). phenotypic complexity and inter species homogeneity hinder the identification by commercial systems used in laboratories. The molecular approaches including PCR and DNA Sequencing use lead to refined identification and limited all discrepancies associated with biochemical identification of organisms (Beaz et al., 2010). Vibrio species are Gram negative bacteria that can be isolated from water. This study was undertaken to investigate the clonal relatedness of the most common Vibrio spp. strains from fish with environmental water isolates by ERIC-PCR, to answer the question whether Vibrio spp. infection environmental water water borne disease.

## 2. MATERIAL AND METHODS

This study based on 92 sample on Tilapia fish and water as:

40 from Edfina (20 fish/20water)  
 40 fromElmahmoudia (20fish/20water)  
 12 from brakish water (6fish/6water)  
 Isolation and identification of bacterial pathogens from fish was carried out according to (Durmaz,Y.and Turkey ,N(2009).

While water sampling for bacteriological examination was carried out from the centrifuged to be cultivated on different media according to (Colakoglu et al .2006) and (Moubarak,S.T.S.(1989).

Bacteria isolated from fish organs (skin,gills, intestine,kidney and water) were cultivated according the following:

Isolation of coliforms according to (ISO 4833:2003).

Isolation of Salmonellaaccording to (ISO-standard 6579:1993 techniques).

Isolation of Pseudomonas and Aeromonas according to (Quinn et al., (2002) and (Buller., 2004).

Isolation and identification of Vibrio spp according to (Cruz et al., (2013) and williams et al., (2013).

Biochemical identification of Vibrio spp.according to (Overman et al. 1985).

Molecular Identification of Vibrio spp.: using ERIC-PCR

**3.RESULTS**

The phenotypic and API.20. biochemical characterization showed the following:

A higher incidence of bacteria in water samples observed in Vibrio as its incidences were 16 (80 %)

in Elmahmoudia, followed by its incidences in marine water 4 (66.67 %), and the lower incidences observed in Edfina 13 (65 %). The incidences of **Coliformis** showed a higher level in El-mahmoudia collected samples 10 (50 %) m followed by Edfina samples 9 (45 %) and samples collected from and in marine water 1 (16.67 %). The incidences of **Aeromonas** showed a higher level in marine water 3 (50 %), followed by Edfina samples 6 (30 %), followed by samples collected from El-mahmoudia 5 (25 %). The incidences of Pseudomonas showed a higher level in samples collected from El-mahmoudia 5 (25 %), followed by Edfina collected samples 4 (20 %) and in marine water 1 (16.67 %). The samples collected from the different localities not recorded any isolates of Salmonellae. In general, the higher incidences of bacteria observed in the samples collected from Elmahmoudia followed by Edfina and the lower in the samples collected from marine water. The higher incidences of bacteria in fish samples observed in Vibrio as its incidences were 18 (90 %) in Elmahmoudia 18 (90 %), followed by its incidences in Edfina 17 (85 %) and the lower incidences observed in marine water 5 (83.34 %). The incidences of Coliformis showed a higher level in El-mahmoudia collected samples 16 (80 %) followed by Edfina samples 14 (70 %) and samples collected from and in marine water 2 (33.30 %).

The incidences of Aeromonas showed a higher level in marine water 4 (66.67 %), followed by Edfina samples 8 (40 %),

**Table .1** The Incidences of bacteria in fish and waer samples

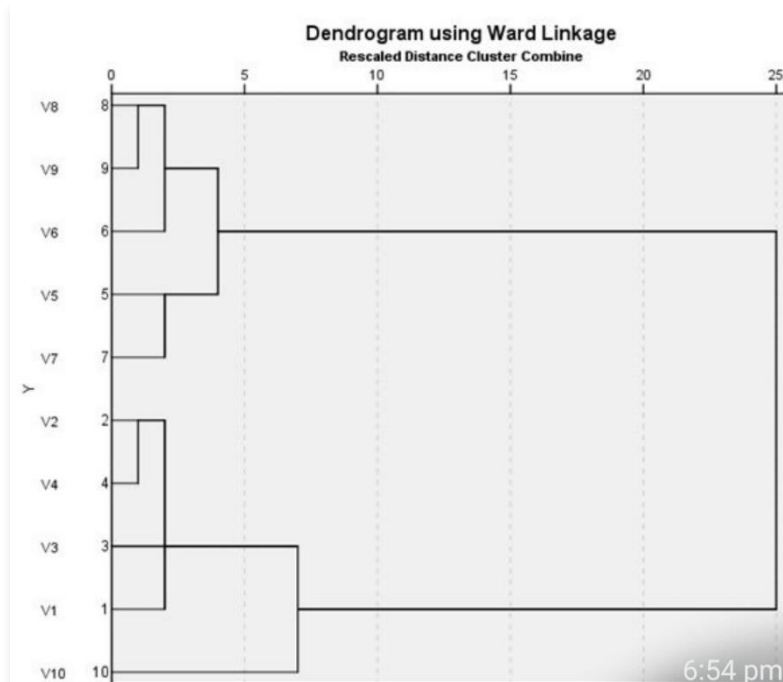
Bacteria	Edfina		Elmahmoudia		Marine water	
	Fish	Water	Fish	water	Fish	Water
92	20	20	20	20	6	6
Coliformis	14	9	16	10	2	1
Salmonella	1	0	1	0	0	0
Aeromonas	8	6	6	5	4	3
Pseudomonas	6	4	7	5	3	1
Vibrio	17	13	18	16	5	4

followed by samples collected from El-mahmoudia 6 (30 %). The incidences of Pseudomonas showed a higher level in samples collected collected from marine

water 3 (50 %), followed by samples collected from El-mahmoudia 7 (35 %) and the lower incidences observed in samples collected from Edfina 6 (30 %).

The lower incidences of bacteria observed in **Salmonella** as its higher incidences observed in fish samples collected from Edfina 1 (5 %) and in El-mahmoudia 1 (5 %) while, in marine water fish no incidences recorded in the fish samples. fish.

In general the higher incidences of bacteria observed in the samples collected from Elmahmoudia followed by Edfina and the lower bacterial incidences observed in the samples collected from marine



ERIC-PCR genetic profiles of all positive *V.parahaemolyticus* strains (n=10).the genetic patterns comprised by ERIC\_PCR provide evidenc for genetic relationship within the analyzed isolated of *V.parahaemolyticus* .

The data related to similarity were studied and bacterial isolates were grouped in one main cluster (dendogram)(drawn with un weight average paire group method “UPGMA”) .The height of the link that is between each strains decides the similarity between them example :sample number 2,4 have the smallest link height. Also, this diagram give an idea about the order of profile clusters as it divided them

into two major clusters in which each group are mpre related similarity in between than compared with other cluster.

Also the previous data obtained from ERIC-PCR can be represented and analyzed using asimilarity index to determine related bands

between samples and each other as the number of bands differe among the isolates, it ranged between 2-7.and to determine the identity pattern rate between isolates ranging from 0.2(20%)to 1(100%) In case of rate between sample (6,10) percent 20%(0.2) In case of rate between sample (8,9) percent 100%(1). samples(strains) and each other ranging from 2-7 bands.

#### 4. DISCUSSION

This study was done to investigate phenotypically the presence of some bacterial pathogens in fresh water fish and it’s environmental water “at Elmahmoudia ,Edfina”and brakish water fish and it’s water at “spans” focusing on *Vibrio* spp. using ERIC\_PCR. To know if this pathogen is water born or not. confirmed molecularely *V.parahaemolyticus* . Water borne transmission of pathogenic *Escherichia coli*,*Salmonella* maltophilia has been documented specially recreational water and underground water (Fredericlo de M.P et al.,2002 and Engelbrecht,J.F.B.2005). Acomplex group of pathogenic bacteria that widely distributed such as *Coliform*,*Pseudomonas*,*Aeromonas*,*Salmonella*,*Vibrio* spp. were repeatedly isolated from environmental water and fish .Although bacterial spp. isolated from them were similar phenotypically .The presence of the most

pathogenic species in water and fish at Mahmoudia canal, based upon the phenotypic and biochemical identification protocol and genetically by PCR based technique tracked *Vibrio parahaemolytica* strains isolated from fish *Tilapia nilotica* and their environmental water at Mahmoudia, Edfina, Spans by ERIC-PCR. ERIC\_PCR. Use to investigate the genetic results elucidated similarity of most strains (80%) phenotypically. Only one identical relation of strain from *V. parahaemolytica* diseased fish and environmental water and two strains from water gave similar bands isolated from. This findings may nominate at least pen point water one of the main source of fish infection. The ring between water environment, fish infection but cannot confirm *Vibrio* spp infection is water borne disease. However, regarding the bacteriological quality of the studied environmental water sources may indicate continuous sources of these bacteria introduced to water. The living mode of these bacteria Coliform, *Aeromonas*, *Pseudomonas*, *Salmonella*, *Vibrio* lack of certain essential nutrient may explain these conclusion

The ERIC\_PCR was less effective for tracking *V. parahaemolytica*, since the sequence may not be widely distributed in *Vibrio* species genome as most of the isolates produced identical profiles reflects the genetic diversity of the strains. The random amplified polymorphic DNA typing of clinical and environmental strains like (Ramalivhana, J.N et al (2010); Rathen asamy, S. et al, (2014), The clonal structure and diversity within the strains of *A. hydrophila* proved a greater heterogeneity among them had been demonstrated by ERIC\_PCR RAPD. We add also that this is the case in *V. parahaemolytica* having genetically heterogeneous. The high bacterial incidence at Mahmoudia hospital on Mahmoudia canal may represent a source of many pathogens in their wastewater, may have an impact on environment and human health (Jolibios, B. and Guerber, M. 2005). Generally the presence of *Aeromonas*, family *Vibrionaceae* suspect their source as healthy hazardous (Desai, S.V. and Varadara, T.M.C 2009

## Acknowledgements

Intendants and dr. walid Amer are thankful for help me

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