Detection and description of a novel Psychrobacter glacincola infection in some Red Sea marine fishes, Hurghada, Egypt

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Abstract

Background: Aquaculture is an important food-producing sector in Egypt, but several pathogenic microorganisms lead to high mortalities and significate economic losses, challenge this sector. The present study investigated the occurrence of *Psychrobacter glacincola* infection among 180 wild Snubnose emperor (*Lethrinus borbonicus*), Blackspot snapper (*Lutjanus ehrenbergii*), Marbled spinefoot (*Siganus rivulatus*), Haffara seabream (*Rhabdosargus haffara*), Blue-barred parrotfish (*Scarus ghobban*) and Broomtail wrasse (*Cheilinus lunulatus*) marine fishes collected from the Red sea at Hurghada, Egypt. The prevalence rate of disease was 6.7%. The recovered isolates were then subjected to bacteriological, biochemical, and molecular identification. The study also investigated pathogenicity and the antibiogram profile of the recovered isolates.

Results: The clinical examination of the infected fish revealed various signs that included lethargy and sluggish movement, hemorrhages and ulcers on the body and the operculum, scale loss, fin congestion and rot, especially at the tail fin. In addition to, congestion of the liver, spleen, and kidney was observed during postmortem examination. Interestingly, 12 isolates were recovered and were homogenous bacteriologically and biochemically. Alongside the biochemical and bacteriological tests, the phylogenetic analysis based on 16S ribosomal RNA (16S rRNA) gene fragment confirmed that the MRB62 identified strain was closely related to members of the genus *Psychrobacter* and identified as *P. glacincola*. Furthermore, the recovered *Ps. glacincola* (MRB62) strain was pathogenic to *Rhabdosargus haffara* fish, causing 23.3% mortality combined with reporting a series of clinical signs similar to that found in naturally infected fishes. In addition, the recovered strain was sensitive to amikacin, streptomycin, ciprofloxacin, gentamycin, chloramphenicol, tobramycin, and ofloxacin.

Conclusions: Our findings add to the body of knowledge about the occurrence of pathogenic *P. glacincola* infection in Egyptian marine fishes and its potential effects on fish. The study also suggests future large-scale surveys for exploring this bacterium among other freshwater and marine fishes in Egypt that would be helpful for the implementation of effective strategies for prevention and control of this infection.

1. Introduction

Marine fish represent an important source of income in many developing countries, besides being one of the major investment choices for national fishermen [1]. Taken into account, Egypt is bordered by the Red Sea on the east, which is 2250 km in length with an average depth of 490 m. Importantly, the Red Sea has a unique composition of fish species, which consists of 1166 species. The marbled spinefoot (*Siganus rivulatus*), Blackspot snapper (*Lutjanus ehrenbergii)*, Snubnose emperor (*Lethrinus borbonicus*), Blue-barred parrotfish (*Scarus ghobban*), Haffara seabream (*Rhabdosargus haffara*), and Broomtail wrasse (*Cheilinus lunulatus*) are some of the most economically important Red Sea marine fish species in Egypt. However, this important sector is challenged by a wide range of serious pathogenic organisms, affecting diverse marine fishes and shellfish [2]. Among others, bacteria are the most prevalent cause of morbidity and mortality among wild populations of fish, resulting in major economic losses in this sector [3]. Taken into consideration, several bacterial strains are normally present in aquatic environments and their simple presence in marine environments is insufficient to cause a disease outbreak, but they might become highly pathogenic under some stressful conditions [4].

Among others, *Psychrobacter* species have been isolated from various marine environments and seawater, which is considered a good habitat for these species [5]. The presence of *Psychrobacter* spp. was also significantly associated with other environmental factors, i.e., temperature, pH close to neutrality, high salinity, higher concentrations of potassium and magnesium [6], and hydrocarbons contaminated aquatic environments [7, 8]. Furthermore, members of the genus *Psychrobacter* have been isolated from the gastro-intestinal tract (GIT), skin and gills of apparently healthy Atlantic salmon [9], Atlantic cod [10], juvenile grouper [11] and Atlantic mackerel [12]. *Psychrobacter* spp. have been used as probiotics to enhance the growth rate and immune system efficiency in fish [13], since they inhibit the growth of some pathogenic bacteria and enhance the growth of many beneficial or neutral bacteria in the gut of fish [14]. Some *Psychrobacter* strains were recorded as opportunistic pathogenic microorganisms for some fish species. Some previous studies [15, 16] reported *P. immobilise* infection in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon. *Psychrobacter* isolates such as *P. immobilis* and *P. phenylpyruvicus* have been isolated in clinical samples from brain tissue, urine, ears, wounds, cerebrospinal fluid, and blood

and have been reported to be opportunistic pathogens in humans [17–21]. Another species named *P. glacincola* was isolated as a novel strain from sea ice cores in Antarctica [22], mud of the Shetland Islands [23], processed fresh edible sea urchin in Tokyo [24], red tanner crab [16], water of aquaculture and agriculture run off of Ria de Aveiro [25], and sediment samples of King George Island, Antarctica [26], respectively.

Revising the available literature, the prevalence of bacterial diseases was documented in several cultured and wild freshwater fish species from Egypt. However, only a few bacteriological surveys were carried out on marine fish species to track disease outbreaks. More importantly, no available data was reported about the *P. glacincola* infection among fishes all over the world. Given the above information, the present study investigated the occurrence of *P. glacincola* among some fish species inhibited by the Red Sea in Hurghada, Egypt, through clinical examination, bacteriological isolation, phenotyping, biochemical and molecular identification of the isolated strains. The study also included a pathogenicity test for verification of the pathogenicity of the isolated strains, combined with antimicrobial susceptibility testing of the recovered *P. glacincola* isolates.

2. Material And Methods

2.1. Study Area and Sampling

A total of 180 fish were obtained from Hurghada, Red Sea governorate, Egypt, during the period from October 2019 to March 2020. The examined fish species were: Snubnose emperor (*Lethrinus borbonicus*), Blackspot snapper (*Lutjanus ehrenbergii*), Marbled spinefoot (*Siganus rivulatus*), Haffara seabream (*Rhabdosargus haffara*), Blue-barred parrotfish (*Scarus ghobban*) and Broomtail wrasse (*Cheilinus lunulatus*). The rate of sampling was 30 fish from each species. Fish were collected and transported immediately to the indoor aquarium at the National Institute of Oceanography and Fisheries in Hurghada, Egypt for clinical and bacteriological examinations.

2.2. Clinical and postmortem examination

Clinical and PM examinations of fish were carried out to detect external and internal clinical abnormalities according to the method described by Schaperclaus et al. (1992) [27]. Fishes were anaesthetized with tricaine methanoesulfonate (MS222-Sigma-Aldrich) prior to examination.

2.3. Bacterial isolation

Bacteriological samples from the liver, kidney, and spleen were collected by sterile loop under completely aseptic conditions. The collected samples were then inoculated into Brain Heart Infusion Broth supplemented with 1.5% NaCl (Oxoid, England) and incubated aerobically at 22°C for up to 48 hours, then streaked onto tryptone soya agar (Oxoid, England) supplemented with 1.5% NaCl and incubated at 22°C for up to 48 hours [28]. The recovered isolates were then preserved at -80°C in Tryptone Soya broth supplemented with 25% glycerol for further identification.

2.4. Phenotyping and Biochemical identification of the isolated bacteria

The suspected isolates were identified through their morphological characteristics, Gram staining, motility test, oxidase test and API 20E system (BioMerieux, France) according to the manufacturer's instructions.

2.5. Molecular Identification of the Recovered Isolates and sequence analysis

The bacterial DNA was extracted from the recovered isolates using the 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 use. Later on, the PCR reaction was conducted to amplify the hypervariable segment of 16S rRNA using a set of universal primers [29], which are shown in Table 1. The PCR reaction was conducted in 50 µl mixtures, according to the mastermix manufacturer's instructions, which contained 25 µl of mastermix, 2 µl of each primer, 4 µl of the extracted DNA, and Nuclease-Free Water up to 50 µl. As shown in Table 1, the PCR reaction was conducted in a thermocycler (Applied Biosystems, USA) under the conditions previously described [30]. In brief, the initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of

denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1.5 minutes, followed by a final extension step at 72°C for 10 minutes. The amplicons (1500 base pairs) were purified and sequenced by the 3500 Genetic Analyzer (Applied Biosystems, USA). The draft genome sequence of strain MR-B62 was sequenced at Solgent Co., Ltd. Bio industry development site (South Korea) using Sanger dideoxy sequencing technology. The sequences of the recovered isolates were analysed using MEGA 7.0 software and compared to those available in the GenBank database. Evolutionary distances were computed using the maximum compo-site-likelihood method. A phylogenetic tree based on 16S rRNA gene sequences was reconstructed by the neighbor-joining method [31].

Table 1
Primer sets used in molecular characterization of Ps. glacincola isolates

Genes	Primers sequence (5′-3′)	PCR conditions	Product sizes/ (bp)
16S rRNA	F27 5 $AGAG \top TGATCMTGGCTCAG \top GT\mathbb{C}GGG \top GTACTCGTC3$ R1492; 5$GG \top A\mathbb{C} \top G \top ACGAC \top$	• Initial denaturation 95 C / 5 min. • 35 cycles of denaturation 94 C/60 s • Annealing at 55 C / 1 min • Extension at 72 C / 1.5 min	1500

2.6. Pathogenicity testing

A total of 40 acclimated healthy Haffara seabream (*Rhabdosargus haffara*) fish with an average body weight 50 ± 5g were obtained from the National Institute of Oceanography and Fisheries, Hurghada, Egypt. These fish were experimentally infected with the bacterial suspension (*P. glacincola*) recovered from naturally infected marine fish. Fish were divided into 4 equal groups; the 3rd groups were injected Intraperitoneal (IP) with *P. glacincola* suspension at a dose of 0.1 mL of (3×10^7 CFU) [32], and the fish of the 4th group were injected IP with 0.1 ml of sterile saline and used as a control. Fish were closely observed daily for 2 weeks, and the clinical signs and mortalities were recorded. Freshly dead fish were subjected to post-mortem examination, bacteriological isolation, and identification of *P. glacincola* from the liver, spleen, and kidney.

2.7. Antimicrobial Susceptibility Test of the recovered *P. glacincola* isolates

The antimicrobial susceptibility of the recovered isolates was determined using the Kirby-Bauer disc diffusion method. The following antibiotic discs were used: tetracycline (30µg), ciprofloxacin (5µg), ofloxacin (5µg), oxolonic acid (2µg), erythromycin (15µg), chloramphenicol (30µg), amoxicillin/clavulanic acid (30µg), cephalothin (30µg), amikacin (30µg), streptomycin (10µg), cefotaxime (30µg), trimethoprim/sulphamethoxazole (25µg), Gentamycin (10µg), clindamycin(2µg), Flucloxacillin (5µg), Tobramycin(5µg), The recovered isolates were streaked into Mueller-Hinton agar (Oxoid, England), the antibiotic discs were placed, and the inoculated plate was incubated at 25°C for 48 h. Diameters of the inhibition zones were measured and interpreted according to the Clinical and Laboratory Standards Institute (2012) (33).

2.9. Statistical Analysis

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

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

3. Results

3.1. Clinical sings of *P. glacincola* infection

The infected marine fishes showed a series of clinical signs that included lethargy and sluggish movement, haemorrhages and ulcers on the body and on the operculum, sometimes scale loss was noticed, fin congestion and rot, especially on the tail fin (Fig. 1A & B). Furthermore, congestion of the liver, spleen, and kidney were noticed during the PM examination (Fig. 1C).

3.2. Prevalence of *P. glacincola* infection

The clinical examination of 180 Red Sea fish revealed a total prevalence of 6.7% of *P. glacincola* infection among all examined fish. Furthermore, the prevalence rates were 16.7, 10 and 13.3% among Blackspot snapper (*Lutjanus ehrenbergii*), Haffara seabream (*Rhabdosargus haffara*) and Broomtail wrasse (*Cheilinus lunulatus*) fish, respectively. On the other hand, Snubnose emperor (*Lethrinus borbonicus*), Marbled spinefoot (*Siganus rivulatus*), Blue-barred parrotfish (*Scarus ghobban*) fishes were not susceptible to infection (Table 2).

Table 2
Occurrence of P. glacincola **among six marine fish species of Red Sea**.

Fish species		**No. of examined Fish**	***Psychrobacter glacincola* infection**	
Scentific name	**English name**		**No. of infected fish**	**Percentage of infected (%)**
Lutjanus ehrenbergii (Peters, 1869)	Blackspot snapper	30	5	16.7
Lethrinus borbonicus (Valenciennes, 1830)	Snubnose emperor	30	0	0
Siganus rivulatus (Forsskål & Niebuhr, 1775)	Marbled spinefoot	30	0	0
Rhabdosargus haffara (Forsskål, 1775)	Haffara seabream	30	3	10
Scarus ghobban (Forsskål, 1775)	Blue-barred parrotfish	30	0	0
Cheilinus lunulatus (Forsskål, 1775)	Broomtail wrasse	30	4	13.3
Total		**180**	**12**	**6.7**

3.3. Bacteriological identification and morphological characters of Colonies

The colonies of these isolates were diplococci, cream-colored to un-pigmented, smooth and opaque with a buttery consistency after incubation at 22°C for up to 48 hours on Tryptone Soya agar. These microorganisms were gram –ve, non-motile and did not result in hemolysis of the blood agar, while they grew to yellowish on MacConkey agar. Colonies also were able to grow at 1.5, 3, 7 and 10% Nacl and at 4°C, 22°C and 37°C.

3.4. Biochemical identification

As shown in Table 3, the twelve (12) isolates were biochemically homogeneous and positive for Cytochrome oxidase, Catalase, Citrate utilization (CIT), variable for Urease (URE) and Voges Proskauer (VP) tests, all strains were negative for O-Nitrophenyl-β-D-Galactopyranoside (ONPG), Arginine dihydrolase (ADH), Lysine de-carboxylase (LDC), Ornithine decarboxylase (ODC), Indole production (IND), H_2S production (H2S), Tryptophane deaminase (TDA), Gelatin liquefaction tests and acids from all sugars (Glucose (GLU), Mannitol(MAN), Inositol (INO), Sorbitol (SOR), Rhamnose (RHA), Sucrose (SUC), Melibiose (MEL), Amygdaline (AMY) and Arabinose (ARA).

Table 3
Biochemical characteristics of recovered strain in the present study *P. glacincola*.

Test	**Result**	**Test**	**Result**
Oxidase test	+ve	MacConky agar	+ve
Catalase	+ve	Blood haemolysis	-ve
Motility	+ve	NaCl 1.5%	+ve
Swarming	-ve	3%	+ve
Growth at 4°C	+ve	7%	+ve
37°C	+ve	10%	+ve
API20E Tests			
Test	Result	Test	Result
ONPG	+ve	GEL	-ve
ADH	-ve	GLU	-ve
LDC	-ve	MAN	-ve
ODC	-ve	INO	-ve
CIT	+ve	SOR	-ve
H_2S	-ve	RHA	-ve
URE	V	SAC	-ve
TDA	-ve	MEL	-ve
IND	-ve	AMY	-ve
VP	+V	ARA	-ve

-ve : Negative + ve : positive V : variable

3.5. Molecular identification

Compared with the GenBank database, the nucleotide sequences of the 16S rRNA gene could detect the isolates of bacteria at the species level according to levels of homology (Fig. 3). The recovered strain was identified and named MRB62. The draft genome sequence of strain MRB62 was deposited into NCBI and assigned accession number (MZ413384.1). Interestingly, high similarity of the 16S rRNA gene sequences of the MRB62 isolate to that of *P. glacincola* T (Accession No. AB334769.1) with

100% identity is a remarkable finding. Furthermore, a closed genetic relationship was detected for the recovered strain (the strain MRB62 of the genus *Psychrobacter*) in the present study with some other strains in Genbank. In this concern, an identity of 99.71%, 99.64%, 99.43%, and 98.86% was reported with that of *P. glacincola* LMG 21274T (Accession No. AJ 430830.1), *P. glacincola* DSM 12194T (Accession No. NR 042076.1), *P. adeliensis* DSM 15333T (Accession No. 117634.1), and *P. immobilis* NBRCT 15733 (Accession No. AJ NR113805.1), respectively (Fig. 2).

3.6. Pathogenicity test

The *P. glacincola* experimentally infected Haffara seabream (*Rhabdosargus haffara*) showed 23.3% mortalities and clinical signs similar to those recorded in the naturally infected fish. These clinical signs included skin hemorrhages, scale loss, tail fin rot, congestion, and liver congestion (Fig. 3), (Table 4). *P. glacincola* was also isolated and identified from the internal organs of the experimentally infected fish. The bacterial pathogen was re-isolated from the induced kidney.

Table 4
Number of dead fish and mortality rate of Haffara seabream (*Rhabdosargus haffara*), experimentally challenged with *P. glacincola*

Rhabdosargus hafara groups	Fish number	Number of dead fish/day									Total number of dead fish	Mortality rate % (at 1st week end)	Mortality rate % (at 2nd week end)
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th to 14th			
Challenged groups	30	3	0	3	6	3	0	3	3	0	21	60%	70%
Control group	10	0	0	0	0	0	0	0	0	0	0	0%	0%

3.7. Antibiotic sensitivity test

In accordance with the antibiotic sensitivity test, the recovered isolates *P. glacincola* were sensitive to Amikacin, Streptomycin Ciprofloxacin, Gentamycin, Chloramphenicol, Tobramycin and Ofloxacin and resist to Tetracycline, Cephalothin, Cefotaxime, Erythromycin, Oxolonic acid, Trimethoprim/Sulphamethoxazole, Clindamycin, Flucloxacillin and Amoxicillin/Clavulanic acid

4. Discussion

Aquaculture represents an important sector in the Egyptian national income structure [34]. However, this sector is challenged by a wide range of opportunistic pathogens that result in high mortalities and considerable economic losses. Bacterial pathogens are among these pathogens that are naturally present in the fish environment, and under some stressful conditions, they become the pathogens of the most important diseases in aquaculture [35]. Among others, *Psychrobacter* spp. could be considered as a potential bacterial pathogen in fish that results in high mortalities and considerable economic losses [15, 16]. Clearly, providing updates and baseline information about the occurrence of *Psychrobacter* spp. infection among marine fishes, together with investigation of the pathogen characteristics, seems crucial for implementation of the appropriate measures to prevent and control the infection. Revising the available literature, no previous studies revealed the occurrence of the novel pathogenic strain of *P. glacincola* infection in fish either at a national or international level. However, several previous studies identified *P. glacincola* as a novel strain from sea ice cores in Antarctica [22], mud of the Shetland Islands [23], processed fresh edible sea urchin in Tokyo [24], and red tanner crab [16]. In addition, other previous studies [23, 36] registered *P. glacincola* in NCBI from marine environment and sea urchin. Given the above information, the present work provides a novel contribution in

relation to the occurrence of a novel pathogenic strain of *P. glacincola* in wild marine fishes in Egypt through the isolation, identification, and characterization of the bacterium by bacteriological, biochemical, and molecular methods.

In the present work, the bacteriological examination of the examined fish revealed that the overall prevalence of *P. glacincola* infection among examined fish was 6.7%, while the individual prevalence rates were 16.7%, 10%, and 13.3% among *Lutjanus ehrenbergii, Rhabdosargus haffara*, and *Cheilinus lunulatus* fish, respectively. On the other hand, *P. glacincola* could not be detected among *Lethrinus borbonicus, Siganus rivulatus* and *Scarus ghobban*. Taken into consideration, no previous studies reported *P. glacincola* infection among fishes all over the world, and the possible explanation for this prevalence may be attributed to the difference in susceptibility of fish species to the infection [37, 38]. In accordance with their clinical impact, the infected species showed several clinical signs and PM lesions as a result of *P. glacincola* infection. In this regard, Blackspot snapper (*Lutjanus ehrenbergii*), Haffara seabream (*Rhabdosargus haffara*) and Snubnose emperor (*Lethrinus borbonicus*) fishes of the Red Sea at Hurghada city showed lethargy and sluggish movement, haemorrhages and ulcers on the body and operculum, scale loss, fin congestion, and rot, especially tail fins, combined with congestion of the liver, spleen, and kidney.

It is noteworthy to state that there are no available literatures reported the clinical signs of *P. glacincola* infection, but our results were agreed to some extent with that of Hisar et al., (2002)[15], who found skin darkness, gills paleness and abnormal swimming, internal organ congestion in rainbow trout infected with other *Psychrobacter* spp. The *P. glacincola* infected fishes showed frayed fins and fin rot that adversely affected the swimming activities and foraging behaviour of the diseased fish, leading to loss of condition and weakness [39–41]. The diffused haemorrhages on the fish body could be attributable to the secretion of some enzymes such as elastase enzyme and hemolysin that damage the blood vessels, leading to blood leakage [42]. Also, the clinical signs and PM lesions of the diseased fishes may be attributed to the extracellular products of *Psychrobacter* spp. such as proteases and hyaluronidase, that are involved in the development of clinical pathology and lesions [43, 44].

Isolation and identification of the causative agents remain one of the main lines for infection control [45]. Phenotyping is commonly used in combination with genotyping to identify and characterize bacterial pathogens [45–47]. Likewise, biochemical characterization has been proved to be a valuable method for the typing and differentiation of several bacterial fish pathogens [48–50]. In this study, the phenotyping of the recovered isolates showed that the morphological characteristics of the colonies were cream-colored, un-pigmented, smooth and opaque with a buttery consistency. In addition, colonies formed yellowish colonies on MacConkey agar with no hemolysis on blood agar. Biochemically, the isolates were homogeneous and positive for cytochrome oxidase, catalase, and citrate utilization, while negative for lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, indole production, H_2S production, tryptophane deaminase, gelatin liquefaction test, and acid from all sugars. Our findings are in accordance with the results reported by Bowman et al. (1997) [22] and Garcia-Lopez et al. (2014) [51], who reported similar bio-chemical reactions with *P. glacincola*. Taken into account, the variation in any biochemical characteristic may be attributed to presence or absence of plasmid(s) or mobile genetic elements that controls its metabolic traits [52].

In accordance with the molecular methods, the phylogenetic analysis based on 16S rRNA gene sequence is an important tool, which confirmed the genetic relatedness and stands alongside the biochemical tests and bacteriological tests for accurate and quick identification of bacteria [53–55]. The 16S rRNA gene sequence alongside the biochemical tests provide accurate and rapid identification for the bacterial pathogen [4, 54, 56] and the phylogenetic analysis of 16S rRNA gene allows and confirms the identification of unknown bacterial isolates [57]. In this study, the phylogenetic analysis identified the recovered strain as *P. glacincola* (MRB62) based on 16S rRNA gene sequence. Comparing the 16S rRNA gene sequence of the present strain (*P. glacincola* MR B62) with known 16S rRNA gene sequences of *Psychrobacter* spp. on GenBank databases revealed closed similarity of 100% with *P. glacincola* T (Accession No. AB334769.1) [24], and the draft genome sequence of this strain was deposited into NCBI and assigned accession number MZ413384.1.

Based on those results, the present study reports for the first time the occurrence of *P. glacincola* infection among Snubnose emperor (*Lethrinus borbonicus*), Haffara seabream (*Rhabdosargus haffara*) and Broomtail wrasse (*Cheilinus lunulatus*) marine fishes of the Red Sea at Hurghada city, Egypt. Furthermore, the recovered strain (*P. glacincola* MR B62) of the present study

revealed an identity of 99.71%, 99.64%, 99.43%, and 99.07% with that of *P. glacincola* LMG 21274T (Accession No. AJ 430830.1) [22], *P. glacincola* DSM 12194T (Accession No. NR 042076.1) [22], *P. adeliensis* DSM 15333T (Accession No. 117634.1) [58], and *P. immobilis* NBRCT 15733 (Accession No. AJ NR113805.1) [59], respectively. The present findings confirm the hypothesis that bacteria with an identity of more than 98.7% in the 16S rRNA gene sequence are considered to be the same species [60].

In accordance with the results of the pathogenicity test, the pathogen was isolated and identified from the experimentally challenged Haffara seabream (*Rhabdosargus haffara*) fish to fulfil Koch's postulates. The present study proved that the present *P. glacincola* isolate was pathogenic to Haffara seabream. In this concern, the challenged fish showed 23.3% mortality rates and exhibited clinical signs similar to those of the naturally infected fishes that included skin hemorrhages, scale loss, tail fin rot and congestion, and liver congestion. The recorded clinical signs may be attributed to the extracellular products such as cytotoxins, hemolysin, protease, collagenase, and hyaluronidase that were released during the infection [61, 62]. The present study also showed that *P. glacincola* isolates were sensitive to Amikacin, Streptomycin Ciprofloxacin, Gentamycin, Chloramphenicol, Tobramycin and Ofloxacin and resist to Tetracycline, Cephalothin, Cefotaxime, Erythromycin, Oxolonic acid, Trimethoprim/Sulphamethoxazole, Clindamycin, Flucloxacillin and Amoxicillin/Clavulanic acid. Some of our results agreed with a previous study [5], where *P. glacincola* was sensitive to streptomycin and gentamycin and resist tetracycline and ampicillin. Generally, the high variations in the antibiotic sensitivity test results may be due to the dramatic anti-microbial resistance growth and the bacterial isolate variations.

5. Conclusions

Given the above information, the present study reported for the first time a novel pathogenic bacterial isolate named *P. glacincola* from naturally diseased Snubnose emperor (*Lethrinus borbonicus*), Haffara seabream (*Rhabdosargus haffara*) and Broomtail wrasse (*Cheilinus lunulatus*) marine fishes. The isolated strains were identified by their morphological and biochemical characteristics. In addition, the phylogenetic analysis of the 16S rRNA gene sequence of the present study MR B62 isolate revealed 100% identity with *P. glacincola* T (Accession No. AB334769.1). The recovered strain, *P. glacincola*, was also pathogenic to Haffara seabream (*Rhabdosargus haffara*) and sensitive to amikacin, streptomycin, ciprofloxacin, gentamycin, chloramphenicol, tobramycin, and ofloxacin. The present data suggest large-scale surveys of *P. glacincola* infection in the fish sector in Egypt, which might be helpful for the implementation of effective control strategies for combating this infection.

Declarations

6.1. Ethics approval and consent to participate

We conall of the experimental protocols and methods carried out in accordance with the relevant rules and regulations of the National Institute of Oceanography and Fisheries Committee for ethical care of marine organisms and experimental animals (NIOF-AICUC), and all methods reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

6.2. Consent for publication

Not applicable

6.3. Availability of data and materials

The datasets generated and/or analysed during the current study are available in the [NCBI] repository, [https://www.ncbi.nlm.nih.gov , Accession number MZ413384.1].

Competing interests

All authors who attributed to the article "Detection and description of a novel *Psychrobacter glacincola* infection in some Red Sea marine fishes, Hurghada, Egypt" declare that they have no known competing financial interests or personal relationships

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6.5. Authors' contributions

M.R.E-S., A.M.E., and M.A.A involved in the conception of the idea, methodology design, performed data analysis and interpretation. A. E. O. and H.H.A contributed their scientific advice, prepared the manuscript for publication and revision. All authors read and approved the final manuscript.

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Figures

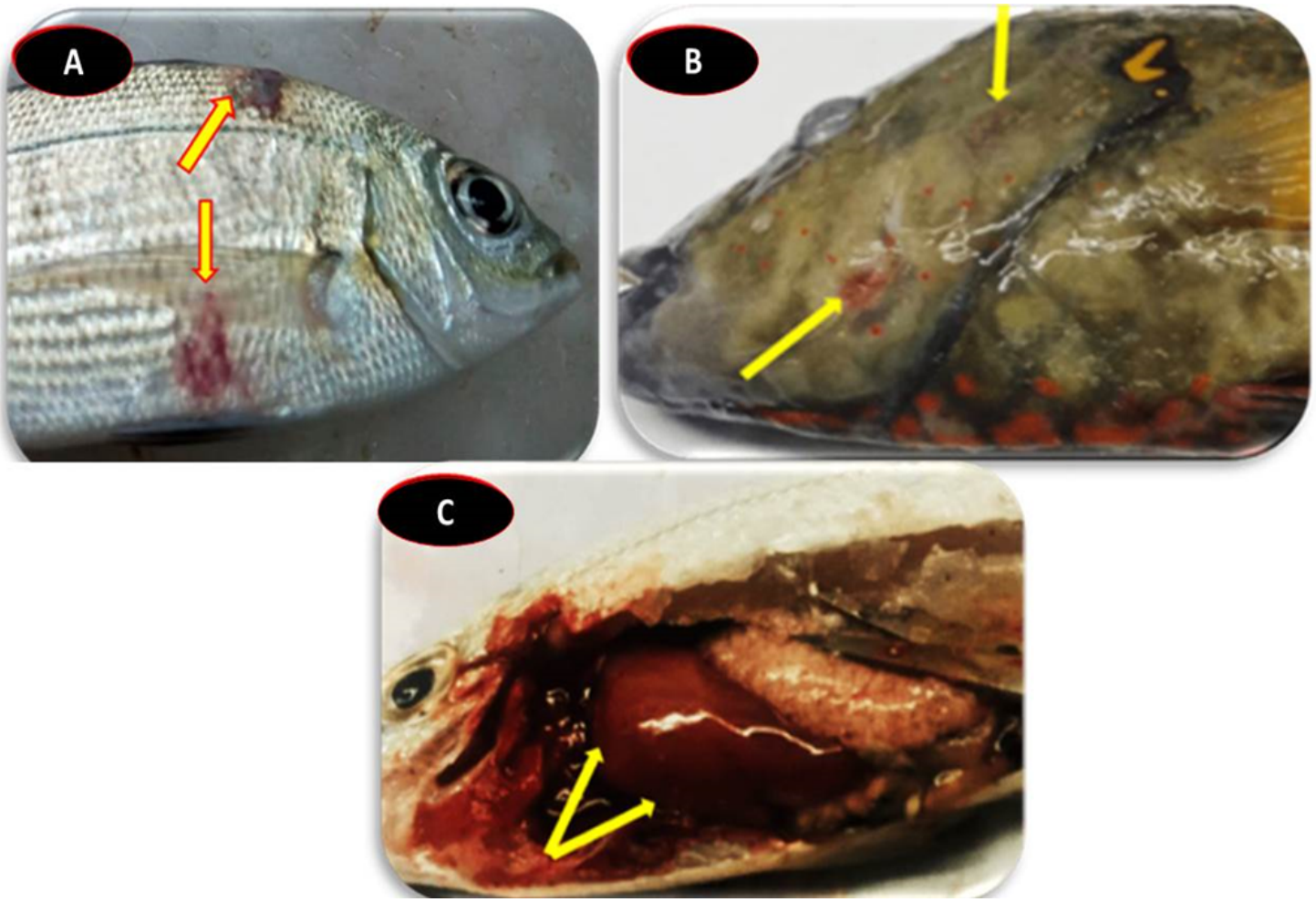

Figure 1

Clinical signs of *P. glacincola* infection of some red sea fishes: (A) *Rhabdosargus haffara* hemorrhagic body ulcer; (B) *Cheilinus lunulatus* hemorrhagic body ulcer; (C) *Rhabdosargus haffara* liver congestion

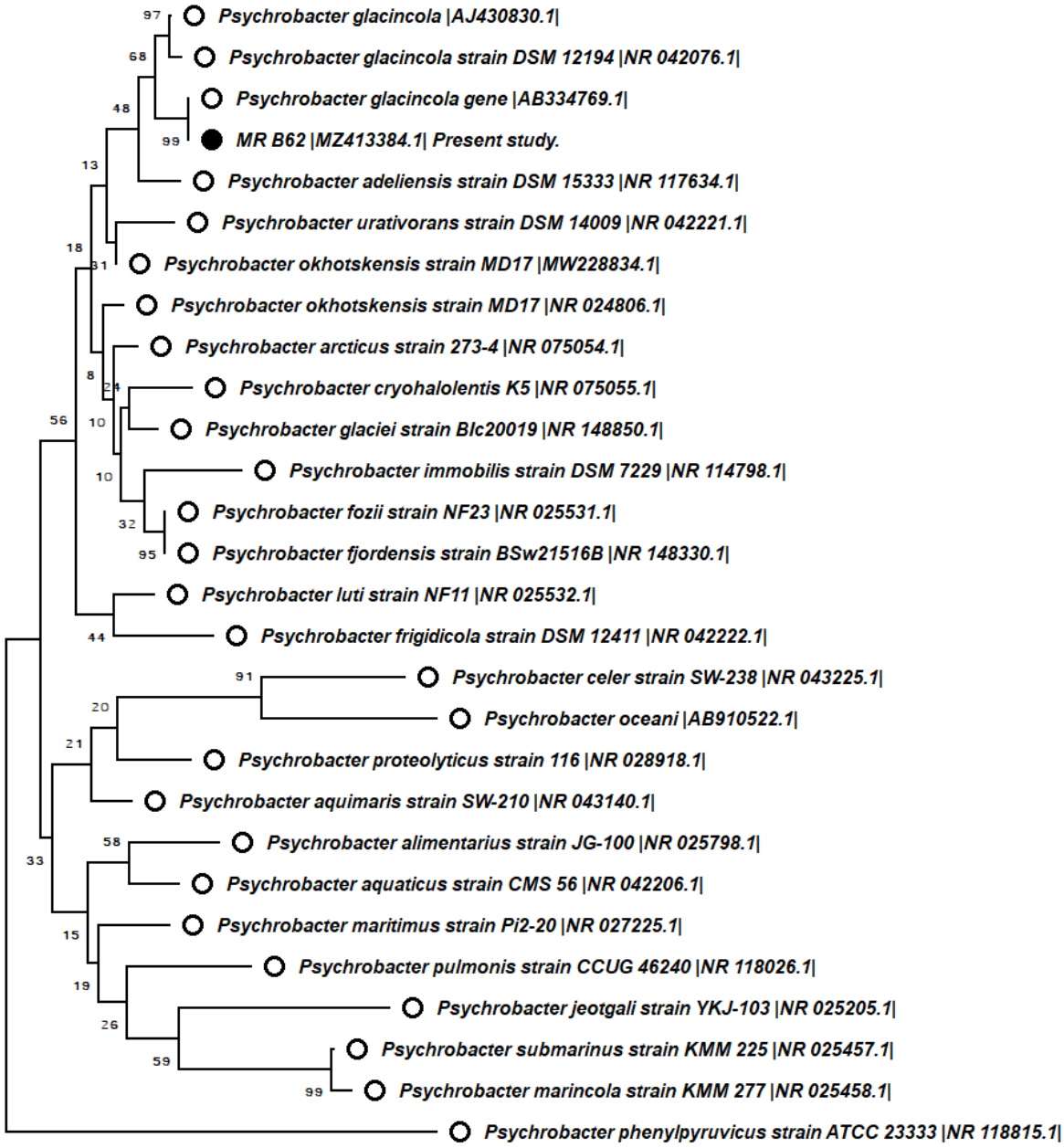

Figure 2

Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain MRB63 and related members within the genus Psychrobacter, showing the evolutionary history. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches.

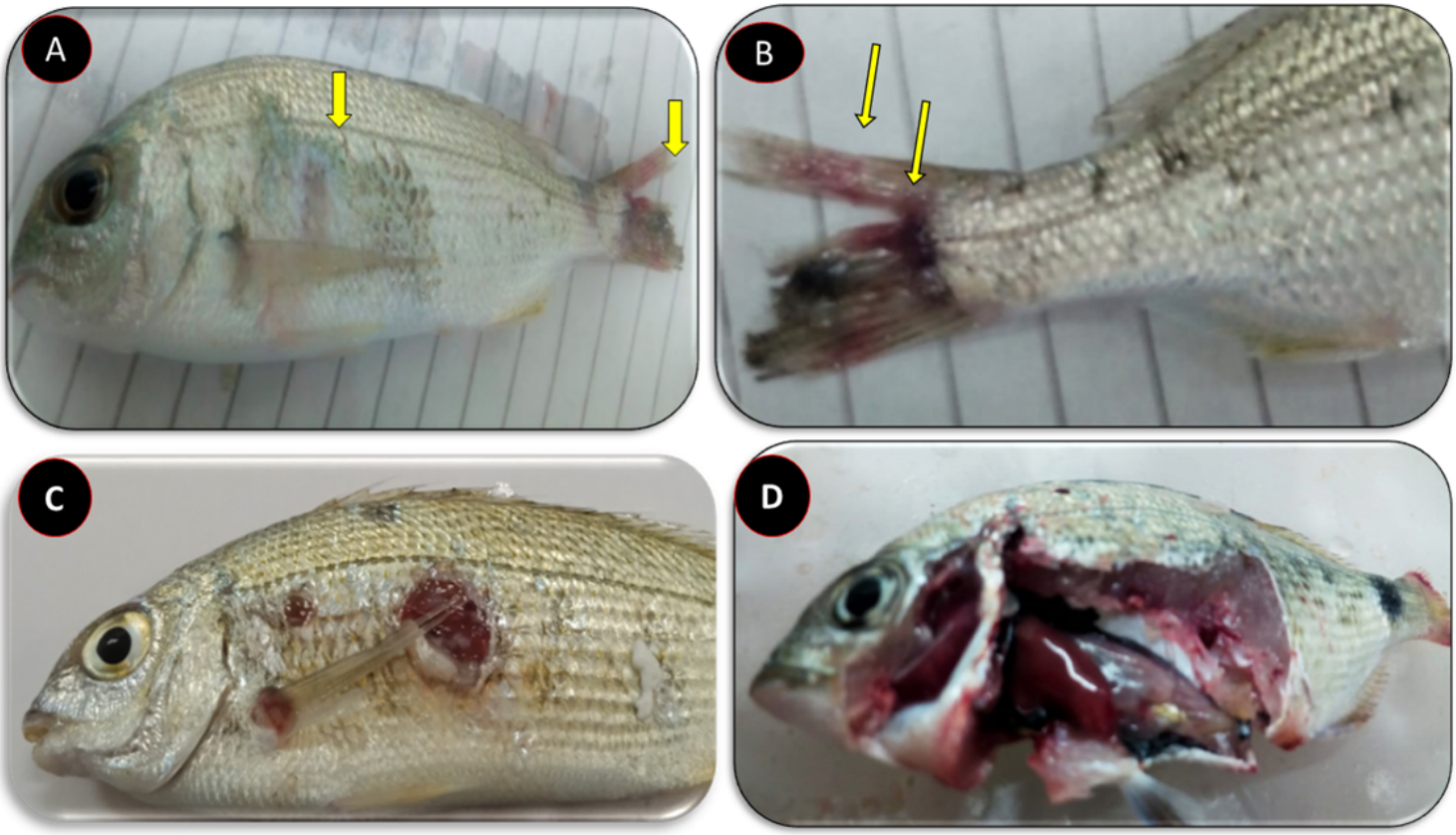

Figure 3

Clinical signs of Haffara seabream (*Rhabdosargus haffara*), experimentally challenged with *P. glacincola*, showing (A) scale loss and tail fin rot; (B) tail fin rot; (C) ulcer on the body; and (D) liver congestion.