Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Phenotypic and Genotypic Characterization of *Streptococcus* Species Isolated From Fish

Thesis

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Abstract

The prevalence of streptococcus species isolated from a total number (80) of tilapia fish was 21.25% with total number of isolates 17 (6 isolates of *E. faecalis* (7.5%), 3 isolates of *S. pluranimalium* (3.75%), 2 isolates of *A. viridans* and *E. gallinarum* (2.5% for each), one isolate of *S. dysgalactiae, S. anginosus, L. garvieae* and *Granulicatella elegans* (1.25% for each)). Isolates were tested for growth in different environmental conditions "10° C, 45° C, 6.5% salinity and 40% bile salts" *Streptococcus anginosus* and *Streptococcus dysgalactiae* showed no growth at 10°C, *Enterococcus gallinarum* and *Streptococcus anginosus* showed no growth at 45°C, *Lactococcus garvieae, Streptococcus dysgalactiae* and *Streptococcus anginosus* showed no growth in 6.5% salinity, and none of the isolates could grow in 40% bile salts. Isolates were identified biochemically using "Vitek" and the results were confirmed by applying PCR technique using by the guidance of the specific 16s rRNA primers for Enterococcus species, *S. pluranimalium, L. garvieae, S. anginosus,* and *A. viridans.* Species specific gene "*ddl* $_{E.\ faecalis}$" was identified in 100% of *E. faecalis* isolates. Virulence gene "*esp*" was detected only in one isolate of *E. faecalis* (16.7%), antibiotic resistance genes "*vanA* and *vanC1*" were detected in *E. gallinarum* with 100% and 50% respectively while 16.7% and 0% respectively in case of *E. faecalis.* Also by using the primer of penicillin resistance gene "*pbp2b*" in case of *S. anginosus*, the result was positive. 100% of the isolates were resistant to tetracycline, 50% to vancomycin and erythromycin, while most of them were susceptible to penicillin. Experimental infection was carried out, all groups showed clinical signs but the most virulent isolates were *L. garvieae* and *S. dysgalactiae* causing 100% mortality. Experimentally infected groups were examined histopathologically revealing; different stages of degenerative changes in liver, kidney, spleen and brain and thrombosis in kidney and liver.

Key words: (Streptococcus - Vitek - antibiotic sensitivity – virulence gene – histopathology)

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Introduction

Fishes are one of the major sources of healthy, easily digested and high quality animal proteins meant for human consumption. In developing countries, about 60% of consumers relay on fish as a source of animal protein, this percent increases to 80% in developed countries (FAO 2013).Rearing fish in a polluted environment results in not only fish health endangers, but also reflects negatively on human health consumers (Akoachere, et al. 2009). Fish can be humbled by various microbial agents including serious bacterial infections and some bacterial infections go beyond fish infection to be either of communicable or zoonotic nature, as *Aeromonas*, *Vibrio, Mycobacteria,* and *Streptococcus* (Russo, et al. 2012 and Verner-Jeffreys, et al. 2012).

Streptococcosis (Strep disease) or pop eye disease is a septicemic disease that affects both fresh and marine water fishes, in both farmed and feral populations, it results in high economic loses estimated by hundred million dollars annually (Karsidani, et al. 2010).Strep disease was first reported in 1957, during an outbreak with high mortalities and absence of specific clinical picture in rainbow trout culture facility in Japan (Hoshina et al. 1958). Since then, numerous other fish species of edible and ornamental character have been found susceptible to this infection, including mullet, eel, tilapia, sturgeon, striped

bass, rainbow sharks, red-tailed black sharks, rosey barbs, and danios, it was observed in 2 forms acute or chronic disease (Yanong and Francis-Floyd, 2013). Stress is usually one of the predisposing factors resulting in streptococcosis outbreaks such as poor environmental condition: rising of the environmental temperature, harvesting, handling, or transportation, poor water quality (high ammonia, and nitrite levels) (Yanong and Francis-Floyd, 2013). Bergey's Manual of Systemic Bacteriology in 1984 listed about seven genera facultative anaerobic gram-positive cocci (GPC) which are: *Aerococcus* (Evans and Schultes, 1969), *Leuconostoc* (Garvie, et al. 1986), *Micrococcus* (Schleifer, 1986), *Pediococcus* (Garvie, et al. 1986), *Staphylococcus* (Schleifer, 1986), *Streptococcus* (Schleifer, et-al. 1985), and *Stomatococcus* (Schleifer, 1986), but before its publication the genus *Streptococcus* was split into three genera: *Streptococcus*, *Enterococcus* (Fecal Streptococci), and *Lactococcus* (Lactic acid Streptococci) (Schleifer, and Kilpper-Balz, 1984 and 1987).

Streptococcus species are gram positive bacteria, where *Streptococcus* is adapted from a Greek word "Streptos" which means easily twisted, or bent (twisted chain) (Ryan and Ray, 2004), it multiplies along one axe forming a chain, contrast this with staphylococci, which divide along multiple axes and generate grape-like clusters of cells (Facklam, and Elliott. 1995). *Streptococcus* is spherical or oval in shape