Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 1, 180-186

Multiple antibiotic resistant *Streptococcus agalactiae* and *Streptococcus iniae* in Nile tilapia aquaculture

Noura Kelany, Hosnia Abdel-Mohsein*, Saber Kotb, Abd El-Moez Ismail

Department of Animal, Poultry Hygiene and Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University, 71526 Egypt.

ARTICLE INFO

Recieved: 23 October 2023

Accepted: 25 December 2023

***Correspondence:**

Corresponding author: Hosnia Abdel-Mohsein
E-mail address: hosnia18@aun.edu.eg

Keywords:

Tilapia
Streptococcus
PCR
Antibiotic resistance
Biofilm

ABSTRACT

Streptococcosis is a zoonotic bacterial disease in tilapia and other fresh that creates economic losses, caused mainly by *Streptococcus agalactiae* and *Streptococcus iniae*. The hazard of the disease is that it may present apparently healthy as well as its presence in culture water. So, this work aimed to detect streptococci in Nile tilapia farms located in Assiut and Minia Governorates, Egypt. *Streptococcus agalactiae* (42 isolates) and *Streptococcus iniae* (59 isolates) were identified. Two *Streptococcus agalactiae* isolates were molecularly confirmed and exhibited resistance to most antibiotics and a multiple antibiotic resistant index of 0.9-1. The identified isolates could not form biofilm. Identification of these hemolytic, resistant *Streptococcus* poses a potential hazard for human health; hence, alternative antimicrobials should be searched against infections caused by these bacteria.

Introduction

Fish offers the most promising source of safe, highly nutritious protein food (Rasco *et al*., 2015). Nile tilapia (*Oreochromis niloticus*) is among the most important farmed aquatic species in the world, cultured in more than 170 country worldwide (Cai *et al*., 2019; FAO, 2020). Interestingly, the world production of farmed tilapia reached 6.03 million tons in 2018, which further consolidated tilapia as the second-most important farmed finfish group (Miao and Wang, 2020). Egypt is the topmost tilapia producer in Africa with more than 1,000,000 tons produced in 2018 (FAO, 2020), representing a cornerstone of tilapia fish farming (Ali *et al*., 2020b). Tilapia have many characteristics that make them an ideal candidate for aquaculture, such as their herbivorous feeding nature, fast growth, tolerance to a wide range of environmental conditions and ability to reproduce in a short generation time (El-Sayed, 2006). However the culture of tilapia under intensive systems can pose a stressful conditions (Van Doan *et al*., 2019). These stressors unfavorably alter the fish immune system, making it vulnerable to infection (Portz *et al*., 2006).

Streptococcus infection represents the most important threat to tilapia culture in Egypt, causing several outbreaks with devastating mortalities mainly in summer season (Alazab *et al*. 2022). In Egypt, *Streptococcus agalactiae* and *Streptococcus iniae* have been considered the two primary species causing streptococcosis in tilapia culture resulting in significant economic losses in the aquaculture industry (Younes *et al*., 2019; Ghetas *et al*., 2021). Globally, streptococcosis has become one of the most violent aquatic diseases, resulting in massive mortality of up to 50% and economic loss yearly as high as $150 million (Jansen *et al*., 2019; Niu *et al*., 2020). *Streptococcus* adherence, colonization, and invasion are related to their virulence factors, as surface pili and proteins (Kaminska *et al*., 2020). These virulence factors contribute to *Streptococcus* biofilm formation (Konto-Ghiorghi *et al*., 2009). Bacterial biofilms facilitate bacterial persistence under environmental stresses and provide protection against antibiotics (Kaur *et al*., 2009;, Rosini and Margarit, 2015).

Moreover, *Streptococcus iniae* and *Streptococcus agalactiae* are human pathogens (Erfanmanesh *et al*., 2012; Zhang *et al*., 2017). Zoonotic Streptococcosis infections occur due to direct contact with a diseased or dead fish and indirect contact with contaminated water resulting in the development of cellulite, endocarditis, meningitis, severe systemic infections, and rarely death in human (Koh *et al*., 2009; Ziarati *et al*., 2022). Additionally, fish consumption has been associated with an increased risk of *Streptococcus agalactiae* colonization in humans (Foxman *et al*., 2007). Therefore, fish farmers are forced to use antibiotics to avoid the threat of infection, which may pose a risk to the aquatic environment and consumer health (Rico *et al*., 2014). Misuse of antibiotics not only induces bacterial resistance but also plays a role in their biofilm formation capabilities (Kaplan, 2011).

Due to insufficient diagnostic facilities at the fish farms, the majority of causative agents responsible for diseases remained uncharacterized, leading to an inappropriate treatment process that further increases fish mortality (Akter *et al*., 2021). Due to *Streptococcus* phenotypic diversity, it is not possible to be identified accurately, so it is essential to search for accurate diagnostic methods to apply the effective treatment (Franco-Duarte *et al*., 2019). Polymerase chain reaction is a highly specific molecular tool for *Streptococcus* identification (Abdelsalam *et al*., 2017; Deng *et al*., 2019). So, this study aimed to isolate and identify *Streptococcus* species from tilapia fish and their aquaculture water of tilapia cultured

ISSN: 2090-6277/2090-6269/

ponds in Assiut and Minia Governorates. Additionally, to evaluate their antibiotic susceptibility and biofilm-producing abilities.

Materials and methods

Ethical approval

The examined tilapia fish in the current research were caught, transported, and examined with permission (Number: 06/2023/0114) of the ethical committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Study area

Fish and water samples were collected during the period from December 2021 to June 2022 in routine farm fishing. The investigation was carried out in three fish farms through four visits, two farms in Assiut (A, B) and one in Minia (C), Egypt.

Water and tilapia samples

A total of seventy water samples in sterile falcon tubes and seventy-nine apparently healthy tilapia fish with water in sterile containers were transported in an ice box to the Animal and Poultry Hygiene and Environmental Sanitation Department laboratory, Faculty of Veterinary medicine, Assiut University, Egypt.

Streptococcus isolation

In the laboratory, total and standard lengths, and weights were measured for each fish. Tilapia bodies were cleaned from dirt with sterile distilled water, and their scales were removed. The fish skin was disinfected by alcohol (70%), then their bellies were aseptically opened with sterile scissors. Swabs were collected aseptically from the liver, kidney, intestine, spleen, and gills of each fish. A sterile loop was inserted through the sterilized area, and then the inoculum was transported into trypticase soya enrichment broth (Biomark Laboratories, India). The inoculated tubes were incubated at 37oC with 5% CO_2 for 24 hours. Then inoculums from the enriched broth were streaked into *Streptococcus* selective media (Biolife Laboratories, Italiana) supplemented with 5ml/L sheep blood. For each sample, a maximum of four colonies, which presumptively appeared pen-headed, white opaque in color, circular, with entire raised edges, and glistening appearance were picked up and inoculated in trypticase soya agar slants for identification. Water samples were examined according to Standard Methods for the examination of water and wastewater examination (APHA, 2017), where one ml from each sample was transferred aseptically to a sterile trypticase soya broth tube, then incubated at 37°C with 5% CO_2 for 24 hours.

Streptococcus biochemical identification

Presumptive identification was performed according to Buller (2004), using conventional biochemical characters including catalase negative, Gram positive, urease negative, citrate negative, urease production, and hemolytic activity in sheep blood agar. Further identification of the isolates was performed according to the methods described in Bergey's Manual® of Systematic Bacteriology (Vos *et al*., 2011). Identified isolates were kept at −20°C in trypticase soya broth with 25 % glycerol for further molecular identification.

Molecular identification of Streptococcus species

The species diversity of *Streptococcus* fish pathogens highlights the troubles of confirmed identification of *Streptococcus* based only on phenotypic traits, so it is important to molecularly identify these pathogens (Baeck *et al*., 2006). After biochemical identification, twenty-three *Streptococcus agalactiae*, and thirteen *Streptococcus iniae* isolates were selected for molecular identification.

DNA extraction

Genomic DNA of the isolates was extracted using the boiling method as described by Queipo-Ortuño *et al*. (2008). One µL from the extracted DNA was used for estimating the purity and concentration using the GeneQuant® 1300 Spectrophotometer (Bioscience, Sweden). The A260/A280 ratio was calculated and ratios between 1.8 and 2.0 were accepted for DNA purity.

PCR conditions and amplification

PCR reactions were carried targeting the intergenic space of *Streptococcus agalactiae* using 16-23S intergenic space primer STRA-AgI: 5′AAGGAAACCTGCCATTTG-3′, STRA AgII:5′TTAACCTAGTTTCTTTAAAACTAGAA-3′ (Phuektes *et al*., 2001). Lactate oxidase (lctO) gene of *Streptococcus iniae* was investigated using lactate oxidase (lctO) gene with the following sequence; LOX-1:(5′AAGGGGAAATCGCAAGTGCC-3′, LOX-2: 5′ATATCTGATTGGGCCGTCTAA-3` according to Mata *et al*. (2004). Amplification of each DNA was performed in a reaction mixture containing 5 µL of Willowfort Master Mix, 1 µL (10 µM) of each forward, and reverse primer, 3 µl of DNA template, and DNase-free water up to a total volume of 20 µL. The amplifications were carried out in a Veriti thermocycler (Applied Biosystem) with the following conditions: primary denaturation at 95°C for 5 minutes, followed by 40 cycles at 95°C for 1 minute, annealing temperature at 58°C for 30 seconds, extension at 72oC for 45 seconds and final extension at 72°C for 10 minutes for the 16-23S intergenic spacer region. While for the lactate oxidase (lctO) gene, the reaction was carried out with initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 1 minute, varying annealing temperatures at 50, 52, 56, 58, and 61°C for 60 seconds, extension at 72°C for 2 minutes and final extension 72°C for 10 minutes.

The amplified PCR products of both bacterial isolates were separated by electrophoresis on a 1% agarose gel. The gel was placed in a UV band illuminator and photographed. For evaluation of PCR products, the band appeared at 270 bp for the 16-23S intergenic spacer region primer, and 870 bp for lactate oxidase primer.

Antibiotic sensitivity testing

The confirmed two isolates were evaluated for their antibiotic sensitivity for the following antibiotics: chloramphenicol (30 mcg), streptomycin-S (10 mcg), doxycycline–D (30 mcg), colistin-CT (10 mcg), trimethoprim/sulfamethoxazole-SXT (1.25/23.75mcg), norfloxacin-NOR (10mcg), ciprofloxacin-C (30 mcg), amoxicillin-AX (25 mcg), and novobiocin-NV (30 mcg). The sensitivity test was carried out according to Verma *et al*. (2023) with modifications. Briefly, homogenous bacterial suspensions of 0.5 McFarland turbidity standards were prepared from fresh bacterial cultures incubated for 18 hours. Each bacterial suspension was spread on Mueller–Hinton agar with 5% sheep blood using a sterile cotton swap. After dryness, specific antibiotic discs were placed on the lawn using sterile forceps then the plates were incubated overnight at 37°C. The developed zone of inhibition was manually measured and the results were interpreted as susceptible, intermediate or resistant according to the interpretative criteria following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2013; 2017; EUCAST, 2023). The multiple antibiotic index (MAR index) was calculated according to Krumperman (1983) to determine the dissemination and prevalence of bacterial resistance in the isolated strains (Kinge *et al*., 2010).

Biofilm evaluation assays

Biofilm evaluation assays were performed as described by Silvestre *et al.* (2020), with modifications. Briefly, bacterial suspension was prepared in trypticase soya broth from overnight cultures in blood agar. Biofilm experiments were performed in 96-well flat- bottom polystyrene cell culture plates using trypticase soya broth with or without 1% glucose. Each strain was inoculated in triplicates where 180 µl of sterile broth was added to the wells and then 20 µl of the bacterial suspension was added. Two hundred microliter of broth-only (with or without glucose) were used as blank. The microplates were incubated at 37°C under 5% CO2 for 48 hours. After incubation, the well content was removed, and each well was vigorously washed thrice with sterile distilled water. The attached bacteria were fixed with 200 µl methanol for 15 minutes then washed three times with distilled water and stained for 15 minutes with 0.2% crystal violet. The stained wells were washed three times with distilled water to remove excess dye and allowed to dry at room temperature. The plate was inspected for the development of biofilms on the bottom and walls of the wells.

Statistical analysis

The result was represented as a percentage using Excel (Microsoft Office 2013). The obtained data were subjected to statistical analysis using the SPSS software, version 22. The data weren`t normally distributed and weren`t homogenous.

Results

The tested fish lengths and weights are presented in Table 1.

Streptococcus species on *Streptococcus* selective media supplemented with blood appeared as small pinpoint colonies with a smooth, white regular edge surrounded by a beta-hemolytic zone. The bacterial isolates were biochemically identified, and only Gram-positive cocci, non-motile, catalase-negative, and hemolytic colonies were further differentiated into *Streptococcus agalactiae* and *Streptococcus iniae* based on their variable reactions in different reactions like mannitol fermentation, hippurate hydrolysis, and esculin hydrolysis. Of the 79 tilapia, 70 isolates (69.3%) were identified as *Streptococcus* species, and from the 70 water samples 31 isolates (30.69%) were identified as *Streptococcus* species. In tilapia, the highest percentage of isolates were identified from the intestine (27.14%), then the kidney (22.85%), followed by the gills (21.42%), then the liver (20%), and finally, the spleen (8.57%) as shown in Table 2.

According to Table 3, *Streptococcus agalactiae* accounts for 41.6% of all *Streptococcus* isolates. The gut had the largest amount of *Streptococcus agalactiae* isolates (29.6%), followed by the gills (25.9%), the liver (22.2%),

Table 1. Values of tilapia fish total and standard lengths and weights.

Visits	Total length/cm			Standard length/cm			Weights/g		
	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
V I	21.2±2.3	18.5	27.5	17.4±1.9	15.0	23.0	190.4±65.5	122.0	435.0
V II	20.9±3.2	17.0	30.0	17.5±2.9	13.0	25.5	158.9±46.6	98.0	285.0
V III	25.1±0.7	24.0	26.0	20.8±0.3	20.5	21.0	251.2±14.3	231.0	275.0
V IV	22.5±1.0	20.5	24.0	18.3±1.0	17.0	20.0	187.4±21.4	145.0	230.0

Table 2. Prevalence of biochemically positive *Streptococcus* species in Nile tilapia aquaculture.

	Streptococcus species																
Visits	In Nile tilapia fish[a]													In aquaculture water			Total isolates	
	Tested	liver		Kidney		Intestine		Spleen		Gills		Total fish isolates		Tested	Isolates		No.	%[c]
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		No.	%[b]		
I	25	0	0	4	5.71	2	2.85	0	0	6	8.57	12	17.14	10	10	32.25	22	21.78
II	14	5	7.1	2	2.85	2	2.85	3	4.28	5	7.14	17	24.28	20	5	16.12	22	21.78
III	20	0	0	2	2.85	3	4.28	0	0	2	2.85	7	10	20	7	22.58	14	13.86
IV	20	9	12.9	8	11.42	12	17.14	3	4.28	2	2.85	34	48.57	20	9	29.03	43	42.57
Total	79	14	20	16	22.85	19	27.14	6	8.57	15	21.42	70	69.3	70	31	30.69	101	

a. Data presented as percentage to the total number of isolates of tilapia (70). b. Data presented as percentage to the total number of isolates of water (31). c. Data presented as percentage to the total number of isolates (101).

Table 3. Prevalence of biochemically positive *Streptococcus agalactiae* in Nile tilapia aquaculture.

	Streptococcus agalactiae															
Visits	Nile tilapia fish[a]												Aquaculture water		Total isolates	
	liver		Kidney		Intestine		Spleen		Gills		Total fish isolates					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%[b]	No.	%[c]
I	0	0	0	0	1	3.7	0	0	2	7.4	3	11.1	6	40	9	21.42
II	3	11.1	0	0	1	3.7	1	3.7	3	11.1	8	29.6	1	6.7	9	21.42
III	0	0	0	0	2	7.4	0	0	1	3.7	3	11.1	2	13.3	5	11.9
IV	3	11.1	3	11.1	4	14.8	2	7.4	1	3.7	13	48.1	6	40	19	45.23
Total	6	22.2	3	11.1	8	29.6	3	11.1	7	25.9	27		15		42	

a. Data presented as percentage to the total number of isolates of tilapia (27). b. Data presented as percentage to the total number of isolates of water (15). c. Data presented as percentage to the total number of isolates (42).

and the kidney and spleen (11.1%). Additionally, *Streptococcus agalactiae* was recovered from the aquaculture water of the three ponds; six isolates were found in visits I and IV, two isolates in visit III, and one isolate in visit II. *Streptococcus iniae* could be isolated from tilapia aquaculture and represented 58.41% of the total *Streptococcus* species isolates. The total *Streptococcus iniae* isolated was 59 isolates: 15 from the water and 43 from the fish as shown in Table 4. In tilapia fish, the kidney isolates showed the highest percentage, followed by the intestine, and finally the spleen.

The suspected *Streptococcus* isolates were subjected to molecular identification (PCR) using species-specific 16-23S intergenic space primer to identify *Streptococcus* agalactiea and lactate oxidase (*lctO*) gene primer for *Streptococcus iniae*. PCR reaction for *Streptococcus agalactiae* identification was performed for twenty-three suspected isolates at an annealing temperature 58°C and only two isolates were identified as positive *Streptococcus agalactiae* at base pair 270: one isolate from the liver in visit I and the other from the kidney in visit III. The identified two isolates represent 8% (2/23) of the tested isolates. Also, thirteen biochemically identified *Streptococcus iniae* isolates were tested the using lactate oxidase (*lctO*) gene. Although wide range of annealing temperatures (50, 52, 56, 58, 61°C) were applied during the PCR reaction, no isolate was confirmed as *Streptococcus iniae*.

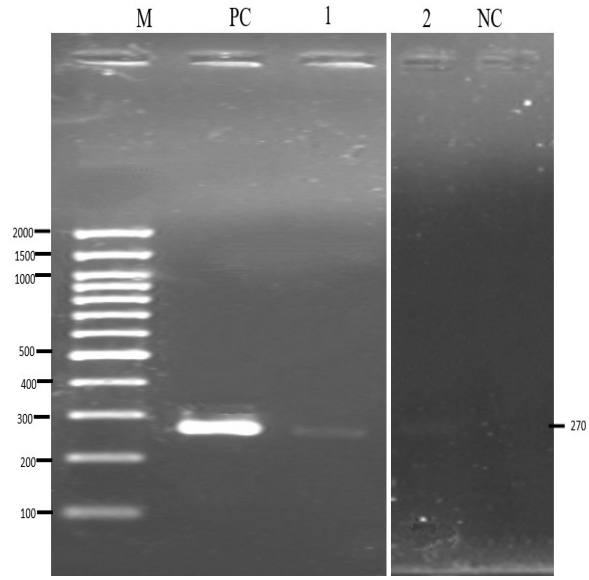

Fig. 1. Gel electrophoresis of *Streptococcus agalactiae*; Lane M: 100 bp DNA marker, Lane (PC): positive control, lane (1-2): positive isolates 270 bp, NC: negative control.

Table 4. Prevalence of positive biochemically positive *Streptococcus iniae* in tilapia aquaculture.

	Streptococcus iniae															
Visits	Nile tilapia fish[a]												Aquaculture water		Total isolates	
	Liver		Kidney		Intestine		Spleen		Gills		Total					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%[b]	No.	%[c]
I	0	0	4	9.3	1	2.3	0	0	4	9.3	9	20.9	4	25	13	22
II	2	4.6	2	4.6	1	2.3	2	4.6	2	4.6	9	20.9	4	25	13	22
III	0	0	2	4.6	1	2.3	0	0	1	2.3	4	9.3	5	31.3	9	15.3
IV	6	14	5	11.6	8	28.6	1	2.3	1	2.3	21	48.8	3	18.7	24	40.7
Total	8	18.6	13	30.23	11	25.58	3	6.9	8	18.6	43		16		59	

a. Data presented as percentage to the total number of isolates of tilapia (43). b. Data presented as percentage to the total number of isolates of water (16). c. Data presented as percentage to the total number of isolates (59).

Table 5. Antibiotic resistance of the isolated *Streptococcus agalactiae* (2 isolates).

Mode of action	Class	Antibiotic	Sensitive		Intermediate		Resistant	
			No.	%	No.	%	No.	%
Cell wall inhibitors	B- lactams	Amoxicillin-AX	1	50%	-	-	1	50%
Cell membrane disruptors	Colistin	Colistin-CT	-	-	-	-	2	100%
Protein synthesis inhibitors	Chloramphenicol	Chloramphenicol-C	-	-	-	-	2	100%
	Tetracycline	Doxycycline–D	-	-	1	50%	1	50%
	Aminoglycoside	Streptomycin-S	-	-	-	-	2	100%
Nucleic acid synthesis disruptors	Amincoumarine	Novobiocin-NV	-	-	-	-	2	100%
	Floroquinoline	Norfloxacine-NOR	-	-	-	-	2	100%
		Ciprofloxacin-CIP	-	-	-	-	2	100%
	Sulfonamides	Trimethoprim/Sulphamethoxazole (SXT)	-	-	-	-	2	100%

Table 6. Percentage of resistance and multiple antibiotic index.

Isolate code	Isolate origin	AX	CT	C	D	S	NV	NOR	CIP	SXT	Multiple antibiotic index[a]
1	liver	s	r	r	r	r	r	r	r	r	0.9
2	kidney	r	r	r	i	r	r	r	r	r	1
Number of resistant isolates		1	2	2	1	2	2	2	2	2	
Percentage of resistance		50%	100%	100%	50%	100%	100%	100%	100%	100%	

a. calculated through dividing the number of antibiotics the isolate is resistant against by the number of the tested antibiotics.
AX (Amoxicillin), CT (Colistin), C (Chloramphenicol), D (doxycycline), S (Streptomycin), NV (Novobiocin), NOR (norfloxacin), CIP (Ciprofloxacin), and SXT (trimethoprim/sulfamethoxazole).

In this study, the two confirmed *Streptococcus agalactiae* isolates were tested for their antibiotic susceptibility. Each was exposed to nine antibiotics in a disk diffusion test. The developed zones of inhibition were measured. Both tested isolates exhibited complete resistance (100%) to colistin, chloramphenicol, streptomycin, novobiocin, norfloxacin, ciprofloxacin, and trimethoprim/sulphamethoxazole. Moreover, one isolate was resistant to amoxicillin and doxycycline. On the other hand, the other isolate was susceptible to amoxicillin and moderately resistant to doxycycline. The two strains exhibited a multiple antibiotic resistance index of 0.9-1.

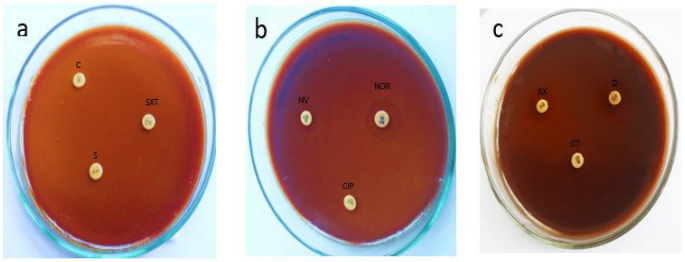

Fig. 2. *Streptococcus agalactiae* sensitivity zone for antibiotic. (a); C (Chloramphenicol), SXT (trimethoprim/sulfamethoxazole), and S (Streptomycin), (b); NV (Novobiocin), NOR (norfloxacin), and CIP (Ciprofloxacin), and (c); AX (Amoxicillin), CT (Colistin), and D (doxycycline).

The biofilm formation ability of the resistant isolates was assessed quantitatively using the crystal violet polystyrene microtiter plate method. After 48 hours incubation period, the two strains failed to form biofilm.

Discussion

Streptococcal infections in fish, in particular those produced by the pathogens *Streptococcus iniae*, and *Streptococcus agalactiae* have increased markedly with the intensification of aquaculture practices (Amal and Zamri-Saad, 2011). A diversity of fish species are susceptible to *Streptococcus* infection and *Streptococcus* species have been identified worldwide from different freshwater fish as Amur sturgeon in China (Yang and Li, 2009), red garra in Ireland (Ruane *et al*., 2013), catfish in China (Tavares *et al*., 2018), Siberian sturgeon in China (Deng *et al*., 2017), freshwater seabass in Thailand (Piamsomboon *et al*., 2020), Adriatic sturgeon in Northern Italy (Colussi *et al*., 2022), and *Streptococcus* species were detected in different marine fish around the world (Nho *et al*., 2009; Pourgholam *et al*., 2011; Erfanmanesh *et al*., 2012; Tsai *et al*., 2015). Streptococcosis of fish should be regarded as a significant non-apparent hazard because of the subclinical form caused by *Streptococcus iniae* and *Streptococcus agalactiae* (Sun *et al*., 2016; Rahmatullah *et al*., 2017)

Noticeably, with the expanding industry of tilapia aquaculture in Egypt, Streptococcosis has become an important and significant problem. Streptococcosis frequently occurred in the ponds in northern Egypt such as Kafr El-Sheikh, Port Said, Dakahlia, and Damietta Governorates mainly during the summer seasons (Saleh *et al*., 2017; Eissa *et al*., 2021; Alazab *et al*., 2022). There are many reasons that make Egyptian tilapia susceptible to Streptococcosis. The first, is that tilapia aquaculture is mainly distributed in northern Governorates, because of the availability of water there as well as the suitable temperature for the growth of tilapia is 28–32oC (El-Sayed, 2006). The second, is that tilapia is susceptible to *Streptococcus* during the hot season of each year because the water temperature is usually rise which may increase the tilapia's susceptibility (Saleh *et al*., 2017; Eissa *et al*., 2021). The third is the management system as with the high-density, and low quality feed the fish culture would be at the risk of Streptococcosis. Different *Streptococcus* species have been found to be the causative agent of Streptococcal infection, mainly *Streptococcus agalactiae* and *Streptococcus iniae* (Amal and Zamri-Saad, 2011).

One hundred and one *Streptococcus* species isolates from tilapia aquaculture could be identified: seventy from tilapia and thirty-one from the aquaculture water. In tilapia, the highest percentage of isolates were identified from the intestine, followed by the kidney. This result was similar to that obtained by Ali *et al*. (2020a) and close to that recorded by Auzureen *et al*. (2016) who identified 144 *Streptococcus* species isolates from hybrid red tilapia in local wet markets. This recorded result was lower than that reported by Abdelsalam *et al*. (2017) who isolated nine isolates from moribund red tilapia. Furthermore, Chitambo *et al*. (2023) recorded one *Streptococcus* species isolated from healthy Nile tilapia.

Thirty-one isolates of the *Streptococcus* species were obtained from the water, accounting for 30.96% of all isolates. This result was similar to that reported by Ali *et al*. (2020a) who found isolates of 20 different *Streptococcus* species in tilapia farming water..

Twenty-seven *Streptococcus agalactiae* isolates were detected in tilapia fish this result was higher than that recorded by Abbas *et al*. (2022) who identified fourteen bacterial isolates from naturally infected Nile tilapia as *Streptococcus agalactiae*, but lower than that recorded by Sudpraseart *et al*. (2021) who reported fifty-nine isolates from Nile tilapia. Also, one hundred and forty-six isolates were confirmed to be *Streptococcus agalactiae* according to the biochemical profiles by Sun *et al*. (2016) from healthy tilapia fish. Furthermore, among the *Streptococcus agalactiae* isolates found in the aquaculture water of the three ponds, fifteen isolates were found in this investigation, accounting for 35.7% of all isolates. This percentage is comparably higher than that reported by Ali *et al*. (2020a), who identified three *Streptococcus agalactiae* isolates. While, Reyes *et al*. (2018) obtained 250-270 CFU/mL of *Streptococcus agalactiae* count in tilapia pond water in Lubao, Pampanga, Philippines. Forty-three *Streptococcus iniae* isolates were identified from tilapia fish in Assiut and Minia Governorates and this was nearly similar to the recovered forty-seven isolates by Saleh *et al*. (2017) from mortality suffering Nile tilapia. Younes *et al*. (2019) identified fourteen isolates from diseased Nile tilapia. Also, in our study, sixteen *Streptococcus iniae* isolates were identified from the water. Compared to our results, Hardi *et al*. (2018) identified two *Streptococcus iniae* isolates from tilapia culture water and fish feces.

Two *Streptococcus agalactiae* isolates (8%) were molecularly confirmed: one isolate from the tilapia liver and the other from the kidney. The water isolates tested for molecular identification failed to give the required band (270bp). Alazab *et al*., (2022) molecularly confirmed 21 *Streptococcus agalactiae* isolates with 23.6% (21/89) of the suspected isolates from tilapia as follows: 10 (47.6%) from the liver; 6 (28.57%) from the kidney; 2 (9.5%) from each eye and gill samples; 1(4.85) from spleen samples using specific primers targeting the *Streptococcus agalactiae*-specific dltS gene. Also, Auzureen *et al*., (2016) confirmed eight *Streptococcus agalactiae* isolates using PCR with a band size 220 bp. While, Abbas *et al*., (2022) confirmed a higher number representing (100%) of the studied *Streptococcus agalactiae* isolates in tilapia fish using 16sRNA universal primer. The lactate oxidase gene (lctO) has been stated to be able to detect *Streptococcus iniae* with superior specificity than the 16S rRNA gene (Mata *et al*., 2004), so this species-specific lox- primer was used in the molecular identification of thirteen *Streptococcus iniae* isolates. Despite of its high specificity (Mata *et al*., 2004), no isolate achieved the band 870bp. Similar to our result, Oviedo-Bolaños *et al*. (2021) couldn`t identify *Streptococcus iniae* using the same primer in infected tilapia in Costa Rica. In contrast to our data, Legario *et al*. (2020) succeeded in identifying seven *Streptococcus iniae* isolates from tilapia aquaculture using the same lctO gene primer of *Streptococcus iniae*. In a follow-up investigation, Younes *et al*. (2019) used a species-specific 16S RNA primer to confirm that the isolates of *Streptococcus iniae* recovered from cultured Nile tilapia produced the desired 300 bp band in every test; additionally, Saleh *et al*. (2017) verified that three of the four isolates were in fact *Streptococcus iniae* at 300 base pair. Using a 16S-RNA primer, El Tawab *et al*. (2022) were also successful in validating 15 *Streptococcus iniae* out of 30 *Streptococcus* isolates. The gene encoding lactate oxidase is very unusual among *Streptococcus* species and related genes, indicating its specificity to *Streptococcus iniae* (Al-Harbi, 2011). The difficulty in *Streptococcus iniae* identification may be related to the lack of a serological identification system because *Streptococcus iniae* is non-Lancefield grouped (Pretto Giordano and Scarpassa, 2015). Also, the struggle of *Streptococcus iniae* accurate identification can be linked to its phenotypic diversity and the unavailability of commercial identification systems includes (Mata *et al*., 2004). Moreover, there is a close homology (98%) in 16S rRNA gene between *Streptococcus iniae* and *Streptococcus* parauberis (Täpp *et al*., 2003).

The extensive misuse of antibiotics has resulted in the emergence of antibiotic resistance in terrestrial and aquatic bacterial species, which is an international issue (Rico *et al*., 2014). Recently, the occurrence of antibiotic resistance among bacterial isolates has highlighted the importance of continuous monitoring of antibiotic resistance patterns. The tested two *Streptococcus agalactiae* isolates were highly resistant to all tested antibiotics except that one isolate was inhibited by amoxicillin and the other was slightly inhibited by doxycycline. Aleru-Obogai *et al*. (2022) found that all tested *Streptococcus agalactiae* isolated from cage-cultured red hybrid tilapia were susceptible to all antibiotics used. While, *Streptococcus agalactiae* strains isolated by Alazab *et al*. (2022) exhibited 95% resistance against ampicillin and erythromycin, followed by trimethoprim-sulfamethoxazole and cefotaxime (76% each), ceftriaxone (72%), tetracycline (66%), gentamicin (62%), penicillin (57%), and finally, 43% against ciprofloxacin. In the current study, the two strains tested were resistant to at least eight antibiotics tested, indicating the presence of multi-antibiotic

resistance profile with a multiple antibiotic resistance index of 0.9-1. Multiple antibiotic resistance in bacteria happens by acquiring and accumulating resistant plasmids, integrons or transposons of multiple genes that each encode resistance to a specific agent and/or by the activity of efflux pumps. *Streptococcus agalactiae* resistance to macrolide (norfloxacin, ciprofloxacin) has been stated to be mainly caused by efflux pumps encoded by the mefA/E genes (Verma *et al*., 2023). The resistance of *Streptococcus agalactiae* versus beta-lactams including amoxicillin has been reported to be due to mutations in penicillin-binding proteins, which affect the binding capacity of penicillin (Metcalf *et al*., 2017). While doxycycline resistance of *Streptococcus agalactiae* is due to the presence of tetM and tetO genes or drug efflux mediated by efflux pumps or target protection mediated by ribosomal protection proteins (Liu *et al*., 2023). The cell wall of the Gram-positive *Streptococcus* species is intrinsically less permeable to aminoglycosides; therefore, *Streptococcus agalactiae* is resistant to aminoglycosides at low concentrations, also ribosomal mutations contribute to its resistance to aminoglycosides (streptomycin) (Liu *et al*., 2023; Zakerifar *et al*., 2023).

Streptococcus species are ubiquitous in culture water and can also be isolated from healthy fish and apparently healthy fish, moreover, *Streptococcus* infection occurs in certain instances as stress conditions (Sun *et al*., 2016; Kannika *et al*., 2017). *Streptococcus* infection from the natural route of infection through direct contact can be systemically disseminated, affecting mainly the brain, kidney, and intestine and causing cell death (Iregui *et al*., 2016, Liu *et al*., 2016). The same in human *Streptococcus* bacteria present commensally in the gastrointestinal and genital tract of healthy women as part of the normal flora (Wernecke *et al*., 2009; Zakerifar *et al*., 2023). The bacteria can climb up through the birth canal and reach the newborn resulting in sepsis and meningitis in 1% of infants while 10% die and a significant percent of survived infants suffer from multiple nervous problems (Horváth-Puhó *et al*., 2021). Unfortunately, fishermen, farm workers, seafood handlers, as well as fish consumers are at risk of *Streptococcus* infection through contact with infected fish or contaminated water. The routine action in case of infection, is that antibiotics are used. However, every time these antibiotics are applied, antibiotic resistance develops. So, great attention should be paid to search for antibiotic alternatives.

Biofilms are aggregates of microbial communities in which bacteria adhere, cluster to a surface, and are surrounded by an extracellular matrix of carbohydrates or exopolysaccharides (Silvestre *et al*., 2020). This matrix protects from host immune defenses and antibiotics (Vestby *et al*., 2020). Therefore, biofilm formation by *Streptococcus agalactiae* can enhance its pathogenicity. Several studies investigated *Streptococcus* biofilm assembly in vitro (Dilrukshi *et al*., 2021; Verma *et al*., 2023). The biofilm formation for the two identified isolates was carried out according to Silvestre *et al*., (2020), however the two isolates could not form biofilm. The inability of the tested *Streptococcus agalactiae* to form biofilm may be related to the conflicting data regarding their biofilm-forming capacity, the glucose concentration, or the pH value (Kaur *et al*., 2009; Silvestre *et al*., 2020). Although higher biofilm production was described, under acidic pH conditions (Ho *et al*., 2013; D'Urzo *et al*., 2014), their inability to form biofilm at low pH values has also been reported (Dilrukshi *et al*., 2021). Glucose also seems to be important for the biofilm production by *Streptococcus agalactiae* (Silvestre *et al*., 2020), however in the absence of glucose, the biofilms of *Streptococcus* are formed (Kaur *et al*., 2009). Furthermore, several studies showed a greater ability of *Streptococcus agalactiae* to produce biofilm with a longer incubation time (48 hours) (Silvestre *et al*., 2020), but the biofilms were formed after 24 hours of incubation and lower (Parker *et al*., 2016; Verma *et al*., 2023). Also, there was a correlation between *Streptococcus agalactiae* biofilm formation and their virulence (Verma *et al*., 2023), but biofilm-forming strains were identified in asymptomatic carriers (Kaur *et al*., 2009; Parker *et al*., 2016). This may explain the inability of our isolates to assemble in biofilms, so further studies are required to find the optimum conditions for *Streptococcus agalactiae* biofilm formation.

Conclusion

Streptococcus bacteria is an opportunistic ubiquitous bacterial pathogen. Two *Streptococcus* isolates could be identified in healthy Nile tilapia. The identified isolates exhibited resistance to most of the tested antibiotics. Due to the outbreaks caused by *Streptococcus* every year around the world and Egypt, there is a critical need to search for and develop of new antimicrobials against these bacteria.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abbas, H.H., Soliman, W.S., Elgendy, M.Y., Youins, N.A., Abu-Elala, N.M., Marzouk, M.S., 2022. Insight on the potential microbial causes of summer mortality syndrome in the cultured Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Biology and Fisheries 26, 133-149.

Abdelsalam, M., Elgendy, M.Y., Shaalan, M., Moustafa, M., Fujino, M., 2017. Rapid identification of pathogenic streptococci isolated from moribund red tilapia (*Oreochromis* spp.). Acta Veterinaria Hungarica 65, 50–59.

Akter, T., Foysal, M.J., Alam, M., Ehsan, R., Paul, S.I., Momtaz, F., Siddik, M.A.B., Tay, A.C.Y., Fotedar, R., Gupta, S.K., Islam, T., Rahman, M.M., 2021. Involvement of *Enterococcus* species in streptococcosis of Nile tilapia in Bangladesh. Aquaculture 531, 735790

Al-Harbi, A.H., 2011. Molecular characterization of *Streptococcus iniae* isolated from hybrid tilapia (*Oreochromis niloticus*×*Oreochromis aureus*). Aquaculture 312, 15–18.

Alazab, A., Sadat, A., Younis, G., 2022. Prevalence, antimicrobial susceptibility, and genotyping of *Streptococcus agalactiae* in Tilapia fish (*Oreochromis niloticus*) in Egypt. Journal of Advanced Veterinary and Animal Research 9, 95.

Aleru-Obogai, C.P., Ollor, A.O., Mbata, C.A., Bartimaeus, E.S., 2022. Phenotypic and genotypic comparison between pathogenic Group B *Streptococcus* revealed the possibility of cross transmission between fish and human. Openeprints. Uk, IJTDH, pp. 21–29.

Ali, G.I. E., Abd El-Hady, H.A.M., Abou Zeid, M.A.M., 2020. Rapid Detection of Streptococci in Cultured Tilapia Fish Using PCR and Chemical Analysis. World's Veterinary Journal 10, 286–296.

Ali, S.E., Jansen, M.D., Mohan, C.V., Delamare-Debouteville, J., Charo-Karisa, H., 2020. Key risk factors, farming practices and economic losses associated with tilapia mortality in Egypt. Aquaculture 527, 735438.

Amal, M.N.A., Zamri-Saad, M., 2011. Streptococcosis in tilapia (*Oreochromis niloticus*): a review. Pertanika J. Trop. Agric. Sci. 34, 195–206.

APHA, 2017. Standard methods for the examination of water and wastewater examination 23RD Edetion. American Public Health Association, American Water Works Association and the Water Environment Federation.

Auzureen, A.M., Hamdan, R. H., Jamaludin, M.H., 2016. Identification and Antibiotic Resistance of *Streptococcus agalactiae* from Red Hybrid Tilapia (*Oreochromis niloticus*) in Local Wet Markets. In: International Seminar on Livestock Production and Veterinary Technology, pp. 561-566.

Baeck, G.W., Kim, J.H., Gomez, D. K., Park, S.C., 2006. Isolation and characterization of *Streptococcus* sP.from diseased flounder (Paralichthys olivaceus) in Jeju Island. Journal of Veterinary Science 7, 53–58.

Buller, N.B., 2004. Bacteria from fish and other aquatic animals: A Practical Identification Manual. CABI Publishing, Wallingford, Oxfordshire. Cai, J.,

Zhou, X., Xue, Y., Lucentea, D., Laganaa, C., 2019. Top 10 species groups in global aquaculture 2017. Food and Agriculture Organization of the United Nations. https://www.fao.org/documents/card/fr/c/ca5224en/

Chitambo, B., Munyeme, M., Hang'Ombe, B., 2023. Identifying Bacteria with Public Health Significance from Farmed Nile Tilapia (*Oreochromis niloticus*), Zambia. International Journal of Microbiology 2023, 6650378.

CLSI., 2013. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

CLSI., 2017. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. Appendix H. Using molecular Assays for resistance.

Colussi, S., Pastorino, P., Mugetti, D., Antuofermo, E., Sciuto, S., Esposito, G., Polinas, M., Tomasoni, M., Burrai, G.Pietro, Fernández-Garayzábal, J.F., Acutis, P.L., Pedron, C., Prearo, M., 2022. Isolation and Genetic Characterization of *Streptococcus iniae* Virulence Factors in Adriatic Sturgeon (Acipenser naccarii). Microorganisms 10, 1–11.

D'Urzo, N., Martinelli, M., Pezzicoli, A., De Cesare, V., Pinto, V., Margarit, I., Telford, J.L., Maione, D., Melin, P., Decheva, A., Petrunov, B., Kriz, P., Berner, R., Büchele, A., Hufnagel, M., Kunze, M., Creti, R., Badassari, L., Berardi, A., Efstratiou, A., 2014. Acidic pH strongly enhances in vitro biofilm formation by a subset of hypervirulent ST-17 *Streptococcus agalactiae* strains. Applied and Environmental Microbiology 80, 2176–2185.

Deng, L., Li, Y., Geng, Y., Zheng, L., Rehman, T., Zhao, R., Wang, K., OuYang, P., Chen, D., Huang, X., He, C., Yang, Z., Lai, W., 2019. Molecular serotyping and antimicrobial susceptibility of *Streptococcus agalactiae* isolated from fish in China. Aquaculture 510, 84–89.

Deng, M.L., Yu, Z. H., Geng, Y., Wang, K.Y., Chen, D.F., Huang, X.L., Ou, Y.P., Chen, Z.L., Zhong, Z. J., Lai, W.M., 2017. Outbreaks of Streptococcosis associated with *Streptococcus iniae* in Siberian sturgeon (*Acipenser baerii*) in China. Aquaculture Research 48, 909–919.

Dilrukshi, G.N., Kottahachchi, J., Dissanayake, T., Weerasekera, M., Peiris, M., Medis, S., Fernando, N., 2021. *In-vitro* Biofilm Formation of Vaginal Isolates of *Streptococcus agalactiae*; Effect of pH and Culture Media. Brazilian Archives of Biology and Technology 64, 1–9.

Eissa, A.E., Attia, M.M., Elgendy, M.Y., Ismail, G.A., Sabry, N.M., Prince, A., Mahmoud, M.A., El-Demerdash, G.O., Abdelsalam, M., Derwa, H.I. M., 2021. *Streptococcus*, *Centrocestus* formosanus and *Myxobolus tilapiae* concurrent infections in farmed Nile tilapia (*Oreochromis niloticus*). Microbial Pathogenesis 158, 105084.

El-Sayed, A.F.M., 2006. Tilapia culturE.In Tilapia Culture. Academic Press.

El Tawab, A.A., Hofy, F.El, Ali, N., Saad, W., El-Mougy, E., Mohammed, A., 2022. Antibiotic resistance genes in *Streptococcus iniae* isolated from diseased *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries 26, 413–428.

Erfanmanesh, A., Soltani, M., Pirali, E., Mohammadian, S., Taherimirghaed, A., 2012. Genetic Characterization of *Streptococcus iniae* in diseased farmed rainbow trout (Onchorhynchus mykiss) in Iran. The Scientific World Journal 2012, 594073.

EUCAST., 2023. The European Committee on Antimicrobial Susceptibility TestinG.Breakpoint tables for interpretation of MICs and zone diameters. Version 13.0, 2023.

FAO., 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. https://www.fao.org/3/ca9229en/ca9229en.pdf

Foxman, B., Gillespie, B. W., Manning, S.D., Marrs, C.F., 2007. Risk factors for group B streptococcal colonization: potential for different transmission systems by capsular type. Annals of Epidemiology 17, 854-862

Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K.S., Salehi, B., Bevilacqua, A., Corbo, M.R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Tintino, S.R., de Souza, V. C.A., Sharifi-Rad, J., Coutinho, H.D. M., Martins, N., Rodrigues, C.F., 2019. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms 7, 130.

Ghetas, H., Neiana, A., Khalil, R., AM, H., Khallaf, M., 2021. *Streptococcus agalactiae* Isolation and Characterization in Nile Tilapia (*Oreochromis niloticus*) with Histopathological Studies. Journal of Current Veterinary Research 3, 70–79.

Hardi, E.H., Nugroho, R. A., Saptiani, G., Sarinah, R., Agriandini, M., Mawardi, M., 2018. Identification of potentially pathogenic bacteria from tilapia (*Oreochromis niloticus*) and channel catfish (*Clarias batrachus*) culture in Samarinda, East Kalimantan, Indonesia. Biodiversitas 19, 480–488.

Ho, Y. R., Li, C.M., Yu, C.H., Lin, Y. J., Wu, C.M., Harn, I. C., Tang, M.J., Chen, Y. T., Shen, F.C., Lu, C.Y., Tsai, T. C., Wu, J.J., 2013. The enhancement of biofilm formation in Group B streptococcal isolates at vaginal pH. Medical Microbiology and Immunology 202, 105–115.

Horváth-Puhó, E., Snoek, L., van Kassel, M.N., Gonçalves, B. P., Chandna, J., Procter, S.R., van de Beek, D., de Gier, B., van der Ende, A., Sørensen, H.T., Lawn, J.E., Bijlsma, M.W., Søgaard, K.K., Hahné, S.J.M., 2021. Prematurity Modifies the Risk of Long-term Neurodevelopmental Impairments After Invasive Group B *Streptococcus* Infections During Infancy in

Denmark and the Netherlands. Clinical Infectious Diseases 74(Suppl 1), S44–S53.
Iregui, C.A., Comas, J., Vásquez, G.M., Verján, N., 2016. Experimental early pathogenesis of *Streptococcus agalactiae* infection in red tilapia *Oreochromis* spp. Journal of Fish Diseases 39, 205–215.
Jansen, M.D., Dong, H.T., Mohan, C.V., 2019. Tilapia lake virus: a threat to the global tilapia industry? Reviews in Aquaculture 11, 725–739.
Kaminska, D., Ratajczak, M., Szumała-Kąkol, A., Dlugaszewska, J., Nowak-Malczewska, D. M., Gajecka, M., 2020. Increasing resistance and changes in distribution of serotypes of *Streptococcus agalactiae* in poland. Pathogens 9, 1–14.
Kannika, K., Pisuttharachai, D., Srisapoome, P., Wongtavatchai, J., Kondo, H., Hirono, I., Unajak, S., Areechon, N., 2017. Molecular serotyping, virulence gene profiling and pathogenicity of *Streptococcus agalactiae* isolated from tilapia farms in Thailand by multiplex PCR. Journal of Applied Microbiology 122, 1497–1507.
Kaplan, J.B., 2011. Antibiotic-induced biofilm formation. International Journal of Artificial Organs 34, 737–751.
Kaur, H., Kumar, P., Ray, P., Kaur, J., Chakraborti, A., 2009. Biofilm formation in clinical isolates of group B streptococci from north India. Microbial Pathogenesis 46, 321–327.
Kinge, C.N.W., Ateba, C.N., Kawadza, D. T., 2010. Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the mmabatho locality, North-West Province, South Africa. South African Journal of Science 106, 44–49.
Koh, T. H., Sng, L.H., Yuen, S.M., Thomas, C.K., Tan, P.L., Tan, S.H., Wong, N.S., 2009. Streptococcal cellulitis following preparation of fresh raw seafood. Zoonoses and Public Health 56, 206–208.
Konto-Ghiorghi, Y., Mairey, E., Mallet, A., Duménil, G., Caliot, E., Trieu-Cuot, P., Dramsi, S., 2009. Dual role for pilus in adherence to epithelial cells and biofilm formation in *Streptococcus agalactiae*. PLoS Pathogens 5, e1000422.
Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology 46, 165–170.
Legario, F.S., Choresca, C.H., Turnbull, J.F., Crumlish, M., 2020. Isolation and molecular characterization of streptococcal species recovered from clinical infections in farmed Nile tilapia (*Oreochromis niloticus*) in the Philippines. Journal of Fish Diseases 43, 1431–1442.
Liu, G., Zhu, J., Chen, K., Gao, T., Yao, H., Liu, Y., Zhang, W., Lu, C., 2016. Development of *Streptococcus agalactiae* vaccines for tilapia. Diseases of Aquatic Organisms 122, 163–170.
Liu, Z., Jiang, X., Li, J., Ji, W., Zhou, H., Gong, X., Miao, B., Meng, S., Duan, L., Shi, Q., Han, X., Gao, P., Chang, C., Dong, A., Li, J., 2023. Molecular characteristics and antibiotic resistance mechanisms of clindamycin-resistant *Streptococcus agalactiae* isolates in China. Frontiers in Microbiology 14, 1–10.
Mata, A.I., Blanco, M.M., Domínguez, L., Fernández-Garayzábal, J.F., Gibello, A., 2004. Development of a PCR assay for *Streptococcus iniae* based on the lactate oxidase (*lctO*) gene with potential diagnostic value. Veterinary Microbiology 101, 109–116.
Metcalf, B. J., Chochua, S., Gertz, R. E., Hawkins, P.A., Ricaldi, J., Li, Z., Walker, H., Tran, T., Rivers, J., Mathis, S., Jackson, D., McGee, L., Beall, B., Glennen, A., Lynfield, R., Reingold, A., Brooks, S., Randel, H., Miller, L., Langley, G., 2017. Short-read whole genome sequencing for determination of antimicrobial resistance mechanisms and capsular serotypes of current invasive *Streptococcus agalactiae* recovered in the USA. Clinical Microbiology and Infection 23, 574.e7-574.e14.
Miao, W., Wang, W., 2020. Trends of aquaculture production and trade: Carp, tilapia, and shrimp. Asian Fisheries Science 33(S1), 1–10.
Nho, S.W., Shin, G.W., Park, S.Bin, Jang, H. Bin, Cha, I. S., Ha, M.A., Kim, Y. R., Park, Y. K., Dalvi, R. S., Kang, B. J., Joh, S.J., Jung, T. S., 2009. Phenotypic characteristics of *Streptococcus iniae* and *Streptococcus parauberis* isolated from olive flounder (*Paralichthys olivaceus*). FEMS Microbiology Letters 293, 20–27.
Niu, G., Khattiya, R., Zhang, T., Boonyayatra, S., Wongsathein, D., 2020. Phenotypic and genotypic characterization of *Streptococcus* spp. isolated from tilapia (*Oreochromis* spp.) cultured in river-based cage and earthen ponds in Northern Thailand. Journal of Fish Diseases 43, 391–398.
Oviedo-Bolaños, K., Rodríguez-Rodríguez, J.A., Sancho-Blanco, C., Barquero-Chanto, J.E., Peña-Navarro, N., Escobedo-Bonilla, C.M., Umaña-Castro, R., 2021. Molecular identification of *Streptococcus* sP.and antibiotic resistance genes present in Tilapia farms (*Oreochromis niloticus*) from the Northern Pacific region, Costa Rica. Aquaculture International 29, 2337–2355.
Parker, R. E., Laut, C., Gaddy, J.A., Zadoks, R. N., Davies, H.D., Manning, S.D., 2016. Association between genotypic diversity and biofilm production in group B *Streptococcus*. BMC Microbiology 16, 1–12.
Phuektes, P., Mansell, P.D., Browning, G.F., 2001. Multiplex polymerase chain reaction assay for simultaneous detection of *Staphylococcus aureus* and Streptococcal causes of bovine mastitis. Journal of Dairy Science 84, 1140–1148.
Piamsomboon, P., Thanasaksiri, K., Murakami, A., Fukuda, K., Takano, R., Jantrakajorn, S., Wongtavatchai, J., 2020. Streptococcosis in freshwater farmed seabass Lates calcarifer and its virulence in Nile tilapia *Oreochromis niloticus*. Aquaculture 523, 735189.
Portz, D. E., Woodley, C.M., Cech, J.J., 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16, 125–170.
Pourgholam, R., Laluei, F., Saeedi, A.A., Zahedi, A., Safari, R., Taghavi, M.J., Nasrollhzadeh Saravi, H., Pourgholam, H., 2011. Distribution and molecular identification of some causative agents of streptococcosis isolated from farmed rainbow trout (*Oncorhynchus mykiss*, Walbaum) in Iran. Iranian Journal of Fisheries Sciences 10, 109–122.
Pretto Giordano, L.G., Scarpassa, J.A., 2015. *Streptococcus iniae*: An Unusual Important Pathogen Fish in Brazil. Journal of Aquaculture Research and Development 6, 9–11.
Queipo-Ortuño, M.I., De Dios Colmenero, J., Macias, M., Bravo, M.J., Morata, P., 2008. Preparation of bacterial DNA template by boiling and effect of immunoglobulin g as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clinical and Vaccine Immunology 15, 293–296.
Rahmatullah, M., Ariff, M., Kahieshesfandiari, M., Daud, H.M., Zamri-Saad, M., Sabri, M.Y., Amal, M.N.A., Ina-Salwany, M.Y., 2017. Isolation and pathogenicity of *Streptococcus iniae* in cultured red hybrid tilapia in Malaysia. Journal of Aquatic Animal Health 29, 208–213.
Rasco, B., Down, K., Ovissipour, M., 2015. Humane Harvesting Initiative: The Influence of Harvest and Post-Harvest Handling Practices on Fish Welfare and Product Quality. Journal of Aquaculture Research and Development 6, 1000303.
Reyes, A.T., Bullanday, M.J.C., Fajardo, L.J., 2018. Antibiotics susceptibility of *Streptococcus agalactiae* Isolated from tilapia pond water in Lubao, Pampanga, Philippines. International Journal of Biology, Pharmacy and Allied Sciences 7, 1702–1716.
Rico, A., Oliveira, R., McDonough, S., Matser, A., Khatikarn, J., Satapornvanit, K., Nogueira, A.J.A., Soares, A.M.V. M., Domingues, I., Van den Brink, P.J., 2014. Use, fate and ecological risks of antibiotics applied in tilapia cage farming in Thailand. Environmental Pollution 191, 8–16.
Rosini, R., Margarit, I., 2015. Biofilm formation by *Streptococcus agalactiae*: Influence of environmental conditions and implicated virulence factor. Frontiers in Cellular and Infection Microbiology 5, 6.
Ruane, N.M., Collins, E.M., Geary, M., Swords, D., Hickey, C., Geoghegan, F., 2013. Isolation of *Streptococcus agalactiae* and an aquatic birnavirus from doctor fish Garra rufa L.Irish Veterinary Journal 66, 2–5.
Saleh, H., Sabry, N., AlRazik, M., Mohamed, F., Ibrahim, M., 2017. Pathogenicity and Characterization of Streptococcosis in Egyptian Nile Tilapia (*Oreochromis niloticus*) in Kafr Elshikh Governorate. Alexandria Journal of Veterinary Sciences 52, 173.
Silvestre, I., Borrego, M.J., Jordão, L., 2020. Biofilm formation by ST17 and ST19 strains of *Streptococcus agalactiae*. Research in Microbiology 171, 311–318.
Sudpraseart, C., Wang, P.C., Chen, S.C., 2021. Phenotype, genotype and pathogenicity of *Streptococcus agalactiae* isolated from cultured tilapia (Oreochromis spp.) in TaiwaN.Journal of Fish Diseases 44, 747–756.
Sun, J., Fang, W., Ke, B., He, D., Liang, Y., Ning, D., Tan, H., Peng, H., Wang, Y., Ma, Y., Ke, C., Deng, X., 2016. Inapparent *Streptococcus agalactiae* infection in adult/commercial tilapia. Scientific Reports 6, 26319.
Täpp, J., Thollesson, M., Herrmann, B., 2003. Phylogenetic relationships and genotyping of the genus *Streptococcus* by sequence determination of the RNase P RNA gene, rnpB. International Journal of Systematic and Evolutionary Microbiology 53, 1861–1871.
Tavares, G.C., de Queiroz, G.A., Assis, G.B. N., Leibowitz, M.P., Teixeira, J.P., Figueiredo, H.C.P., Leal, C.A.G., 2018. Disease outbreaks in farmed Amazon catfish (Leiarius marmoratus x Pseudoplatystoma corruscans) caused by *Streptococcus agalactiae*, S. iniae, and S. dysgalactiae. Aquaculture 495, 384–392.
Tsai, M.A., Wang, P.C., Yoshida, T., Chen, S.C., 2015. Genetic characteristics of *Streptococcus* dysgalactiae isolated from cage cultured cobia, Rachycentron canadum (L.). Journal of Fish Diseases 38, 1037–1046.
Van Doan, H., Hoseinifar, S.H., Chitmanat, C., Jaturasitha, S., Paolucci, M., Ashouri, G., Dawood, M.A.O., Esteban, M.Á., 2019. The effects of Thai ginseng, Boesenbergia rotunda powder on mucosal and serum immunity, disease resistance, and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture 513, 734388.
Verma, S., Kumari, M., Pathak, A., Yadav, V., Johri, A.K., Yadav, P., 2023. Antibiotic resistance, biofilm formation, and virulence genes of *Streptococcus agalactiae* serotypes of Indian origin. BMC Microbiology 23, 176.
Vestby, L.K., Grønseth, T., Simm, R., Nesse, L.L., 2020. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics 9, 52.
Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B., 2011. Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes. Springer Science and Business Media.
Wernecke, M., Mullen, C., Sharma, V., Morrison, J., Barry, T., Maher, M., Smith, T., 2009. Evaluation of a novel real-time PCR test based on the *ssrA* gene for the identification of group B streptococci in vaginal swabs. BMC Infectious Diseases 9, 148.
Yang, W., Li, A., 2009. Isolation and characterization of *Streptococcus dysgalactiae* from diseased Acipenser schrenckii. Aquaculture 294, 14–17.
Younes, A.M., Gaafar, A.Y., Abu-Bryka, A.E.D. Z., Mohamed, L.A., Bayoumy, E.S.M., 2019. Genotyping and pathogenicity of *Streptococcus iniae* strains recovered from cultured *Oreochromis niloticus* at Kafr El-Shiekh governorate, Egypt. Egyptian Journal of Aquatic Biology and Fisheries 23, 467–474.
Zakerifar, M., Kaboosi, H., Goli, H.R., Rahmani, Z., Peyravii Ghadikolaii, F., 2023. Antibiotic resistance genes and molecular typing of *Streptococcus agalactiae* isolated from pregnant women. BMC Pregnancy and Childbirth 23, 1–13.
Zhang, Z., Yu, A., Lan, J., Zhang, Y., Zhang, H., Li, Y., Hu, M., Cheng, J., Wei, S., Lin, L., 2017. Draft Genome Sequence of an Attenuated *Streptococcus agalactiae* Strain Isolated from the Gut of a Nile Tilapia (*Oreochromis niloticus*). Genome Announc. 5, e01627-16.
Ziarati, M., Zorriehzahra, M.J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., Emran, T. Bin, Dhama, K., Chaicumpa, W., Shamsi, S., 2022. Zoonotic diseases of fish and their prevention and control. Veterinary Quarterly 42, 95–118.