Egyptian Veterinary Medical Society of Parasitology Journal

EVMSPJ. Vol (20) 2024:133-143

Original Article

Prevalence of crustaceans and bacteria isolated from fish species in Ismailia governorate, Egypt.

Islam A.K. El-waziry[1], Alaa Eldin Eissa[2*], Reham M. El-Tarabili[3], Eman M. Aboulhassan[4], Hadeer Aboelnaga[5], Amina A. Dessouki[5]

[1]Department of Aquaculture Diseases Control, Fish Farming and Technology Institute, Suez Canal University, Ismailia 41522, Egypt

[2]Department of Aquatic Animal Medicine and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211 Egypt

[3]Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522 Egypt

[4]Department of Parasitology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522 Egypt

[5]Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522 Egypt

*Corresponding author:
Alaa Eldin Eissa
Tel.:+20106753010
E-mail address:
aeissa2005@gmail.com

Abstract:

Fish are frequently exposed to a variety of pathogenic agents in the aquatic environment, causing mixed infections. In the present study; we investigated the prevalence of parasitic crustaceans and bacterial pathogens of fish collected from Ismailia governorate, Egypt during the period from March 2022 to February 2023. A total number of 400 fishes belonging to four genera were collected randomly. Parasitological examination showed a total of three crustacean species were isolated from the gills, skin, and fins of different fish spp. (*Levonica redmani, Caligus* and *Lernanthropus* species*)* in addition the bacterial examination revealed the presence of *Psedumonas aeruginosa.*

Key words: Crustaceans, Fish, Bacteria, Prevalence

Introduction

Fish is the cheapest sources of protein after meat and is a major part of human protein supplements in many countries of the world (Shahriar et al., 2019). Fish supplements an estimate of 60% world's protein. As the world's population increases inevitably at a rate of almost 2% per year, the demand for seafood as a source of animal protein will increase (Huicab-Pech et al., 2017). Fish parasitology as an indispensable tool in aquatic health studies and a basic understanding of the richness of community parasitism in many localities is essential for instituting control. Many ectoparasites negatively affect the appearance and reduced production of species of economically important fish, both from the wild and fish farms, thus making them difficult to market (Moravec, 2007; Moravec et al., 2017). The great majority of parasitic crustaceans on marine fish belong to groups such as copepoda and isopoda (Helna et al., 2024). Previous reports showed that marine fishes are highly susceptible to parasitization with copepods and isopods (Nashad et al., 2021). Rhode (2005) reported that isopods are considered a large ectoparasitic crustacean group on marine fish, are diverse and occur in fish worldwide. Isopods live in the sea, in freshwater, or on land, and most are small greyish or whitish animals with rigid, segmented exoskeletons, they have two pairs of antennae, seven pairs of jointed limbs on the thorax, and five pairs of branching appendages on the abdomen that are used in respiration. Females brood their young in a pouch, under their thorax. Copepods are the most prevalent parasitic group of fish (Bowker et al., 2012). Unlike the generous-sized isopoda and branchiura, copepoda are small to microscopic-sized crustacean parasites that were free-living during the early life stages, then the adults, in most cases, become fish pathogenic and leave high mortalities in fish farms (Misganaw and Getu, 2016). Several copepod members were reported to parasitize numerous fish species worldwide (Luque et al., 2013).

Several bacterial and viral pathogens and parasites are opportunistic and occur in the environment or as asymptomatic carriers on some fish, which renders aquaculture facilities highly susceptible to disease outbreaks and hinders the development of an efficient, cost-effective, and stable aquaculture process(Jørgensen, 2020). The current study aimed to identify both the crustaceans and bacteria isolated from fish species in Ismailia governorate, Egypt.

Material and Methods:

Sampling and Parasitological examination

400 fish (*Pomadasys stridens(*striped piggy, *Sparus aurata(*Denis*), Tilapia Zilli(*Bolti*),* and *Dicentrarchus labra(*Karous) were collected from Ismailia governorate from March 2022 to February 2023. The fish samples were transferred to the laboratory (Department of Aquaculture Diseases Control, Fish Farming and Technology Institute, Suez Canal University, Ismailia), they were macroscopically and microscopically examined, for the detection of any abnormalities and for the presence of crustaceans in the various fish body sections (skin, fins, gills and mouth parts), the identification of the crustaceans was according to (Tadros et al., **2020**). All parasites were identified using selected identification keys of Yamaguti (1958, 1961, and 1971).

Bacteriological examination

Fish samples were transmitted to the laboratory in sealed well aerated plastic bags, to undergo additional clinical and bacteriological analyses.

Isolation and identification

A loopful of the harvested internal organs was directly spread onto cetrimide agar and MacConkey's agar (Oxoid, UK), and incubated at 37 °C for 24 hours aerobically. *Pseudomonads* are frequently linked to the synthesis of a yellowish-green luminous

pigment (Lamont and Martin, 2003). All colonies that were suspected were collected and purified to analyze their phenotypic and biochemical properties. All recovered isolates were confirmed using primers targeting 16S rRNA gene of *P. aeruginosa* (PaF: 5′-GGGGGA TCTTCGGACCTCA-3′; PaSR: 5′-TCCTTAGAGTGCCCACCCG-3′) (Spilker et al., 2004).

Antibiotic susceptibility testing.

Two recovered representatives *P.aeruginosa* isolates were tested for susceptibility to ten antibiotics (Himedia) including Amoxicillin (AX,25 µg), Meropenem (MEM,10µg), amoxicillin/ clavulanic acid (AMC,20/10 µg), Gentamicin (CN,10 µg), Erythromycin (E,15 µg), Tetracycline (TE,10 µg), Levofloxacin (LEV,5 µg), Ceftriaxone (CRO,30 µg), Rifamycin SV (RF,30 µg), and Fosfomycin (F0-200 µg) by disk diffusion method (CLSI 2017).MAR index was calculated.

Molecular diagnosis

Extraction of DNA and PCR methodology

Extraction of DNA was done according to QIAamp DNA Mini kit (Qiagen, Germany, GmbH) instructions. PCR mixture was done according to Emerald Amp Max PCR Master Mix (Takara, Japan), Code No. RR310Akit, the temperature and time conditions of the primers during PCR.

Agarose gel electrophoresis

The PCR products were separated using 5 V/cm gradients on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at ambient temperature. 15 µl of the PCR products were placed into each gel slot for gel analysis. The fragment sizes were determined using a gene ruler 100 bp DNA Ladder (Fermentas, Thermo Scientific, Germany) and a Gel model 100 bp DNA Ladder (Qiagen, Germany, GmbH). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra), and computer software was used to analyze the data.

Results

Parasitological examination

A total of 3 parasitic crustaceans (*Caligus* spp. and *Lernanthropus* spp. and *Levonica redmani)* were detected.

Prevalence of parasitic crustacean infestation among examined fishes*:*

The analysis of 400 fish samples revealed that the total prevalence of crustacean infestation was 23.25%. The highest percentage was in *Pomadasys stridens* 38% followed by *Dicentrarchus labrax* 29%, *Sparus aurata* 16% and the lowest percentage was in *Tilapia zilli* 10%.) **(Fig. 1). (Fig. 2)** showed the seasonal prevalence of crustacean's infestations, revealing that the highest infestation was in spring, the parasites were identified as (*Levonica, Caligus,* and *Lernanthropus* spp.) **(Fig.3& 4).**

Bacteriological examination

Isolation and morphological characterization

Samples were taken from the kidney, spleen, and liver under complete aseptic conditions and plated on maCckongy agar and the result revealed:

- **Pale (non-lactose fermenter)** on MacConkey medium which was identified later as *P. aeruginousa* as shown in **table 1.**
- Gram staining of bacterial isolates showed that *P.aeurginosa* are gram-negative rod-shaped, arranged in pairs or short chains and non-spore-forming, as shown in **table 1.**

Prevalence of bacterial infection among examined fishes:

The results of the bacteriological identification revealed that, (2/50) *P.aeurginosa* with a percentage of 4%.

Biochemical identification of retrieved bacterial isolates in examined fish spp:

Results of the biochemical tests for *P.aeurginosa* isolated from fish and as shown in **table 2.**

<u>Antibiotic sensitivity results</u>

Depending on the phenotypic antibiotic resistance, for *P.aeurgionosa* one isolate exhibited multi-drug resistance (MDR) and another exhibited extensive drug resistance (XDR).All isolates showed MAR index values in this study ≥ 0.2 which indicates multiple resistant patterns and this indicates recovered isolates originated from high-risk contamination as shown in Error! Reference source not found.**).**

Molecular Identification of *P.aeurginosa*

Two recovered isolates were selected and identified by specific PCR primers for 16S rRNA gene as *P.aeurginosa*. All isolates were positive with a fragment size of 956 bp **(Fig.5)**

Discussion

The main purpose of the current study is to determine the prevalence of both crustacean and bacteria affecting four species of fishes (*Pomadasys stridens, Sparus aurata, Tilapia zilli,* and *Dicentrarchus labra*). Concerning the total prevalence of crustaceans in these fish species, the highest rate of infection was in *Pomadasys stridens* (38%) followed by *Dicentrarchus labrax* (29%). Seasonally, the prevalence of the collected crustaceans from the four species has the maximum values in spring (43%) and summer, while their minimum in winter and autumn, these results agreed with (Eissa et al., 2017) who recorded a high infestation rate of crustacean in spring 84%, and summer 80% and with (El-Deen et al., 2013) who recorded high prevalence of crusteacea in the summer season, followed by spring season and absent in autumn and winter seasons, this result is in disagreement with that obtained by (Samak and Said, 2008; Tadros et al., 2020), they found that the infestation rates with the crustacean parasites reached their maximum values in both autumn and winter compared with summer and spring. This change in the prevalence may be attributed to the differences in the area from which the fishe were collected or due to changes in the immune response of the fish according to the temperature and food (Eissa et al., 2017).

Pathogens and parasites have adverse effects on the physiological and reproductive activities of the host fish (Vismanis and Kondratovics, 1997). *Pseudomonas aeruginosa*, a common bacterium, causes septicemia in freshwater fish, causing huge economic losses in fish-producing countries. All isolated isolates of *P. aeruginosa* have typical morphological, cultural, and biochemical features. In this study,4% of isolates recovered from fish only. Geographical distribution, environmental conditions, host vulnerability, and sample collection season may affect prevalence. Several antimicrobials are used worldwide to treat and prevent fish bacterial infections. Antibiotic overuse and antibiotic-resistance genes could cause MDR strains (Algammal et al., 2020). Routine antibiotic sensitivity testing is important to identify an appropriate antibiotic and solve this problem (Algammal et al., 2020)

Conclusion

The high predominance of isopods and copopde in fish presence of MDR *P.aeruginosa* ender aquaculture sector which effect on humans.

Ethics

Ethical approval was obtained from the ethical committee, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Algammal, A.M., Mabrok, M., Sivaramasamy, E., Youssef, F.M., Atwa, M.H., El-Kholy, A.W., Hetta, H.F., Hozzein, W.N., 2020. Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor opr L and tox A virulence genes and bla TEM, bla CTX-M,

and tet A antibiotic-resistance genes. Scientific reports 10, 15961.

Bowker, J.D., Carty, D.G., Wandelear, N., Schaffer, J., Swee, W., LaPatra, S.E., 2012. Efficacy of SLICE premix (0.2% emamectin benzoate) for reducing infestations of Salmincola spp. on freshwater-reared rainbow trout. North American Journal of Aquaculture 74, 428-437.

Eissa, I., El-Lamie, M., Ali, S., Khaled, N., 2017. Studies on Parasitic Crustaceans among Some Cultured Marine Fishes in Ismailia Governorate. Suez Canal Veterinary Medical Journal. SCVMJ 22, 185-205.

El-Deen, A., Mahmoud, A., Hassan, A., 2013. Field studies of caligus parasitic infections among cultured seabass (Dicentrarchus labrax) and mullet (Mugil cephalus) in marine fish farms with emphasis on treatment trials. Glob. Vet 11, 511-520.

Helna, A.K., Aneesh, P.T., Kumar, A.B., Ohtsuka, S., Maran, B.A.V., 2024. Site-specific Parasitism of Crustaceans on the Black Pomfret, Parastromateus niger (Carangidae) from Indian Waters. Thalassas: An International Journal of Marine Sciences, 1-12.

Huicab-Pech, Z., Castaneda-Chavez, M., Lango-Reynoso, F., 2017. Pathogenic bacteria in Oreochromis niloticus var. Stirling tilapia culture. Fisheries and Aquaculture Journal 8, 1I-1I.

Jørgensen, L.v.G., 2020. Zebrafish as a model for fish diseases in aquaculture. Pathogens 9, 609.

Lamont, I.L., Martin, L.W., 2003. Identification and characterization of novel pyoverdine synthesis genes in Pseudomonas aeruginosa. Microbiology 149, 833-842.

Luque, J.L., Pavanelli, G., Vieira, F., Takemoto, R., Eiras, J., 2013. Checklist of Crustacea parasitizing fishes from Brazil. Check list 9, 1449-1470.

Misganaw, K., Getu, A., 2016. Review on major parasitic crustacean in fish. Fisheries and Aquaculture Journal 7, 1-6.

Moravec, F., 2007. Nematode parasites of fishes: Recent advances and problems of their research. Parassitologia 49, 155-160.

Moravec, F., Chaabane, A., Neifar, L., Gey, D., Justine, J.-L., 2017. Species of Philometra (Nematoda, Philometridae) from fishes off the Mediterranean coast of Africa, with a description of Philometra rara n. sp. from Hyporthodus haifensis and a molecular analysis of Philometra saltatrix from Pomatomus saltatrix. Parasite 24.

Nashad, M., Aneesh, P.T., Kumar, A.B., Bineesh, K., 2021. A new species of branchial fish parasitic isopod, Norileca Bruce, 1990 (Crustacea: Isopoda: Cymothoidae), from the Andaman Islands, India. Marine Biology Research 17, 669-679.

Rhode, K., 2005. Marine parasitology. CABI, Australia. Folia parasitol 53, 77-78.

Samak, O.A.A., Said, A.E., 2008. Population Dynamics of the Monogeneans, Diplectanum Aequans and d. Laubieri and the Copepod, Lernanthropus Kroyeri Infesting the Gills of the Sea Bass, Dicentrarchus labrax in Egypt. Res. Gate 56, 47-50.

Shahriar, A., Akter, T., Kobra, A.T., Emran, T.B., Mallick, J., Dutta, M., 2019. Isolation of

pathogenic and non-pathogenic microbial stains from different types of sea fish samples and their quality assessment with antibiogram properties. Journal of Advances in Microbiology 19, 1-10.

Spilker, T., Coenye, T., Vandamme, P., LiPuma, J.J., 2004. PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. Journal of clinical microbiology 42, 2074-2079.

Tadros, S.W.A, K El-Gayar, A., M Abouelhassan, E., 2020. Prevalence of ecto- and endoparasites of the Sea bass Dicentrachus Labrax in Port Said Governorate, Egypt. Egyptian Journal of Aquatic Biology and Fisheries 24, 629-643.

Vismanis, K., Kondratovics, E. 1997. The parasites and disease of the Baltic cod. In: Proceeding of the 13th Baltic marine Biologists Symposium Jurmala, Latvia, Sept, 4-31.

Yamaguti, S. 1958. Systema helminthum Vol. I The digenetic trematodes.Part I and II Interscience publishers Inc New York and London.

Yamaguti, S. 1961. Systema helminthum Vol. III, parts 1 and 2 the nematodes of Vertebrates Iinterscience Publication, New York.

Yamaguti, S. 1971. Synopsis of Digenetic trematodes Vertebrates.Yokyo, Keigakyu Publ., 1074 pp.

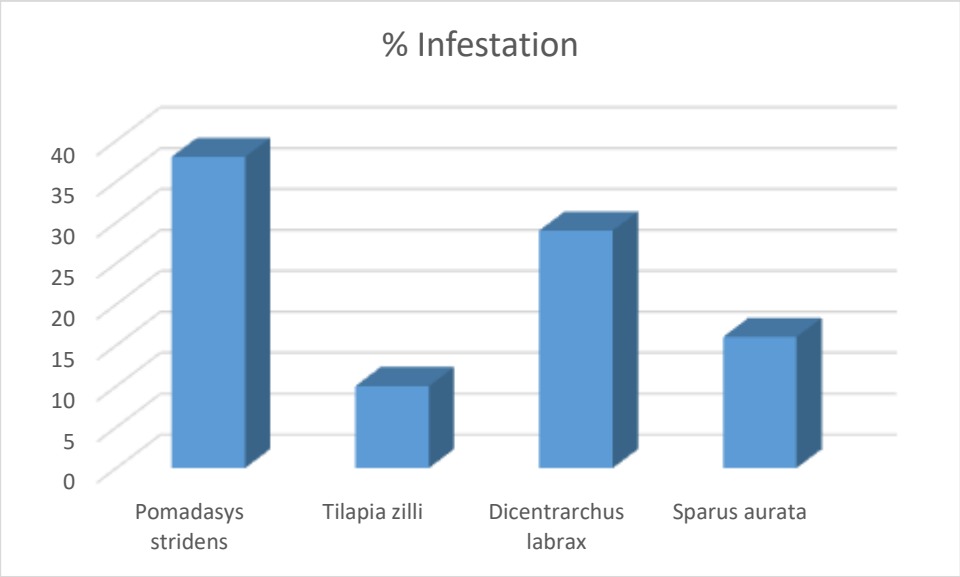

Fig. 1. Total Prevalence of Crustacean infestation in examined fish spp.

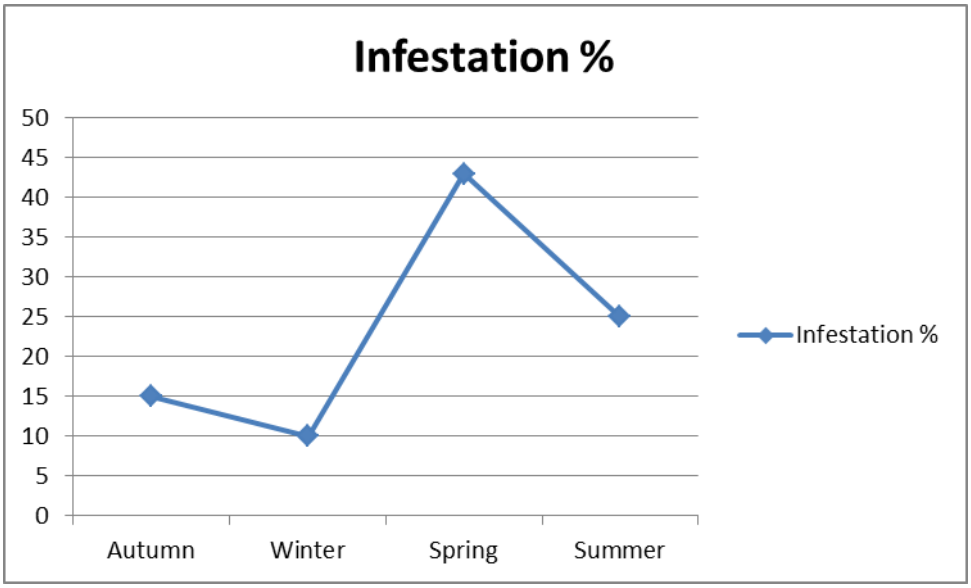

Fig. 2. Total Prevalence of Crustacean infestation in examined fish spp. during different seasons of the year.

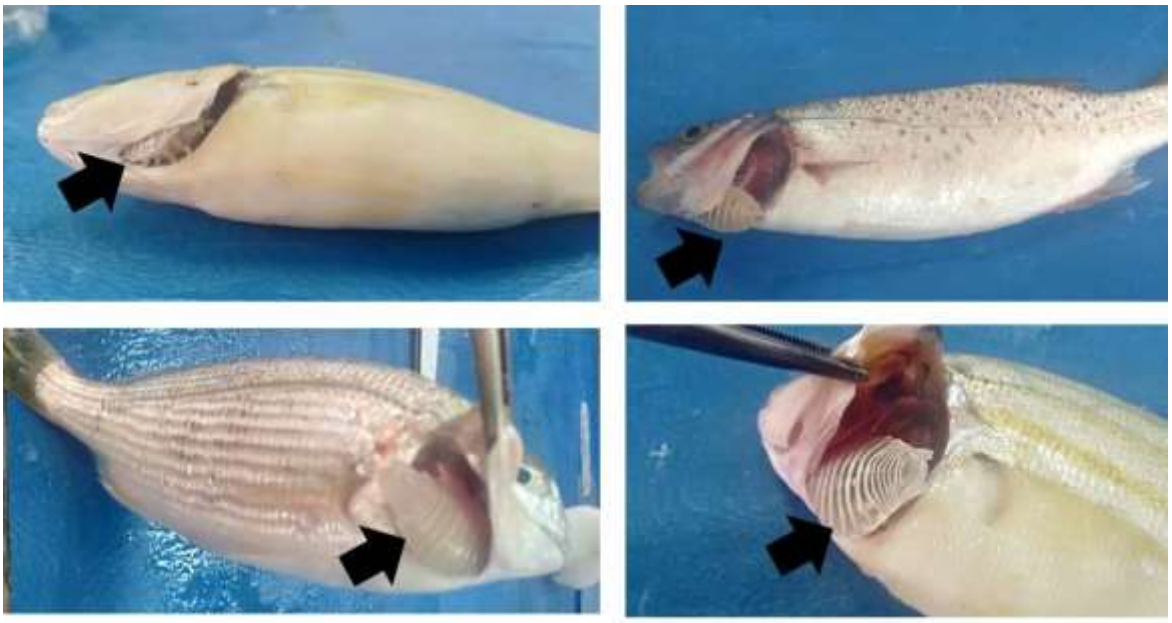

Fig.3. The gill attachment of *Levonica redmani* of different fish species

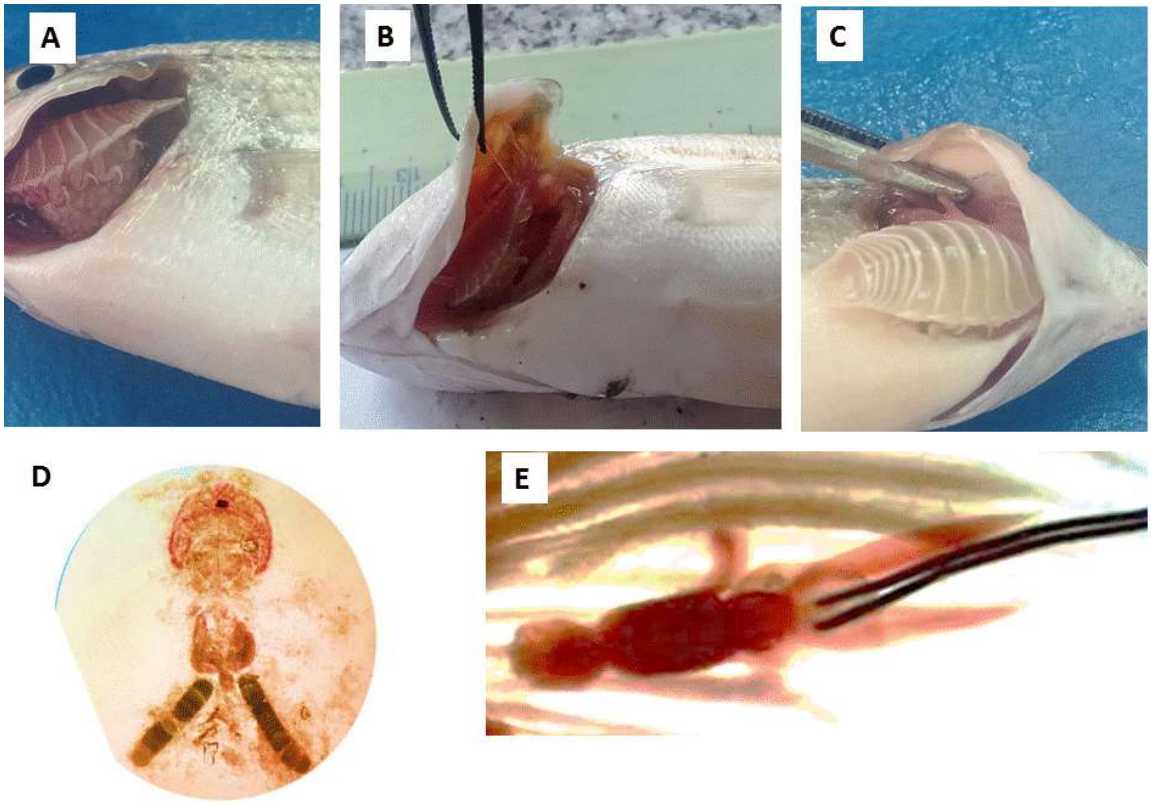

Fig.4. (A, B, C) The gill attachment of *Levonica redmani* of different fish species
(D,E) Crustacean copepodes.

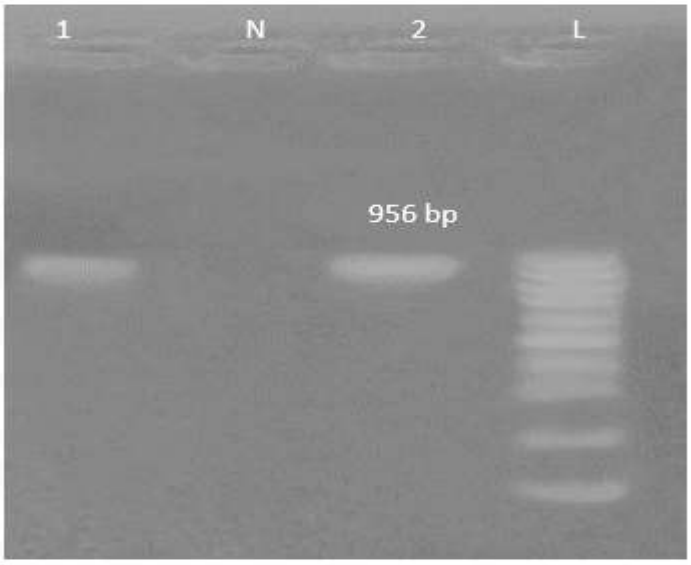

Fig.5. Ethidium bromide-stained agarose gel of PCR products representing amplification of 956 bp amplicons of 16S rRNA gene of *P.aeurginosa* revealed the presence of two positive isolates.

L: Ladder, N: Negative control, 1,2show positive results

Table 1. Morphological characters of bacterial isolates.

Test	***Psedumonas aeruginosa***
Gram´s stain	Gram-negative
Shape	Rod-shaped
Arrangement	arranged in pairs or short chain
Spores	Not spore-forming
Motility	Motile
Growth on MacConkey	Pale non-lactose fermenter colonies

Table 2. Biochemical tests of bacterial isolates.

Test	***P. aeruginosa***
Catalase	+
Oxidase	+
MacConkey agar	***Non lactose fermenter***
Motility	+

Gelatin liquefaction	+
Indol	-
Simmsons citrate	+
Methyl red	-
Vogus- Proskauer	-
Arginine dihydrolase	+
Lysine decarboxyalse	-
Ornithine decarboxylase	-
TSI	***K/K***
Urea	-
ONPG	-

+ = Positive - = Negative A = Acid K=Alkline V = Variable reaction

Table 3: Antibiotic resistance patterns of *P. aeruginosa*

Isolates No.	Phenotypic resistance antibiotic	Type of resistant	MAR
P1	AX,MEM,AMC,TE,CN,FO	MDR	0.60
P2	AX,MEM, AMC, TE, CN, CRO, RF,FO,E	XDR	0.90

ملخص عربي

معدل انتشار القشريات والبكتيريا المعزولة من بعض أنواع الأسماك في محافظة الإسماعيلية، مصر.

اسلام الوزيري ،علاء الدين عيسى، ريهام مختار الطرابيلي ، ايمان محمد ابوالحسن،هدير أبو النجا ، امينة الدسوقي.

تتعرض الأسماك في كثير من الأحيان لمجموعة متنوعة من مسببات الأمراض والطفيليات في البيئة المائية، مما يسبب عدوي مختلطة. في هذه الدراسة؛ قمنا بدراسة انتشار القشريات الطفيلية ومسببات الأمراض البكتيرية للأسماك التي تم جمعها من محافظة الإسماعيلية، مصر خلال الفترة من مارس 2022 إلى فبراير 2023. تم جمع إجمالي 400 سمكة من أربعة أنواع وهى القاروس والبلطى ةالدنيس والبيجى المخطط بشكل عشوائي. أظهر الفحص الطفيلي أنه تم عزل ثلاثة أنواع من القشريات من الخياشيم والجلد والزعانف لأسماك مختلفة. (أنواع *Levonica redmani* و *Caligus* و *Lernanthropus*) بالإضافة إلى ذلك أظهر الفحص البكتيري وجود بكتيريا.*Psedumonas aeruginosa*

الكلمات المفتاحية: القشريات، الأسماك، البكتيريا، الانتشار