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Characterization and susceptibility of streptococci and enterococci isolated from Nile tilapia (*Oreochromis niloticus*) showing septicaemia in aquaculture and wild sites in Egypt

Kamelia M. Osman^{1*}, Khalid S. Al-Maary², Aymen S. Mubarak², Turki M. Dawoud², Ihab M. I. Moussa², Mai D. S. Ibrahim³, Ashgan M. Hessain⁴, Ahmed Orabi¹ and Nehal M. Fawzy⁵

Abstract

Background: The present investigation was an endeavor into the elucidation of the disease-causing pathogen of streptococcosis in Nile tilapia (*Oreochromis niloticus*) in Egypt affecting adult fish cultured and wild fish in the Nile river. Fish were obtained from commercial fishermen, collected as part of their routine fishing activities. The researchers observed the routine fishing process and selected fish for use in the study, at the point of purchase from the fisherman.

Results: Diseased fish showed exophthalmia with accumulation of purulent and haemorrhagic fluid around eyes, and ventral petechial haemorrhages. The Post mortem examination revealed, abdominal fat haemorrhage, pericarditis and enlargement of the liver, spleen and kidney. Gram-stained smears revealed the presence of Gram-positive cocci, β -hemolytic, oxidase and catalase negative. Analysis of the 16S rRNA gene confirmed that the 17 tilapia isolates studied were 6/17 *Enterococcus faecalis*, 2/17 *Enterococcus gallinarum*, 3/17 *Streptococcus pluranimalium*, 2/17 *Aerococcus viridans*, 1/17 isolate of each *Streptococcus dysgalactiae*, *Streptococcus anginosus*, *Lactococcus garvieae* and *Granulicetella elegans/Leuconostoc mesenteroides cremoris*. It should be noted that there was no mixed infection. Multiple resistance was observed and the most frequent antibiotic combination was penicillin, ampicillin, vancomycin, chloramphenicol, rifampicin, ofloxacin, clindamycin, erythromycin and tetracycline representing eight classes.

Conclusions: Consequently, we concluded that *Streptococcus* species are an emerging pathogen for Nile tilapia aquaculture in Egypt and to be considered as a new candidate in the warm water fish diseases in Egypt with special reference to *L. garvieae*, *S. dysgalactiae* in addition to *L. mesenteroides cremoris* which was not reported before from tilapia and taking into consideration their zoonotic implications for public health.

Keywords: *Streptococcus* species, Tilapia (*Oreochromis* spp.), Lancefield C, *L. Garvieae*, *S. Dysgalactiae*, *L. Mesenteroides cremoris*, Antibiotic resistance

* Correspondence: kamelia-osman@hotmail.com

¹Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Full list of author information is available at the end of the article



Background

The common name Nile tilapia (*Oreochromis niloticus*) is a species of Tilapia (*Oreochromis* sp.) which in turn is the regular name for about a hundred species of cichlid fish from the tilapiine cichlid lineage [1]. They are freshwater fish species, local to Africa and Middle East areas [2], and the third biggest group of bony fish. Delineations from Egyptian tombs underscored that Nile tilapia have been developed for more than 4000 years [3]. By the second half from the twentieth century, tilapia were brought into a myriad of tropical, sub-tropical and mild locales of the globe [4] becoming a standout asset amongst the most refined fish species adding to the global nourishment in the fish industry [5] where the sustenance of more than half a billion people in the Third World are supported by fisheries and aquaculture [6]. The market price of farmed tilapia boosted up from 154 million USD in 1984 to 4000 million USD in 2010 [7]. China, with a production capacity of 806,000 MT represents almost 50% of the total global production positioned itself as the largest Nile tilapia producer, with Egypt to follow with 200,000 MT, the Philippines with 111,000 MT, Thailand with 97,000 MT and Indonesia with 72,000 MT. Five other countries come next in their capacity for Nile tilapia production, The Lao People's Democratic Republic, Costa Rica, Ecuador, Colombia and Honduras [8]. Also, although significant quantities of tilapia are also produced annually by Cuba, Israel, Malaysia, the USA, Viet Nam and Zimbabwe, yet it should be noted that, their production is reported to FAO as 'tilapias nei' (which may include other tilapia species) and 'freshwater fishes nei' [8].

Streptococcosis is a worldwide threat and epidemiological studies revealed 500 streptococcal isolates in more than 50 sites in 13 countries (<http://www.thefishsite.com/articles/190/streptococcus-in-tilapia/#sthash.G4T0Xzsl.dpuf>). Streptococcosis, also known as 'pop-eye', is contagious with high mortality and has assumed its importance due to being the most crushing threat as it can bring about huge number of deaths of large size fish causing heavy commercial losses in Australia, Israel, Italy, Japan, Korea, South Africa, Colombia, Indonesia and USA [9, 10]. The global commercial losses estimated to reach 250 million USD in 2008 [11]. Within 3 to 7 days, acute *Streptococcus* infections in fish induce >50% mortality rates [12] while chronic infections the mortalities could extend to several weeks, with a daily death of one or two of the fish. In most cases, the clinical symptoms of *Streptococcus* infection, with no species differences (<http://www.thefishsite.com/articles/190/streptococcus-in-tilapia/#sthash.G4T0Xzsl.dpuf>), is usually in the form of lethargic, erratic swimming (spiraling or spinning swimming), dark skin pigmentation, exophthalmia with opacity and haemorrhage in the eye, abdominal

distension, diffused haemorrhage in the operculum, around the mouth, anus and base of the fins and enlarged blackened spleen [8, 10, 13–15].

Streptococcosis is not limited to geographic boundaries or/and host range causing a global flare-up in aquaculture farms [16]. A diversity of fish species are susceptible to infection, including sturgeon, various ornamental fish, including rainbow sharks, red-tailed black sharks, rosey barbs, danios, some cichlids including Venustus (*Nimbochromis* ("Haplochromis") *venustus*) and *Pelvicachromis* sp. and several species of tetras, coho salmon, yellowtail (*Seriola quinqueradiata*), Jacopever (*Sebastes schlegeli*), salmonids, Japanese eel (*Anguilla japonica*), ayu and tilapia (*Oreochromis* spp.), striped bass (*Morone saxatilis*), Atlantic croaker (*Micropogon undulatus*), blue fish (*Pomatomus saltatrix*), channel cat fish, golden shiner (*Notemigonus chrysoleuca*), hardhead (sea) cat fish (*Arius felis*), menhaden (*Brevoortia patronus*), pin fish (*Lagodon rhomboides*), sea trout (*Cynoscion regalis*), silver trout (*Cynoscion nothus*), spot (*Leiostomus xanthurus*), stingray (*Dasyatis* sp.), striped bass (*Morone saxatilis*), striped mullet (*Mugil cephalus*) and turbot (*Scophthalmus maximus*) [17, 18].

The most relevant *Streptococcus* species that cause disease in the tilapia farming globally are *S. iniae*, *S. agalactiae*, *S. dysgalactiae* and *Lactococcus garviae* [19, 20]. Since the first description of a streptococcal infection in rainbow trout (*Oncorhynchus mykiss*) [21], the diversity and the inherent characteristics of the bacterial species incriminated with streptococcosis was debatable [9, 17]. High and low temperatures affect the virulence agents at which streptococcosis is induced [22]. Warm water streptococcosis implicates species such as *L. garviae* (synonym *E. seriolicida*), *S. iniae* (synonym *S. shiloi*), *S. agalactiae* (synonym *S. difficilis*) or *S. parauberis* inducing mortalities at temperatures above 15 °C. Differently, *Vagococcus salmoninarum* or *L. piscium* and *S. phocae* are incriminated in cold water streptococcosis at temperatures below 15 °C. The ability of *S. phocae* to cause cold water streptococcosis was evident when researchers were able to isolate it from marine mammals inhabiting cold waters, including Cape fur seal (*Arctocephalus pusillus pusillus*), ringed seal (*Phoca hispida*) and harbor porpoise (*Phocoena phocoena*) and gray seal (*Halichoerus grypus*), harbor seal (*Phoca vitulina*) and cetaceans [9, 10]. Pathogenic fish *Streptococcus* species have been associated with *S. agalactiae*, *S. difficilis*, *S. dysgalactiae*, *S. equi*, *S. equisimilis*, *S. (= E.) faecium*, *S. ictaluri*, *S. iniae*, *S. milleri*, *S. parauberis*, *S. phocae*, *S. pyogenes* and *S. zooepidemicus*. In addition, *E. faecalis* NCTC 775 T, *E. faecium* NCTC 7171 T, *L. lactis* NCFB 604, *S. mutans* NCFB 2062 provoke streptococcosis in Atlantic salmon and rainbow trout [9, 17].

Zoonotically, streptococcosis is of great significance to delineate the infectious etiology of streptococcosis in Nile tilapia as a potential cause of disease in humans [16, 17] which caused a significant increase in the use of antibiotics in aquaculture. The present investigation was an endeavor into the elucidation of the disease-causing pathogen of streptococcosis in Nile tilapia (*O. niloticus*) in Egypt affecting adult fish cultured and wild fish in the Nile river by studying the phenotypic, antibiotic resistance and molecular characterization of the streptococcal strains isolated from Nile tilapia demonstrating septicemia.

Methods

Fish sampling

Fish were obtained from commercial fishermen, collected as part of their routine fishing activities. The researchers observed the routine fishing process and selected fish for use in the study, at the point of purchase from the fisherman the fish were deceased and as such no ethical approval was required for this study. A total number of 80 Nile tilapia were collected as freshly dead or moribund fish (Fig. 1a-d) presenting at least one or more of the clinical signs of septicemia (eye lesions: in the form of unilateral or bilateral eye redness/opacity, skin lesions: detached scales, extensive skin congestion, ulcers, hemorrhage, or dark discoloration in the form of strips, fins: congestion at the base of fins, or even

hemorrhages, abdomen: slightly distended in some cases, anal opening: congested protruded anal opening) were collected from different locations in Egypt (River Nile in Giza, Kafr Elzayat, Bani-sweif and a breeding farm at El-Tal El-Kebir) during the period 2013–2014. Selection of locations and farms from which the diseased fish were collected depended on their availability at time sampling, accessibility and proximity. After collection, the sampled fish were placed in expanded polystyrene boxes, covered with a plastic film, transported refrigerated to the laboratory and processed for microbiological analysis within 3 h in order to detect *Streptococcus* spp.. Septicaemic fish samples were submitted to the Fish Disease Department, Animal Health Research Institute, Dokki, Egypt.

Isolation and phenotypic characterization of bacteria

The swabs from the fish matrix that were collected (liver, kidney, brain, spleen and ascitic fluid) were immediately streaked onto Columbia agar supplemented with 5% defibrinated sheep blood (CBA) (Oxoid Ltd., Basingstone, England) plates and incubated at 30 °C for 24 h in 5% CO₂ atmosphere. Suspected colonies were picked, purified and subjected to phenotypic differential analysis by conventional procedures by their morphological, physiological and biochemical plate and tube tests and in addition to the Gram-positive identification (GPI) cards of the Vitek 2 system (bioMérieux, France) which was

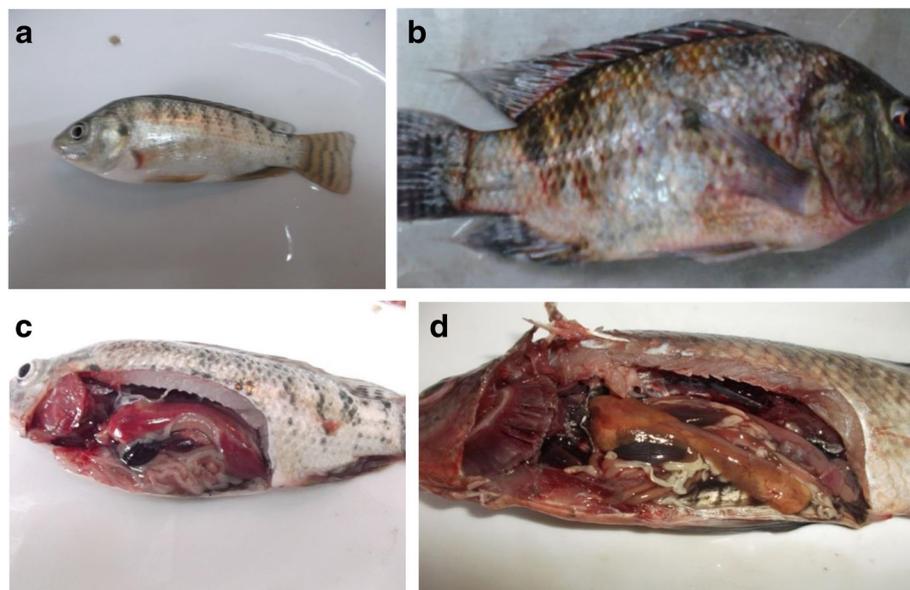


Fig. 1 **a** Body shape of Tilapia fish laterally compressed to oval. Lateral line interrupted with cycloid scales. Caudal fin has distinct vertical stripes and strong body vertical bands. Healthy transparently clear and spherical eye lenses. Normal glistening skin with no pathological lesions observed. **b** Tilapia fish showing externally eye redness, hemorrhage and ulcers on different parts of the body, hemorrhagic dorsal and tail fins, extensively detached scales and distended abdomen with signs of bleeding beneath the skin appearing as small dots the size of a pinprick or as a patch. **c** Shows normal appearance of Tilapia fish with normal liver, kidney, gall bladder and intestines with bright red gills. **d** Tilapia fish postmortem internal findings revealed typical signs of septicemia in which severely congested gill, kidney, spleen, intestine and heart, dark gall bladder, in addition to hemorrhagic ascites, friable pale liver, muscle redness, and parts of the intestine were devoid of food

used following the manufacturer's instructions. The ability of the strains to grow at 10 and 45 °C was tested in Brain-heart infusion medium over a period of 10 days.

Preparation of genomic DNA and genetic characterization

The suspected *Streptococcus* isolates were cultured in Todd Hewitt Broth at 25 °C for 24 h, and genomic DNA was extracted using the Qiagen Genomic-tip 500/G kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was estimated spectrophotometrically before use. The extracted DNA was used in the process of amplification of the 16S rRNA gene of the *Streptococcus* species -specific isolates with a pair of specific primers for each species using polymerase chain reaction (PCR) as outlined in Table 1. Seven µl of the amplified products was electrophoresed using 1.0% (w/v) agarose gel in 1× TBE electrophoresis buffer (0.1 mM Tris/HCl, 0.1 mM boric acid, 0.002 mM EDTA, pH 8.3). The gel was stained with GelRed Nucleic Acid Gel Stain. A negative control (no template DNA) and positive controls consisting of *E. gallinarum* ATCC 49673, *E. faecalis* ATCC 19433, *L. garvieae* ATCC 43921, *S. pluranimalium* ATCC 700864^T, *A. viridans* ATCC 11536 T (accession no. M58797), *S. anginosus* ATCC33397 were included in each run.

Serological assays

The Lancefield streptococcal group of the isolated strains was identified using the Oxoid Streptococcal Grouping Kit DR0585 (Oxoid Ltd., Basingstone, England) following the manufacturer's instructions.

Antimicrobial susceptibility testing

The resistance of the isolated strains to antibiotics was assayed by the disc diffusion method [18, 29–31]. *E. faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658, *Pseudomonas aeruginosa* ATCC 27853 were used as Quality control strains for disk diffusion susceptibility testing of aquatic bacterial isolates [32]. The upcoming antibiotic discs (Oxoid, Ltd., Basingstoke, England) selected for testing are the most frequently used and prescribed in Egypt which are inhibitors of protein synthesis: phenicols (chloramphenicol), tetracyclines (tetracycline) and macrolides (erythromycin) for both *Enterococcus* and *Streptococcus* species, while aminoglycosides (gentamicin, streptomycin and amikacin) only for *Enterococcus* species; cell wall synthesis: penicillins (penicillin, ampicillin, amoxicillin-clavulonic acid and piperacillin-tazobactam) and glycopeptides (vancomycin) for both *Enterococcus* and *Streptococcus* species and polymyxins (colistin) for *Enterococcus* species; nucleic acid synthesis: ansamycins (rifampicin) and nitrofurantoin (nitrofurantoin) were used for both *Enterococcus* and *Streptococcus* species, fluoroquinolones (ciprofloxacin for *Enterococcus* species and ofloxacin for *Streptococcus* species), quinolones (nalidixic acid for *Enterococcus* species) and lincosamides (clindamycin) for *Streptococcus* species.

Statistical analysis

The experimental data obtained was subjected to multiple linear regressions using Microsoft Excel 2007 to evaluate the statistical analysis between the percentage of antimicrobial resistance (y- range) and actual concentration (x- axis) for all antibiotics to enable us to plot percent inhibition versus drug concentration for each strain.

Table 1 Oligonucleotide primers sequences and size of the PCR-targeted products

Microorganism	Target Gene	Bp fragment	Primer sequence (5' - 3')	Annealing Temp (°C)	References
<i>E. faecalis</i> / <i>E. gallinarum</i>	16S rRNA (Genus -specific primers)	112 bp	F- TAC TGA CAA ACC ATT CAT GAT G R- AAC TTC GTC ACC AAC GCG AAC	59 (<i>E. faecalis</i>) 50 (<i>E. gallinarum</i>)	[23]
<i>E. faecalis</i>	<i>ddl</i> _{<i>E.faecalis</i>} (Species-specific primers)	941 bp	F- ATC AAG TAC AGT TAG TCT R- ACG ATT CAA AGC TAA CTG	55	[24]
<i>L. garvieae</i>	16S rRNA (species-specific primers)	1100 bp	F- CAT AAC AAT GAG AAT CGC R- GCA CCC TCG CGG GTTG	58	[25]
<i>S. pluranimalium</i>	16S rRNA (species-specific primers)	1500 bp	F- AGA GTT TGA TCC TGG CTC AG R- ACG GCT ACC TTG TTA CGA CTT	52	[26]
<i>A. viridans</i>	16S rRNA gene (species-specific primers)	540 bp	F- GTG CTT GCA CTT CTG ACG TTA GC R- TGA GCC GTG GGC TTT CAC AT	55	[27]
<i>S. anginosus</i>	MIL (<i>pbp2b</i>) (Anginosus Group specific)	275 bp	F- TGC TGC AAC GGT AGC TAA TGG R- GAA AGG TTT CTG CTG TCC CTG	58	[28]
	16srRNA (Species-specific primers)	105 bp	F- GCG TAG GTA ACC TGC CTA TTA GA R- CGC AGG TCC ATC TAC TAGC	58	

Table 3 Prevalence and Types of *Enterococcus*, *Lactococcus* and *Streptococcus* species isolated from diseased fish organs using 16 s rRNA

Matrix	Total No.	Species																Total	
		<i>E. faecalis</i>		<i>S. pluranimalium</i>		<i>A. viridans</i>		<i>E. gallinarum</i>		<i>S. anginosus</i>		<i>L. garvieae</i>		<i>S. dysgalactiae equisimilis</i>		<i>Granulicetella elegans / Leuconostoc mesenteroides cremoris</i>		n	%
		n	%	n	%	n	%	n	%	n	%	n	%	n	%				
Liver	80	3	3.8	2	2.5	1	1.3	1	1.3	1	1.3	1	1.3	0	0	0	0	8	10.0
Kidney	80	1	1.3	0	0	1	1.3	1	1.3	0	0	0	0	0	0	0	0	3	3.8
Brain	80	0	0	0	0	0	0	0	0	0	0	0	0	1	1.3	0	0	1	1.3
Spleen	80	1	1.3	1	1.3	0	0	1	1.3	0	0	0	0	0	0	1	1.3	4	5
Ascitic fluid	3	1	33.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	33.3
Total	323	6	1.9	3	0.9	2	0.6	2	0.9	1	0.3	1	0.3	1	0.3	1	0.3	17	5.3

n, number of isolates

clindamycin, erythromycin and tetracycline (7/17, 41.2%) representing eight classes (Table 5).

Discussion

In this work, a collection of 17 strains of *Streptococcus* species isolated from diseased tilapia were biochemically, serologically and genetically characterized and data on their prevalence of the disease in Egypt. In contrast to other results [18], 16 of our isolates could not be serologically typed to any Lancefield group and in the Vitek 2 inconclusive results were obtained, a phenomenon which was also previously observed by Figueiredo et al. [33]. On the other hand, our sole isolate of *S.*

dysgalactiae subspecies *equisimilis* which was grouped in the C group was also identified in Japan by Nomoto et al. [34, 35] and Abdelsalam et al. [36], in the amberjack (*Seriola dumerili*) and yellowtail (*Seriola quinqueradiata*) and in Amur sturgeon (*Acipenser schrenckii*) in China [37] and in Nile tilapia (*Oreochromis niloticus* (L.) in Brazil [20].

Our detection of *Streptococcus* species in the three organ tissues (liver, spleen and kidney) implicates them as the target organs of the organism [38–40]. Fish pathogenic strains have been described, as either an α - or β -haemolytic or as non-haemolytic [41]. Superficially, this data could surmise heterogeneity amid the pathogens,

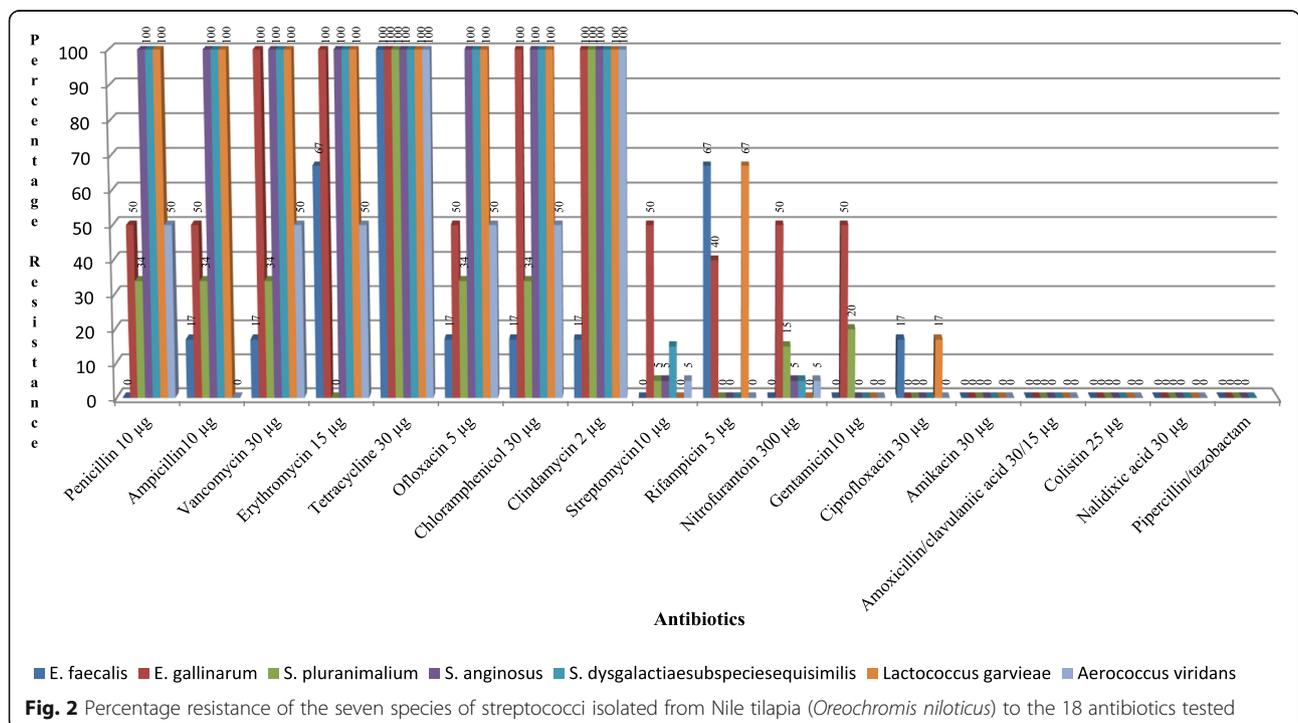


Table 4 Statistical analysis between the percentage of antimicrobial resistance (y- range) and actual concentration (x- axis) of antibiotics for the isolated seven strains

	<i>E. faecalis</i>	<i>E. gallinarum</i>	<i>S. pluranimalium</i>	<i>S. anginosus</i>	<i>S. dysgalactiae subspecies equisimilis</i>	<i>L. garvieae</i>	<i>A. viridans</i>
<i>E. faecalis</i>	1						
<i>E. gallinarum</i>	0.534252	1					
<i>S. pluranimalium</i>	0.434469	0.696294	1				
<i>S. anginosus</i>	0.458603	0.7774	0.693549	1			
<i>S. dysgalactiae subspecies equisimilis</i>	0.455604	0.783461	0.692309	0.999059	1		
<i>L. garvieae</i>	0.593849	0.767848	0.650752	0.953986	0.950886	1	
<i>A. viridans</i>	0.555896	0.785501	0.873946	0.824254	0.823841	0.784558	1

inspite of the fact that some confirmed ranks such as *S. agalactiae*, incorporates both α - and β -haemolytic strains. It should be noted that, many features are common within the predominance of fish pathogens [9]. Yet, this does not exclude some discrepancies reported in isolates recovered from the Transvaal in South Africa [42] and other isolates from Japan [43–45]. Such discrepancies could be due to the dissimilarity and absence of a standardized test protocols and/or point to heterogeneity in the species composition of the organisms.

The development and application of molecular methodology has been of great benefit and advantage through facilitating identification precision, classification of streptococci [17] and time-saving. Thus, our use of the PCR in the species -specific analysis of the 16S rRNA gene was of great assistance in the present investigation and unequivocally demonstrated that all of them belonged to the *Streptococcus* species. To a certain degree, geographical discrepancies have been reported from South Africa [42], Japan [46] and Italy [47]. It is of interest to reveal that according to the available literature, *L. garvieae* although being rarely isolated from tilapia with streptococcosis, it was identified in two countries only, Brazil [19] and Indonesia [10]. This makes our detection of *L. garvieae* in our isolates, the third report. Interestingly, the isolation of *Leuconostoc mesenteroides cremoris* in our investigation was not reported before from tilapia.

Aquaculture is a developing field where antibiotics are intensely used, either specifically to the water or in fish sustenance, as prophylactics to control infections, which has brought about the blooming of AR genes in the aquatic environment [48]. The measure of AMR in aquaculture is under dispute as there is an absence of consent on a standard indicator organism. Ratably, the utilization of antimicrobials in aquaculture seems, by all accounts, to be of substantially more prominent greatness when compared with terrestrial animals. A shortage of solid ratable and subjective information on antimicrobial use (AMU) in different animal production systems over the area is a noteworthy hole in the exploration. Subjective and ratable accessible information (for the most part AMU recurrence of particular antimicrobials) from particular examinations recommends a high assorted variety of antimicrobials utilized both as development promoters (AGPs), and in addition for prophylactic and treatment purposes, despite the fact that outcomes are hard to look at crosswise over investigations. As a result of the extensive contrasts in underway frameworks, sampling and assay techniques, phenotypic evaluations of AMR pervasiveness in this audit should be deciphered with extraordinary care. Generally, the collection of samples are liable to have an expansive testing mistake. The antibiotics used in aquaculture represent different classes of antibiotics, some of which are classified as “Highest Priority Critically Important Antimicrobials” in medical treatment in the WHO list of

Table 5 Antibiotic resistance combinations profile of the 17 *Streptococcus* species isolated from Nile tilapia

Antibiotic resistance combination profile	number of isolates/17	% of isolates	number of antibiotics	Number of classes
P, Am	1	5.9	2	1
P, Am,VA	1	5.9	3	2
P, Am,VA, C	2	43	4	3
P, Am,VA, C, RD	1	5.9	5	4
P, Am,VA, C, RD, OFX	1	1.9	6	5
P, Am,VA, C, RD, OFX, DA	1	5.9	7	6
P, Am,VA, C, RD, OFX, DA, E	3	17.6	8	7
P, Am,VA, C, RD, OFX, DA, E, TE	7	41.2	9	8

P penicillin, Am ampicillin, Va vancomycin, C chloramphenicol, RD rifampicin, OFX ofloxacin, DA clindamycin, E erythromycin, TE tetracycline

Critically important antimicrobials for human medicine (CIA list) such as fluoroquinolones, 3rd and 4th Generation cephalosporins, Macrolides and ketolides and Glycopeptides [49]. Fortunately, the isolated strains in the present investigation were not resistant to any of the CIA list with the exception of erythromycin. Globally, the use of antimicrobial agents is regulated differently from country to country, being either very strict or under-regulated. Possible hazards associated with drug abuse in fish farming are the presence of residues in food and the development of antibiotic resistance in the bacterial population. In agreement, to our surprise numerous multi-ABR- bacteria (49%) were observed among our isolates, irrespective of their traditional resistance to beta lactam antibiotics [50, 51]. It was theorized that, resistance to these antibiotics could be a result of their overuse or/and misuse in the aquaculture industry, could arise from gene mutations or by acquisition of transferable genetic elements such as integrons [50, 52]. The property of horizontal spread of resistance genes was incriminated to be responsible for the recorded levels of multi-resistance. Notwithstanding AMU in aquaculture, the basic routine with regards to releasing fertilizer from earthbound creatures into water frameworks leaves the aquatic environments especially powerless against the improvement of AMR.

Conclusion

Worldwide death of fish caused by streptococcal infection remains an extortionate issue for growers of Nile tilapia and other warmwater fish, yet can be handled effectively with a cohesive, supportable scheme incriminating vaccines and new-generation antibiotics developed specifically for aquaculture. Strains of *Streptococcus* associated with infection in humans can cause people handling affected whole raw fish, primarily tilapia, to become at risk of developing neonatal meningitis and sepsis (early-onset and late-onset disease), cellulitis, subdural empyema, endocarditis or arthritis following a puncture wound [53–55]. Substantially, our results highlight the necessity to encourage conscientious fish producers, efficient husbandry performances and judicious practise of antibiotics in aquaculture. It should be emphasized that, *S. dysgalactiae* has emerged as a causative agent in fish disease, with a highly medical significance in mammalian and human health as alpha-hemolytic Lancefield group C *S. dysgalactiae* of fish origin, was implicated in ascending upper limb cellulitis in humans [56].

Abbreviations

AGPs: Antimicrobial growth promoters; AMU: Antimicrobial usage; CBA: Columbia blood agar; CIA: Central Intelligence Agency; FAO: Food and Agriculture Organization; GPI: Gram-positive identification; MT: Metric tons; USD: United States Dollar; WHO: World Health Organization

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Availability of data and materials

Raw data will not be shared.

Authors' contributions

KO developed the concept, designed experiments, collected, and analyzed data and prepared the manuscript; KAM, IM, MI and AH contributed their scientific advice during the work and manuscript revision; AO and NF performed the experiments. All authors discussed the results and implications and commented on the manuscript at all stages. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The researchers observed the routine fishing process and selected fish for use in the study, at the point of purchase from the fisherman the fish were deceased and as such no ethical approval was required for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author details

¹Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. ²Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia. ³Department of Public Health, College of Applied Medical Science, King Khalid University, Abha, Kingdom of Saudi Arabia. ⁴Department of Health Science, College of Applied Studies and Community Service, King Saud University, Riyadh, Kingdom of Saudi Arabia. ⁵Department of Fish Diseases and Management, Animal Health Research Institute, Dokki, Giza, Egypt.

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