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Antibiotic resistance genes in *Streptococcus iniae* isolated from diseased *Oreochromis niloticus*

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ABSTRACT

Streptococcus iniae is one of the most hazardous fish pathogens infecting both farmed and wild fish species. The antibiotic resistance genes (ARGs) were inspected in *Streptococcus iniae* (*S. iniae*) isolates obtained from the cultured Nile tilapia, *Oreochromis niloticus* (*O. nloticus*), collected from fish farms in Kafr El-Shiekh Governorate, Egypt. The present study aimed to isolate and identify *S. iniae* and monitor the presence of some antimicrobial resistance genes. A total number of 30 *Streptococcus* isolates were recovered and then biochemically identified in the current study. Fifteen isolates (50%) were identified as *S. iniae* by polymerase chain reaction using 16S rRNA specific primers. Sensitivity of *S. iniae* isolates was recorded for erythromycin, tetracycline, norfloxacin, ciprofloxacin, oxytetracycline, streptomycin, sulfamethoxazole-trimethoprim, amoxicillin, and florfenicol. *S. iniae* showed high resistance to erythromycin and tetracycline. The tetracycline-resistant isolates contained the *tet*(M/O) gene, of which 3 and 2 isolates contained the *tet*(M) and *tet*(O) genes, respectively. Among the erythromycin-resistant isolates, 3 contained the *erm*(B) gene. The studied isolates showed a multiple antibiotic resistance profile, indicating difficulty in treating such infection. Thus, a need to develop a new strategy for controlling *Streptococcus* outbreaks in infected farms like vaccination is recommended.

INTRODUCTION

Fish is considered the most important animal protein source for human consumption, particularly in poor African countries **(Hussain *et al.*, 2011; Aboyadak *et al.*, 2015)**. Aquaculture has become the most effective source to substitute the shortage in global fisheries production and meet the expanded demand of animal protein for human consumption **(Aboyadak *et al.*, 2017; Mohamed *et al.*, 2017)**.

The Nile tilapia is the second most extensively farmed fish species after carp due to its fast growth rate, acclimatization with different environmental conditions and good marketability **(Hai, 2015; Dawood *et al.*, 2019; Ali et al., 2021a)**. The global tilapia production has increased steadily, reaching 5.4 million metric tons in 2016 **(FAO, 2018)**. The Nile tilapia (*Oreochromis niloticus*) is the most important cultured fish species in Egypt. The Egyptian production exceeded 1.08 million tons in 2019; more than 80.5% of the total Egyptian fish production comes from aquaculture **(GAFRD, 2020)**.

Bacterial pathogens including *Streptococcus* sp., *staphylococcus* sp., *Edwardsiella* sp., *Francisella orientalis* and *Vibrio* sp., *Aeromonas* sp. and *Pseudomonas* sp. are associated with septicemia and high mortality in farmed fish **(Ali et al., 2018; Lee *et al.*, 2019)**.

Gram positive cocci infection represents a major concern in the Egyptian aquaculture sector contributing to a huge financial loss. *Staphylococcus epidermidis* and *Staphylococcus aureus* was isolated from diseased *Dicentrarchus labrax* and *Diplodus sargus* **(Aboyadak *et al*., 2016a; Ali *et al*., 2019; Ali *et al*., 2021b)**.

In addition, Streptococcosis is a serious bacterial disease affecting cultured *O. niloticus* in many countries; streptococcal infection resulted in 400 million US dollars losses in China **(Chen *et al.*, 2012)**. **Aboyadak *et al*. (2016b)** and **Saleh *et al*. (2019)** isolated *Streptococcus iniae* from the cultured tilapia, and the diseased fish showed increased mortality, nervous signs and the presence of hemorrhagic patched on the external body surface. Additionally, the internal gross lesions were observed in the enlarged congested liver and posterior kidney.

Streptococci is a facultative anaerobic Gram-positive non-sporing bacterium **(Maisak *et al.*, 2008; Liamnimitr *et al.*, 2017)**. Streptococcosis, is a septicemic disease caused mainly by *Streptococcus agalactiae* or *Streptococcus iniae*, this disease has a major negative impact on aquaculture **(Acar *et al.*, 2015)**.

Several classes of antibiotic families are extensively used in the treatment of diseased fish such as tetracyclines, macrolides, beta lactams as penicillin and cephalosporins, while quinolones are less frequently used **(Aboyadak *et al*., 2016b; Zhang *et al.*, 2020)**. However, multiple drug-resistant bacterial pathogens can arise from the misuse of antibacterial drugs by fish farmers, and this could be fatal for human if the antibiotic resistance genes were transported to zoonotic bacteria as streptococci **(Ghittino *et al.*, 2003; Wu *et al.*, 2013)**.

Polymerase chain reaction (PCR) is highly specific molecular tool, which is not restricted to identifying bacterial fish pathogens to genus and species level **(Aboyadak *et al*., 2017; Cui *et al.*, 2019; Deng *et al.*, 2019)**. PCR can be a valuable tool in screening bacterial isolates for the presence of antibiotic resistance genes **(Ortega *et al.*, 2018; Seyfried *et al.*, 2010)**; addingly, PCR can explain the mechanism of resistance according to the type of detected gene.

Many antibiotic resistance genes have been discovered and detected by PCR as resistance to tetracycline that is governed by *tet* genes. This gene is protecting bacterial cell through the active efflux of the drug out of the bacterial cell, or protecting the ribosome against antibiotic. Moreover, it may be responsible for the production of certain enzyme modifying the drug structure **(Giovanetti *et al.*, 2003)**. There is a various type of *tet* genes, viz. *tet*(A), *tet*(B), *tet*(D), *tet*(E) and *tet*(G) that are reported in Gram-negative bacteria **(Jones *et al.*, 2006)**. **Roberts (1996)** reported that Gram-positive bacteria have different types of tetracycline resistance genes, including *tet*(K), *tet*(L), *tet*(M), *tet*(O), and *tet*(S).

The *erm* and *mef* gene family are associated with erythromycin resistance; this gene exerts the effect through the modification of the ribosomal target of erythromycin through methylation, mutation or active drug efflux out of bacterial cell **(Sapkota *et al.*, 2006)**. Erythromycin resistance gene is widely distributed in many *Streptococcus, Enterococcus* and *Staphylococcus* sp.

The present study focused on the isolation and identification of *Streptococcus iniae* from the diseased cultured *O. niloticus*, determining the antimicrobial susceptibility of recovered isolates, followed by molecular screening of the antimicrobial resistance genes.

MATERIALS AND METHODS

Ethical approval

The present study was authorized by the animal ethics committee of Kafrelsheikh University.

2.1. Fish sampling

A total of 140 clinically diseased *O. niloticus* were randomly sampled from several tilapia farms in Kafrelsheikh Governorate over the seasons of 2019. Sampled fish showed one or more of the classical disease signs, such as exophthalmia, eye opacity, septicemia, skin ulceration, scales detachment, hemorrhage all over the external body surface and dorsal and caudal fin erosions. Samples were immediately transported to and refrigerated at the central diagnostic lab, Faculty of Veterinary Medicine, Kafrelsheikh University. Each fish sample was packed in a sterile polythene bag and maintained in ice pox and then subjected to bacteriological examinations under complete aseptic condition.

2.2. Bacterial isolation and culture conditions

For bacteriological examination, tissue samples were taken aseptically from fish eyes, liver, kidney and brain. Small pieces of tissue samples from each fish were homogenized following the descriptions of **Aboyadak *et al*. (2016a)**. Afterwards, they were initially pre-enriched on tryptic soy broth (Difco, Detroit, MI, USA) and incubated for 18-24h at 37°C. A loopful from each broth sample was streaked on tryptic soy agar (TSA) and incubated for 24h at 37˚C. For selective *streptococcus* isolation, one colony was taken on Tryptic soy broth, and after 24h of incubation a loopful was streaked on

enriched modified Edwards Medium, Oxoid®, UK as described in the studies of **Quinn *et al.* (2002)** and **Markey *et al.* (2013)**. Enriched media was prepared by the addition of 5% bovine blood and colistin sulphate (5 mg/L). Cultured plates were incubated for 24h at 37°C.

2.3. Biochemical identification

Pure cultures were stained with Gram stain and examined microscopically for phenotypic identification. Further biochemical tests such as motility, oxidase, catalase, methyl red, Voges Proskauer, indole, esculin, and gelatin hydrolysis, as well as oxidation-fermentation and sugar utilization tests were carried out for the identification and strain differentiation of bacterial isolates. The obtained results were compared to *S. iniae* reference strain present in central diagnostic laboratory, Faculty of Veterinary Medicine, Kafrelsheikh University. The phenotypic and biochemical characteristics of each *S. iniae* isolate was recorded. The recovered *S. iniae* isolates were stored in 25% glycerol at -85°C, and another copy stabbed in semi-solid agar was preserved in the refrigerator at 4°C for further use.

2.4. Molecular study

2.4.1. DNA extraction

Bacterial isolates were cultured in tryptic soy broth for 18h at 37°C. Then, bacterial cells were collected by centrifugation, and multi-genomic DNA extraction kit (Qiagen, Germany) was used for DNA extraction, following the guidelines of the manufacturing company. Afterwards, Genomic DNA was quantified at A 260:280 using nanodrop spectrophotometer.

2.4.2. PCR reaction mixture

PCR reactions used for detection of *S. iniae* and the resistant genes were performed in a total volume of 25µl, and each reaction volume consisted of 12.5 µl of 2xMaster Mix (Intron, Korea), 5µl of DNA extract, 1.25µl of forward primer, 1.25 µl of reverse primer and finally 4.5µl of nuclease-free water as described by **El-Bahar *et al.* (2019)**. The PCR reaction was performed using Peltier Thermal Cycler model MG 960T enzyme® - USA,

2.4.3. PCR identification of *S. iniae*

The biochemically identified *S. iniae* isolates were subjected to PCR amplification of 16S rRNA gene, using the specific primer designed by **Zlotkin *et al.* (1998)** and represented in Table (2). PCR programing starts with the initial denaturation at 94°C for 5min, followed by 35 cycles of (denaturation at 94°C for 30s, annealing at 50°C for 30s, and extension at 72°C for 30s). Then, a final extension step was performed at 72°C for 10min for generating the characteristic 300 base pairs (bp) amplicons specific for *S. iniae*.

2.4.4. Detection of antibiotic resistance genes by PCR

The oligonucleotide primer sets used for detecting common macrolide resistance gene *erm*(B) and tetracycline resistance genes of *tet*(M) and *tet*(O) are presented in Table (1). The amplification of the *tet*(M) and *tet*(O) genes was performed as described by **Ng**

***et al.* (2001)**; while, the amplification of the *erm*(B) gene was performed according to **Sapkota *et al.* (2006)**.

Thermal cycling programing for the amplification of the studied resistance genes was started with initial denaturation at 96°C for 3min, followed by 35 cycles of denaturation at 96°C for 30s, annealing at 55°C for 1.0min, and extension at 72°C for 2min, followed by a final extension step at 72°C for 10min.

Table 1. Primer sequence used in the molecular study

Target	Primer Name	Oligonucleotide Sequence (5' - 3')	Amplicon size (bp)	Reference
Streptococcus iniae 16S rRNA	Sin1 Sin2	CTAGAGTACACATGTACTAAG GGATTTTCCACTCCCATTAC	300	**Zlotkin *et al.* (1998)**
Tetracyclines *tet*(M)	F R	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	406	**Ng *et al.* (2001)**
Tetracyclines *tet*(O)	F R	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	515	
Macrolides (erythromycin) *erm*(B)	F R	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	**Sapkota *et al.* (2006)**

2.4.5. Detection of target amplicon

Five microliters of each PCR product were electrophoresed on 2% agarose gel containing 0.5µg/ml of ethidium bromide. A volume of 10µl of DNA Ladder (Intron, Korea) was added to the first well. Power supply was adjusted at 80V for 1h, and then DNA bands were visualized by C200 gel documentation system, Azure Biosystems, USA.

2.6. Antimicrobial susceptibility test

The antimicrobial substitutability profile of the tested isolates was determined by agar disc diffusion test, following the descriptions of **Ali *et al*. (2018)**. Briefly, overnight cultured broth was adjusted to 0.5 McFarland standard, and then one ml was streaked over Mueller–Hinton agar plate surface. Seeded plate was kept for 10min to absorb the excess bacterial suspension, and antibiotic discs were carefully added, followed by a 24h of incubation at 35°C. Susceptibility of *S. iniae* was determined using antibiotic discs for Erythromycin (E 15µg), Tetracycline (TE 30µg), Norfloxacin (NOR 5µg), Ciprofloxacin (CIP 5µg), oxytetracycline (OTC 30µg), Streptomycin (S 10µg), Amoxicillin (AX 25µg), Sulfamethoxazole -trimethoprim (SXT 23.75/1.25µg) and Florfenicol (F 10µg). The produced zone of inhibition diameter was measured by a ruler to the nearest mm and interpreted following the guidelines of the Clinical and Laboratory Standards Institute **(CLSI, 2018)**.

RESULTS

3.1. Results of initial and biochemical identification

Thirty isolates were successfully grown on modified Edwards's medium and biochemically identified as genus *Streptococcus* revealing that the streptococcal infection represent 21.4% of disease etiology in the infected *Oreochromis niloticus* (30/140); the biochemical profile of the recovered isolates is showed in Table (2).

Table 2. Phenotypic and biochemical characteristics of the recovered *Streptococcus iniae* isolates

Biochemical test	Isolated *S. iniae*	Reference *S. iniae*
Gram staining	+ve	+ve
Morphology	Cocci	Cocci
Motility	−ve	−ve
Catalase	−ve	−ve
Oxidase	−ve	−ve
Aesculin	−ve	−ve
Gelatinase	−ve	−ve
Hemolysis	*β-haemolytic*	*β-haemolytic*
Tryptic soy agar	+ve	+ve
Blood agar	+ve	+ve
Nutrient agar	+ve	+ve
Brain heart infusion broth	+ve	+ve
Oxidative–fermentative (OF) test	F	F
Indole test	+ve	+ve
Methyl Red test	−ve	−ve
Voges–Proskauer test	+ve	+ve
α-galactosidase	−ve	−ve
β-galactosidase	−ve	−ve
β-glucuronidase	+ve	+ve
Acidification (ribose)	+ve	+ve
Acidification (arabinose)	−ve	−ve
Acidification (mannitol)	+ve	+ve
Acidification (sorbitol)	−ve	−ve
Acidification (lactose)	−ve	−ve
Acidification (trehalose)	+ve	+ve
Acidification (inulin)	−ve	−ve
Acidification (raffinose)	−ve	−ve

3.2. Results of molecular study

Molecular study indicated the presence of 15 *S. iniae* out of the thirty *Streptococcus* isolates that represents 50%; the characteristic amplicon specifically for *S. iniae* appeared at 300 bp target (Fig. 1). The PCR screening of resistance genes among *S. iniae* isolates is showed in Table (3). Results indicate the presence of three isolates (20%) with *erm*(B) gene (Fig. 2). Thus, they are resistant to erythromycin; while, there was another five isolates (33.3%) resistant to tetracyclines; three of them contained *tet*(M) and another 2 contained *tet*(O) genes (Figs. 3, 4).

Table 3. Incidence of resistance genes in the recovered *S. iniae* isolates

No. of tested isolates	*tet*(M)		*tet*(O)		*erm*(B)	
	No.	%	No.	%	No.	%
15	3	20	2	13.3	3	20

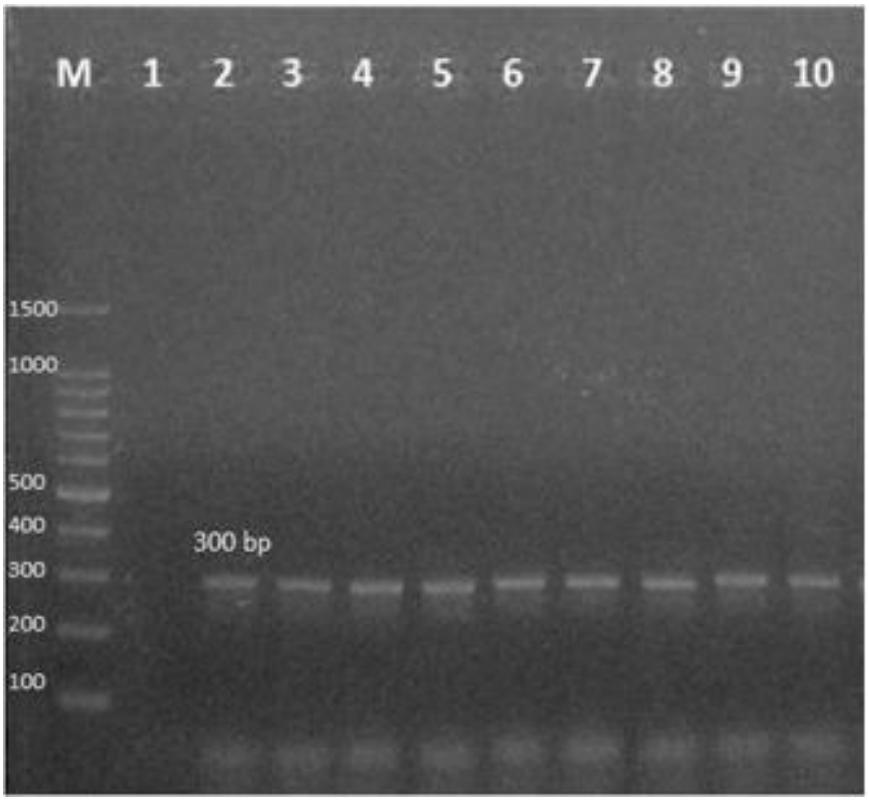

Fig. 1. Gel electrophoresis of *S. iniae* amplicon Lane M: 100 bp DNA ladder, Lane (1): negative control, lane (2-3-4-5-6-7-8-9-10): positive samples 300 bp

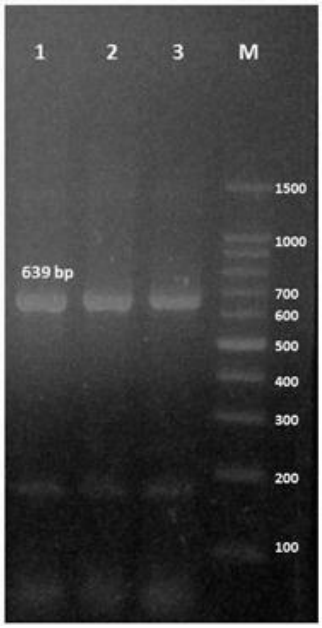

Fig. 2. Gel electrophoresis of *erm (B*) resistant gene amplicon Lane M: 100 bp DNA ladder, lane (1& 2 & 3): positive samples 639 bp

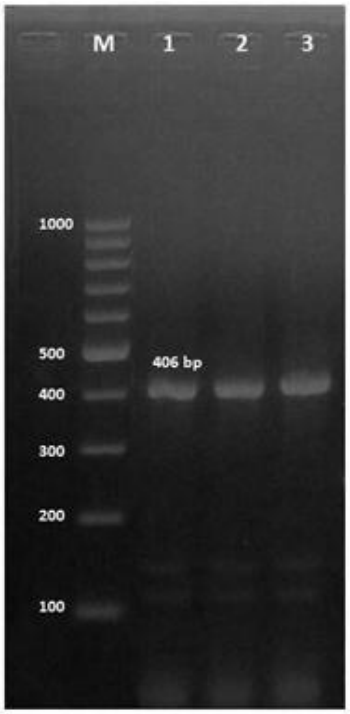

Fig. 3. Gel electrophoresis of *tet*(*M*) resistant gene amplicon Lane M: 100 bp DNA ladder, lane (1& 2 & 3): positive samples 406 bp

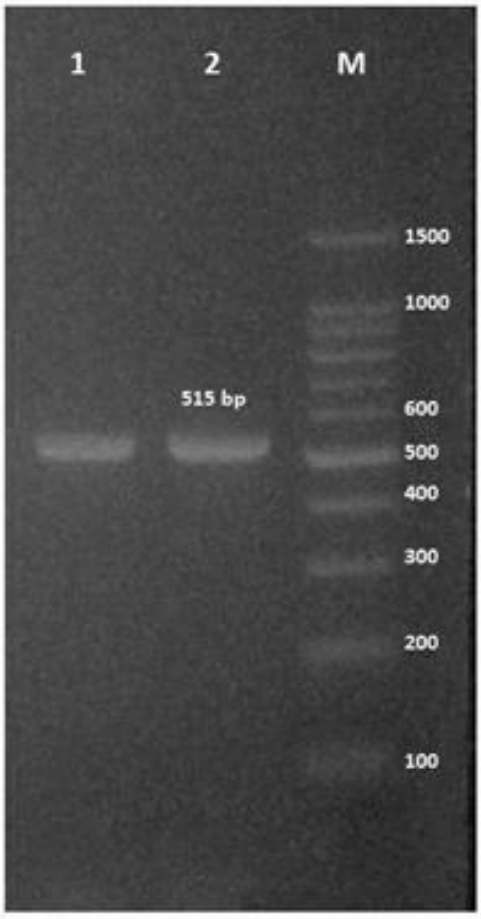

Fig. 4. Gel electrophoresis of *tet*(*o*) resistant gene amplicon Lane M: 100 bp DNA ladder, lane (1& 2): positive samples 515 bp

3.3. Results of antimicrobial susceptibility test

The antimicrobial susceptibility of *S. iniae* to different antibiotics is presented in Table (4), indicating the presence of multi antibiotic resistance profile. The tested *S. iniae* isolates were highly susceptible to Florfenicol (100%), while the majority of these isolates (46.7%) were resistance to Tetracycline and Oxytetracycline, followed by Erythromycin (33.3%). About 26.7% of studied isolates was resistant to both Streptomycin and Norfloxacin, followed by Amoxicillin (20%), Ciprofloxacin (13.3%) and Sulfamethoxazole-trimethoprim (13.3%).

Table 4. Results of antimicrobial susceptibility patterns of *S. iniae* isolates

Isolates	CIP	NOR	TE	OTC	AX	S	SXT	F	E
S_1	S	S	R	R	S	R	S	S	S
S2	I	S	S	S	R	S	I	S	R
S3	S	R	R	R	S	I	S	I	I
S4	R	R	S	S	R	R	R	S	S
S5	I	I	R	R	I	S	S	S	R
S6	S	S	I	I	S	I	I	S	I
S7	S	S	S	S	I	S	S	S	R
S8	S	S	R	R	S	S	S	I	S
S9	I	I	R	R	S	S	S	S	S
S10	S	S	R	R	I	I	R	S	S
S11	S	S	S	S	R	S	I	S	I
S12	R	R	I	I	S	R	S	S	S
S13	S	S	I	I	S	R	S	S	R
S14	S	R	S	S	S	S	S	I	R
S15	S	S	R	R	I	S	S	S	S
NRI	2	4	7	7	3	٤	2	0	5
%	13.3%	26.6%	46.7%	46.7%	20%	26.6%	13.3%	%٠	٣٣.٣

S: Sensitive, **R:** Resistance, **I:** Intermediate, **NRI:** number of resistant isolates, **%:** percent of resistant isolates, **TE**: Tetracycline, **OTC:** Oxytetracycline, **F:** Florfenicol, **NOR:** Norfloxacin, **CIP:** Ciprofloxacin, **E:** Erythromycin, **S:** Streptomycin, **AX:** Amoxicillin, **SXT:** Sulfamethoxazole-trimethoprim

DISCUSSION

Streptococci is considered the most common Gram-positive bacterial pathogen affecting the cultured tilapia worldwide. In this study, thirty streptococci were isolated and biochemically identified from the diseased *Oreochromis niloticus*, representing 21.4% of the total bacterial infection of the diseased fish. In the present study, Fifteen *S. iniae* was further identified by amplifying 16S rRNA gene at the target size (300 bp), and similar results were described in the studies of **Dangwetngam *et al.* (2016)** and **Karen *et al.* (2021)**. The authors diagnosed *S. iniae* isolated from the tilapia through the amplification of 16S rRNA gene. Meanwhile, **Rahmatullah *et al.* (2017)** used *lct*O gene in the molecular detection of *S. iniae* in the infected tilapia tissues. The present work revealed that *S. iniae* is the most common streptococcal species affecting diseased tilapia. In the studied farms, many other researchers such as **Saleh *et al*. (2019)** isolated *S. iniae* from the diseased tilapia. Moreover, **Jantrakajorn *et al.* (2014)** and **Ortega *et al.* (2018)** isolated and identified this pathogen from diseased fish tissues.

The misuse of antibiotics in the veterinary field gives rise to drug fastness and multiple antibiotic resistance among animal pathogens. This can be explained through

horizontal transfer of antibiotic resistance genes between bacterial population **(Rodriguez-Mozaz*et al.*, 2015)**.

The *tet*(M) gene is the most common tetracycline resistant gene found in screened *S. iniae* samples with 20%. Meanwhile, the *tet*(O) gene was found in only 13.3% (2 out of 15). These findings contradicted with those of **Karen *et al.* (2021)** who found that, *tet*(O) gene was present in 29.1%, while only 12.7% of isolates had *tet*(M) gene. In accordance with the current results, **Zeng *et al.* (2006)** reported the presence of *tet*(M) gene in 80.7% of *S. agalactiae* isolates, while *tet*(O) gene was present in only 4.9% of the same isolates. In harmony to the present findings, **Zeng *et al.* (2006)**, **Suanyuk *et al.* (2008)**, **Tamminen *et al.* (2011)**, **Dangwetngam *et al.* (2016), Nguyen *et al.* (2017)**, **Higuera-Llanten *et al.* (2018)** and **Liang *et al.* (2018)** confirmed the abundant distribution of tetracyclines resistance genes, particularly *tet*(*M*) in bacterial isolates recovered from cultured fish farms. This could be attributed to the over use of oxytetracycline in the treatment of bacterial fish diseases **(Aboyadak *et al.*, 2016b)**. Tetracycline resistant genes are present in bacterial plasmid, explaining the rapid distribution and transportation between bacterial populations **(Higuera-Llantén *et al.*, 2018)**.

The current study indicated the presence of *erm*(B) in only three samples (20%); this result was higher than that of **Karen *et al.* (2021)**. In accordance with the present results, **Zeng *et al.* (2006)** recognized *mef* gene (macrolides resistance gene) and erythromycin resistance gene (*erm*) in the bacterial samples collected from cultured fish. **Zeng *et al.* (2006)** and **Nguyen *et al.* (2017)** found *erm*(B) gene in 6.6% and 6.3% of the collected isolates. Multiple antibiotic resistance recorded in this work could be attributed to overuse of antibiotics in the treatment of affected fish farms with possible development of acquired resistance. On the other hand, this can be related to the fertilization of fish ponds with poultry farms droppings, mainly containing some antibiotic residues from the the long-term administration of antibiotics during the production cycling.

CONCLUSION

In this study, fifteen *S. iniae* isolates were identified using 16SrRNA specific primer, tetracyclines resistance genes, particularly *tet*(M) and *tet*(O). In addition, the erythromycin resistant gene *erm*(B) was detected in the collected isolates. Sensitivity test indicated multi antibiotic resistance profile of studied *S. iniae* isolates. It is highly recommended to reduce the misuse of antibiotics in veterinary filed to avoid the development of antibiotic resistant bacterial pathogens.

REFERENCES

Aboyadak, I. M.; Ali, N. G. M.; Goda, A. M. A-S.; Aboelgalagel, W. H. and Alnokrashy, A. M. E. (2015). Molecular Detection of *Aeromonas hydrophila* as

the Main Cause of Outbreak in Tilapia Farms in Egypt. Journal of Aquaculture and Marine Biology, 2(5): 00045. DOI: 10.15406/jamb.2015.02.00045.

Aboyadak, I. M.; Ali, N. G.; Abdel-Aziz, M. M.; Gado, M. S. and El-Shazly, K. A. (2016b). Role of Some Antibacterial Drugs in Control *Streptococcus iniae* Infection in *Oreochromis niloticus*. Journal of Pharmacology and Clinical Research, 1(5): 555573. DOI: 10.19080/JPCR.2016.01.555573.

Aboyadak, I. M.; Sabry, N. M.; Ali, N. G. and El-Sayed, H. S. (2016a). Isolation of *Staphylococcus epidermidis*, *Bacillus cereas* and, *Pseudomonas stutzeri* from diseased European sea bass *(Dicentrarchus labrax)* for the first time in Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 20(4): 103-114.

Acar, U.; Kesbic, O. S.; Yılmaz, S.; Gultepe, N. and Turker, A. (2015). Evaluation of the effects of essential oil extracted from sweet orange peel *(Citrus sinensis)* on growth rate of tilapia *(Oreochromis mossambicus)* and possible disease resistance against *Streptococcus iniae*. Aquaculture, 473: 282-286.

Ali, N. G. M.; Aboyadak, I.M. and El-Sayed, H. S. (2019). Chemotherapeutic control of Gram-positive infection in white sea bream (*Diplodus sargus*, Linnaeus 1758) broodstock, Veterinary World, 12(2): 316-324. doi: 10.14202/vetworld.2019.316-324.

Ali, N. G.; Aboyadak, I. M. and Gouda, M. Y. (2018). Rapid Detection and Control of Gram-negative Bacterial Pathogens Isolated from Summer Mortality Outbreak Affecting Tilapia Farms. Journal of Biological Sciences, 19(1):24-33. DOI: 10.3923/jbs.2019.24.33.

Ali, N. G.; Ali, T. E.; Aboyadak, I. M. and Elbakry, M. A. (2021a). Controlling *Pseudomonas aeruginosa* infection in *Oreochromis niloticus* spawners by cefotaxime sodium. Aquaculture, 544, 737107, https://doi.org/10.1016/j.aquaculture.2021.737107.

Ali, N. G.; El-Nokrashy, A. M.; Gouda, M. Y. and Aboyadak, I. M. (2021b). Summer Mortality Syndrome Affecting Cultured European Seabass at Kafrelsheikh Province, Egypt. Front. Mar. Sci., 8:717360. doi: 10.3389/fmars.2021.717360.

Chen, M.; Li, L.P.; Wang, R.; Liang, W.W.; Huang, Y. and Li, J. (2012). PCR detection and PFGE genotype analyses of streptococcal clinical isolates from tilapia in China. Vet Microbiol 159(3–4), 526–530. https://doi.org/10.1016/J.VETMIC.2012.04.035.

Clinical and Laboratory Standards Institute (CLSI) (2018). Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100-S28. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA.

Cui, M.; Zhang, H.; Li, J.; Liu, R.; Wu, M.; Xu, D. and Zhang, Q. (2019). Differential PCR detection of *Streptococcus agalactiae* and *Streptococcus iniae* in tilapia (Oreochromis niloticus) by a single primer pair. J Fish Dis.42(8), 1211.https://doi.org/10.1111/jfd.13014.

Dangwetngam, M.; Suanyuk, N.; Kong, F. and Phromkunthong, W. (2016). Serotype distribution and antimicrobial susceptibilities of *Streptococcus agalactiae* isolated from infected cultured tilapia (*Oreochromis niloticus*) in Thailand: nine-year perspective. J. Med. Microbiol., 65(3), 247–254.https://doi.org/10.1099/jmm.0.000213

Dawood, M. A. O.; Eweedah, N. M.; Moustafa, E. M. and Shahin, M. G. (2019). Symbiotic Effects of *Aspergillus oryzae*and β-Glucan on Growth and Oxidative and Immune Responses of Nile Tilapia, *Oreochromis niloticus*. Probiotics and Antimicrobial Proteins, 8: 1-2.

Deng, L.; Li, Y.; Geng, Y.; Zheng, L.; Rehman, T. and Zhao, R. (2019). Molecular serotyping and antimicrobial susceptibility of *Streptococcus agalactiae* isolated from fish in China. Aquaculture, 510, 84–89. https://doi. org/10.1016/j.aquaculture.2019.05.046.

El-Bahar, H. M.; Ali, N. G.; Aboyadak, I. M.; Khalil, S. A. and Ibrahim, M. S. (2019). Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in *Oreochromis niloticus*. International Microbiology, doi: 10.1007/s10123-019-00075-3.

Food and Agriculture Organization of the United Nations, FAO (2018). The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. FAO.

GAFRD (2020). Fish Statistics Yearbook 2018. Egypt: General Authority for Fish Resources, Ministry of Agriculture Publications

Ghittino, C.; Latini, M.; Agnetti, F.; Panzieri, C.; Lauro, L.; Ciappelloni, R. and Petracca, G. (2003). Emerging pathologies in aquaculture: effects on production and food safety. Vet Res Commun., 1:471-479. doi: 10.1023/b:verc.0000014204.37722.b6.

Giovanetti, E.; Brenciani, A.; Lupidi, R.; Roberts, M.C. and Varaldo, P. E. (2003). Presence of the *tet*(O) gene in erythromycin and tetracycline resistant strains of *Streptococcus pyogenes* and linkage with either the *mef*(A) or the *erm*(A) gene. Antimicrob. Agents Chemother., 47, 2844–2849.

Hai, N. V. (2015). Research findings from the use of probiotics in tilapia aquaculture: a review. Fish & Shellfish Immunology, 45: 592-597. doi: 10.1016/j.fsi.2015.05.026.

Higuera-Llantén, S.; Vásquez-Ponce, F.; Barrientos-Espinoza, B.; Mardones, F. O.; Marshall, S. H. and Olivares-Pacheco, J. (2018). Extended antibiotic treatment in salmon farms select multi-resistant gut bacteria with a high prevalence of antibiotic resistance gene. PLoS One, 13(9): e0203641. https://doi.org/10.1371/journal.pone.0203641.

Hussain, S. M.; Javed, M.; Javid, A.; Javid, T. and Hussain, N. (2011): Growth responses of *Catla catla, Labeo rohita* and *Cirrhina mrigala* during chronic exposure of iron. Pak J. Agric. Sci., (1): 48: 225-230.

Jantrakajorn, S.; Maisak, H. and Wongtavatchai, J. (2014). Comprehensive investigation of Streptococcosis outbreaks in cultured Nile tilapia, *Oreochromis niloticus*, red tilapia, *Oreochromis sp*., of Thailand. J. World Aquacult. Soc., 45(4), 392–402. https://doi.org/10.1111/jwas.12131.

Jones, C.H.; Tuckman, M.; Murphy, E. and Bradford, P. A. (2006). Identification and sequence of a *tet*(M) tetracycline resistance determinant homologue in clinical isolates of *Escherichia coli*. J. Bacteriol., 188 (20), 7151–7164. doi: 10.1128/JB.00705-06

Karen, O. B.; Jorengeth, A. R. R.; Carolina, S. B.; Juan, E. B. C.; Nelson, P. N.; Cesar, M. E. B. and, 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. *Aquacult Int* 29, 2337–2355 (2021). https://doi.org/10.1007/s10499-021-00751-0

Lee, J. M.; Jang, W. J.; Hasan, M. T.; Lee, B. J.; Kim, K. W.; Lim, S. G.; Han, H. S. and Kong, I. S. (2019). Characterization of a *Bacillus sp*. isolated from fermented food and its symbiotic effect with barley β-glucan as a biocontrol agent in the aquaculture industry. Applied Microbiology and Biotechnology, 103: 1429-1439. doi: 10.1007/s00253-018-9480-9.

Liamnimitr, P.; Mon-on, N.; Jaemwimon, P.; Dachavichitlead, W. and Surachetpong, W. (2017). Detection of *Streptococcus iniae* and *Streptococcus agalactiae* in tilapia (*Oreochromis sp*.) in Thailand. Thai. J. Vet. Med. Suppl., 47: 285-286.

Liang, J.; Huang, L.; Wei, M.; Ma, S.; Li, S.; Wen, Y.; Meng, W.; Huang, S. and Huang, J. (2018). Detection of antibiotics resistance and tetracycline resistance genes in *Streptococcus agalactiae* from tilapia in Guangxi. Journal of Southern Agriculture, 49(10): 2077–2086. https://doi.org/10.3969/j.issn.2095-1191.2018.10.26

Maisak, H.; Patamalai, B.; Amonsin, A. and Wongtavatchai, J. (2008): Streptococcosis in Thai culture tilapia *Oreochromis niloticus*. Thai. J. Vet. Med., 38:85-86.

Markey, B. K.; Leonard, F.C.; Archambault, M.; Cullinane, A. and Maguire, D. (2013). Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Mohamed, H. M.; Emeish, W. F.; Braeuning, A. and Hammad, S. (2017). Detection of aflatoxin-producing fungi isolated from Nile tilapia and fish feed. EXCLI journal, 16: 1308-1318 – ISSN 1611-2156.

Ng, L. K.; Martin, I.; Alfa, M. and Mulvey, M. (2001). Multiplex PCR for the detection of tetracycline resistant genes. Mol. Cell Probes., 15, 209–215.

Nguyen, T. T. T.; Nguyen, H. T.; Tsai, M. A.; Byadgi, O.; Wang, P. C. and Yoshida T. and Chen, S. C. (2017). Genetic diversity, virulence genes, antimicrobial resistance of *Streptococcus dysgalactiae* isolates from different aquatic animal sources. Aquaculture 479, 256–264. https://doi.org/10.1016/J.AQUACULTURE.2017.06.002

Ortega, C.; García, I.; Irgan, R.; Fajardo, R.; Tapia-Cammas, D.; Acosta, J. and Avendaño-Herrera, R. (2018). First identification and characterization of *Streptococcus iniae* obtained from tilapia (*Oreochromis aureus*) farmed in Mexico. J. Fish Dis. 41(5), 773–782. https://doi.org/10.1111/jfd.12775

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W. J. and Leonard, F. C. (2002). Veterinary microbiology and microbial disease. First Published Blackwell Science Company, Iowa, State University Press.

Rahmatullah, M.; Ariff, M.; Kahieshesfandiari, M.; Daud, H.M.; Zamri-Saad, M. and Sabri, M. Y. (2017). Isolation and pathogenicity of *Streptococcus iniae* in cultured red hybrid tilapia in Malaysia. J. Aquat. Animal Health, 29(4): 208–213. https://doi.org/10.1080/08997659.2017.1360411

Roberts, M. C. (1996). Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol. Rev., 19, 1–24.

Rodriguez-Mozaz, S.; Chamorro, S.; Marti, E.; Huerta, B.; Gros, M. and Sànchez-Melsió, A. (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 69, 234–242. https://doi.org/10.1016/J.WATRES.2014.11.021.

Saleh, H., Ali, N. G., Aboyadak, I. M., Nadia, S. A. (2019). Subcellular degenerative changes in hepatopancreas and posterior kidney of *Streptococcus iniae* infected Nile tilapia using Transmission Electron Microscope. Egyptian Journal of

Aquatic Biology and Fisheries, 23(1): 305-316. DOI: 10.21608/EJABF.2019.28099.

Sapkota, A. R.; Ojo, K. K.; Roberts, M. C. and Schwab, K. J. (2006). Antibiotic resistance genes in multidrug-resistant Enterococcus spp. and Streptococcus spp. recovered from the indoor air of a largescale swine-feeding operation. Lett. Appl. Microbiol., 43 (5), 534–540. doi: 10.1111/j.1472-765X.2006.01996.x.

Seyfried, E. E.; Newton, R. J.; Rubert, K. F.; Pedersen, J. A. and McMahon, K. D. (2010). Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. Microbl. Ecol., 59(4), 799–807. https://doi.org/10.1007/s00248-009-9624-7

Suanyuk, N.; Kong, F.; Ko, D.; Gilbert, G.L. and Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia Oreochromis sp. and Nile tilapia *O. niloticus* in. Thailand relationship to human isolates. Aquaculture, 284:(1–4), 35–40. https://doi.org/10.1016/j.aquaculture. 2008.07.034

Tamminen, M.; Karkman, A.; Lõhmus, A.; Muziasari, W. I.; Takasu, H. and Wada, S. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environ. Sci. Technol., 45(2): 386–391. https://doi.org/10.1021/es102725n

Wu, Y.; Gong, Q.; Fang, H.; Liang, W.; Chen, M. and He, R. (2013). Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT *Oreochromis niloticus*) and disease resistance against Streptococcus agalactiae. Fish and Shellfish Immunology, 34(1): 220–227. https://doi.org/10.1016/ j.fsi.2012.10.020

Zeng, X.; Kong, F.; Wang, H.; Darbar, A. and Gilbert, G. L. (2006). Simultaneous detection of nine antibiotic resistance related genes in *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization assay. Antimicrob. Agents Chemother., 50(1), 204–209. https://doi.org/10.1128/AAC.50.1.204-209.2006

Zhang, Z.; Li, Y.; Hu, M. and Yu, A. (2020). Comparative transcriptome profiling reveals a mechanism of *Streptococcus agalactiae* resistance to florfenicol. Microb.Pathog., 142, 104098. https://doi.org/10.1016/j.micpath.2020.104098.

Zlotkin, A.; Hershko, H. and Eldar, A. (1998). Possible transmission of *Streptococcus iniae* from wild fish to cultured marine fish. Applied and Environmental Microbiology, 64, 4065–4067. doi: 10.1128/AEM.64.10.4065-4067.1998.