Cairo University
Faculty of Veterinary Medicine
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Advanced Studies on Control of Some Bacterial Diseases Affecting Seabream and Seabass

Thesis Presented by

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For the degree of Ph.D. of Veterinary Medical Science
(Fish Diseases and Management)

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Approval sheet

This is to approve that the dissertation presented by **Mahmoud Mehani Oan Abou Okada** to Cairo University titled: **"Advanced Studies on Control of Some Bacterial Diseases Affecting Seabream and Seabass"** for the Ph.D. degree in Veterinary Medical Science (Fish Diseases and Management) has been approved by the examining committee:

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Abstract

European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are marine species of great economic importance and these species are vulnerable to seasonal serious mortality episodes causing severe economic losses, particularly in Mediterranean aquaculture. Seasonal mortality episodes targeting both seabream and seabass cultured in semi-intensive earthen pond and intensive concrete pond, Damietta province, Egypt was conducted during 2016 and 2017. During summer mortality episode, fifty freshly dead and moribund sea bream fish were collected while thirty freshly dead and moribund sea bass were collected during winter mortalities. Clinically, the collected fish revealed hemorrhages around the mouth, operculum, at the base of pectoral and anal fins, inflammation of anal opening, tail fin erosion and ulceration at caudal peduncle area. Morpho-chemical tests were performed to describe the phenotypic picture and biochemical properties of pure colonies using API®20NE and API®20E system. Definitive confirmation of bacterial isolates was performed by PCR targeting 16SrRNA, collagenase, toxR, aerolysin and 16SrDNA genes revealed 39 isolates (*Vibrio* species), 31 (*Vibrio alginolyticus*), 8 (*Vibrio parahemolyticus*), 8 (*Aeromonas hydrophila*) and 5 (*Pseudomonas fluorescens*), respectively. *Vibrio alginolytcus* was the most prevalent bacterial pathogen for sea bass and sea bream 56.7% and 28%, respectively followed by *Vibrio parahemolyticus* 18% in sea bream then *Aeromonas hydrophila*, and *Pseudomonas fluorescens* 26.7% and 16.7% in sea bass. Antibiogram of isolates showed sensitivity to oxytetracycline, doxycycline, flumequine, and trimethoprim-sulfamethoxazole. sharp increase in ammonia and nitrite, decreased dissolved oxygen and ability to scavenge iron from iron-binding proteins are the most possible triggering factors for initiation, establishment and spread of infections. Infectivity test of isolates revealed that the LD_{50} of *Vibrio alginolyticus* (VAS7) and *Vibrio parahemolyticus* (VP3) for sea bream was 3×10^5 CFU/g fish and 1×10^6 CFU/g fish, respectively while the LD50 of *Vibrio alginolyticus* (VAK16), *Aeromonas hydrophila* (AHK6) and *Pseudomonas fluorescens* (PFK12) for sea bass was 5×10^4 CFU/g fish, 7.5×10^5 CFU/g fish and 1.7×10^6 CFU/g fish. Experimental immunization of sea bass by formalized polyvalent bacterin applied by different routes for control of some bacterial diseases showed RPS% of 88.9%, 50%, 33.3% and 25% for intraperitoneal, prolonged immersion, dip immersion and oral routes, respectively.

Key words: ***Vibrio* species - *Aeromonas hydrophila* – *Pseudomonas fluorescens* – ToxR gene - LD_{50} - Polyvalent bacterin**

DEDICATION

Dedicated to

My parents

, My wife

, My daughters

, My brothers

, My sisters

& my close friends

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1. INTRODUCTION

Aquaculture has a great potential to meet the growing demand for seafood and animal protein. Increased fish production of the country through aquaculture can be considered as a recent success story. Aquaculture represents about 74% of the total fish production. In 2012, the share of sea bass and sea bream had increased to (13,798 tons) and (14,806 tons), respectively **(GAFRD, 2013).**

European sea bass *(Dicentrarchus Labrax)* and gilthead sea bream *(Sparus aurata)* are currently produced in most countries bordering the Mediterranean Sea. The main producers are Greece, Turkey, Spain, Italy and France. Smaller producers are Portugal, Croatia, Cyprus, Israel, Malta, Egypt, Tunisia and Morocco **(FAO, 2011)**.

Bacterial pathogens consider the main causes of outbreaks in cultured Sea bream and Sea bass, these pathogens are *Vibrio sp*., *Pseudomonas sp*., *Photobacterium damselae sp. Piscicida*, *Photobacterium damselae sp. damselae*, *Tenacibaculum maritimum*, *Streptococcus sp.*, *Staphylococcus sp*. and *some Aeromonads* **(Toranzo *et al.*, 2005)**

The onset of outbreaks of fish diseases may be attributed to the suppression of fish immune system. Such immune-suppression is positively linked to the abrupt increase in water temperature, un-ionized ammonia, iron, copper, nitrite and salinity associated with

uprising depletion of dissolved oxygen. Immunologically, the above-mentioned water quality results are well known to have direct impact on adaptive, innate and cellular immune responses **(Abdel-Aziz *et al.*, 2013).**

Vibriosis is one of the most serious bacterial diseases in mariculture causing high mortality up to 100% with severe economic losses throughout the Mediterranean area **(Sarjito *et al.*, 2009)**

Vibrio alginolyticus (V. alginolyticus) is a major fish pathogen within the genus *Vibrio* and has caused severe vibriosis with massive mortality in various fish species as sea bass and sea bream throughout the world. *V. alginolyticus* is always associated with deteriorating culture conditions or physical damage of cultured fish; and therefore, it is always considered as an opportunistic pathogen. The virulent properties of *V. alginolyticus* vary from species to species, and in some cases, virulence even varies within the same fish species **(Balebona *et al.*, 1998 and Zhu *et al.*, 2000).**

Aquaculture activities are suffering mortalities due to several disease outbreaks by bacterial pathogens. Among, *Aeromonas hydrophila* (*A. hydrophila*) considered as a major threat and easily spread through accidental abrasions, known as many symptoms namely, hemorrhagic septicemia, ulcers, exophthalmia and abdominal distension **(Sahoo *et al.*, 2011** and **Austin and Austin, 2012).**

Pseudomonas fluorescens (Ps. fluorescens) is a common aquaculture pathogen, infecting both invertebrate and vertebrate