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Biochemical and Molecular Identification of the Most Common Streptococci Affecting Common pandora (*Pagellus erythrinus* Linnaeus, 1758) From the Mediterranean Coast of Tripoli

Alaa Eldin Eissa[1*], Abdulatif A. Asheg[2], Abdelsalam Abu Mhara[2], Mahmoud S. Sharaf[1], Awad A. Abdelbaky[1], Amira S. A. Attia[3] Tarek D. Dakhil[2], Alkhateib Y. Gaafar[4], Eman M. Ismail[5], Rasmia H.M. Abu Leila[6], Heba A. Abdel Hady[7], Emad A. Afiffy[8], Abdelbary Prince[9], Reham H. Ragab[1] and Khalid Shahin[10]

[1]Department of Aquatic Animal Medicine and Management, Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, University of Tripoli, Libya
[3]Department of Veterinary Publi Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[4]Hydrobiology Department, Veterinary Research Institute, National Research Centre, Dokki 12622, Giza, Egypt
[5]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt
[6]Department of Fish Diseases, Animal Health Research Institute, Agriculture Research Center, Dokki, Egypt
[7]Department of Virology and Serology, Animal Health Research Institute, Alexandria Provincial Laboratory, Egypt
[8]DSM Nutritional Products Ltd, Wurmisweg 576, 4303 Kaiseraugst, Switzerland
[9]Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt
[10] National Institute of National Institute of Oceanography and Fisheries, Cairo, Egypt
***Corresponding Author:** Alaa Eldin Eissa, **E-Mail:** aeissa2012@cu.edu.eg

ABSTRACT

Streptococcosis is one of the septicaemic bacterial diseases of public health concern due to its zoonotic potential. Its luxurious existence in wild marine fish from an open water body strongly suggests presence of massive sewage pollution. The aim of the current study was to identify and characterize most common streptococci affecting common pandora (*Pagellus erythrinus*) inhabiting the Mediterranean coast of Tripoli. A total number of 270 common pandora were clinically examined for possible streptococcal infection and non-streptococcal infection. The fishes were collected from the area extending from Tripoli to Tajoura (east to Tripoli) during the three seasons, summer, autumn, and spring. No fish samples were available during winter due to the bad climate / storms along the entire western Libyan Mediterranean coasts. *Streptococcus iniae, Streptococcus dysgalactiae, Streptococcus phocae, Enterococcus faecalis,* and other non-streptococcal species, such as *Aeromonas hydrophila, Pseudomonas anguilliseptica,* and *Photobacterium damselae sub species damselae* were biochemically identified. Regardless of the season, *S. iniae* and *E. faecalis* were the most prevalent streptococcal species (13% and 8.88%, respectively). In contrast, the most retrieved non-streptococcal species were *A. hydrophila,* followed by *Pseudomonas anguilliseptica*, with a prevalence of 10%, and 14.44%, respectively. The highest infections were recorded during autumn, followed by summer, then spring with percentages of 53.34%, 50%, and 43.4%, respectively. The majority of the isolates were sensitive to erythromycin, florfenicol, and sulfamethoxazole-trimethoprim. The molecular screening using the sequences of 16S rRNA genes has confirmed the phylogenetic relationship to *S. dysgalactiae, E. faecalis, S. iniae,* and *S. phocae* isolates with similarity percentages exceeding 99.6%. The sequences were deposited in the GenBank with accession numbers (OK033868, OK033869, OK033870, and OK033871). To sum up, the obtained results highly suggests that the common pandora fish from Tripoli coast of the Mediterranean is biologically contaminated with various zoonotic streptococci which could be considered as a biological indicator for municipal sewage pollution.

Keywords: Common pandora, Sewage pollution, South Mediterranean, *Streptococci*, Tripoli.

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INTRODUCTION

The direct dumping of sewage into the seashores of several Mediterranean countries has a tremendous negative impact on the aquatic fauna and flora of The Mediterranean Sea **(Abdallah and Abdallah, 2008)**. The direct discharge of chemical wastes; oil refineries, agrochemicals, car industries, shipping run-offs, and ballistic water; has been accused of being the major immunosuppressive factor in aquatic vertebrates and invertebrates **(Claudet and Fraschetti, 2010)**. These adverse effects on the integrity of the immune barriers in aquatic animals have facilitated the direct biological invasion with an enormous number of bacterial, viral, mycotic, and parasitic invaders **(Shahidul Islam and Tanaka, 2004)**.

Streptococcus species are among the top list of invasive biological pollutants to the marine environment as well as freshwater resources worldwide **(Yanong and Francis-Floyd, 2002; Abdelsalam *et al.*, 2013; Abdelsalam *et al.*, 2015a; Abdelsalam *et al.*, 2015b; Sherif et *al.*, 2020, Numberger *et al.*, 2021)**. Streptococcal diseases in fish were originally identified in 1957 in Japan, affecting farmed rainbow trout **(Hoshina *et al.*, 1958)**. Salmon, Mullet, Golden shiner, Pinfish, Eel, Sea trout, Tilapia, Sturgeon, Striped bass, Seals, Whales, and Turtles have all been proven to be susceptible to infection since then **(Bonar *et al.*, 2003)**. *Streptococcus* has also been recovered from ornamental fish, including Rainbow sharks, Red-tailed black sharks **(Russo *et al.*, 2006),** *Oreochromis niloticus*, and *Mugil cephalus* **(Sherif *et al.*, 2020)**.

Streptococcal infections in fish can result in significant mortality rates (more than 50%) within 3 to 7 days **(Yanong and Francis-Floyd, 2002)**. On the other hand, Some outbreaks are more chronic, with mortalities lasting several weeks and only a few fish dying each day **(Austin and Austin, 2016)**. A typical history indicating that *Streptococcus* is the cause of the disease in a group of fish, may include reports of abnormal swimming behavior, commonly described as spinning **(Yanong and Francis-Floyd, 2002)**.

Streptococcus iniae is a threatening fish pathogen that induced invasive diseases and massive outbreaks in fish farms **(Weinstein *et al.*, 1997)**. Streptococcal infections have been linked to a considerable morbidity and mortality in marine mammals. Several streptococcal species have been isolated, including *Streptococcus iniae* from captive Amazon river dolphins (*Inia geoffrensis*) **(Bonar *et al.*, 2003)**, *Streptococcus equi* subsp. *zooepidemicus* from harbor seals (*Phoca vitulina*), Grey seals (*Haliochoerus grypus*) **(Akineden *et al.*, 2007)**.

The haem-agglutination activity of *S. dysgalactiae* may be attributed to its fimbria-like structures, but in the case of *L. garvieae*, it's unclear which function these structures serve **(Abdelsalam *et al.*, 2009)**. *S. dysgalactiae* showed high adherence to the EPCs, while *L. garvieae* strains didn't satisfactorily adhere to the EPCs. streptolysin S (SLS); a pore-forming secreted cytotoxin; that has been shown to be a virulence factor in the human pathogen *S. pyogenes*, has a homolog in *S. iniae* **(Locke *et al.*, 2007).**

Several candidates, such as surface proteins, capsular polysaccharides, and extracellular secreted products, have been identified by recent molecular studies into factors that contribute to the virulence of *S. iniae*. Furthermore, the recently completed sequencing of *S. iniae's* genome will expedite the identification of new virulence factors and serve as a guide for the development of effective vaccine targets for farmed fish, thereby mitigating the risk of zoonotic infection **(Baiano and Barnes, 2009)**.

The bacterial resistance from different ecosystems is rising at an alarming rate and is becoming a major global concern. The prevalence of resistant bacteria in the environment is determined by the bacteria's exposure to various antibiotics, which results in the development of certain strains of resistant bacteria to specific antibiotics **(Al-Bahry *et al.*, 2009)**. The discovery of resistant *Enterococci* in The Mediterranean aquaculture site shows the existence of a marine reservoir of antibiotic resistances that could be transmitted to virulent strains affected by mariculture in an antibiotic-independent way **(Di Cesare *et al.*, 2012)**. Several reports of antibiotic-resistant *Streptococci* have been reported among marine fishes exposed to long-acting sewage pollution **(Rani *et al.*, 2013; Sherif *et al.*, 2020)**.

One of the biggest issues facing aquaculture is the emergence of antimicrobial resistance (AMR) in farmed fish. Due to the high frequency of bacterial infections in fish, antibiotics are often used, which prolongs their survival in the aquatic environment and promotes the growth of the antibiotics' resistant bacteria **(Preena *et al.*, 2020)**. Aquaculture-related AMR has the potential to spread horizontally by gene transfer to clinically significant natural environment strains, impacting the entire ecosystem **(Kim *et al.*, 2006)**. Antibiotic-resistant bacteria are present in the majority of cultivated fish, even ornamental varieties. The complexity of antibiotic resistance in aquaculture can be untangled with a detailed understanding of gene transfer mechanisms such plasmids, transposons, integrons, and gene cassettes **(Baiano and Barnes, 2009)**.

Sørum (2005) has indicated that fish diseases caused by *Streptococci* are rare. Nonetheless, there have been multiple documented streptococcal outbreaks. It was discovered that a *Streptococcus* species obtained from Japanese yellowtail farms was resistant to tetracyclines, chloramphenicol, lincomycin, and macrolides **(Dallaire-Dufresne *et al.*, 2014)**. Some isolates may spread their drug resistance to other *Streptococci*.

The rise in antibiotic resistance among bacterial pathogens is a significant challenge in human medicine. Researchers studying antibiotic resistance in aquaculture have produced findings that could shed light on how pathogens found in hospitals and other clinical settings pick up and modify genetic structures necessary for their survival when subjected to antibiotic therapy **(Sørum, 2005)**. Resistance genes are most likely regularly transported into hospitals and other comparable settings by human clinical pathogens. The 51-kb R plasmid pTP10 from the multiresistant clinical isolate *Corynebacterium striatum* M82B could serve as an example to illustrate such scenario. DNA fragments originally discovered in soil bacteria as well as in pathogens of plants, animals, fish (*P. damselae subsp. piscicida*), and humans make up this R plasmid **(Kim *et al.*, 2006)**. In bacteria from terrestrial, animal, and human bacterial floras and environments, R plasmids of fish pathogens and aquatic bacteria, like the IncU plasmid, have been found **(Sørum, 2005; Kim *et al.*, 2006)**.

The current study was performed to identify and characterize most common streptococci affecting common pandora (*Pagellus erythrinus*) inhabiting the Mediterranean coast of Tripoli.

MATERIALS AND METHODS

1. Fish sampling

A total of 270 live common pandora (*Pagellus erythrinus*) were captured by fishermen through autumn, summer and spring seasons from the coastal areas between Tajoura (east to Tripoli) to Janzour (west to Tripoli). Live fish were anaesthetized in an anaesthetic bath containing a sedative dose of MS-222 **(AL-Taee *et al.*, 2021)** (Sigma Aldrich Co., St. Louis, MO, USA) before sample collection and clinical examination to minimize the possible handling stress Samples were divided equally over three seasons (summer, autumn, and spring) (90 fish / season). The captured fish' average weights and lengths were 250±15 g and 20±5 cm respectively.

It is worthy to mention that winter season was void of samples due to bad climatic conditions including wind-driven sea waves and storms through the western Libyan Mediterranean coast. Euthanized fish were kept on crushed ice in an insulated icebox and sent to the Poultry and Fish Diseases laboratory, Faculty of Veterinary Medicine, University of Tripoli 2-4 hours after collection at most.

2. Clinical examination

To remove external contaminants, the fish were swapped with 70% ethanol. Using the three lines technique, fish was cut open from the left side to expose the liver and other internal organs **(Eissa, 2016)**. Before sampling, fish were checked externally and internally for any apparent gross findings. Any observed clinical findings were directly photographed with a digital camera.

3. Bacteriological examination

Following clinical examination, loopfuls from kidneys and brains were streaked onto blood agar (BA) (CM0055, Oxoid, UK) supplemented with 2 % NaCl, and KF *Streptococcus* agar base (SAB) plates (BO0914T,Fisher-Scientific, Carlsbad , USA) with triphenyl tetrazolium chloride (TTC) supplement. The inoculated plates were then incubated at 25-37°C for 24-72 hrs. The bacteriological isolation protocol was adopted from **Eissa *et al.* (2021)**. The colonial characteristics were compared to the standard criteria published by **Austin and Austin (2016)**.

4. Biochemical identification

The recovered isolates were biochemically identified following identification scheme descried by **Austin and Austin (2016)** morpho-chemical criteria for *Streptococcus* / *Enterococcus* species. The retrieved isolates were presumptively characterized using conventional biochemical tests (Gram reaction; oxidase; catalase; hemolysis pattern on blood agar; salt tolerance (2%, 3%, and 6.5%); culture at 25°C, and 37°C; growth on bile esculin agar; carpenicillin sensitivity), which were further confirmed using the commercial miniaturized API 20 Strep system (Biomérieux, France) according to the manufacturer's instructions.

5. Antibiogram

The antibiotic susceptibility of the retrieved bacterial isolates was evaluated using the Kirby Bauer disc diffusion method described by **Bauer (1966),** and **NCCLS (1999)**. Mueller-Hinton agar (MHA) (Oxoid, Hampshire, UK) supplemented with 2% (w/v) sodium chloride was used for in vitro antimicrobial susceptibility testing , which was incubated at 25°C and 37°C for 24-48 hrs. Antibiotic inhibition zones were measured in mm using a measuring caliber at the end of the incubation time.

6. Sequencing of 16S rRNA

The extraction of bacterial DNA was performed using the PrepMan® Ultra Sample Preparation Reagent protocol (Applied Biosystems, USA). The PCR amplification of the 16S rRNA gene was performed using the universal pair primers (F-5′-AGAGTTTGATCCTGGCTCAG-3′) and (R-5′-AAGGAGGTGATCCAGCC-3′); described by **Gross *et al.* (2012)**. PCR reactions was done according to the protocol described by **Eissa *et al.*, (2020)**. PCR products were purified using GeneJET™ Gel extraction kit (Thermo Scientific, Waltham, USA). The sequencing process of the 16S rRNA genes of four bacterial strains was performed in two directions using ABI 3730XL DNA sequencer at Macrogen sequencing company (Macrogen, Seol, South Korea). The raw sequences were edited and assembled using Bio Edit version 7.0 **(Hall, 2004)**. The identity of bacterial isolates was confirmed by aligning the assembled sequences with other interrelated isolates deposited in the database of GenBank. The neighbor-joining phylogenetic tree was created using MEGA version X, and the evolutionary distance calibrated by the Kimura2-parameter method with 1000 replicates of bootstrap value was applied **(Kumar *et al.*, 2016)**.

7. Prevalence of infection among sampled common pandora

The prevalence of infection among different *P. erythrinus* collected through different sampling intervals were estimated by calculating the number of infected fishes divided by the total number of examined fish multiplied by 100.

8. Ethical declaration

The current study was ethically carried in compliance with University of Tripoli Animal Care Committee and following regulations for the care of animals in research. All the examined fishes were anaesthetized in an anaesthetic bath containing a sedative dose of MS-222 (Sigma Aldrich Co., St. Louis, MO, USA) before the clinical examination and the sample collection to minimize the possible handling stress **(AL-Taee *et al.*, 2021)**. Fish euthanasia for sample collection was done according to the most recent guidelines of **AVMA (2020).**

RESULTS

1. Clinical examination

There were no apparent pathognomonic signs, and most of the examined fish were apparent healthy and close to normal. However, some fish showed fin congestion and erosions **(Fig.1)**. Internally, the clinical examination showed moderate hemorrhage in the kidney, stomach, and swim bladder, as well as congestion with moderate hemorrhage in brains of the examined fish **(Fig. 2)**.

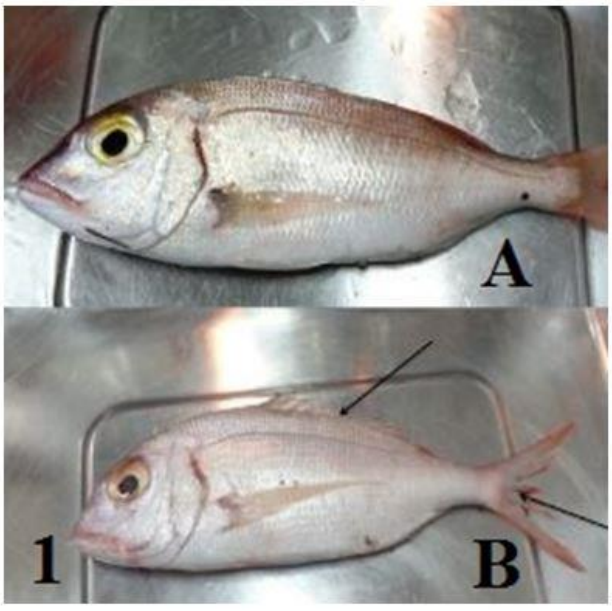

Fig. 1: An apparently healthy *P. erythrinus* (A), *P. erythrinus* with fin erosion and congestion (B).

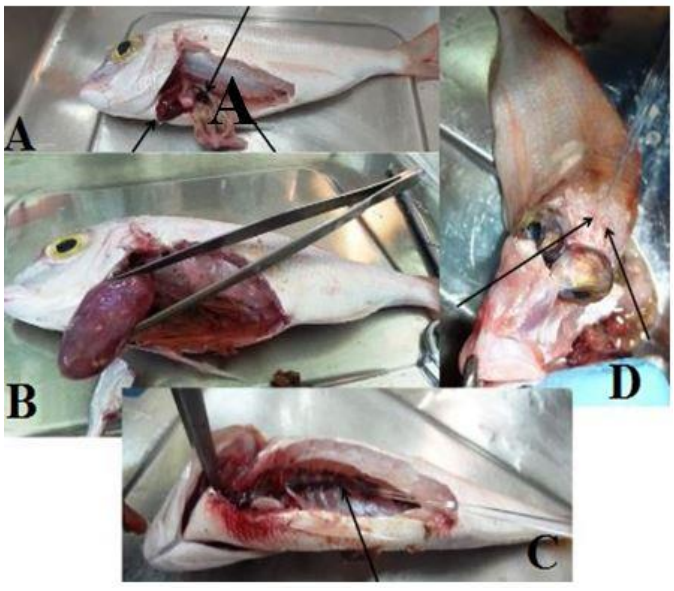

Fig. 2: Mildly congested internal organs of *P. erythrinus* (A), stuffed and congested stomach; congested swim bladderof sampled *P. erythrinus* (B), *P. erythrinus* with hemorrhagic kidney (C), *P. erythrinus* with a congested brain (D).

2. Prevalence of *Streptococci* in the examined *P. erythrinus*

Some bacterial species were recovered from *P. erythrinus* fish with different prevalence percentages in the coastal area of Tripoli, namely; *Streptococcus iniae, S. dysgalactiae, S. phocae, Enterococcus fecalis,* and other non-streptococcal spp **(Table 1)** . *Aeromonas hydrophila, Pseudomonas,* and *Photobacterium damselae. S. iniae* was the highest prevailed streptococcal spp during summer (13 %) and spring (10 %) while *E. fecalis* was the highest in autumn (8.88 %). Among non-streptococcal spp, *A. hydrophila* was the most prevailed species during summer (10 %) and spring (7.77 %), while in autumn; *Pseudomonas* was the most recovered (14.44 %) **(Table 1).**

Table 1: Percentages of retrieved isolates from sampled *P. erythrinus* (n=90/season).

Bacterial Groups	isolates identity	Summer		Winter	Spring		
		Number of isolates/90 fish	%	Number of isolates/90 fish	%	Number of isolates/90 fish	%
Streptococcal	*S. iniae*	12	13.33	7	7.77	9	10
	S. dysgalactiae	8	8.88	6	6.66	7	7.77
	S. phocae	5	5.55	10	1.11	5	5.55
	E. fecalis	7	7.77	8	8.88	6	6.66
No growth	No growth	45	50	42	46.66	51	56.6
Non Streptoccal	*A. hydrophila*	9	10	4	4.44	7	7.77
	Pseudomonas	0	0	13	14.44	4	4.44
	Photobacterium damselae	4	4.44	0	0	1	1.11

3. Biochemical identification

The results of biochemical examination using the conventional biochemical test as well as the miniaturized API 20 Strep test strips have revealed that the retrieved *Streptococci* were categorized into 4 primary isolates *S. iniae, S. dysgalactiae, S. phocae,* and *Enterococcus faecalis* **(Table 2).**

Table 2: Phenotypic and biochemical characteristics of the retrieved *Streptococci.*

Criteria	*Streptococcus iniae*	*Streptococcus dysgalactiae*	*Streptococcus phocae*	*Enterococcus faecalis*
Sheep blood agar with 2% NaCl	*β*-hemolysis	*α*- hemolysis.	*β*-hemolysis	*ɣ*- hemolysis
Gram staining	+	+	+	+
Catalase	-	-	-	-
Oxidase	-	-	-	-
Voges–Proskauer test	-	-	+	+
Hydrolysis of Esculin	+	-	-	+
α-Galactosidase	-	-	-	-
β-Glucuronidase	+	+	+	-
β-Galactosidase	-	-	-	-
Alkaline phosphatase	+	+	+	-
Arginine hydrolysis	+	-	+	+
Acid from Ribose	+	+	+	+
Acid from Arabinose	-	-	-	-
Acid from Mannitol	+	-	+	+
Acid from Lactose	-	+	-	-
Acid from Sucrose	-	+	-	-

4. Antibiogram of the retrieved *Streptococci*

The retrieved isolates of *S. iniae* and *S. phocae* were sensitive to erythromycin, florfenicol, and sulfamethoxazole-trimethoprim. Likewise, *S. dysgalactiae* isolates were sensitive to florfenicol, kanamycin, erythromycin, and sulfamethoxazole-trimethoprim. *E. faecalis* isolates were only sensitive to florfenicol, kanamycin, erythromycin, and sulfamethoxazole-trimethoprim.

5. Molecular identification of the retrieved *Streptococci*

The sequences of 16S rRNA genes belonging to *S. dysgalactiae, E. faecalis, S. iniae,* and *S. phocae* isolates were deposited in the GenBank with the following accession numbers (OK033868, OK033869, OK033870, and OK033871), respectively. The identity of these isolates was confirmed by sequencing 16S rRNA and comparing the oligonucleotide sequences with other relevant genes deposited in GenBank. The accession number (OK033868) yielded 1461-bp and showed 99.79 % similarity with *S. dysgalactiae* (AB537916 and AB537914), and 99.73% similarity with *S. dysgalactiae* (CP033166 AP018726, AB537917, and AB102730). Therefore, the accession number OK033868 was confirmed to be *S. dysgalactiae*. On the other hand, the accession number (OK033869) was 1311-bp and exhibited 99.69% similarity with *E. faecalis* (JX536104- CP028724- LR962620), and 99.62% similarity with *E. faecalis* (MW175606- MW175607- LR961966- KC465417). Therefore, the accession number OK033869 was confirmed to be *E. faecalis*. The accession number (OK033870) yielded 1482-bp and showed 99.86% similarity with *S. iniae* (LC378582 and NR_025148), and 99.80% similarity with *S. iniae* (CP032401, CP032400, LC378579, and KY118915). Therefore, the accession number OK033870 was confirmed to be *S. iniae*. The accession number (OK033871) yielded 1414-bp and displayed 99.86% similarity with *S. phocae* (HM032023), and 99.79% similarity with *S. phocae* (MK841551, EF599165, and NR_133868). Consequently, the accession number OK033871 was confirmed to be *S. phocae*. The neighbor-joining phylogenetic analysis showed that the four bacterial strains (*S. dysgalactiae, E. faecalis, S. iniae,* and *S. phocae*) were clustered with their correlated accession numbers of relevant bacterial species and separated from each other **(Fig.3)**.

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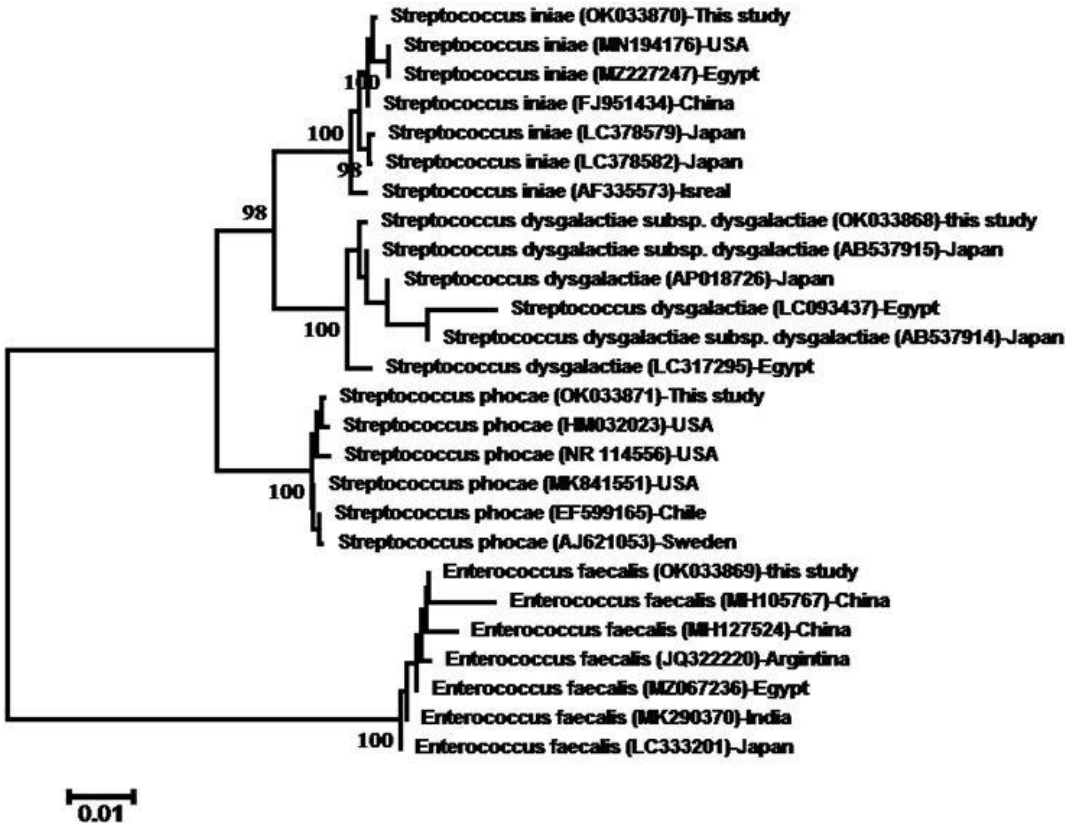

Fig. 3: The neighbor-joining phylogenetic tree showed the comparative analysis of the 16S rRNA gene sequence of *S. dysgalactiae, S. iniae, S. phocae, E. faecalis,* and other interrelated bacterial strains on GenBank.

DISCUSSION

Streptococcus. iniae has not yet been placed to a Lancefield group and is *β*-hemolytic on blood agar, with some clinical strains reported from Asia being more mucoid than others **(Lau *et al.,* 2006)**. Furthermore, due to their abundance in faeces and lengthy survival in the environment, *Enterococcus* and *Streptococcus* species have been considered as biomarkers of faecal pollution in water. Despite the fact that the ratio of faecal coliforms to faecal *Streptococci* has been ruled out as an indicator **(Rivera *et al.,* 2010)**, species identification associated with a specific environment or host may provide additional information about the origin of isolates and the source of faecal contamination **(Gerba, 2009)**.

This study proposes a method for quickly identifying clinical and environmental *Enterococcus* species. That was determined by 12 biochemical tests. With some exceptions, the identification limit was more than 99 % (87, 91.5, and 97 % for *E. avium*, *E. hirae*, and *E. durans*, respectively). The use of sucrose fermentation to discriminate *E. avium* from *E. raffinosus* reduced the identification threshold in the key.

Enterococcus faecalis isolates were morpho-chemical confirmed based upon consistency with standard criteria published by **Blanch *et al.* (2003),** and **Austin and Austin, (2016)**. Interestingly, the prevalence of *E. faecalis* in the sampled *P. erythrinus* during spring (6.66 %), and summer (7.77%) was less than that achieved in autumn (8.88 %). These results apparently contradicted the achieved results in several epidemiological studies where summer and spring were commonly the highest seasons in *E. faecalis* prevalence **(Blanch *et al.,* 2003; Austin and Austin, 2016)**.

In the current study, the achieved morpho-chemical criteria for *S. iniae* suspect isolates were consistently accordant with the standard criteria of ATCC *S. iniae* isolates and many other *S. iniae* confirmed isolates described in several fish microbiological literatures edited by **Buller (2014)**. The prevalence of the retrieved *S. iniae* isolates was the highest in summer (13.33 %), followed by spring (10%), and autumn of the same year, which represented the lowest prevalence (7.7%). Summer season allows an enriched media for the establishment, growth, and spread of parasites which are famous vectors for transmitting bacterial fish pathogens, including *S. iniae*. Moreover, summer is the peak of the breeding cycle of the *P. erythrinus*, where they exist in larger numbers in the coastal areas of Tripoli with a consequent increase in the number of exposed fish to biological pollutants sheded from parasitic vectors heavily attached to them, or from the continuously dumped sewage. However, the higher temperature is a detrimental factor in the dynamics and water chemistry of the sea, whereas levels of oxygen are highly declined in hot water together with a gradual increase of ammonia. Together with the external parasites' breakdown, these deteriorated water chemistries would have triggered remarkable immunosuppression in the cohabitating fishes with an ultimate heavy invasion with *S. iniae* and other seasonal bacteria such as *Photobacteriaum damsalae* subspecies *damselae*, which have been concurrently isolated from the sampled *P. erythrinus* during summer, 2019. These assumptions coincided with a similar epidemiological survey of the most prevalent pathogens in gilthead seabream and sea bass from The Mediterranean coasts of Egypt **(Moustafa *et al.,* 2014; Moustafa *et al.,* 2015)**.

ß-haemolytic *S. phocae* was isolated in seals and has been linked to Atlantic salmon disease in Chile since 1999; Chilean isolates are genetically, serologically, and phenotypically homogeneous **(Valdés *et al.,* 2009)**. Our retrieved isolates were Gram-positive cocci arranged in chains and hemolytic on blood agar supplemented with 5% sheep RBCs in the ongoing study. The isolates were biochemically capable of generating acid from D-Fructose, Maltose, N-Acetylglucosamine, and Ribose. Isolates were positive for alkaline phosphatase production. No growth was obtained on 40 % bile, 10°C, and 45°C. The isolates were susceptible to Bacitracin. The above-mentioned results coincided with the standard morpho-chemical criteria of *S. phocae* **(Romalde *et al.,* 2008)**. The highest prevalence of *S. phocae* was reported in autumn (11 %), while that in summer, and spring were equal (5.5 %).

This result is not epidemiologically understandable except if the harbor host (seals, otters, dolphins) are gathered after their migration during the autumn season at closer distances to areas where *P. erythrinus* was collected than in summer or spring. Also, in autumn, *P. erythrinus* are scanty in number in the deep zones of The Mediterranean Sea, where they travel to warmer coastal zones for food and stabilization. This would have triggered seals to travel to more coastal zones where their prey fish are available. Consequently, the exposure time between *S. phocae* harbor hosts (seals) would have increased with the ultimate increase in prey fish infection or at least carrying the pathogen on their mucous or skin. This assumption is relatively consistent with the epidemiological patterns of *S. phocae* in seals reported by **Skaar *et al.,* (1994)**.

Streptococcus dysgalactiae is the only pyogenic *Streptococcus* that is not *β*-hemolytic **(Igwe**

***et al.*, 2003)**. The causative agent was identified as the α-hemolytic Lancefield group C *Streptococcus dysgalactiae* subsp. *dysgalactiae* [GCSD]. The syndrome caused by GCSD is characterized by a systemic multifocal inflammatory reaction, micro-abscessation, acute septicemia, and significant mortality rates in fishes with pathognomonic necrotic ulcers at the caudal peduncle area **(Abdelsalam *et al.*, 2010b; Sherif *et al.*, 2020).** GCSD has been identified as the primary cause of various mammalian diseases, including streptococcal mastitis/endometritis in domestic mammals, as well as skin lesions, meningitis, and bacteremia in humans **(Koh *et al.*, 2009)**. In the current study, our isolates were α hemolytic on blood agar supplemented with 5 % sheep RBCs which have turned into *β*-hemolytic after a long time. Also, there were no growths at 10, 45ºC or pH 9.6. All suspect isolates were able to grow in the presence of 40 % bile salts. The isolates were negative for V.P., bile esculin, sodium hippurate hydrolysis, pyridinyl amidase, and α or *β*-galactosidase **(Yang and Li, 2009)**. They were cable of producing acid from Trehalose and amygdalin.

The above-mentioned results were accurately consistent with the standard morpho-chemical criteria of *S. dysgalactiae* reported by **Yang and Li (2009)**. The achieved prevalence of the retrieved *S. dysgalactiae* was the highest in summer (8.88 %), followed by spring (7.77 %), then autumn (6.6 %). These prevalence are consistent with similar results obtained by **Abdelsalam *et al.*, (2010a),** and **Buller, (2014)**. Those studies have confirmed that the highest prevalence of *S. dysgalactiae* is remarkably high during the summer season and, to a small extent, the late spring. Further, the deteriorated water quality during the summer season, as previously described, would have resulted in favoring the growth of *S. dysgalactiae*, and *E. faecalis* more than *S. iniae*, and *S. phocae*. Since the beginning of this century, *Enterococci*, formerly known as faecal *Streptococci*, have been recognized as being of faecal origin.

Enterococcus species typically live in the intestines of humans and other animals. *Enterococci*. On the other hand, they are ubiquitous and can be found free-living in soil, on plants, or in dairy products **(Fortina *et al.*, 2004; Naser *et al.*, 2005; Gerba, 2009)**. *Enterococci* are part of the normal intestinal microbiota of humans and animals, and they are regarded as indicators of fecal contamination of recreational water. However, they can also be isolated from natural ecosystems that haven't been contaminated by faecal material **(Wright *et al.*, 2009)**. Depending on the disease type, infections are treated with a course of antimicrobial drugs such as penicillin, ampicillin, amoxicillin, cloxacillin, cefazolin, and/or gentamicin, doxycycline, and trimethoprim/sulfamethoxazole for 1 to several weeks **(Koh et *al.*, 2004; Koh *et al.*, 2009)**. This could explain the type of antibiotic resistances recorded in this study.

CONCLUSION

The uprising dangers of sewage pollution through the southern Mediterranean coasts were confirmed to have very deleterious effects on living aquatic animals including fish, shellfish and marine mammals occupying these coasts. The luxurious isolation of pathogenic of pathogenic *Streptococci* originating from marine mammal (*S. phocae*, *S.iniae*), human / animal (*S. dysgalactiae*), and sewage (*E. feacalis*) explains the magnitude of sewage pollution through the Southern Mediterranean coasts. Moreover, the reported bacterial resistance against wide-spectrum of commercial antibiotics is an alarming indicator about the potentials/magnitudes of Multidrug resistances expected to occur among consumers of marines fishes caught from these polluted Mediterranean coasts. Ultimately, the current study highlighted the urgent need for large scale regional study to determine the actual potentials of Multidrug resistant bacteria and their public health impacts on inhabitants of The North African Mediterranean coasts.

Availability of data and materials

All data are included in the manuscript.

Conflicts of interest

All authors declare that they have no conflict of interest.

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Author's Contributions

All authors equally contributed to the manuscript.

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