Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Fisheries and Aquatic Science

ISSN 1816-4927
DOI: 10.3923/jfas.2017.253.263

Research Article

Detection of the Most Common *Vibrios* Affecting Common Pandora (*Pagellus erythinus*) from the Coasts of Tripoli, Libya

[1,2]Alaa Eldin Eissa, [2]Mohamed Basheer Altakaly, [3]Said Kamal Abolghait, [4]Mona Mahmoud Ismail and [2]Abdelsalam Abumhara

[1]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, 11221 Giza, Egypt
[2]Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya
[3]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt
[4]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt

Abstract

Background and Objective: *Vibrios* are ubiquitous in environmentally polluted seawater with special reference to municipal sewage. In the current study, four main species of *Vibrios* had been frequently isolated/identified from the Common Pandora fish (*Pagellus erythrinus*) collected through summer 2014 to spring 2015. **Materials and Methods:** A panel of specific morpho-chemical conventional and semi-automated tests (API 20E) were used to uncover the identities of the retrieved isolates. The degree of similarity within API 20E identification charts as well as the taxonomical matching against standard criteria stated at the Bergey's Manual of Systematic Bacteriology were concomitantly used for final confirmation of the retrieved isolates. Statistical analysis was done by two-way ANOVA using SPSS. **Results:** The four fully confirmed species were *Vibrio vulnificus* (*V. vulnificus*), *Vibrio parahaemolytices* (*V. parahaemolyticus*), *Vibrio fluviales* (*V. fluviales*) and *Vibrio alginolyticus* (*V. alginolyticus*). The research has revealed that *V. fluviales* was the highest isolated species (33.33%) in Summer 2014 and Spring 2015 (27.77%), while *V. vulnificus* was the lowest isolated through summer 2014 (4.44%) and spring 2015 (5.55%). In winter season 2014, *V. parahaemolyticus* presented the highest percentage of infection (20%), while *V. alginolyticus* was associated with the lowest percentage of infection (2.22%). Antibiogram has revealed that most of the retrieved *V. alginolyticus* and *V. parahaemolyticus* were sensitive to doxycyclin, polymyxin and resistant to oxytetracyclin, florfenicol and ampicillin. However, *V. fluviales* isolate were sensitive to all tested antibiotics with the exception of ampicillin. Interestingly *V. vulnificus* were sensitive to doxycyclin, polymyxin, oxytetracyclin, while resistant to florfenicol, sulfamethoxzole-trimethoprim and ampicillin, which was remarkably different from the antibiogram recorded for other retrieved *Vibrio* species. **Conclusion:** Conclusively, the achieved results throughout the current study were indicative of a consistently growing municipal sewage pollution across the western coasts of Tripoli, Libya.

Key words: *Pagellus erythrinus*, Common Pandora, vibriosis, *Vibrio* infection, biological pollution

Citation: Alaa Eldin Eissa, Mohamed Basheer Altakaly, Said Kamal Abolghait, Mona Mahmoud Ismail, Abdelsalam Abumhara, 2017. Detection of the most common *Vibrios* affecting Common Pandora (*Pagellus erythinus*) from the coasts of Tripoli, Libya. J. Fish. Aquat. Sci., 12: 253-263.

Corresponding Author: Alaa Eldin Eissa, Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, 11221 Giza, Egypt
Tel: +2-01065753010

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

Mediterranean is a semi-closed sea that is characterized by a relatively limited rate of water exchange. From fisheries perspectives, the Mediterranean is characterized by the presence of diversified large number of aquatic species, which represent 5.5% of the world's marine fauna[1,2]. The Mediterranean sea has not been a good location for the development of an industrial fishing throughout past centuries. The Mediterranean coasts extends for more than 4,000 km crossing fairly large number of South European countries and Arab countries[1]. The ongoing chronic degradation of the marine habitat throughout the Mediterranean region has colossal negative effects on the fisheries industry in most of the North African coasts[3].

Marine aquaculture in North Africa is an expanding industry and important production sector, which has been focused on some species such as seabass, seabream and mullet. The Pandora fish (*Pagellus erythrinus*), is a popular member of the seabream family for North African customers[4]. The Common Pandora currently represents a moderately large portion of the marine fisheries harvest at the South Mediterranean coasts. Recently, like other seabream fishes, *Pagellus erythrinus* has been cage-cultured in several areas across the Mediterranean coasts of North Africa [5].

Sewage, industrial effluents, crude oil refineries, oil exploration though the coasts of North African countries including Libya have great negative impacts on the Mediterranean aquatic environment[6,7]. Data extracted from a study performed by Guidetti *et al.*[8] suggested that coastal fish negatively respond to the impact caused by sewage discharge into the surrounding water basin. Such negative response may include bacterial pathogens invasion (*Vibrios*, *Streptococcus*, *Aeromonas*, *Pseudomonas*), parasitic invasion (nematodes, digeneans, acanthocephalans) and heavy metal pollutants (lead, cadmium and mercury)[9,10]. Previous studies performed by marine biologists about 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[11] and several fish populations[12,13]. Being a pathogen of public health concern, the *Vibrio* species gain global interest of the microbiology community and zoonotic diseases, experts[14].

Vibriosis is a worldwide aquatic animal disease that present an actual danger for both aquatic species and human consumers. 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*[12]. However, *V. parahaemolyticus*, *V. vulnificus*, *V. anguillarum*, *V. alginolyticus*, *V. ordalli* and *V. harveyi* are more linked to fish and shellfish[14].

Vibrios come on the top list of pathogens with direct jeopardy to mariculture development due to high mortalities associated with their invasion to fishes[15,12]. It is crucial to know that, *Vibrios* are ubiquitous to marine environment, while clinical disease outbreaks only occur when a sharply stressed fish get exposed to the flaring up infectious agent[14]. 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[16]. *Vibrio alginolyticus* and *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[17,18]. *Vibrio alginolyticus* causes many epizootic out breaks among the Gilthead seabream and European seabass populations, which possess high economic value at the Mediterranean communities[17,18]. Morphologically, *Vibrios* are Gram negative, asporogenous rods that are straight or curved and are motile with a single polar flagellum when grown in liquid medium[19].

In Libya, there has been a scarcity in literatures discussing the magnitude of *Vibrio* infections among the Seabream populations in general and Common Pandora in specific throughout the entire Tripoli coast of the Mediterranean. Thus, the current study was planned to detect the most Common *Vibrios* affecting Common Pandora populations at the coastal Mediterranean area of Tripoli.

MATERIALS AND METHODS

Fish sampling: A total number of 270 Common Pandora fish (*Pagellus erythrinus*) were collected through the period of April, 2014-2015 in a manner that samples were equally collected through three seasons (summer, autumn and spring). For every sampling season a total of 90 fish with an average weight of 135 g and 20 cm average total length were collected. Fishes were kept on crushed ice in an insulated ice box and transferred as soon as possible (2-4 h after collection at maximum) to the laboratory of Poultry and Fish Diseases (PFDL), Faculty of Veterinary Medicine, University of Tripoli.

Fig. 1: Geographical location of sampling sites from Tajurah through Janzour at the Greater Tripoli Mediterranean coast

Sampling locations: The fishes were collected from the area extending from Tajoura (East to Tripoli) to Janzour (West to Tripoli). Specified sampling locations are illustrated on Fig. 1.

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 other internal organs using the method adopted from Stoskopf[20] and Eissa[21]. Fishes were externally and internally examined for any possible lesions before being sampled for bacteriological examination. Any reported lesions were documented using direct photographing with a digital camera (RX100 V The premium 1.0-type sensor compact camera with superior AF performance, Saint Diego, CA, USA).

Bacteriological isolation: After clinical examination, loop fulls from kidney were inoculated into in alkaline peptone water (APW) tubes and incubated at 25°C for 24 h. Aliquots from the inoculated APW tubes were then streaked onto thiosulphate citrate bile salt sucrose agar (TCBS) plates. Inoculated plates were then incubated at 25°C for 24-72 h 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[14].

Purification and preservation of 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[14] and with slight modification (adding 2% salt to the purification medium) from similar protocol described by Whitman[22]. A fresh pure colony from the tertiary culture were inoculated into 0.5% soft TSA slants for short term preservation in refrigerator (4°C) and 20% glycerol peptone saline for long term preservation in deep freezer (-80°C).

Biochemical identification: Purified isolates were biochemically characterized according to the diagnostic scheme described by Whitman[22] and Austin and Austin[14]. Biochemical identification were achieved using conventional biochemical tests (Gram reaction, Oxidase, Catalase, TSI, O/F, O129 sensitivity). Confirmation of biochemical profiles of the retrieved isolates was performed using the commercial miniaturized API 20E test kit (Analytical Profile Index) (BioMereux, France) according to the manufacturer's instructions.

Antibiogram: Antibiotic susceptibility of the retrieved bacterial isolates was determined using the Kirby Bauer disk diffusion method according to Bauer *et al*.[23]. The following antimicrobial discs (Oxoid, UK) were used: Ampicillin 10 µg (AML 10), O/129 150 µg (129/150), Oxytetracycline 30 µg (TE 30), Doxycyclin (DO 30), florfenicol, polymyxin B (Pb 300), Trimethoprim/Sulfamethazole 25 µg (SXT 25). *In vitro* antimicrobial susceptibility was screened on Mueller-Hinton agar (MHA) (Oxoid, Hampshire, UK) supplemented with 1.5% (w/v) sodium chloride. At the end of incubation period, antibiotic inhibition zones were measured in mm using a measuring caliber.

Statistical analysis: Statistical analysis was done using two-way ANOVA, complete randomized block (CRB) utilizing 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 and prevalence of *Vibrio* spp. in Pandora fish: No significant differences were noticed between percentages of infected fish in different season of collection (Fig. 2). External examination of mori bund collected fish revealed fin congestion and/or rot (Fig. 3a) while internal examination showed congestion in liver, severe hemorrhage in kidney and mild hemorrhage in swim bladder (Fig. 3b, c).

Microbiological examination and identification: Result is revealed that four *Vibrio* species were isolated from Common Pandora collected during summer and winter 2014 and spring 2015. Retrieved isolates were morpho-chemically identical to the standard morpho-chemical criteria of the recorded species in Bergy's Manual of Systematic Bacteriology. It was very obvious that *V. fluviales* and *V. parahaemolyticus* were the most prevalent *Vibrio* species through all collection seasons. In addition, other fish specific non-*Vibrio* species have been isolated aside of the retrieved *Vibrios* such as *Aeromonas hydrophila*, *Pseudomonas flouresence*, *Photobacterium damselae* and *Tenacibaculum maritimum*. The later was the most common non-*Vibrio* organism recorded in summer and spring season with prevalence of 22% (Table 1).

Morpho-chemical characteristics of the retrieved *Vibrio* isolates: Cultural characteristics of the retrieved *Vibrio* isolates on TCBS medium are shown in Fig. 4. Briefly, retrieved colonies were rounded green colonies with blue center, ranged from pin point to 2 m for *V. vulnificus* and *V. parahaemolyticus*, respectively and rounded yellow colonies of 2-3 mm for *V. Fluvialis* and *V. alginolyticus* (Fig. 4a-d).

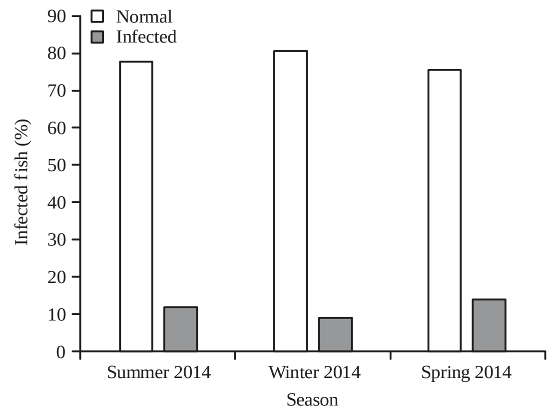

Fig. 2: Percentages of Common Pandora fish infected with *Vibrio* spp at different collection seasons

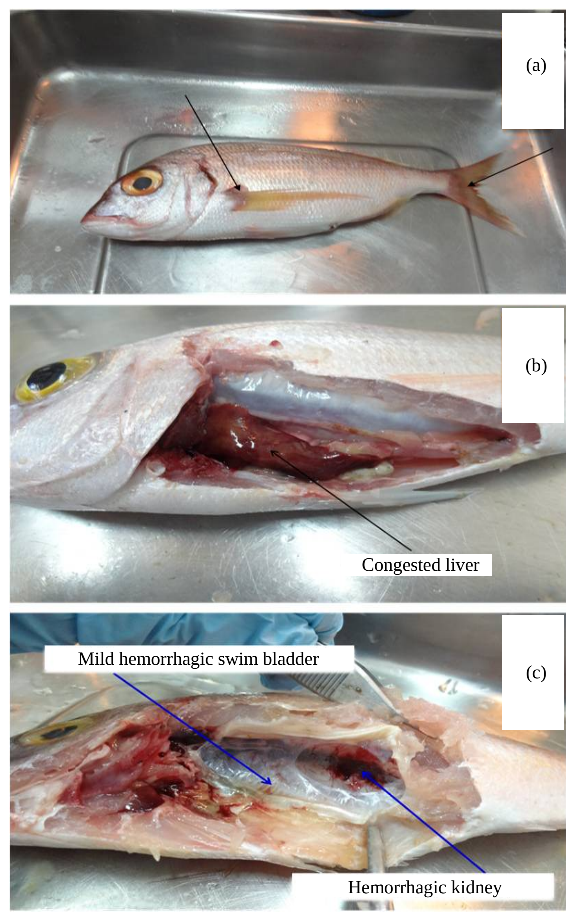

Fig. 3(a-c): Common Pandora fish with (a) Congested fins, (b) Congested liver and (c) Severe hemorrhage in kidney and mild hemorrhage on swim bladder

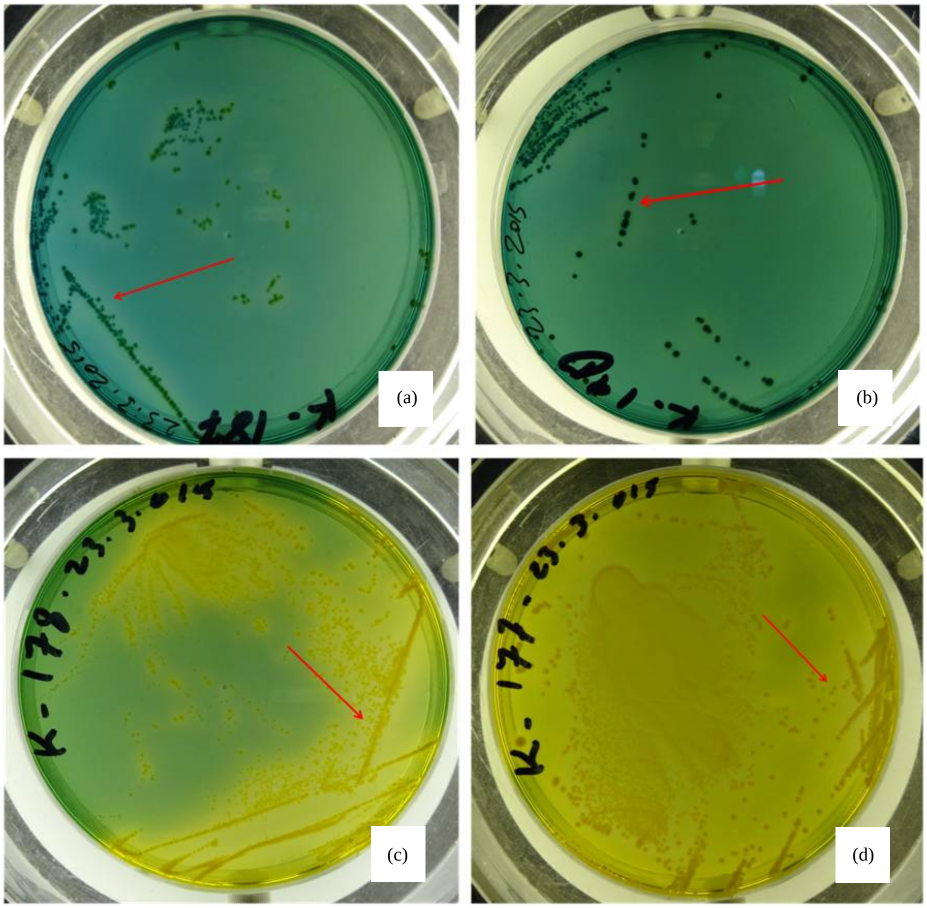

Fig. 4(a-d): Cultural characteristics of the retrieved *Vibrio* isolates on TCBS medium. The retrieved colonies were rounded green colonies with blue center, ranged from pin point to 2 m for (a) *V. vulnificus* and (b) *V. parahaemolyticus*, respectively and (c) Rounded yellow colonies of 2-3 mm for *V. fluvialis* and (d) *V. alginolyticus*

Table 1: Identified bacterial isolates from sampled Common Pandora collected during different seasons 2014/2015

		Number and percentages of retrieved isolates		
Bacterial groups	Retrieved isolates identity	Spring 2015	Winter 2014	Summer 2014
Vibrio isolates	*Vibrio fluvialies*	30 (33.3%)	15 (17%)	25 (27.7%)
	Vibrio alginolyticus	13 (14.4%)	2 (2%)	7 (7.7%)
	Vibrio parahaemolyticus	10 (11.1%)	18 (20%)	22 (24.4%)
	Vibrio vulnificus	4 (4.4%)	8 (9%)	7 (5.5%)
No growth	No growth	18 (20%)	33 (37%)	5 (7.7%)
Other non *Vibrio* isolates	*Photobacterium demselae*	10 (11.1%)	0	0
	Tenacibaculum maritimum	5 (5.5%)	0	20 (22.2%)
	Enterococcus facales	0	0	2 (2.2%)
	Aeromonas hydrophila	0	0	2 (2.2%)
	Pseudomonas florescence	0	14 (15%)	0
	Total	90 (100%)	90 (100%)	90 (100%)

Biochemical characterization 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 4 main isolates, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* and *V. fluviales*. The detailed biochemical characteristic of the retrieved isolates is described in Table 2.

Antibiogram: With the exception of *V. fluviales* that showed sensitivity to all tested antibiotics including ampicillin, it

Table 2: Morpho-chemical characteristics of the retrieved *Vibrio* isolates from sampled Common Pandora fish

Criteria	*Vibrio parahaemolyticus*	*Vibrio vulnificus*	*Vibrio alginolyticus*	*Vibrio fluviales*
API 20 E ID	4366107	5306005	4347324	3046126
Culture character on T.C.B.S	Green colonies 2-3 mm in diameter	Green colonies 2-3 mm in diameter	Yellow colonies 2-3 mm in diameter	Yellow colonies 2-3 mm in diameter
Gram stain character	Gram –ve rods	Gram –ve curved rods	Gram –ve rods	Gram –ve rods
Oxidation/fermentation	+/+	+/+	+/+	+/+
Oxidase	+	+	+	+
Catalase	+	+	+	+
ONPG	-	+	-	+
ADH	-	-	-	+
LDC	+	+	+	-
ODC	+	+	+	-
CIT	+	+	+	-
H_2S	-	-	-	-
URE	-	-	-	-
TDA	+	-	-	-
IND	+	-	+	+
VP	-	-	+	-
GEL	+	+	+	+
GLU	+	+	+	+
MAN	+	-	+	+
INO	+	-	+	-
SOR	-	-	-	-
RHA	-	-	-	-
SAC	-	-	+	+
MEL	-	-	-	-
AMY	+	+	-	-
ARA	+	-	-	+
Nitrate	+	+	+	+

ONPG: O-nitrophenyl-β-D-galactoside, ADH: Arginine dihydrolase, LDC: Lysine decarboxylase, ODC: Ornithine decarboxylase, CIT: Citrate utilization, H_2S production, URE: Urea hydrolysis, TDA: Tryptophan deaminase, IND: Indole production, VP: Voges-Proskauer (acetoin production), GEL: Gelatinase, GLU: Glucose fermentation/oxidation, MAN: Mannitol, INO: Inositol, SOR: Sorbitol. RHA: Rhamnose SAC: Sucrose, MEL Melonine, AMY: Amylase and ARA: Arginase

Table 3: Antibiogram for the retrieved *Vibrio* isolates

	Standard inhibition zones of the antibiotics used in the antibiogram					
Antibiotic	Dox-30	Poly-300	Oxy-30	Flor-30	Su/tr-25	Amp-10
Resistant	<8	<8	<15	<16	<11	<12
Mildly sensitive	8-12	8-12	15-18	16-21	11-15	12-13
Sensitive	>12	>12	>18	>21	>15	>13
Antibiotic sensitivity for the retrieved *Vibrio* isolates						
Sensitivity (*V. alginolyticus*)						
Inhibition zone (mm)	22	18	13	10	15	0
Interpretation	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
Sensitivity (*V. parahaemolyticus*)						
Inhibition zone (mm)	13	14	12	12	9	0
Interpretation	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
Sensitivity (*V. fluviales*)						
Inhibition zone (mm)	30	21	25	30	24	0
Interpretation	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
Sensitivity (*V. vulnificus*)						
Inhibition zone (mm)	15	16	15	10	8	0
Interpretation	Sensitive	Sensitive	Mild sensitive	Resistant	Resistant	Resistant

Dox-30: Doxycyclin, Poly-300: Polymyxin, Oxy-30: Oxytetracycline, Flor-30: Florfenicol, Su/tr-25: Sulfamethaxzole-trimethoprim and Amp-10: Ampicilin

appears that all *Vibrio* spp., are sensitive to doxycyclin and polymyxin and resistant to florfenicol, sulfamethoxzole-trimethoprim and ampicillin (Table 3).

Detection of the parasites during clinical examination of the sampled fish: Clinical examination of the Common Pandora fish samples revealed the presence of systemic helminths

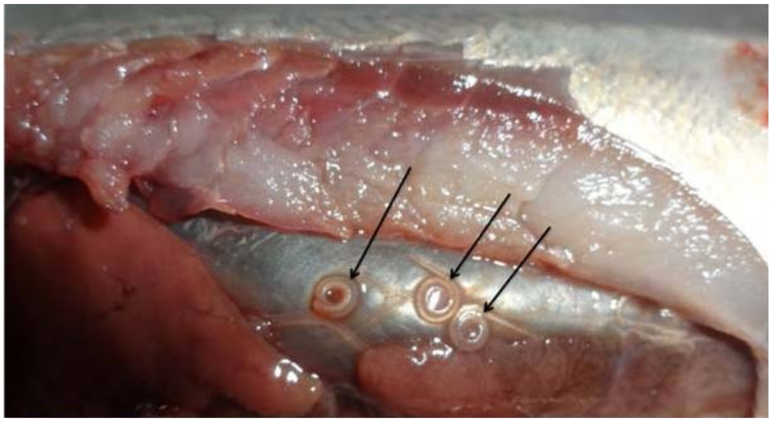

Fig. 5: Nematode worms attached to swim bladder and peritoneal cavity of the sampled Common Pandora

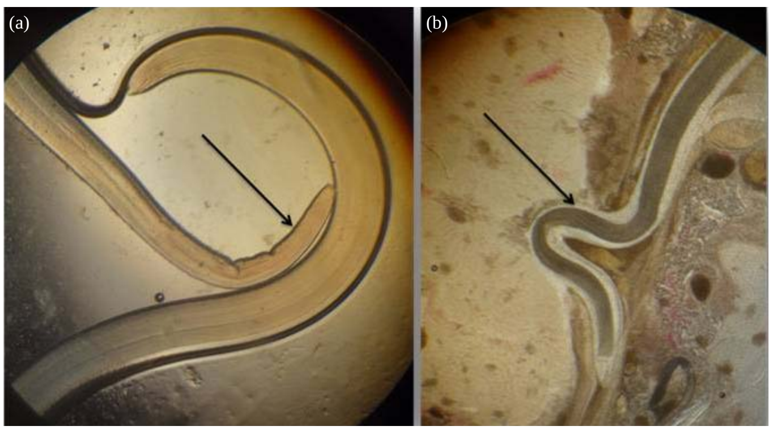

Fig. 6(a-b): (a) Ultra magnification of nematode worms attached to swim bladder and (b) Peritoneal cavity of the sampled Common Pandora

infestation in many cases. Nematodes (e.g., *Anisakis pegreffii*) (Fig. 5, 6a-b) and digenean trematodes were among the worms found encysted or free wondering throughout the fish viscera.

DISCUSSION

Disease is an interaction between three dynamic factors, host-environment-pathogen in a definite period of time[24,25]. Environmental changes, especially habitat degradation by anthropogenic pollutants and oceanographic alterations induced by climate change, can influence pathogen-host interaction. The direct dumping of municipal sewage into the Mediterranean shallow basin is a classical regime that is adopted by many countries like Egypt, Libya, Tunisia and Morocco and many other bordering countries[3]. Such irresponsible anti-environment behavior is representing a continuous evolving source of biological/chemical sorts of pollution with consequent degrading effects to the natural marine environments as well as its biodiversity[25]. Municipal sewage may possibly transfer vulnerable zoonotic biological agents from land to the sea[26]. The zoonotic bacterial pathogens interaction models are vulnerably increasing at the South Mediterranean basin due to the continuous direct dumping of municipal sewage into the sea coasts[27]. Moreover, several bacterial infections of public health concerns such as vibriosis, streptococcosis and mycobacteriosis have been reported in several Mediterranean fishes[28]. Health-wise, the potentially pathogenic vibrios a critically serious pathogens, as they are associated with fatal diseases in humans and aquatic animals[28].

Despite the fact that higher percentages of the examined Common Pandora (86, 88 and 84% in summer 2014, winter 2014 and spring 2015, respectively) are apparently normal, yet the percentages of the retrieved isolates were partially irrelevant to normal or disease condition of the examined fish.

Several systemic helminths infestation have been recorded throughout the course of the study (e.g., Nematodes and digenetic trematodes). Worms were found wondering at the peritoneal wall as well as at some internal organs like spleen, liver, intestine and swim bladder. At the aquatic environment, the concurrent parasitic/bacterial infections are the real scenarios for infections in the natural environments where water bodies are naturally inhabited by a wide spectrum of ubiquitous bacteria and parasites[26,29]. Sun *et al*.[30] explained an endosymbiotic relationship between *F. columnare* and the parasitic ciliate *Ichthyophthirius multifiliis* in which the bacteria adheres to the parasite through association with the cilia. The case is not only restricted to the endosymbiont relationship between the parasite and bacteria but also extended to a synergistic type of relationship in which the parasite enhances the bacterial invasion into the fish skin with consequent disease development[26,29,31].

From public health perspective, Saha *et al*.[32] have indicated that heavy nematode infection in intestinal loops of Indian diarrheal patients has drastically immune suppressed the local intestinal mucosal immunity in such way that the prevalence of the ubiquitous *V. cholera* pathogenic bacteria was too high concurrently with the intestinal nematode infection. In a very similar way, we would assume an aquatic model of nematode/*Vibrio* concurrent infection in the examined Common Pandora. The carnivorous eating behavior of Common Pandora fish allow them to apprehend as much as they can from the marine mussels, crustaceans and small fishes that contain early larval stages of the highly zoonotic *Anisakis* species. Once get lodged into the gastrointestinal tract of the fish, the early *Anisakis* larvae starts a unique stage of development into 3rd larval stage which is capable of invading intestinal wall to peritoneal cavity, blood circulation and then to different internal organs with special preference to hematogenous organs like kidney and spleen. The larval migration of the 3rd larval stages through the intestine would demolish or at least corrupt the local mucosal resistance of intestinal epithelial of this night eating fish. There is a well-established fact that *Vibrios* are among the symbiotic bacteria living inside the intestine of fishes and other aquatic species with a very facultative way[14]. However, the apparent suppression of the intestinal mucosal immunity have synergized together with the continuously changing intestinal environment pH to favor an acute invasion into the intestine then to the blood circulation. After arriving the circulation, typical case of septicemia is on the way through which the bacterial toxins where secreted and pathogen lodged into internal organs like kidney, spleen and liver[33,34].

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[33,35,36.] These factors likely require concerted expression for pathogenesis to take place. Conclusively, *V. vulnificus* and *V. parahaemolyticus* are complex microorganisms with physiological characteristics that contribute to its survival in the marine environment and in the human host[34,37]. The microscopic wounds induced by the *Anisakis* larval migration through intestinal wall 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[32]. 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[38]. This could describe how *Vibrio* species together with *Anisakis* larvae were synergistically working in a very aggressive way to induce a clinical disease in the target fish[28].

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[37,39]. In summer and late spring, 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[25]. 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[29,30,40]. In the current study, around 15% of the Common Pandora collected from Western coast of Tripoli, Libya have presented typical signs of severe *Vibrio* infection concurrently with a heavy infestation of 3rd larval stage *Anisakis* nematode and digenetic trematodes. We inferentially hypothesized that the damaging effect of *Anisakis* larvae on 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.

The results of the current study indicated that the ubiquitous *V. fluviales*, which is commonly existing in seawater, shellfishes and seabed[14,41] represented the larger sector of the isolated *Vibrios* through the entire study (26% from the total number of *Vibrios* through the three seasons of the study). This results is relatively consistent with similar results obtained by Ashraf and Zafar[41], who related the higher incidence of *V. fluviales* to high salinity above 30 and high sewage pollution. Such deteriorated environmental condition is similar to that reported in this study.

The *V. parahaemolyticus*, is a pathogenic *Vibrio* that is present in plenty loads in the common reservoirs such as shellfishes, mussels and mollusks[14,28]. In the present study, the average percentage of the retrieved isolates of *V. parahaemolyticus* is 14% which is 2nd on list of the isolated *Vibrios* through the entire study. *Vibrio parahaemolyticus* is associated with sewage pollution and it is one of the main food poisoning pathogens linked to raw or inefficiently cooked seafood feeding[14]. The presence of *V. parahaemolyticus* in apparently normal Common Pandora might indicate a chronic carrier case where signs of the disease absent. In fish with disease signs, the pathogen could have reached to a higher loads that was capable of inducing a disease pattern triggered by the concurrent mucosal damage made by nematode and/or digentic trematode infestation[28].

The *V. alginolyticus*, is a ubiquitous nature *Vibrio* that is present in moderate loads in the common reservoirs such as shellfishes and sea water[14]. *Vibrio alginolyticus* causes many epizootic outbreaks among the Gilthead seabream and European seabass populations, which possess high economic value at the Mediterranean communities[12,15,17,18,42]. They are also well known to be a good source of probiotics that is used as immune stimulant in the teleost and shellfish mariculture[14,43]. In the present study, the average percentage of the retrieved isolates of *V. alginolyticus* is 8% which is 3rd on list of the isolated *Vibrios* through the entire study.

The *V. vulnificus*, is a highly pathogenic *Vibrio* that is present in moderate loads in the common reservoirs such as mussels, shellfishes and mollusks[14,28]. *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[28]. They are also well known to be an eminent cause of cellulites and septicemia in fishermen, swimmers and people handling infected fishes[14]. In the present study, the average percentage of the retrieved isolates of *V. vulnificus* is 6% which is 4th on list of the isolated *Vibrios* through the entire study. Eissa *et al.*[28] have confirmed the vulnerable role of *Anisakis* species as a tissue wondering nematode in infection/spread of V. vulnificus to the benthic carnivore black sorpion fish from the Western coast of Tripoli/Libya. These results runs parallel with that of Eissa *et al.*[28], where *Anisakis* species has been detected in *V. vulnificus* infected Common Pandora fish from the same geographic location too.

Some non-*Vibrio* bacterial pathogens have also been isolated concomitantly with *Vibrios* from the same examined Common Pandora during different seasons. *Photobacterium damsalea* was concomitantly isolated from the same fish at a percentage of 11% only during the summer season. The fact that *Photobacterium damselae* was isolated only though he summer season is consistent with similar results obtained by Abdel-Aziz *et al.*[42] and Ashraf and Zafar[41]. Such pathogen is associated with higher salinity above 30 ppm and temperatures above 26°C, which is the typical case here. *Pseudomonas florescence* was common concomitant isolate during the winter season which could be explained by the obligate aerobe nature of such pathogen[14,25]. 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[14,25]. The long Gram-negative marine *flavobacteria*, *Tenacibaculum maritimum* (formerly known as *Flavobacterium maritimus*) was concomitantly isolated during the summer (5.5%) and late spring seasons (22%) which is also expected during higher temperature surge in summer or sharp temperature fluctuation in late spring. High temperatures, high ammonia and deteriorated environmental parameters induced by sewage is a triggering factor in the pathogenicity and invasion of such pathogen to the liable fishes. Results obtained by us is consistent with that obtained by Ashraf and Zafar[41] for high salinity water and Eissa *et al.*[29], for freshwater. Also, the facultative aerobic *Enterococus fecalis* and *Aeromonas hydrophila* have been isolated during the late spring. Such pathogens are mainly derived from the sewage dumping into the sea.

The isolation of the 4 above mentioned *Vibrios, Enterococcus*, *Aeomonas* and *Pseudomonas* species as well as the zoonotic nematode *Anisakis* is highly indicative for the deteriorated water quality of the Western coast of Tripoli which is a continuously evolving dynamic problem triggered by the random dumping of untreated municipal sewage into the seawater 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 20E system and all results were consistent with their morpho-chemical standard criteria described by Austin and Austin[14], Buller[43] and Whitman[22].

The antibiogram of all retrieved *Vibrio* isolates revealed higher sensitivities to two major *Vibriocidal* antibiotics namely, doxycyclin and polymyxin. Most of isolates with the exception of *V. fluviales* were resistant to the traditionally used antibiotics such as oxytetracycline, florfenicol and ampicillin. Controversially, *V. fluviales* were sensitive to all used antibiotics with the exception of ampicillin. The presence of relative resistance to the traditionally human used antibiotics such as ampicillin, florfenicol and oxytetracycline is a bad indicator of the developing antibiotic resistance genes in fishes inhabiting the polluted basin of the Western coast of Tripoli. These results is a critical alarm for the public health authorities to the ongoing antibiotic resistance resulting from the continuous dumping of municipal sewage into seawater. Finally, successful isolation of 4 species of *Vibrios*, zoonotic other non *Vibrio* species and zoonotic nematodes together with the developing antibiotic resistance is an obvious indicator of sewage pollution at the Western coast of Tripoli extending from Tajoura to Janzour.

CONCLUSION

From environmental point of view, the mixed infections with zoonotic pathogens such as *Vibrio* spp, other non-*Vibrio* bacteria and zoonotic helminths (*Anisakis*) are highly indicative of a consistently uprising municipal sewage pollution across the Western coast of Tripoli, Libya. Further, the occurrence of certain degrees of antibiotic resistances was also supporting the assumption that the main source of antibiotics in the open seawater was the dumping of municipal sewage. Ultimately, screening of the antibiotic resistance genes is highly required in the future.

SIGNIFICANCE STATEMENTS

This study was successful in identifying a group of specific fish/public health important pathogens such as *Vibrio vulnificus*/*Vibrio parahaemolyticus*, which are two of the most frequent causes of human diarrhea reported by WHO. The assessment of the infection intensity of these pathogens in fish tissues has given us an indirect assumption about the critical levels of municipal sewage pollution at the Libyan coasts of the Mediterranean which is a novel assumption that were not inferred in any previous study at this geographic region before. Finally, declaring that the TCBS bacteriological medium is not a selective medium for the isolation of *Vibrio* species as originally known, for is a novel scientific hypothesis that has been clearly postulated throughout the current study.

REFERENCES

1. Bianchi, C.N. and C. Morri, 2000. Marine biodiversity of the Mediterranean sea: Situation, problems and prospects for future research. Mar. Pollut. Bull., 40: 367-376.
2. Stefanescu, C., B. Nin-Morales and E. Massuti, 1994. Fish assemblages on the slope in the Catalan sea (Western Mediterranean): Influence of a submarine canyon. J. Mar. Biol. Assoc. UK., 74: 499-512.
3. Abdallah, M.A.M. and A.M.A. Abdallah, 2007. Biomonitoring study of heavy metals in biota and sediments in the South Eastern coast of Mediterranean sea, Egypt. Environ. Monit. Assess., 146: 139-145.
4. FAO., 2005. Review of the state of world marine fishery resources. FAO. Fisheries Technical Paper No. 457, Marine Resources Service, Fishery Resources Division, FAO. Fisheries Department, Food and Agriculture Organization, Rome, Italy, pp: 1-235.
5. Balebona, M.C., M.J. Andreu, M.A. Bordas, I. Zorrilla, M.A. Morinigo and J.J. Borrego, 1998. Pathogenicity of *Vibrio alginolyticusfor* cultured gilt-head sea bream (*Sparus aurata* L.). Applied Environ. Microbiol., 64: 4269-4275.
6. Pant, A. and A.K. Mittal, 2007. Monitoring of pathogenicity of effluents from the UASB based sewage treatment plant. Environ. Monitor. Assess., 133: 43-51.
7. Igbinosa, E.O., L.C. Obi and A.I. Okoh, 2009. Occurrence of potentially pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape province of South Africa. Res. Microbiol., 160: 531-537.
8. Guidetti, P., G. Fanelli, S. Fraschetti, A. Terlizzi and F. Boero, 2002. Coastal fish indicate human-induced changes in the Mediterranean littoral. Mar. Environ. Res., 53: 77-94.
9. Lafferty, K.D. and R.D. Holt, 2003. How should environmental stress affect the population dynamics of disease? Ecol. Lett., 6: 654-664.
10. Austin, B. and D.A. Austin, 2016. *Vibrios*. In: Bacterial Fish Pathogens, Austin, B. and D.A. Austin (Eds.)., Springer International Publishing, Germany, pp: 499-601.
11. El-Sahn, M.A., B.A. El-Banna and A.M. El-Tabey Shehata, 1982. Occurrence of *Vibrio parahaemolyticus* in selected marine invertebrates, sediments and seawater around Alexandria, Egypt. Can. J. Microbiol., 28: 1261-1264.
12. Moustafa, M., A.E. Eissa, A.M. Laila, A.Y. Gaafar, I.M.K. Abumourad and M.Y. Elgendy, 2015. Investigations into the potential causes of mass kills in mari-cultured Gilthead sea bream, (*Sparus aurata*) at Northern Egypt. Res. J. Pharm. Biol. Chem. Sci., 6: 466-477.
13. Abdelaziz, M., M.D. Ibrahem, M.A. Ibrahim, N.M. Abu-Elala and D.A. Abdel-Moneam, 2017. Monitoring of different *Vibrio* species affecting marine fishes in Lake Qarun and Gulf of Suez: Phenotypic and molecular characterization. Egypt. J. Aquatic Res., 43: 141-146.

14. Austin, B. and D.A. Austin, 2012. Vibrionaceae Representatives. In: Bacterial Fish Pathogens, Austin, B. and D.A. Austin (Eds.)., Springer, Netherlands, pp: 357-411.
15. Moustafa, M., A.E. Eissa, A.M. Laila, A.Y. Gaafar, I.M.K. Abumourad and M.Y. Elgendy, 2014. Mass mortalities in mari-cultured European sea bass (*Dicentrarchus labrax*) at Northern Egypt. Res. J. Pharm. Biol. Chem. Sci., 5: 95-109.
16. Avendano-Herrera, R., A.E. Toranzo and B. Magarinos, 2006. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: A review. Dis. Aquatic Org., 71: 255-266.
17. Bakhrouf, A., J.M. and H.E. Ben Ouada, 1995. Detraitement des *Vibrioses* du loup Dicentrarchus labrax dansune zone de Pisciculture, a Monastir, Tunisie. Mar. Life, 5: 47-54.
18. Zorrilla, I., M.A. Morinigo, D. Castro, M.C. Balebona and J.J. Borrego, 2003. Intraspecific characterization of *Vibrio alginolyticus* isolates recovered from cultured fish in Spain. J. Applied Microbiol., 95: 1106-1116.
19. Kaysner, C. and A.J. Depaola, 2004. Food and drugs administration: Bacteriological analytical manual, methods for specific pathogens. *Vibrio* spp. U.S. Department of Health and Human Services, USA.
20. Stoskopf, M.K., 1992. Clinical Pathology, in Fish Medicine. Penn. W.B. Saunders, Philadelphia, pp: 113-131.
21. Eissa, A.E., 2016. Clinical Manual of Fish Diseases. LAP Lambert Academic Publishing, Germany, Pages: 140.
22. Whitman, K.A., 2004. Finfish and Shellfish Bacteriology Manual: Techniques and Procedures. Iowa State Press, Iowa, USA.
23. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
24. Hedrick, R.P., 1998. Relationships of the host, pathogen and environment: Implications for diseases of cultured and wild fish populations. J. Aquatic Anim. Health, 10: 107-111.
25. Eissa, A.E., N.A. Tharwat and M.M. Zaki, 2013. Field assessment of the mid winter mass kills of trophic fishes at Mariotteya stream, Egypt: Chemical and biological pollution synergistic model. Chemosphere, 90: 1061-1068.
26. Perkins, S.E. and A. Fenton, 2006. Helminths as vectors of pathogens in vertebrate hosts: A theoretical approach. Int. J. Parasitol., 36: 887-894.
27. Fernandez-Jover, D., E. Faliex, P. Sanchez-Jerez, P. Sasal and J.T. Bayle-Sempere, 2010. Coastal fish farming does not affect the total parasite communities of wild fish in SW Mediterranean. Aquaculture, 300: 10-16.
28. Eissa, A.E., M. Abdelsalam, A. Abumhara, A. Kammon and F.T. Gammoudi *et al*., 2015. First record of Vibrio vulnificus/Anisakis pegreffi concurrent infection in black scorpionfish (*Scorpaena porcus*) from the South Mediterranean basin. Res. J. Pharm. Biol. Chem. Sci., 6: 1537-1548.
29. Eissa, A.E., M.M. Zaki and A.A. Aziz, 2010. *Flavobacterium columnare Myxobolus tilapiae* concurrent infection in the earthen pond reared Nile tilapia (*Oreochromis niloticus*) during the early summer. Interdiscip. Bio Central, Vol. 2. 10.4051/ibc.2010.2.2.0005.
30. Sun, H.Y., J. Noe, J. Barber, R.S. Coyne and D. Cassidy-Hanley *et al*., 2009. Endosymbiotic bacteria in the parasitic ciliate *Ichthyophthirius multifiliis*. Applied Environ. Microbiol., 75: 7445-7452.
31. Plumb, J.A., 1997. Infectious Diseases of Tilapia. In: Tilapia Aquaculture in the Americas, Costa-Pierce, B.A. and J.E. Rakocy (Eds.). World Aquaculture Society, Baton Rouge, LA., USA., pp: 212-228.
32. Saha, M.R., M.A. Alam, R. Akter and R. Jahangir, 2008. *In vitro* free radical scavenging activity of *Ixora coccinea* L. Bangladesh J. Pharmacol., 3: 90-96.
33. Linkous, D.A. and J.D. Oliver, 1999. Pathogenesis of *Vibrio vulnificus*. FEMS Microbiol. Lett., 174: 207-214.
34. Jones, M.K. and J.D. Oliver, 2009. *Vibrio vulnificus*: Disease and pathogenesis. Infect. Immun., 77: 1723-1733.
35. Thompson, F.L., T. Iida and J. Swings, 2004. Biodiversity of *vibrios*. Microbiol. Mol. Biol. Rev., 68: 403-431.
36. Strom, M.S. and R.N. Paranjpye, 2000. Epidemiology and pathogenesis of *Vibrio vulnificus*. Microbes Infect., 2: 177-188.
37. Oliver, J.D., 2005. *Vibrio Vulnificus*. In: Oceans and Health: Pathogens in the Marine Environment, Belkin, S. and R.R. Colwell (Eds.)., Springer, New York, pp: 253-276.
38. Wright, A.C., L.M. Simpson and J.D. Oliver, 1981. Role of iron in the pathogenesis of *Vibrio vulnificus* infections. Infect. Immun., 34: 503-507.
39. DePaola, A., G.M. Capers and D. Alexander, 1994. Densities of *Vibrio vulnificus* in the intestines of fish from the US Gulf Coast. Applied Environ. Microbiol., 60: 984-988.
40. Bricknell, I. and R. Raynard, 2002. Viral disease risks to and from emerging marine aquaculture species. Proceedings of the 10th Annual New England Farmed Fish Health Management Workshop, (FFHM'02), Eastport, Maine, USA.
41. Ashraf, M. and A. Zafar, 2013. A survey of parasites of wild Rohu in Trimmu headworks, Pakistan. World Aquacult., 5: 50-52.
42. Abdel-Aziz, M., A.E. Eissa, M. Hanna and M.A. Okada, 2013. Identifying some pathogenic Vibrio/Photobacterium species during mass mortalities of cultured Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) from some Egyptian coastal provinces. Int. J. Vet. Sci. Med., 1: 87-95.
43. Buller, N.B., 2004. Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual. CABI Publishing, Wallingford, UK., ISBN-13: 9780851999548, Pages: 361.