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Infection and Molecular Characterization of *Pseudomonas abyssi* MRM71 in Marine Fishes at Red Sea Hurghada, Egypt

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ABSTRACT

A novel pathogenic Gram-negative, rod-shaped bacterium was isolated from *Lutjanus ehrenbergii, Lethrinus borbonicus, Rhabdosargus haffara* and *Scarus ghobban* marine fishes of the Red Sea at Hurghada, Egypt. A total number of 25 out of 180 (13.9%) fishes were clinically diseased and exhibited a series of clinical signs, including skin darkness, hemorrhages especially at the base of fins, exophthalmia, skin ulcers, fin rot, swollen intestine and congestion or paleness of the internal organs. The morphological and biochemical characteristics of the 25 isolates were homogenous and were identified as *Pseudomonas abyssi*. Phylogenetic analysis of the 16S rRNA gene sequence showed that the isolated strains were closely related to the members of the genus *Pseudomonas* and shared the highest sequence identities (100%) with *P. abyssi* MT5 (GenBank accession no. MF962536.1)(100%). Thus, based on 100% identity with *P. abyssi* MT5 from GenBank, this isolate was *P. abyssi*. In the experimental study, it was pathogenic to *Rhabdosargus haffara* and sensitive to chloramphenicol, tetracycline, ciprofloxacin, ofloxacin, tobramycin, clindamycin, gentamycin and amoxicillin/clavulanic acid. Furthermore, AgNPs strongly inhibited *P. abyssi* growth and recorded a minimum inhibitory concentration (MIC) of 8 µg/mL. Blood vessels congestion, degenerative and necrotic lesions were detected in histopathological sections of the hepatopancreatic, splenic and posterior kidney tissues of *Rhabdosargus haffara* experimentally infected with *P. abyssi.*

INTRODUCTION

Pseudomonas infections are among the most important bacterial pathogens affecting several freshwater and marine fishes **(McCarthy *et al.*, 2013; Falaise *et al.*, 2016)**, causing economic losses and may cause potential zoonotic hazards **(Gauthier *et al.*, 2015; El-Gohary *et al.*, 2020).** *Pseudomonas* species have been isolated from various

marine environments, including sediments **(Carrion *et al.*, 2011)**, seawater **(Yoshida *et al.*, 2015)**, and challenger deep part of the Mariana Trench **(Wei *et al.*, 2018)**. Diverse *Pseudomonas* spp. are pathogenic to many fish species, viz. gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*), turbot *(Scophthalmus maximus*) **(Berthe *et al.*, 1995)**, Ayu (*Plecoglossus altivelis*) **(Wakabayashi *et al.*, 1996)**, rainbow trout **(Altinok *et al.*, 2006)** and the Nile tilapia (*Oreochromis niloticus*) **(Eissa *et al.*, 2010)**. In Egypt, *P. fluorescens, P. angulliseptica, P. aeruginosa* and *P. putida* have been described as causative agents of pseudomonas septicemia disease in many fish species **(Clarridge, 2004; El-Nagar, 2010)**, but there is no available literature about the occurrence of *P. abyssi* infection among fishes in Egypt or other countries. One of the main lines of diseases control relies on the accurate and rapid molecular diagnostic techniques such as 16S rRNA gene sequence alongside the biochemical tests which provide accurate and rapid identification of the fish bacterial pathogens **(Kim *et al.*, 2001; Clarridge, 2004; Tringe *et al.*, 2008; Shah *et al.*, 2011 Srinivasan *et al.*, 2015)**. Furthermore, the bacteria can be identified by sequencing the 16S rRNA gene followed by comparing this sequence with known sequences deposited in the NCBI GenBank **(Srinivasan *et al.*, 2015)**.

Egyptian fish production from natural resources was about 391.8 thousand tons, representing 26.7% of the total fish production **(Hassan *et al.*, 2019)**. Fish form an important source of high quality animal protein and a cheap alternative to red meat, reflecting its role in food security and economic development. *Lutjanus ehrenbergii* (Blackspot snapper), *Lethrinus borbonicus* (Snubnose emperor), *Siganus rivulatus* (Marbled spinefoot), *Rhabdosargus haffara* (Haffara seabream), *Scarus ghobban* (Blue-barred parrotfish) and *Cheilinus lunulatus* (Broomtail wrasse) are among the most economically important Red sea marine fishes.

This study aimed to investigate *P. abyssi* infection among the previously mentioned 6 marine fish species inhabiting the Red sea, Hurgada, Egypt, bacteriological isolation of the pathogen and address the phenotyping and biochemical identification of the isolated microorganisms. Moreover, the study focused on the identification of the isolates by molecular techniques and phylogenetic analysis. The pathogenicity test was performed to fulfill Koch's postulates. Furthermore, the antimicrobial susceptibility test was used in combination with the measurement of MIC of AgNPs and ZnONPs against the recovered *P. abyssi*.

MATERIALS AND METHODS

1. Animal ethics

All methods were carried out following relevant guidelines and regulations. Handling of fish and all the experimental protocols were achieved by well-trained scientists according

to the guidelines of National Institute of Oceanography and Fisheries Committee for ethical care of marine organisms and experimental animals (NIOF-AICUC).

2. Study Area and Sampling

A total number of 180 fishes were collected from the Red Sea at Hurghada city, Egypt during the period from October 2019 to March 2020. The examined fish species were *Lethrinus borbonicus Valenciennes* (Snubnose emperor), *Lutjanus ehrenbergii* (Blackspot snapper), *Siganus rivulatus* (Marbled spinefoot), *Rhabdosargus haffara* (Haffara seabream), *Scarus ghobban* (Blue-barred parrotfish) and *Cheilinus lunulatus* (Broomtail wrasse). Thirty fish from each species were transported immediately to indoor aquarium at the National Institute of Oceanography and Fisheries at Hurghada for further clinical, post-mortem and bacteriological examinations.

3. Clinical and postmortem examination

Fishes were anesthetized by tricaine methanoesulfonate (MS222 - Sigma-Aldrich) prior to dissection; clinical and post-mortem examination for detection of the external and internal clinical abnormalities were determined, following the protocol of **Schaperclaus *et al.* (1992)**.

4. Bacterial isolation

Samples from liver, kidney and spleen were collected by sterile bacteriological loops under aseptic condition. The collected samples were immediately inoculated into tryptone soya broth (TSB, Oxoid, England) and incubated aerobically at 22°C for up to 48 hours and streaked onto tryptone soya agar (TSA, Oxoid, England), supplemented with 1.5% NaCl and incubated at 22°C for a period up to 48 hours **(Noga, 2010)**. The recovered isolates were then preserved at -80°C in Tryptone Soya broth, supplemented with 25% glycerol till further identification.

5. Phenotyping and biochemical identification of the isolated bacteria

Suspected colonies were identified through the assessment of their morphological characteristics, Gram-staining, motility test and oxidase test and API 20E system (bioMerieux, France), using the standard laboratory methods described in the study of **Garrity *et al.* (2005)** and following the manufacturer's instructions.

6. Molecular identification of the recovered isolates and sequence analysis

The draft genome sequence of strain MR M71 was sequenced at Solgent Co. Ltd Bio industry development site (South Korea), using Sanger dideoxy sequencing technology. Bacterial DNA was extracted from the recovered isolates, using Gene JET genomic DNA purification kit (Thermo Scientific, EU) according to the manufacturer's instructions. The extracted DNA was then stored at -20°C until further use. The PCR reaction was conducted to amplify the hypervariable segment of 16S rRNA, using a set universal primers **(Frank *et al.*, 2008)**, which are shown in Table (1). The PCR reaction was

conducted in a volume of 50 µl mixtures, according to mastermix manufacturer's instructions, which contained 25 µl of mastermix, 2 µl from each primer, 4 µl from the extracted DNA and Nuclease-Free Water up to 50 µl. As shown in Table (1), PCR reaction was conducted in a thermocycler (Applied Biosystems, USA) under the conditions previously described **(Polz *et al.*, 1998)**. Briefly, the initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycle of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1.5 minutes and followed by a final extension step at 72°C for 10 minutes. The amplicons (1500 base pairs) were purified and sequenced by 3500 Genetic Analyzer (Applied Biosystems, U.S.A.). The sequences of the recovered isolates were analyzed using MEGA 7.0 software and compared to those available at GenBank database. Evolutionary distances were computed using maximum composite-likelihood method. Phylogenetic tree based on 16S rRNA gene sequences was reconstructed by the neighbor-joining method **(Kumar *et al.*, 2016)**.

Table 1. Molecular characterization of *Pseudmonas* abyssi isolate

Gene	Primers sequence (5′- 3′)	PCR coditions	Product
16S rRNA	F27 AGAGTTTGATCMTGGCTCAGTTGTCCGGGTTGTACTCGTC 1492R GGTTACCTTGTTACGACTT	Initial denaturation 95 ^{0} C / 5 min.35 cycles of denaturation at 94 ^{0} C/60 s Annealing at 55^{0} C/1min extension at 72^{0} C/1.5 min	1500

7. Pathogenicity testing

A total of 56 of the acclimated healthy *Rhabdosargus haffara* fish, with an average body weight of 60.0 ± 5.0g were obtained from the National Institute of Oceanography and Fisheries, Hurghada, Egypt. The specimens were used for the experimental infection with the isolated *P. abyssi* recovered from naturally infected marine fish. Fish samples were divided into 4 equal groups; the 1st, 2nd and 3rd groups were injected I/P with *P. abyssi* suspension at a dose of 0.1 mL of (3×10^{7} CFU) **(Austin & Austin 2012; Oh *et al.*, 2019)**. Meanwhile, the 4th group was used as a control positive and injected I/P with 0.1 ml of sterile saline. Fish were immersed in tricaine methane sulfonate (MS222 - Sigma-Aldrich) solution at a concentration of 40-80 mg/L, and immobilization took approximately 5 minutes **(Tonguthai *et al.*, 1999)**. The concentration used in the experimental challenge was selected according to preliminary works. Fishes were observed daily for 2 weeks, and the clinical signs and mortalities were recorded. Freshly dead fish were subjected to post-mortem examination and bacteriolohical isolation and identification of *P. abyssi* was determined from the internal organs.

8. Antimicrobial Susceptibility Test of the recovered *P. abyssi* isolates

The antimicrobial susceptibility of the recovered isolates was determined by Kirby-Bauer disc diffusion method, using the following antibiotics disks: ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5µg), cefotaxime (30 µg), ofloxacin (5 µg), oxolonic acid (2 µg), erythromycin (15 µg), chloramphenicol (30 µg), amoxicillin/clavulanic acid (30 µg), cephalothin (30 µg), amikacin (30 µg), streptomycin (10 µg), clindamycin (2 µg), gentamycin (10 µg), tobramycin (10 µg) and trimethoprim/sulphamethoxazole (25 µg). Briefly, the recovered isolate was streaked into Mueller-Hinton agar (Oxoid, England); the antibiotic disks were placed, and the inoculated plate was incubated at 25°C for 24- 48hrs. Diameters of the inhibition zones were measured and interpreted according to the Clinical and Laboratory Standards Institute (2006).

9. Determination of minimum inhibitory concentration (MIC) of AgNPs and ZnONPs against *P. abyssi* isolates

The antibacterial activities of 20 nm silver nanoparticles (AgNPs; MKnano, Canada) and 100 nm zinc oxide nanoparticles (ZnONPs; Sigma-Aldrich,USA) were tested against the recovered *P. abyssi* isolates. The tested concentrations of AgNPs were 1, 2, 4, 8, 16, 20, 30, 40, and 50 µg/ml; while for ZnONPs, concentrations were 1, 5, 10, 15, 20, 25, 30, 35 and 40 µg/mL . These different concentrations of AgNPs and ZnONPs were prepared in sterile test tubes containing 5mL of Luria-Bertani broth, and one test tube was used as control for each antibacterial. All tubes were inoculated with an equal volume (200 µl) of freshly prepared bacterial suspension diluted to an optical density of 0.5. The inoculated tubes were then incubated at 25°C for 24h. Later on, the absorbance was measured by UV-visible spectrophotometer at 600nm, and the graph was plotted against optical density and the concentration of nanoparticles. The concentration giving the least optical density corresponds to MIC of the used antimicrobials **(Rautela *et al.*, 2019)**.

10.Histopathological examination

Necropsy of the affected fishes was performed, and gross lesions were reported. The samples including skin lesions, liver and spleen were fixed in 70% ethanol solution, embedded in paraffin wax and sectioned at 3-5 µm. The sections were stained with haematoxylin and eosin (H & E) **(Gridley, 1960)**.

11. Statistical analysis

Simple descriptive statistics, i.e., percentages, were used for the analysis of the data. The prevalence of the infection was calculated using the following formula:

Prevalence of infection (%) = No. of infected fish/Total no. of examined fish

RESULTS

1. Clinical and post-mortem examination

The naturally infected *Lutjanus ehrenbergii, Lethrinus borbonicus, Rhabdosargus haffara* and *Scarus ghobban* fishes exhibited clinical signs, viz. skin darkness, hemorrhages on the body, at base of the fins and on gills' cover (Fig. 1A), fin rot (Fig. 1B), corneal opacity (Fig. 1C) and exophthalmia. The postmortem examination showed serous fluid in the abdominal cavity, the liver was enlarged, friable, congested and in some cases pale (Fig. 1D). The intestine was hemorrhagic and inflamed. The spleen was enlarged and congested and in some cases appeared pale.

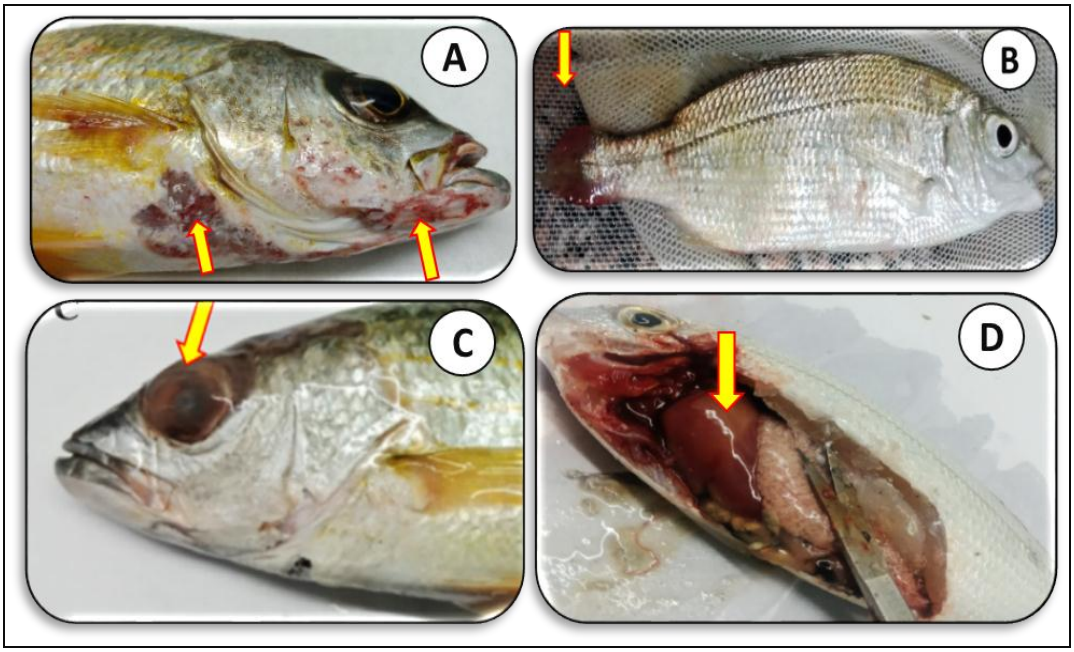

Fig. 1. Clinical signs of naturally infected fishes: **(A)** *Lutjanus ehrenbergii* showing hemorrhages and erosions on the lower jaw and body; **(B)** *Rhabdosargus haffara* showing tail fin rot; **(C)** *Lutjanus ehrenbergii* showing exophthalmia and eye opacity and **(D)** *Rhabdosargus haffara* showing congested and friable liver

2. Bacteriological and phenotypic characterization of the recovered isolates

Twenty-five (N=25) bacterial isolates were recovered from kidney, liver and spleen of the clinically diseased fishes. All isolates were gram-negative, short motile bacilli, produced small, flat, round, creamy-colored colonies and showed gummy consistency on TSA (Fig. 2A), while no hemolysis was detected on blood agar (Fig. 2B). They were identified as *P. abyssi* according to their morphological and biochemical characteristics illustrated in Table (2).

Table 2. Morphological and biochemical characteristics of *P.* abyssi isolates

Test	Result	Test	Result
Oxidase test	+ve	Blood haemolysis	-ve
Catalase	+ve	4°C Temperature	+ve
Motility	+ve	45°C	+ve
Swarming	-ve	4% NaCl	+ve
MacConky	+ve	10% NaCl	+ve
ONPG	-ve	GEL	-ve
ADH	-ve	GLU	-ve
LDC	-ve	MAN	-ve
ODC	-ve	INO	-ve
CIT	+ve	SOR	-ve
H2S	-ve	RHA	-ve
URE	-ve	SAC	-ve
TDA	-ve	MEL	-ve
IND	-ve	AMY	-ve
VP	-ve	ARA	-ve

-ve: negative +ve: positive

4. Sequencing and phylogenetic analysis of the *P. abyssi* 16S rRNA gene fragment

The nucleotide sequence of 16S rRNA of MR M71 was analyzed in comparison with the nucleotide sequences of 16S rRNA gene of *Pseudomonas* in Gen Bank database. The close genetic relationship and identity were assessed between the present isolate sequence and *P. abyssi* MT5 (GenBank accession no. MF962536.1)(100%). The MRM71 strain is *P. abyssi* based on 100% identity with *P. abyssi* MT5 (GenBank accession no. MF962536.1). The draft genome of MRM71 *P. abyssi* was deposited into NCBI and assigned GenBank accession no. MW788678 (Fig. 4).

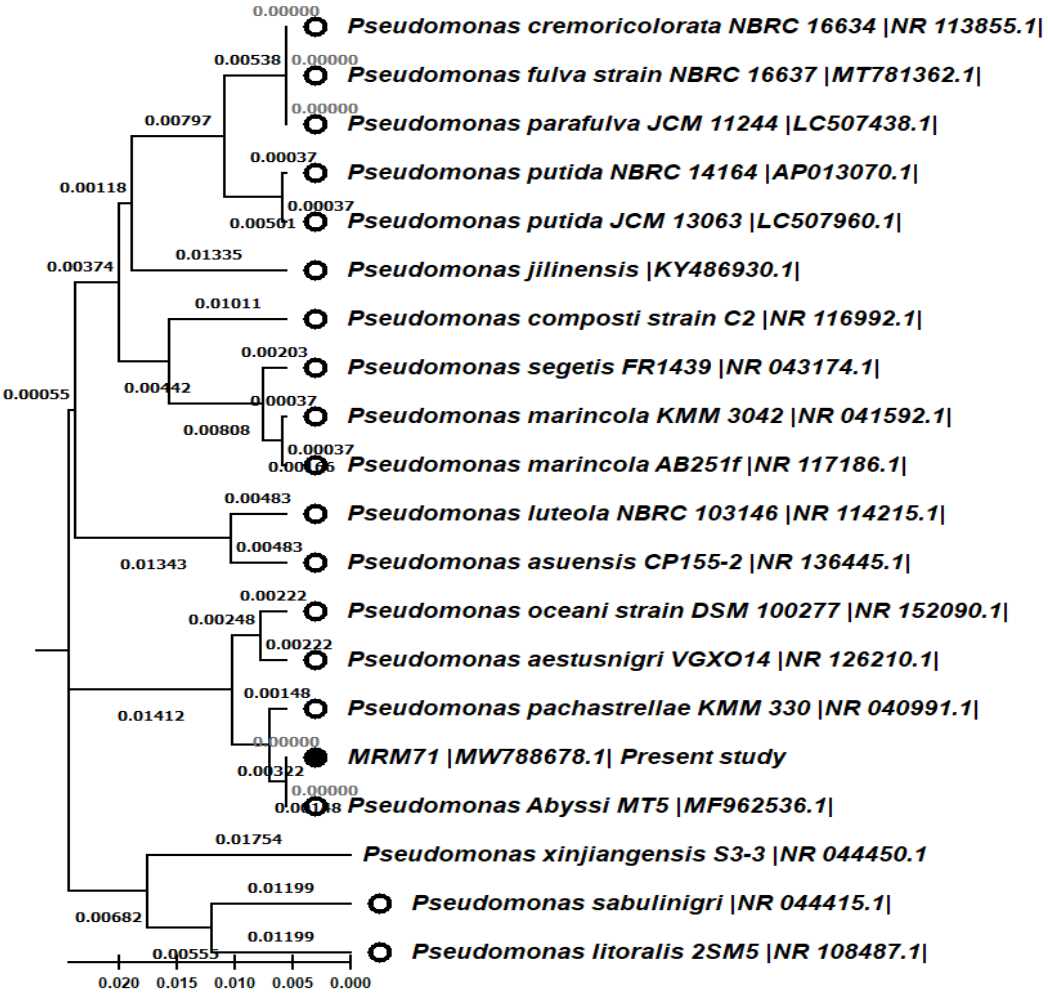

Fig. 4. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain MRM71and related members within the genus pseudomonas. The evolutionary distances were computed using the maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The optimal tree is shown and the tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 20 nucleotide sequences. The evolutionary analyses were conducted in MEGA7 **(Kumar *et al.*, 2016)**

5. Pathogenicity of *Pseudomonas abyssi* in Haffara seabream fish

The experimentally infected *Rhabdosargus haffara* exhibited 70% mortalities and clinical signs similar to that recorded in the natural infected fishes, including skin darkness, hemorrhages and ulcers on the body (Figs. 5A & B), tail fin rot (Fig. 5B) in addition to corneal opacity and exophthalmia. Internally, the intestine was engorged with mucoid fluids (Fig. 5C) and the liver was congested (Fig. 5D). Analysis data obtained revealed that the survival rate was 30% at the end of the second week of the challenge time. *P. abyssi* was isolated and identified from the internal organs of the experimentally infected fish.

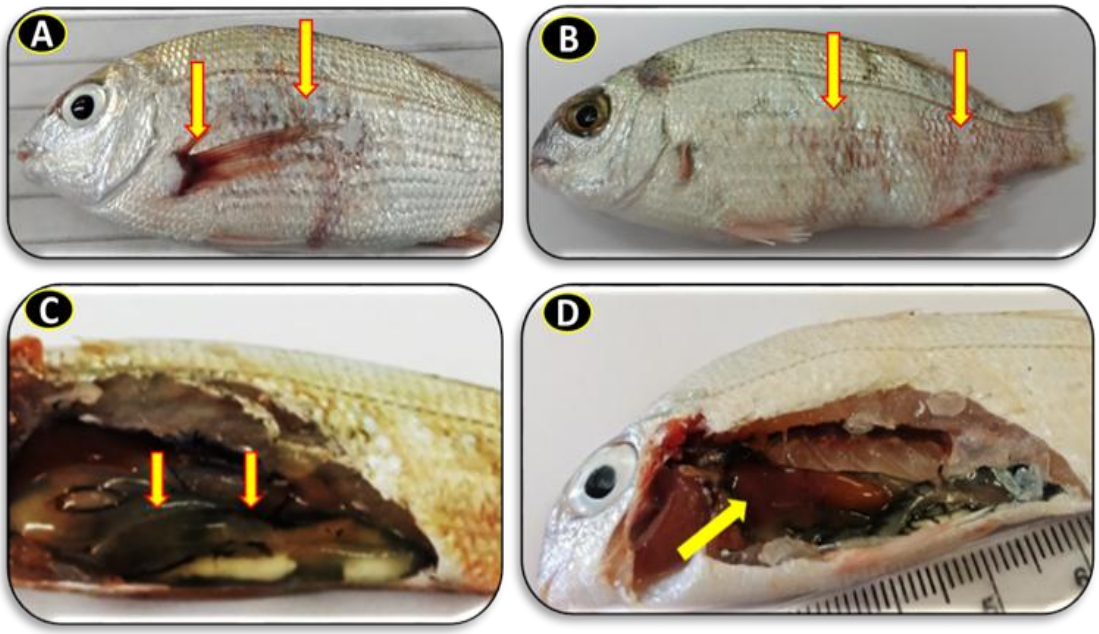

Fig. 5. Clinical signs and PM lesions of *Rhabdosargus haffara* experimentally infected with *P. abyssi* showing **(A)** Hemorrhagic ulcer and erosions; **(B)** Tail fin rot and hemorrhagic ulcers on the body; **(C)** Intestinal distension and **(D)** Liver congestion.

6. Antimicrobial susceptibility test and MIC of AgNPs and ZnONPs against *P. abyssi*

The results revealed that *P. abyssi* was sensitive to chloramphenicol, tetracycline, ciprofloxacin, ofloxacin and amoxicillin/ clavulanic acid, while resistant to ampicillin, cephalothin, amikacin, streptomycin, cefotaxime, erythromycin, oxolonic acid and trimethoprim/ sulphamethoxazole. On the other hand, the MIC of AgNPs and ZnONPs against *P. abyssi* were (8 µg / mL) and (15 µg / mL).

7. Histopathological alterations of *Rhabdosargus haffara* experimentally infected with *P. abyssi*

The hepatopancreatic sections from *Rhabdosargus haffara* fish, experimentally infected with *P. abyssi* showed congested vein (V) and necrotic hepatopancreas (HP). The hepatic tissue lost its normal architecture, with a dissociation of hepatic cords arrangement; hepatocytes (arrows) were shrunken and showed coagulative necrosis (Fig. 6). Spleen sections from *Rhabdosargus haffara*, experimentally infected with *P. abyssi* showed hyperplasia of melanomacrophage centers (circles) and area of necrosis (stars) (Fig. 7). The posterior kidney sections from *Rhabdosargus haffara*, experimentally infected with *P. abyssi* showed congested vein (arrow), hyperplasia of melanomacrophage centers (circles), glomerular atrophy (red arrowheads), necrosis and detachment of renal tubular epithelium (arrows), and interstitial mononuclear inflammatory cellular infiltration (black arrowheads) (Fig. 8).

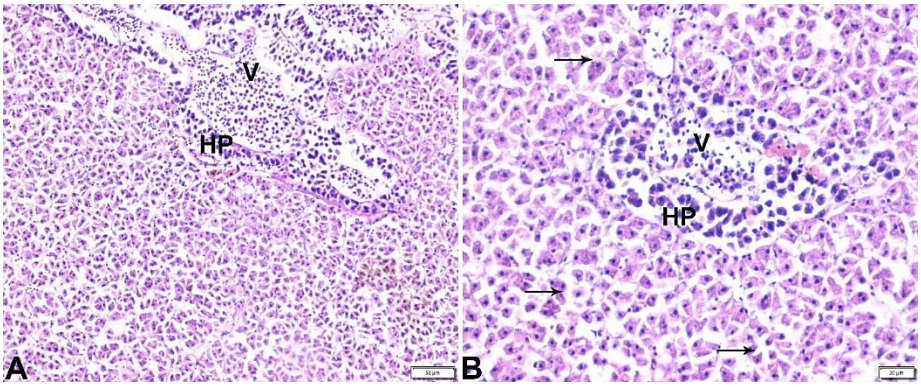

Fig. 6. Rhabdosargus haffara fish, experimentally infected with P. abyssi showed hepatopancreatic sections from congested vein (V) and necrotic hepatopancreas (HP).The hepatic tissue lost its normal architecture, with a dissociation of hepatic cords arrangement; hepatocytes (arrows) were shrunken and showed coagulative necrosis.

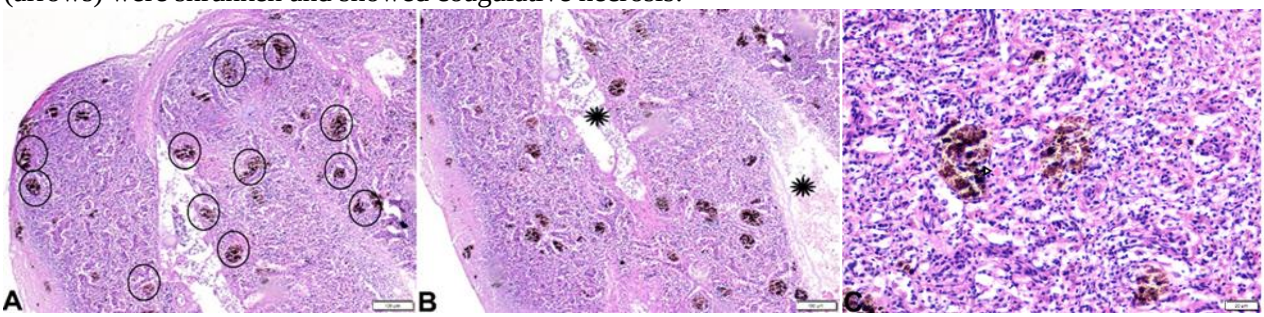

Fig. 7. Rhabdosargus haffara, experimentally infected with P. abyssi showed spleen sections from hyperplasia of melanomacrophage centers (circles) and area of necrosis (stars).

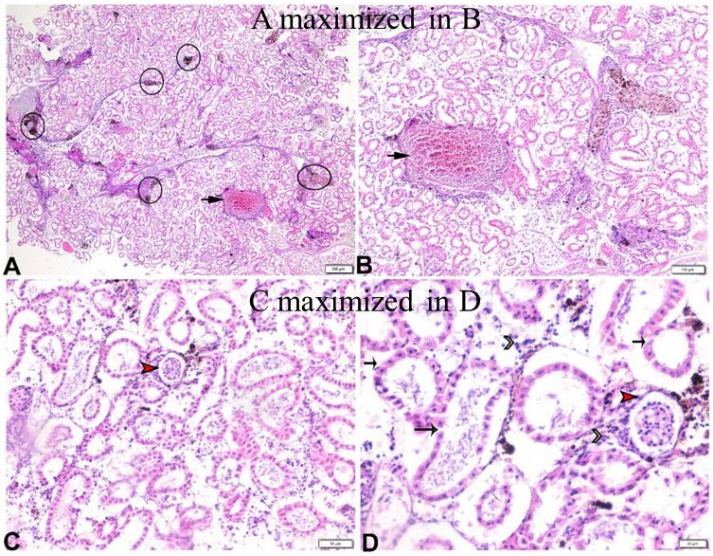

Fig. 8. Rhabdosargus haffara, experimentally infected with P. abyssi showed posterior kidney sections with congested vein (arrow), hyperplasia of melanomacrophage centers (circles), glomerular atrophy (red arrowheads), necrosis and detachment of renal tubular epithelium (arrows), and interstitial mononuclear inflammatory cellular infiltration (black arrowheads).

DISCUSSION

Bacterial pathogens are normally present in aquatic environments, and their presence is insufficient to cause a disease outbreak; however, they become highly pathogenic under some stressful conditions **(Polz *et al.*, 1998; Frank *et al.*, 2008)**. *Pseudomonas* spp. have been considered as serious bacterial fish pathogens, causing high mortalities and considerable economic losses **(Kumar *et al.*, 2016)**. The present study reported for the first time *P. abyssi* infection among *Lutjanus ehrenbergii, Lethrinus borbonicus, Rhabdosargus haffara* and *Scarus ghobban* marine fishes of the Red Sea at Hurghada City. The naturally infected fishes showed several clinical signs and PM lesions, such as skin darkness, hemorrhages and erosions on the lower jaw and body, tail fin rot, hemorrhage on the fins, exophthalmia and corneal opacity beside congested and friable liver. These results are in accordance to those of **Mastan (2013)** and **El-Barbary and Hal (2017)**, who detected skin darkening or discoloration, hemorrhages and ulcers on the skin surface, eye opacity and exophthalmia, fin congestion and rot especially tail fin, ascites and hemorrhagic liver. The frayed fins and fin rot of the diseased fish negatively affect the swimming activities and foraging behavior of the diseased fish **(Khalil & Emeash, 2018)** leading to fish weakness. The diffused hemorrhages all over the body might be attributed to the secretion of elastase enzyme and hemolysin factor by this bacterium which damaged the blood vessels and contributed the haemorrhagic septicemia **(Zhang *et al.*, 2005; Martins *et al.*, 2012)**. In addition, the clinical signs and PM lesions may be attributed to the extracellular products, viz. proteases, lipases, and hyaluronidase enzymes of the pathogen, involved in the clinical pathology or lesions development **(Pemberton *et al.*, 1997; Esteve *et al.*, 2004)**. *P. abyssi* infection was not recorded in *Siganus rivulatus* and *Cheilinus lunulatus* fish, and the possible explanation could be attributed to the difference in fish species susceptibility to the infection **(Lopez *et al.*, 2016)**.

The total prevalence of *P. abyssi* infection among the examined fishes was 13.6%, while the prevalence was 40, 26.7, 16.7 and 16.7% among *Lethrinus borbonicus*, *Lutjanus ehrenbergii, Rhabdosargus haffara* and *Scarus ghobban* fishes, respectively. On the other hand, *Siganus rivulatus* and *Cheilinus lunulatus* fishes were not infected and the possible explanation of the prevalence could be attributed to the difference in susceptibility of different fish species to the infection **(Carol *et al.*, 2013)**.

Regarding the bacteriological identification of the recovered isolates, *P. abyssi* colonies were small, flat, round, creamy-colored and of gummy consistency on TSA agar; while, no hemolysis was detected on blood agar; it was gram-negative short motile bacilli. These findings agree with those of **Wei *et al.* (2018)**, who reported simillar characetresitics of *P. abyssi* isolated for the first time from abyssopelagic water of the

Mariana Trench, China. All recovered isolates were biochemically homogenous and positive for cytochrome oxidase, catalase, citrate utilization tests, while they were negative for arginine dihydrolase, gelatin hydrolysis, H_2S production, urease, indole production, gelatin liquefaction and glucose fermentation tests. These findings are in harmony with the *P. abyssi* biochemical characterization results of **Wei *et al.* (2018)**.

The 16S rRNA gene sequence alongside the biochemical tests provide accurate and rapid identification of bacterial fish pathogens **(Altinok *et al.*, 2006; Shah *et al.*, 2011; Seil & Webster 2012; Carol *et al.*, 2013; El-Barbary & Hal, 2016; Hasan, 2019)**. Phylogenetic analysis of 16S rRNA gene sequence of strain MR M71 revealed the presence of a close relation with members of genus *Pseudomonas* and shared the highest sequence identities with P. abyssi MT5 (GenBank accession no. MF962536.1) (100%). The MR M71 isolate was identified as *P. abyssi*, based on 100% identity with *P. abyssi* MT5 (GenBank accession no. MF962536.1). The MR M71 isolate genome sequence was deposited into NCBI and assigned accession number MW788678**.**

The pathogenicity test of this study showed that the recovered *P. abyssi* strain was pathogenic to *Rhabdosargus haffara* and the challenged fish exhibited clinical signs similar to that of naturally infected fish with 71.4% mortality rate. Furthermore, *P. abyssi* was isolated and identified from the experimentally infected fish according to Koch's postulate. The findings of the pathogenicity assay supported by El-Barbary and Hal **(2017)**, who stated that identical pathological signs were observed in trout species naturally and experimentally infected with *Pseudomonas* spp. Also, Altinok *et al*., **(2006)** reported identical pathological signs in both *P. putida* outbreak and experimental infection. The clinical signs and PM lesions reported in the present work might be attributed to the pathogen's extracellular products such as cytotoxins, hemolysin, protease, collagenase and hyaluronidase that released during the infection **(Pemberton *et al.*, 1997; Esteve *et al.*, 2004; Zhang *et al.*, 2005; El-Barbary &Hal 2016)**.

For the antibiogram profile the current results showed that, *P. abyssi* isolates were sensitive to chloramphenicol, tetracycline, ciprofloxacin, ofloxacin, tobramycin, clindamycin, gentamycin and amoxicillin/clavulanic acid. While, they were resistant to the remaining antibiotics used in the study. Some of these results coincide with those of **El-Barbary and Hal (2016)** and **Hasan (2019)** who reported that *Pseudomonas* spp. were sensitive to ciprofloxacin. Given the fact that the antimicrobial resistance was dramatically grown, it is urgently required to discover new effective antimicrobial agents **(Seil *et al.*, 2012; Swain *et al.*, 2014; Salomoni *et al.*, 2017)**.

The antimicrobial resistance was dramatically grown, making an urgent need to discover new effective antimicrobial agents. Nanotechnology became an alternative for traditional antimicrobials **(Swain *et al.*, 2014)** and several previous reports revealed the bactericidal

and bacteriostatic activities of AgNPs and ZnONPs against a wide range of microorganisms **(Hassani *et al.*, 2015; Salomoni *et al.*, 2017; Khan *et al.*, 2019)**. In the present study, it was found that MIC of AgNPs against *P. abyssi* isolates was 8 µg/mL, while it was 15µg/mL for ZnONPs. Revising the available literatures, no previous studies explored the antibacterial activity of AgNPs and ZnONPs against *P. abyssi*. However, a previous study found that AgNPs inhibited growth of *P. aeruginosa* **(Salomoni *et al.*, 2017)** and ZnONPs at a concentration of 150-158 µg/ml inhibited growth of *P. aeruginosa* **(Hassani *et al.*, 2015)**. The stronger antibacterial efficacy of AgNPs than ZnONPs against *P. abyssi* isolates might be attributed to the difference in the physical properties of the tested agents, including shape, size and stability of nanoparticles **(Padmavathy & Vijayaraghavan, 2008)**.

The histopathological alterations in the hepatopancereas, spleen and kidney in *Rhabdosargus haffara*, experimentally infected with *P. abyssi* were confirmed in the study of **Hossam *et al.* (2015)**, who recorded the congestion of the pancreatic acini and hepatic sinusoid, hepatocytes degeneration and mild pancreatic acini necrosis. The spleen sections showed activation of melanomacrophage centers, lymphoid depletion and apoptosis, with macrophages infiltration. The kidneys exhibited congestion of blood vessel, depletion of hemopiotic elements and mild tubular necrosis in *P. flourescence* experimentally infected Nile tilapia.

CONCLUSION

The present study reported for the first time a novel pathogenic *P. abyssi* from *Lethrinus borbonicus*, *Lutjanus ehrenbergii*, *Rhabdosargus haffara* and *Scarus ghobban* fishes, inhabiting Red Sea with prevalnce 40.0, 26.7, 16.7 and 16.7%, respectivelly. This bacterium was identified using phylogenetic analysis, based on 16S rRNA gene alongside biochemical tests. The analysis data of 16S rRNA gene sequencing of MR M71 isolate revealed 100% identity with *P. abyssi* MT5 (GenBank accession no. MF962536.1). The isolated strain was pathogenic to *Rhabdosargus haffara* and sensitive to chloramphenicol, tetracycline, ciprofloxacin, ofloxacin, tobramycin, clindamycin, gentamycin and amoxicillin/clavulanic acid. These results could be used to treat the infected fish. The AgNPs strongly inhibited *P. abyssi* growth and recorded 8 µg/mL MIC. The effective antibiotics and AgNPs can be used for the treatment of the infected fish. Further studies are recommended to explore the presence of *P. abyssi* infection in other localities and among other marine and freshwater fish species of Egypt.

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