j.Egypt.net.med.Assoc 78, no 3. 385 – 402 (2018)

COMMON VIBRIOS AFFECTING THINLIP GREY MULLET (LIZA RAMADA)

By

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ABSTRACT

Vibrios are a group of Gram-negative septicemic bacteria that mainly affect marine, estuarine fishes and freshwater fishes sometimes. *Vibrios* are ubiquitous in open water resources especially seawater and estuarine habitats. In the current study, four main species of *Vibrios* were commonly isolated/identified from the thin lip grey mullet (*Liza ramada*) collected through spring 2017 to autumn 2017. The identities of the retrieved isolates were confirmed as *Vibrio alginolyticus* (*V. alginolyticus*), *Vibrio fluvialis (V. fluvialis) Vibrio Parahaemolyticus (V. Parahaemolyticus)* and *Vibrio vulnificus (V. vulnificus)* depending on their morphological /biochemical characteristics obtained from the adopted conventional and semi-automated biochemical tests (API20E). All isolates characters were in full accordance with the standard criteria stated by the Bergey's Manual of Systemic Bacteriology. Results has also revealed that *V. alginolyticus* was the highest isolated species (27.50 %) in Summer 2017 and autumn 2017(22.50%) ,while *V. vulnificus* was the lowest isolated through spring 2017 (3.75%) and autumn 2017(3.75%) . In spring season 2017, *V. parahaemolyticus* presented the highest percentage of infection (12.5 %). Antibiogram has revealed that most of the retrieved *V. alginolyticus* were sensitive to Doxycycline and Polymyxin and resistant to oxytetracyclin, florfenicol and Ampicillin. However *V. fluvialis* isolate were sensitive to all tested antibiotics with the exception of Ampicillin. From environmental point of view, the achieved results are indicative of consistently uprising agricultural /municipal drainage pollution across the northern delta provinces (lakes). Further, the occurrence of certain degrees of antibiotic resistances is also supporting the assumption that. the main source of antibiotics in the open water is the dumping of agricultural / municipal drainage. Ultimately, screening of the antibiotic resistance genes is highly required in the future.

Keywords:

Liza ramada, Thinlip grey mullet, *Vibriosis, Vibrio* infection, Antibiotic resistance.

INTRODUCTION

Egypt has been blessed with diverse aquatic environments that are unique in their ecological as well as biological natures. Egyptian fishes are either harvested from natural fisheries as Mediterranean Sea, Suez Canal, Red Sea, River Nile, Northern lakes, Karun Lake or intentionally reared in marine or freshwater inland aquaculture facilities. Seeds of mugil species are collected from the Mediterranean coasts or Northern lakes to be further propagated in inland aquaculture facilities of mainly polyculture or exceptionally monoculture nature. Thus, bringing in the juveniles of Mugil from natural water (Sea, lakes) would present an ideal mean of spread of pathogenic flora from the nature into aquaculture facilities **(Eissa *et al.,* 2016).** The ongoing chronic degradation of the marine / brackish habitat throughout the Mediterranean coasts / Northern lakes has colossal negative effects on the Egyptian fisheries industry **(Abdallah and Abdallah, 2008**).Sewage ,agricultural drainage , industrial effluents , natural gas exploration though the Egyptian Coasts of Mediterranean Sea and Northern lakes will drastically have great negative impacts on fish, shellfish and other aquatic species **(Pant and Mittal 2007; Rai and Igbinosa *et al.,* 2009).** Data extracted from a study performed by **Guidetti *et al*. (2002)** suggests that coastal fish negatively respond to the impact caused by the sewage discharge into the surrounding water basin. Such negative response may include bacterial pathogens invasions (*Vibrios ,Streptococcus, Aeromonas, Pseudomoas*) , parasitic invasion (nematodes , digeneans , acanthocephalans) and heavy metal pollutants (lead,cadmium and mercury) **(Lafferty and Holt , 2003; Austin , 1999).** Previous studies performed by marine biologists on the impact of sewage on the Mediterranean coasts of Egypt with special reference to Alexandria concluded that several Vibrio species are existing within numerous marine species including invertebrates **(Al- Sahn *et al.,* 1982)** and several fish populations **(Abdelaziz *et al.,* 2014; Moustafa *et al.,* 2015).** Being a pathogen of public health concern, the *vibrio* species gain global interest of the microbiology community and zoonotic diseases experts **(Austin and Austin, 2012)**. *Vibriosis* is a worldwide aquatic animal disease that present an actual danger for both aquatic species and human consumer. Several species of *Vibrios* have been recorded to cause disease among fishes and shellfishes. The most frequently occurring *Vibrio* infections in environmental

samples,fish and shellfish are*V.parahaemolyticus* ,*V.vulnificus* , *V. anguillarum* (*Listonella anguillarum*) , *V. alginolyticus* ,*V.carchariae*, *V. cholerae*, *V. damsela*, *V. furnissii*, *V. harveyi*, *V. ordalii* **(Hoa *et al*., 2000; Austin and Austin., 2012).** However, *V. parahaemolyticus*, *V. vulnificus*, *V. anguillarum, V. alginolyticus* and *V, ordalli* and *V. harveyi* are more linked to fish and shellfish **(Austin and Austin. 2012).** *Vibrios* are ubiquitous in marine and brackishwater environments and are commonly present in or / on shellfish and other seafood. They are present in the environment as free-living and associated with different substrata **(Tamplin *et al.,* 1990)**. Epibionts are able to survive in the natural environment longer than free-living forms, and by means of adhesive strategies, they can adapt to adverse conditions, e.g. organic matter limitation **(Roszak and Colwell, 1987; Carman and Dobbs., 1997).** The colonization of planktonic copepod integument by Vibrio spp. is a well-described phenomenon especially in what concerns the attachment to the copepods in fecal polluted and non-polluted coastal zones **(Kaneko and Colwell., 1975; Venkateswaran *et al.,* 1989; Tamplin *et al.,* 1990; Carli *et al*., 1993; Maugeri *et al.,* 2004).** *Vibrios* come on the top list of pathogens with direct jeopardy to mariculture development due to high mortalities associated with their invasion to fishes **(Moustafa *et al.* 2014 and 2015)**. It is crucial to know that Vibrios is ubiquitous to marine environment.Meanwhile clinical disease outbreaks take place when a sharply stressed fish get are exposed to the flaring up infectious agent **(Austin and Austin, 2012).** Septicemia induced by *Vibriosis* is characterized by hemorrhages on the base of pectoral fins, exophthalmia, loss of appetite and edematous lesions on the body surface **(Toranzo *et al.,* 2005).** *Vibrio alginolyticus* (*V. alginolyticus*) and *Vibrio Parahaemolyticus* (*V. Parahaemolyticus*) are responsible for mass mortalities among fish stocks in many marine fish farms throughout the Mediterranean area and severe economic losses in aquaculture worldwide **(Actis, *et al.,*1999)**.*V. alginolyticus* causes many epizootic outbreaks among the Gilthead seabream, European seabass and grey mullet populations, which possess high economic value at the Mediterranean communities **(Zorrilla *et al.,* 2003a).** Thus, the current study was planned to detect the most common Vibrios affecting Mugil fish populations in aquaculture facilities located within Northern Egyptian provinces bordering the Lake Manzalla. The following are the goals of the study: determination of the prevalence of *Vibrios* infection among Mugil fish collected from aquaculture facilities at Northern Egyptian provinces; detection, isolation and identification of the most common *Vibrios* among Collected Mugil fish samples.

MATERIAL AND METHODS

Fish samples collection:

A total number of 240 Thinlip grey mullet (Liza ramada) fish were collected from private fish facilities at Manzalla ,Dakhleya and Damietta through the period of March 2017 to October 2017 in a manner that samples were equally collected through three seasons / two years (spring ,summer , autumn) . For every sampling season, eighty fish with an average weight of 230 g Liza ramada fish were collected. Fish was kept on crushed ice in an insulated icebox then transferred as soon as possible (maximum 2-4 hrs. after collection) to the laboratory of Fish Diseases and Management (FDML), Faculty of Veterinary Medicine, Mansoura / Cairo University.

Sample processing:

Fish were flushed with ethanol 70 % to get rid of external contaminants. Using three line technique (triangular incision) fish were cut opened from the left side to expose liver and her internal organs using the method adopted from **Stosckopf (1993) and Eissa (2016).** All fish were externally and internally examined for any possible lesions before being sampled. Any reported lesions were documented using direct photographing using a digital camera.

Bacteriological isolation:

After clinical examination, swabs or lapful's were taken from kidneys then incubated in alkaline peptone water (APW) tubes at 25°C for 24 hrs. Aliquots from the inoculated APW tubes were then streaked onto Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS) plates. Inoculated plates were then incubated at 25 °C for 24 -72 hrs at maximum. Culture plates were inspected for any possible colonial growths. The cultural characteristics of the retrieved colonies were recorded. Bacteriological isolation protocol was modified from **Austin and Austin (2012).**

Purification o the retrieved isolates:

Gram stained smears from single colonies were done to check for purity of the retrieved colonies. The colonies giving consistent Gram staining results were then re-streaked onto trypticase soya agar (TSA) with 2 % NaCl for purification as a secondary culture. Tertiary cultures were made from the pure colonies. Purification protocol was adopted from **Austin and Austin (2012)** with slight modification according to methods described by **Whiteman, (2004).**

Biochemical identification:

Retrieved isolates were biochemically characterized following the morph-chemical criteria of Vibrio species described by **Whiteman, (2004) and Austin and Austin (2012)**.Presumptive characterization of the retrieved isolates were achieved using conventional biochemical tests (Gram reaction, Oxidase, Catalase, TSI, O/F, O129 sensitivity), which further affirmed using the commercial miniaturized API_20E system according to the manufacturer's instructions. The isolates were identified according to diagnostic scheme described by **Whiteman (2004), Austin, and Austin (2012).**

Antibiogram:

Antibiotic susceptibility of the retrieved bacterial isolates was determined using the Kirby Bauer disk diffusion method according to **Bauer *et al.* (1966).** In vitro antimicrobial susceptibility was screened on Mueller-Hinton agar (MHA) (Oxoid, Hampshire, UK) supplemented with 1.5 % (w/v) sodium chloride, and then incubated at 25 °C for 24 - 48 hrs. At the end of incubation period, antibiotic inhibition zones were measured in mm using a measuring caliber.

Determination of percentage of infection among sampled *Liza ramada* fishes:

The percentages of infection among different Mugil fishes collected through different sampling intervals were estimated by calculating number of infected fishes divided by total number of examined fish and multiplied by 100.

Histopathology:

Renal, hepatic, splenic, and branchial tissue samples from examined *Liza ramada* fish were fixed in 10% neutral buffered formalin solution. Formalin fixed tissues were then processed and embedded in paraffin. Five-micron sections of tissue samples were stained with hematoxylin and eosin (H & E) using methods described by **Bancroft *et al.* (1996).**

Statistics:

Statistical analysis was done using 2 Way ANOVA- Complete randomized block (CRB). Statistical analysis of fish results were achieved using the Statistical Package for Social Sciences (SPSS, 20) software for IBM personal computers. In all cases, $P < 0.05$ was accepted as significance level.

RESULTS

Clinical Examination:

Results of clinical examination of the sampled Thinlip grey mullet (Liza ramada) fish were categorized into:

1.A parentally normal fish:

Fish were showing no prominent clinical findings and them rather close to normal Fig. (1). Most of the apparently normal cases were those examined during spring season.

Fig. (1): An apparently healthy *Liza ramada* fish.

2.Clinically diseased (Moribund fish) :

a.Externally, the fish were showing fin and gills congestion Fig. (2).

b.Internally, the fish were showing variety of lesions such as ascites, congested liver, spleen, haemorrhagic gastroenteritis, and haemorrhage on the swim bladder wall, severe haemorrhage in the kidney Fig. (3, 4).

c.The clinical picture was noticeably remarkable during summer and autumn seasons than spring seasons.

Fig. (2): A Liza ramada fish with fin congestion and external haemorrhage.

Fig. (3): Liza ramada fish with marked ascitis.

Fig. (4):A Liza ramada fish with congested liver and mild haemorrhage on the swim bladder wall.

Microbiological examination:

Table (1): Percentage of retrieved bacterial isolates from sampled Liza ramada *that were* collected during spring season 2016.

Bacterial groups	Retrieved isolates identity	Retrieved isolates	
		Number	%
Vibrio isolates	*Vibrio fluvialis*	14	17.5
	Vibrio alginolyticus	5	6.25
	Vibrio Parahaemolyticus	10	12.50
	Vibrio vulnificus	3	3.75
No growth	No growth	30	37.5
Other Non-Vibrio isolates	*Pseudomonas florescence*	18	22.5
	Total	80	100%

Table (2): Percentages of retrieved bacterial isolates from sampled Liza ramada collected during summer season 2016.

Bacterial groups	Retrieved isolates identity	Retrieved isolates	
		Number	%
Vibrio isolates	*Vibrio alginolyticus*	22	27.5
	Vibrio fluvialis	10	12.5
	Vibrio Parahaemolyticus	7	8.75
	Vibrio vulnificus	5	6.25
No growth	*No growth*	10	12.5
Other Non-Vibrio isolates	*Enterococcus fecales*	11	13.75
	Aeromonas hydrophila	9	11.25
	Tenacibacullum maritimum	6	7.5
	Total	80	100%

Table (3): Percentage of retrieved bacterial isolates from sampled Liza ramada collected during autumn season 2016.

Bacterial groups	Retrieved isolates identity	Retrieved isolates	
		number	%
Vibrio isolates	*Vibrio alginolyticus*	18	22.5
	Vibrio fluvialis	14	17.5
	Vibrio parahemolyticus	10	12.5
	Vibrio vulnificus	3	3.75
No growth	No growth	12	15
Other Non-Vibrio isolate	*Photobacterium damselae*	15	18.75
	Tenacibacullum maritimum	8	10
	Total	80	100%

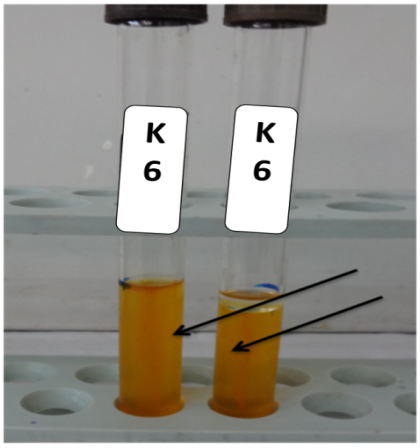

Fig. (5): Oxidation / Fermentation (O/F) test results for the retrieved *Vibrio* isolates: O / F +/+ (Yellow coloration of aerobic and anaerobic tubes).

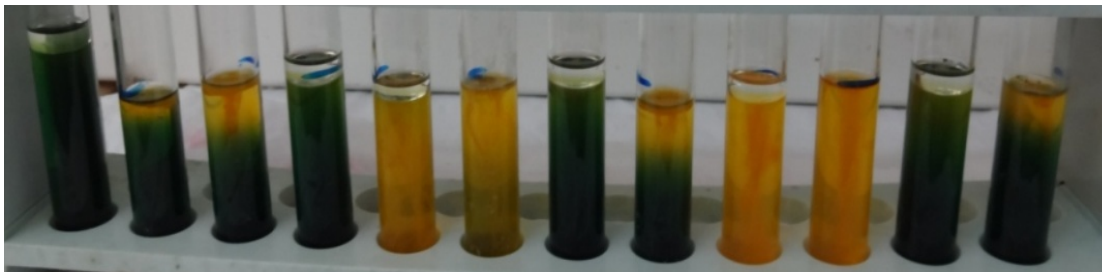

Fig. (6): Oxidation / Fermentation (O/F) test results for the retrieved *Vibrio* (161) and *Photobacterium damsalae* (165) isolates: O/ F +/+ (Yellow coloration of aerobic and anaerobic tubes); O/F +/- for the retrieved *Pseudomonas florescence* (No change of the blue green colour of the aerobic and anaerobic tubes).

Fig. (7): API 20E test kit showing the biochemical profile for the retrieved of *Vibrio* isolates

Fig. (8): API 20E test kit showing the biochemical profile for the retrieved of Pseudomonas florescence.

Biochemical identification:

The results of biochemical examination using the conventional biochemical tests as well as the semi-automated API 20 E test strips have revealed that, the retrieved *Vibrio* isolates were categorized into four main isolates: *V. Parahaemolyticus, V. vulnificus, V. alginolyticus* and *V. fluvialis*. The detailed biochemical characteristic of the retrieved isolates is described in (Table2).

Table (4): Morpho-chemical characteristics of the retrieved *Vibrio* isolates from sampled Common panadora fish.

Criteria	*Vibrio alginolyticus*	*Vibrio fluviales*	*Vibrio parahemolyticus*	*Vibrio vulnificus*
API 20 E ID	**4347324**	**3046126**	**4366107**	**5306005**
Culture character on T.C.B.S	**Yellow colonies 2-3 mm in diameter.**	**Yellow colonies 2-3 mm in diameter.**	**Green colonies 2-3 mm in diameter.**	**Green colonies 2-3 mm in diameter.**
Gram stain character	**Gram –ve rods**	**Gram –ve rods**	**Gram –ve rods**	**Gram –ve curved rods**
Oxidation / fermentation	+/+	+/+	+/+	+/+
Oxidase	+	+	+	+
Catalase	+	+	+	+
ONPG	-	+	-	+
ADH	-	+	-	-
LDC	+	-	+	+
ODC	+	-	+	+
CIT	+	-	+	+
H2S	-	-	-	-
URE	-	-	-	-
TDA	-	-	+	-
IND	+	+	+	-
VP	+	-	-	-
GEL	+	+	+	+
GLU	+	+	+	+
MAN	+	+	+	-
INO	+	-	+	-
SOR	-	-	-	-
RHA	-	-	-	-
SAC	+	+	-	-
MEL	-	-	-	-
AMY	-	-	+	+
ARA	-	+	+	-
Nitrate	+	+	+	+

Antibiogram:

The results of Antibiogram for the retrieved *Vibrio alginolyticus* isolates has revealed that isolates were only sensitive to Doxycycline and Polymyxin , Sulfamethoxazole- trimethoprim while resistant to Oxytetracycline, Florfenicol, and Ampicillin.

Table (5): Antibiogram for retrieved *Vibrio alginolyticus*.

Antibiotic	Standard inhibition zone			*Vibrio alginolyticus*	
	Resistant	Mildly sensitive	Sensitive	inhibition zone	Response
Doxycyclin-30	< 8	8-12	>12	22mm	Sensitive
Polymixin-300	< 8	8-12	>12	18mm	Sensitive
Oxytetracycline -30mm	<15	15-18	>18	13mm	Resistant
Florfenicol-30	<16	16-21	>21	10mm	Resistant
Sulfamethoxazole-trimethoprim -25	<11	11-15	>15	15mm	Sensitive
Ampicilin-10	<12	12-13	>13	0	Resistant

The results of antibiogram for the retrieved *Vibrio fluviales* isolates has revealed that isolates were sensitive to all the used antibiotic discs, while only resistant to Ampicillin.

Table (6): Antibiogram for retrieved *Vibrio fluviales.*

Antibiotic	Standard inhibition zone			*Vibrio fluviales*	
	Resistant	Mildly sensitive	Sensitive	inhibition zone	Response
Doxycyclin-30	< 8	8-12	>12	30mm	Sensitive
Polymyxin-300	< 8	8-12	>12	21mm	Sensitive
Oxytetracycline -30mm	<15	15-18	>18	25mm	Sensitive
Florfenicol -30	<16	16-21	>21	30mm	Sensitive
Sulfamethoxzole-trimethoprim -25	<11	11-15	>15	24mm	Sensitive
Ampicilin-10	<12	12-13	>13	0	Resistant

Histopathology:

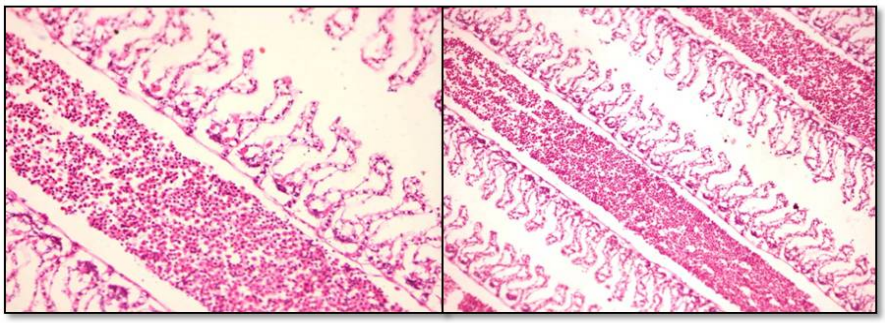

Fig. (9): highly congested branchial blood vessels and hemmorahagic branchitis of Liza ramada **H&E** stained gill tissues **(A. 400 X- B. X200).**

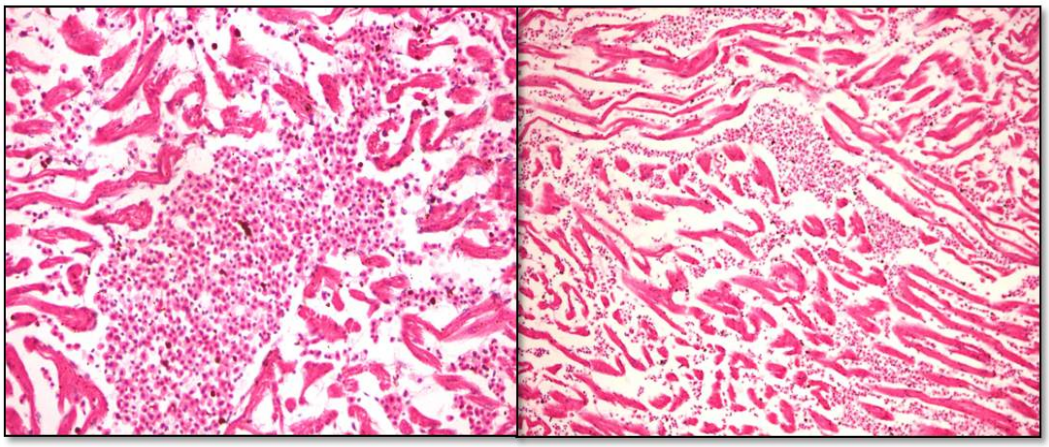

Fig. (10): H& E stained tissue sections of Liza ramada cardiac muscle fibers highly infiltrated with RBCs and inflammatory cells (hemorrhagic myocarditis) tissues **(A. X400-B. 200 X).**

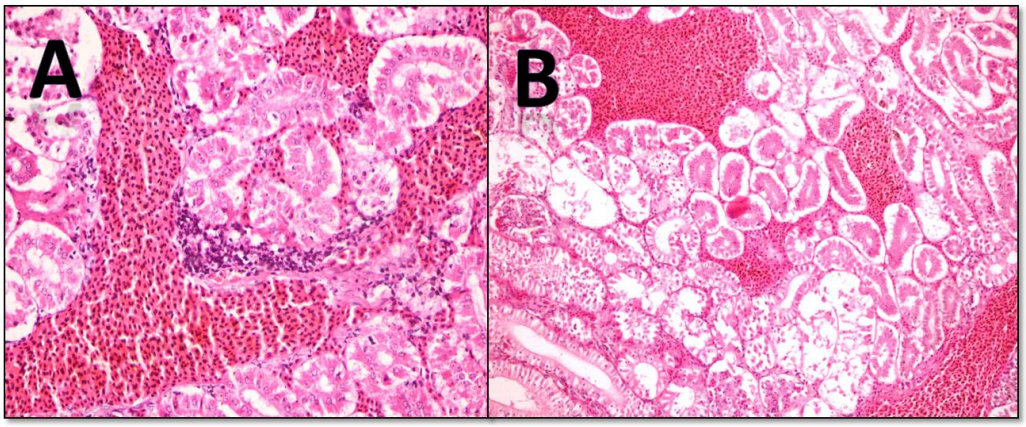

Fig. (11): H& E stained tissue sections of renal tissue with severe interstitial hemorrhage and some inflammatory cells infiltration with tubular degeneration **(A. X400-B. 200 X).**

DISCUSSION

In Egyptian monoculture or polyculture fish farms, seeds of mugil species (Liza ramada or Mugil cephalous) are usually obtained from the Mediterranean Sea then transferred to earthen pond based aquaculture facilities. In Damietta or Dakhleya provinces, Mugil species are raised in mixed water that is mainly fed from agricultural drainage sources which sometimes receives relatively moderate quantities of municipal drainage (Dr. Alaa Eldin Eissa, personal communications). Considering most of fish bacterial pathogens as common seawater / drainage water inhabitants then the possibility of bacterial disease eruptions under stressful environmental conditions (hot climate, high ammonia, high pH, low dissolved oxygen and parasites) is relatively high. Vibrios are among the most common inhabitants of drainage water, sea sediments and bottom feeder filter-feeder invertebrates.Thus, vibrios are commonly isolated from benthic Mediterranean, inland reared brackiswater fishes and northern lake fishes **(Eissa *et al.,* 2015; Moustafa *et al.,* 2015; Eissa *et al.,* 2017).** *Vibrio* possesses a wide array of virulence factors, including acid neutralization, capsular polysaccharide expression, iron acquisition, cytotoxicity, motility, and expression of proteins involved in attachment and adhesion **(Thompson *et al.* 2004; Linkous *et al* .1999, Strom and Paranjpye 2000).** These factors likely require concerted expression for pathogenesis to take place. Conclusively, *V. vulnificus and V. parahemolyticus are* complex microorganisms with physiological characteristics that contribute to its survival in marine environment and in the human host **(Jones *et al* .2009 - Oliver. 2005).** The microscopic wounds induced by skin external parasites , intestinal parasites and other internal organ with consequent microscopical hemorrhages at these organs would produce a reliable amount of iron due to blood cell damage in a very similar way to the action of *Ascaris* in the human intestine **(Saha *et al,.* 2008).** Further, marked undefined generalized hemorrhage in many examined samples could have triggered swift spread of Vibrios within fish tissues. The sequestered iron represents an eminent enhancer for *Vibrio* pathogenicity within the fish body, as the iron acquisition is one of the main pathogenic mechanisms of such pathogen **(Wright *et al.,* 1993).** Such finding could describe how *Vibrio* species as a secondary invader together with primary stress factors were working in a very aggressive way to induce a clinical disease in the target fish **(Eissa *et al.,* 2015).** Histopathological studies severe to moderate cases of hemorrhagic inflammation of different sampled Liza ramada organs including gills, liver, spleen, kidneys

and ovaries are consistent with the septicemic nature of pathogenic *Vibrio*s as well as the iron sequestrating pathogenicity mechanism of such *Vibrios*. It is well documented that temperature rise above 22°C enhances *Vibrio* spp. pathogenicity and virulence by the following mechanisms: increases the bacterial growth rate by an average of 30%; increases the adhesion capacity of the bacterium to the fish tissues **(Oliver, 2005; DePaola *et al.*1994).** In summer and autumn, the sharp decrease in dissolved oxygen due to temperature rise above 25°C jeopardizes the immune system of fish by increasing the potentials of the ubiquitous bacterial invasion **(Eissa *et al.,* 2013).** Surveys combining information on bacteriological and parasitological burden under natural conditions have shown that mixed infections are very common, and hence the etiology of some disease outbreaks or clinical conditions is complex and difficult to ascribe to a single pathogen **(Eissa *et al.* 2010; Sun *et al*.,2009; Bricknell and Raynard. 2002).** In the current study, around 15 % of the mugil fish collected from Damietta and Dakhleya fish farms have presented typical signs of severe *Vibrio* infection concurrently with other bacterial infections such as Enterococcus fecalis , Aeromonas hydrophila, Tenacibaculum maritimum (summer) and Photobacterium damsalae , Tenacibacullum maritimum (autumn) . We inferentially hypothesized that, the damaging effect of other concurrent infectious agents as well as chemical environmental *stressors* on gills, skin, intestinal mucosal immunity and renal humoral / cellular immunity would have presented the main triggering factor in swift invasion of *Vibrio* species to fish tissues and the subsequent enhancement of the pathogenic mechanism of the pathogen. This assumption is highly consistent with similar ones presented by **Eissa *et al.* (2017).** The results of the current study indicated that the ubiquitous *V. alginolyticus and V. fluvialis*, that are commonly existing in seawater, shellfishes, seabed and earthen ponds bottom **(Austin and Austin, 2012; Ashraf, 2013)** represented the first and second largest sector of isolated Vibrios through the entire study (18.75 % and 15.8 % from the total number of isolates through the three seasons of the study). This results is relatively consistent with similar results obtained by **Ashraf (2013)** who related the higher incidence of *V. fluvialis* to high salinity above 30 and high sewage pollution which could have exceptionally happened in Damietta Liza ramada samples. Similar results were also reported in brackish water environments through various earthen pond reared marine / brackish species at the Northern Egyptian provinces in several fish species including Mugil species **(Zorrilla *et al.,* 2003a; Abdelaziz *et al.,* 2013; Moustafa *et al.,* 2014; Moustafa *et al.,* 2015).** The *V. Parahaemolyticus,* is a pathogenic *Vibrio* that is present

in plenty loads in the common reservoirs such as shellfishes, mussels and mollusks **(Austin and Austin, 2012; Eissa *et al.* 2015).** In the present study, the average percentage of the retrieved isolates of *V. Parahaemolyticus* is 11.25 % that is 3rd on list of the isolated vibrios through the entire study. *V. Parahaemolyticus* is associated with sewage pollution and it is one of the main food poisoning pathogens that is linked to raw or inefficiently cooked seafood feeding **(Austin and Austin, 2012).** The presence of *V. Parahaemolyticus* in apparently normal Liza ramada fish samples might indicate a chronic carrier case where signs of the disease absent. In fish with disease signs, the pathogen could have reached to higher loads that were capable of inducing a disease pattern triggered by the concurrent mucosal damage made by other environmental stressors **(Eissa *et al.* 2015).** The *V. vulnificus,* is a highly pathogenic *Vibrio* that is present in moderate loads in the common reservoirs such as mussels, shellfishes and mollusks **(Austin and Austin, 2012; Eissa *et al.,* 2015).** *V. vulnificus* causes many epizootic outbreaks among marine fish populations such as seabream , seabass and most recently black scorpion fish from the western coast of Tripoli / Libya **(Eissa *et al.,* 2015).** They are also well known to be an eminent cause of cellulites and septicemia in fishermen, swimmers and people handling infected fishes **(Austin and Austin. 2012).** In the present study, the average percentage of the retrieved isolates of *V. vulnificus* is 4.6 % that is 4th on list of the isolated vibrios through the entire study. **Eissa *et al.* (2015)** have confirmed the vulnerable role of *Anisakis* species as a tissue wondering nematode in infection / spread of *V. vulnificus* to the benthic carnivore black-scorpion-fish from the western coast of Tripoli / Libya. Our results were concordant with those of **Eissa *et al.* (2017)** who have isolated this type of Vibrio in similarly low percentage (6 %).Some non-Vibrio bacterial pathogens have also been isolated concomitantly with *Vibrios* from the same examined Liza ramada during different seasons. *Photobacterium damsalea* was concomitantly isolated from the same fish at a percentage of 6.25 % only during the autumn season.The fact that *Photobacterium damselae* was isolated only though he autumn season is consistent with similar results obtained by **Eissa *et al.* (2017), Abdel-Aziz (2013) and Ashraf (2013).** Such pathogen is associated with salinity levels below than 26-ppm and temperatures above 29 ˚C, which is the typical case here. Pseudomonas florescence was common concomitant isolate during the winter season (7.5 %) which could be explained by the obligate aerobe nature of such pathogen **(Austin and Austin, 2012; Eissa *et al.,* 2013; Eissa *et al.,* 2017).** In winter, the low water temperature

allows good dissolved oxygen levels in seawater which is critical for *P. florescence* as they can only live , flourish and invade at water containing good levels of dissolved oxygen required for their aerobic metabolism **(Austin and Austin, 2012; Eissa *et al.* 2013).** The sewage pollutant indicator Enterococcus fecalis was isolated during summer season together with Aeromonas hydrophila (3.75 %) and the long Gram negative marine *flavobacteria, Tenacibacullum maritimum* (formerly known as *Flavobacteium maritimus*) was concomitantly isolated during the summer/autumn (5.8 %) is also expected during higher temperature surge in summer or sharp temperature fluctuation in late autumn . High temperatures, high ammonia and deteriorated environmental parameters induced by sewage are a triggering factor in the pathogenicity and invasion of such pathogen to the liable fishes. Results obtained by us are consistant with that obtained by **Eissa *et al.* (2017) and Ashraf (2013)** for saline water and **Eissa *et al.* 2010** for freshwater. The isolation of the four above-mentioned *Vibrios, Enterococcus species, Aeomonas and pseudomonas* species as is highly indicative for the deteriorated water quality of the sampled fishponds that is a continuously evolving dynamic problem triggered by the random dumping of untreated agricultural / municipal drainage into the seawater / lakes water and consequent invasion of the fish. It is worth to mention that all bacterial isolates retrieved throughout the study was confirmed using the semi-automated API 20 E system and all results were consistent with their morpho-chemical standard criteria described by **Eissa *et al.,* (2017), Buller, (2004) and Whiteman, (2004).** The antibiogram of all retrieved Vibrio isolates revealed higher sensitivities to two major vibriocidal antibiotics namely, Doxycycline and Polymyxin. Most of isolates with the exception of *V. fluvialis* were resistant to the traditionally used antibiotics such as Oxytetracyclin, Florfenicol and Ampicillin. Controversially, *V. fluvialis* were sensitive to al used antibiotics with the exception of Ampicillin. The presence of relative resistance to the traditionally human used antibiotics such as Ampicillin, Florfenicol, and Oxytetracyclin is bad indicator of the developing antibiotic resistance genes in fishes inhabiting the northern lakes, Mediterranean Sea and natural water streams in Nile delta. This result is critical alarm for the public health authorities to the ongoing antibiotic resistance resulting from the continuous dumping of municipal/ agricultural drainage water. Finally, successful isolation of 4species of *Vibrios* and zoonotic other non-*Vibrio* species together with the developing antibiotic resistance is an obvious indicator of agricultural/ municipal pollution of the sampling areas. In conclusion, the achieved results are indicative of

consistently uprising agricultural /municipal drainage pollution across the northern delta provinces (lakes). Further, the occurrence of certain degrees of antibiotic resistances is also supporting the assumption that the main source of antibiotics in the open water is the dumping of agricultural / municipal drainage. Ultimately, screening of the antibiotic resistance genes is highly required in the future.

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