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Filed Studies on The most Prevailing Bacterial Diseases Affecting Some Cultured Marine Fishes Egypt

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THE objective of this study was recorded the most prevailing bacterial agents which spreads in the cultured fish in Ismailia province, Eastern Egypt. This study was carried out on 1080 premature fish. 360 *Dicentrarchus labrax* (225±25 g) and 360 *Sparus aurata* (150±25 g), and 360 *Mugil cephalus* (125±25). The examined fishes showed exophthalmia, hemorrhagic area on the skin, around the buccal cavity, head, and on the base of the fines, ulcers varied in their degrees, dark colour, and respiratory signs. The recorded postmortem lesions were septicemic signs with enlargement and congestion of the liver and spleen with distended gall bladder. With the presence of serous to hemorrhagic ascetic fluid, the stomach and intestines displayed congestion, thickening, and inflammation of their walls. The total prevalence of bacterial infection in examined premature fishes was 27.68%. The highest percentage was in *D. labrax* at 38.88%, followed by *S. aurata* at 27.22%, and the lowest percentage in *M. cephalus* at 16.94%. The most prevailing bacterial agents were Vibrio spp and *Pseudomonas* spp.

Keywords: *Dicentrarchus labrax, Sparus aurata, Mugil cephalus*, Bacterial agent, Ismailia province.

Introduction

Aquaculture represents an important sector in the Egyptian economy providing essential food facing the growing demand for animal protein [1]. Global shortage in protein production of animal origin had made most governorates to modern trends to increase the production rate of it. Fish farming whether fresh or marine fishes considered one of the important solutions to meet this problem [2]. In case of farm fishes, the chance of emergency of many diseases, especially bacterial one increase [3]. The main cause of high morbidity and mortality rates in marine fish is a bacterial agent, which affects a variety of marine fish diseases [4]. Fish infections are more severe in farmed fish than in wild fish, which has an adverse effect on the aquatic environment. This environment is a good scenario for many pathogens, and human activity causes these pathogens to become more virulent [5]. According to estimates, bacterial organisms are responsible for more than 20% of these losses (6). Since the beginning of marine fish culture, bacterial diseases have been one of the most significant causes of economic loss. It is important to note that diseases are traditionally thought of as typical in mariculture [7]. Bacterial diseases cause high mortality in both wild and crowded fish. The majority of bacterial pathogens have a narrow host range, infecting and causing disease in just one genus or even one species of fish. Some pathogens are obligatory, whereas others are facultative pathogens that are common aquatic

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dwellers and need stress to help spread disease throughout fish populations [8]. These bacteria are mainly opportunist pathogens that attack the tissues of the fish host, which are made vulnerable to infection by stressors or other disease processes. The vibrio genus is the most important group of these microorganisms in this regard [9]. Recent years have seen an upsurge in the frequency of outbreaks linked to various emergent fish pathogenic bacterial species [10]. The evaluation of the most prevalent bacterial agent found in cultured fish in the province of Ismailia was the goal of this work.

Material and Methods

Fish samples

From assimilatory ponds, 1080 premature fish were collected for analysis, including 360 *D. labrax* (225± 25 g), 360 *S. aurata* (150 ±25 g), and 360 *M. cephalus* (125± 25).With a focus on the feeding rate, swimming condition, and lesions of septicemia that occur on the infected fish. The fish were thoroughly evaluated for the clinical indications of illnesses. Additionally, any post-mortem injuries were reported. Bacteriological Examination according to Tille et al. [11]. The samples were promptly moved under aseptic conditions in a chilled, insulated box to the Hydrobiology Department, National Veterinary Institute, National Research Centre, Giza, Egypt for bacteriological analysis, the clinical examinations, and the postmortem investigations [12].

Bacterial isolation

The infected fish's spleen, liver, kidney, brain, and skin lesions were used to isolate bacteria, which was then inoculated into nutrient broth, MacConkey broth (Tryptic Soya Agar), and Tryptic soya broth with NaCl 2% at 30 ^{0}C for 24 to 48 hours. Finally, the bacteria were cultured into specific media and incubated at 30 ^{0}C for Inoculum was streaked on general and selective the purified colonies were picked up and streaked over a specific medium for additional purification. The purified colonies were then streaked on to Rimler-Shotts medium (R.S. medium), Aeromonas selective agar base with Ampicillin supplement, Pseudomonas selective agar base, Manitol salt agar, S.S. agar, and T.C.B.S. agar, Ordals media, and In order to maintain some pure strains, they were placed in tryptic soy broth (T.S.B.; Bioxon) containing 15% (v/v) glycerol and stored at -80°C [13].

Identification of the isolated bacteria

The isolates were identified in accordance with Elemar et al. [14]. Gram stain was used to generate smears of suspected bacterial colonies from cultivated samples, which were then viewed under a microscope. Suspected purified isolates were then utilized for phenotypic characterization of bacterial isolates (Biochemical identification) by the Vitek2 Compact System (BIOMERIUX, France). Furthermore, the multiplex PCR technique was used to make a bacteriological diagnosis. According to Austin et al. [13]**,** polymerase chain *Pseudomonas* species according to Pirnay et al. [15]

TABLE 1. The oligonucleotide sequence for 16 R S genes of *Pseudomonas* species.

Product name	Sequence (5' to 3')	Product size (bp)
16SF 16SR	5′ AGAGTTTGATCCTGGCTCAG-3′ 5′ CTACGGCTACCTTGTTACGA-3	958

Pseudomonas aeruginosa

TABLE 2. The oligonucleotide sequence for to xR gene of *P. aeruginosa*.

Product name	Sequence (5' to 3')	Product size (bp)
PA-SS-F: PA-SS-R:	5' GGGGGATCTTCGGACCTCA 3' 5' TCCTTAGAGTGCCCACCCG 3'	958

Vibrio species according Cai *et al*. [16].

V. anguillarium

TABLE 3. The oligonucleotide sequence for to xR gene of *Vibrio anguillarum.*

Product name	Sequence (5' to 3')	Product size (bp)
Van-ami8 Van-ami417	5'- ACATCATCCATTTGTTAC-3' 5'- CCTTATCACTATCCAAATTG -3'	429

V. harveyi

TABLE 4. Oligonucleotide primers of the gene of *V. harveyi*

Primer	Target gene	Sequence (5' to 3')	Product size (bp)
M-454	tdh	5'- CGT TGA TTA TTC TTT TAC GA -3'	623
M-441	tdh	5'- TTT GTT GGA TAT ACA CAT -3'	623

Results and Discussion

Regarding the clinical picture, it was discovered that fish with *Pseudomonas* and Vibrio bacteria naturally infected had dark body colouring. On several areas of the body surface, particularly at the ventral section of the abdomen, in the mouth region, along the groove beneath the jaw, around the anus, and at the base of the fins, large irregular haemorrhages were observed. In some fish, exophthalmia with corneal opacity has been observed. It may be attributed to septicaemic reactions [7, 17].

Internal organs

Internal examination of the spleen and liver, kidney and Spleen showed congestions and enlarged suffering from ascetic fluid in the abdominal cavity. The liver was pale and distended gall bladder. Intestines were seen filled with gases, sometimes haemorrhagic. The stomach and intestine showed congestion, enlargement, thickening, and inflammation of their walls in some examined *D. labrax, S. aurata*, and *M. cephalus* (Plates, 4 and 5) [7, 18].

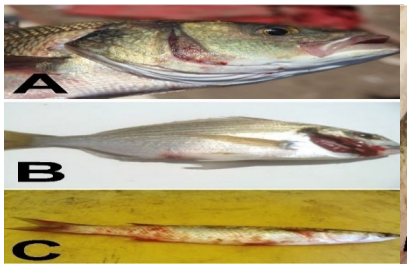

Plate 1. Showing (A) Haemorrhages on the skin of *D. labrax*, *(B) S. aurata* and *(C)) M.cephalus.*

Plate 2. (A) Haemorrhages all-over the body surface and at the head of *M.cephalus*. (B) Showing deep ulcer on the skin of M. *cephalus*.

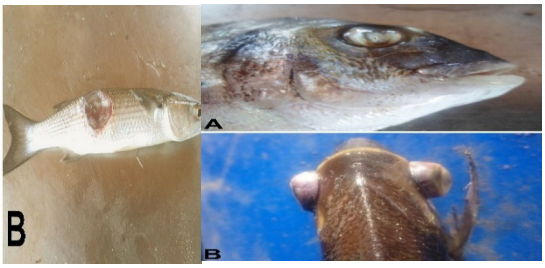

Plate 3. (A) Showing unilateral corneal opacity of *S. aurata*. (B) Bilateral exophthalmia of *S. aurata*.

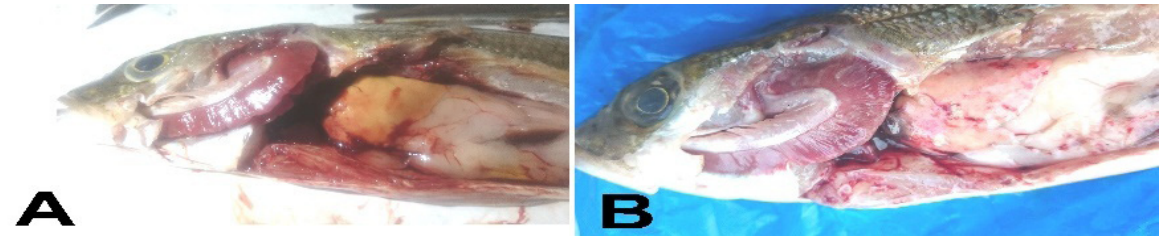

Plate 4. Showing (A&B) D. labrax fish suffered from the palness of the liver.

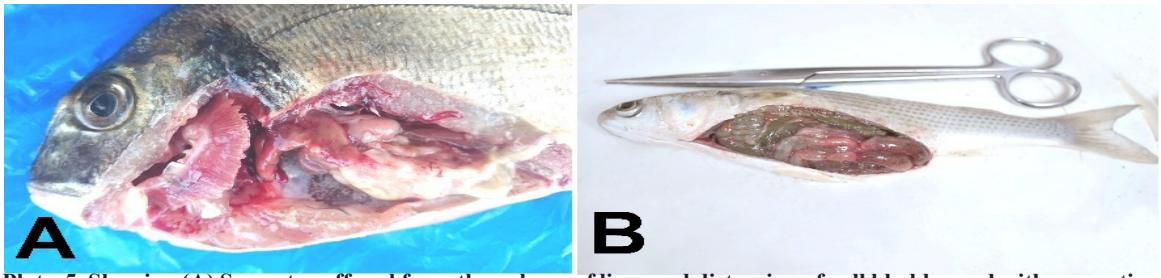

Plate 5. Showing (A) S aurata suffered from the palness of liver and distension of gall bladder and with congestion of gills (B) *M. cephalus* fish congestion of all internal organs.

TABLE 5. Phenotypic and biochemical identification.

Phenotypic and biochemical characteristics of *Pseudomonas* species				
Test Conducted	**Pseudomonas species**			
	P. fluorescens	**P. aeruginosa**	**P. anguilliseptica**	**P.putida**
Colour of colony	Yellowish green	Yellow	Yellow	Yellow
Gram staining	-ve	-ve	-ve	-ve
Shape	R	R	R	R
Fluorescent	+	-	-	-
Motility	+	+	+	+
O/F	O	O	O	O
Catalase test	+	-	+	+
Oxidase	+	+	+	-
Indole test	-	-	+	-
Oxidase reaction	+	+	+	+
Nitrite Reduction	+	+	+	+
Ornithine decarboxylase	+	+	-	-
Arginine dihydrolase	+	+	-	-
β-galactose	+	+	+	+
Urease production	+	+	+	-
H2S production	+	+	+	+
Production of acid from				
Glucose	+	+	+	+
Fructose	+	+	+	+
Dextrose	+	+	-	-
Galactose	+	-	+	+
Sucrose	+	-	+	-
Xylose	+	+	+	+

-: Negative; +: Positive O: Oxidative, V=Variable result

Phenotypic The result of bacterial isolates, (and biochemical characteristics of *V. harveyi and V. alginolyticus*.		
Biochemical tests	*V. harveyi*	*V. alginolyticus*
Gram stain	-ve	-ve
Growth on T.C.B.S.	Yellow colonies	Yellow colonies
Swarming on solid media	ve-	+ve
Motility	+ve	+ve
Oxidase	+ve	+ve
Catalase	+ve	+ve
H2S production	-ve	+ve
Urease	+ve	-ve
Citrate	+ve	-ve
Indole production	+ve	+ve
VP	-ve	+ve
Methyl red	+ve	+ve
Glucose	+ve	+ve
Sucrose	+ve	+ve
Mannitol	+ve	+ve
Lactose	-ve	-ve

Our research on the morphological characteristics of *Pseudomonas spp* showed that they were motile, short bacilli, gram negative, and grew on R.S medium and *Pseudomonas* specific agar at 25°C for 24-48 hours. The bacterium creates colonies that are unique to *Pseudomonas*, which are circular, smooth, moist, convex, about 1-2 mm in diameter, glistening, and spreading as incubation time increases. In the media around it, it produced distinctive diffusible yellow-green fluorescent pigments. Colonies grow characteristically dark green on R.S. medium. Isolates on blood agar were hemolytic, and at 4°C but not at 40°c, grew. These results are in concordance with those obtained by Ma et al.[19].

Regarding the biochemical characteristics and identification of *Pseudomonas* species. The results of this investigation showed that *Pseudomonas* species isolates were resistant to the sensitivity tests for O/129 and Novobiocin. Oxidase, Catalase, ADH, and V-P activity were all positive in the isolates, and they were also motile and oxidative in O/F. On the other hand, isolates were negative in respect to OPNG, LDC, ODC, TDA, and Citrate, H2S production, Urease, Indole and Gelatinase production. Some isolates utilized arabinose and glucose oxidative. These results are in concordance with those obtained by El-Moghazy [20].

The current findings about the morphological characteristics of *Vibrio spp*. revealed that they were motile, straight to slightly curved, gram-negative rods that grew on marine agar and T.C.B.S selective agar and were incubated at 25°C for 24 to 48 hours. On marine agar, suspect colonies were spherical, elevated, smooth, convex, white to creamy, and shiny. colonies are generated as with T.C.B.S selective medium yellow on [21]. Regarding the identification and biochemical characters of Vibrio spp proved to be motile, sensitive to O/129 vibrio static disc and Novobiocin. It was proved to be were positive in respect to ONPG, ADH, Aesculin, Indole, V-P, Catalase, Gelatin, and gave variable results for citrate test. While it was negative for LDC, ODC, TDA, H2S production, Methyl-red and Urease test [22, 23] who observed that yellow and green pigmented colonies on TCBS media, and as creamy colored colonies on TSA media with 2.5% NaCl concentration.

The result obtained identification of *P. aeruginosa* (P. aeruginosa) bacterial isolates by PCR As shown in (Fig. 1-4).

These findings were also reported by Spilker et al. [24], who noted that *Pseudomonas*-specific primers allowed for the successful amplification of a 16S rDNA fragment during PCR. While *Vibrio spp*. was shown to be present in various marine creatures, including *silver sea bream* and *sea mullet prawn* [25]. The research demonstrated that one strain of many virulence factors might be acquired at a time, leading to severe clinical sickness in the marine species, based on genetic analyses of various proteins in *Vibrio spp* [16]. While Eissa et al.[4] reported that the majority of isolates of vibrio from lake Tmsah were molecularly identified using the pvsA gene primers, producing a product size of 338-bp size and 348-bp for *V. alginolyticus* and *V. parahemolyticus*, respectively. These findings may be explained by the type, habitat, and timing of the fish used in the marine examination. According to Alicia et al.[7] and , Samuelsen et al.[26], several fingerlings and juveniles of some marine fish species have been found to be infected with these bacteria (*Vibriosis, Pseudomonas*). Our research on the bacterial pathogens that had been isolated showed that the most often isolated bacterial pathogens were Gram-negative *Vibrio spp* and Gram-positive *Pseudomonas spp.* These findings confirmed those made by Zorrila et al.[22], who identified *Vibrio spp., Pseudomonas spp., and P. piscicida* as the predominant pathogenic bacteria isolated from sick gilthead sea bream in south-western Spain. The total prevalence of bacterial infections in the naturally infected marine fish of the present investigation was recorded (23.24 %). These results are higher than those recorded by. This may be due to the difference in the localities of isolation and different stressors (Toxicants and climatic factors), as the perturbations in the environment may increase the potential for disease occurrence in fish populations [4].

Regarding the prevalence of bacterial infection infections such as *Vibriosis, Pseudomonas* septicemia, they have been observed in several fingerlings and premature of some marine fish species. These results may be attributed to microorganisms cause serious infections once unfavorable aquatic environmental conditions [7, 26].

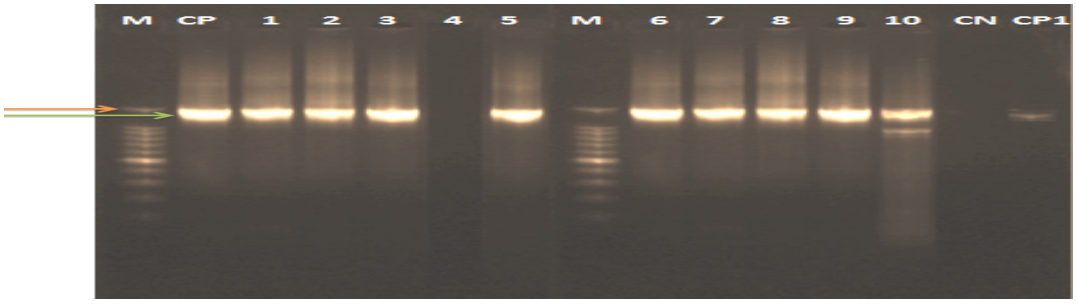

Fig 1. Profile of the amplification product of the 16S rRNA gene of Pseudomonas genus. CP: Positive control, *P. aeruginosa;* CN: Negative control;, M: Marker (100 bp).

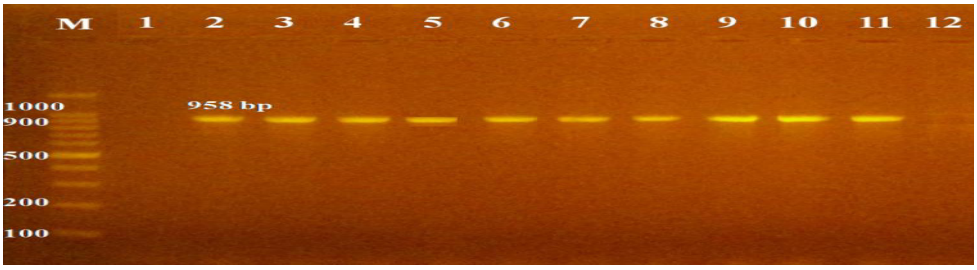

Fig. 2. Agarose gel electrophoresis of PCR amplification of (958 bp) for characterization of *P. aeruginosa* Lane M: 100 bp ladder as molecular size D.N.A. marker. Lane 1 control negative for *P. aeruginosa*. Lane 2 control positive for *P. aeruginosa* Lane 3-11 positive for *P. aeruginosa*, Lane 12 negative for *P. aeruginosa*

Vibrio species

1- *V. anguillarum*

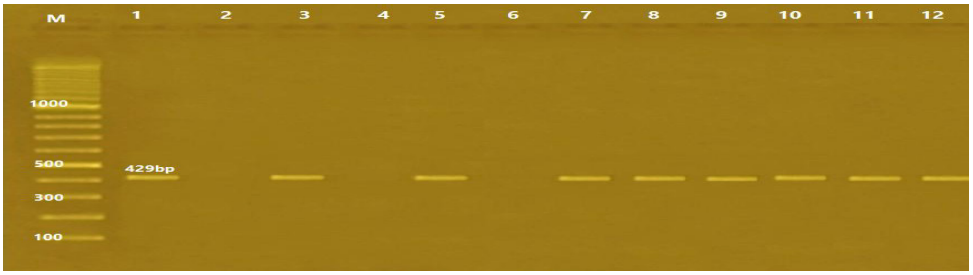

Fig. 3. Agarose gel electrophoresis of PCR amplification of (429 bp) for characterization of *V. anguillarum*. Lane M: 100 bp ladder as molecular size D.N.A. marker. Lane 1 control positive for *V*. anguillarum. Lane 2 control negative for *V. anguillarum*. Lane3,5,7,8-12 positive for *V. anguillarum*. Lane 4,6 negative for *V. anguillarum*

2- *V. harveyi*

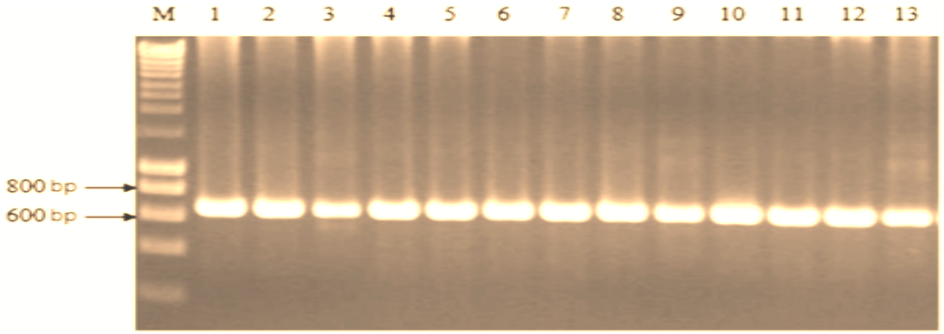

Fig. 4. Detection of partial hemolysin gene from *V. harveyi* strains. Lane M: 100bp D.N.A. Ladder. Lane: 1 to 13.

Our research into the isolated bacterial pathogens showed that Gram-negative bacteria outnumbered Gram-positive bacteria, with *Vibrio* and *Pseudomonas spp*. being the most frequently isolated bacterial pathogens. These findings corroborated those made by Evans [27], who claimed that *Vibrio and Pseudomonas spp*. were the most common pathogenic bacteria found in sick gilthead sea bream in south-western Spain. Also, *M. cephalus* is more infected by pathogenic bacteria [28]. These results may be due to the widespread haemocytic infiltrations in muscle indicate their potential involvement in defense mechanisms against bacterial infections through recognition, phagocytosis and cytotoxic activities (29).

The overall prevalence of bacterial infections was found to be 23.24 percent in the naturally infected marine fish used in the current study. These outcomes surpass those noted by Elgendy et al.[30]. This may be because of the varying isolation locations and stressors (such as toxins and climatic conditions), as changes in the environment may enhance the likelihood that diseases would spread among fish populations. The total prevalence of bacterial in *D. labrax* at 38.88%, followed *by S. aurata* 27.22%, and the lowest percentage in *M. cephalus* at 16.94% (Table, 6).

These outcomes may be explained by the fact that the pathogen's successful transmission is a crucial step in the progression of infectious disease outbreaks that target cultured fish and are accompanied by concurrent bacterial infections and parasitic fish infestations [30].

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Conflict of Interest

The authors declare that no conflict of interest.

TABLE 6. Showing total bacterial infestation in premature examined fish.

Fish species	No of the non-treated examined fish	No infected fish	%
D labrax	360	140	38.88
S aurata	360	98	27.22
M cephalus	360	61	16.94
Total	1080	299	27.68

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دراسات حقليه عن أهم الامراض البكتيرية السائدة التى تؤثر على بعض الأسماك البحرية المستزرعة فى مصر

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[١] قسم بحوث الأحياء المائية – المعهد القومي البيطري – المركز القومي للبحوث - القاهرة - مصر..
[٢] كلية الثروة السمكية والمصايد – جامعة كفر الشيخ - مصر.

يعتبر الإستزراع السمكي واحد من أهم مصادر البروتين من أصل حيوانى فى مصر ونظرا لزيادة الطلب علية دفع معظم الحكومات على توفير هذا البروتين والعمل على زيادة إنتاجه لذلك نجد اهتمام كبير فى مصر بالإستزراع السمكي وخاصة المكثف منه ومع هذا الاهتمام نجد زيادة الاصابة بالأمراض وخاصة البكتيرية منها والتي تؤدى الى زيادة نسبة النفوق وتقليل الانتاجية وخصوصا مع الاستزراع المكثف. ,ويهدف هذا البحث لعمل دراسة لمعرفة أهم المسببات المرضية البكتيرية فى مزارع الاسماك البحرية فى منطقة الاسماعلية مصر ومعرفة مدى انتشارها وتأثيرها على أسماك الدنيس والقاروص والبورى وتم اخذ العينات من عدة مزارع فى محافظة الأسماعلية حيث تم ملاحظة العلامات السريرية للأسماك المصابة وتم عزل وتصنبف البكتريا الموجودة من خلال عمل مزرعة بكتيرية ومن خلال صبغة الجرام ومن خلال تحديد النمط الحركى للبكتيريا ومن خلال التفاعلات البيوكيميائية وعن طريق البيولوجيا الجزيئية (PCR) وقد نتج عنة وجود بكتيريا السيدوموناس والفيبرو وقد تم دراسة تأثيرهذه البكتيريا على الأسماك

الكلمات الدالة: أسماك الدنيس والقاروص والبورى , العوامل البكتيرية , محافظة الاسماعلية.