Suez Canal University

Faculty of Veterinary Medicine

Department of Bacteriology, Immunology and Mycology

Prevalence and Molecular Characterization of *Aeromonas* Species Isolated from Manzala Lake Fish.

Thesis Presented By

Al-Shaimaa Tawfik Hanafy Mahmoud Maklad

(B.V.SC. 2011, Suez Canal University)

(M.V.SC. 2016, Suez Canal University)

For Ph.D. Degree in Veterinary Medical Science

(Bacteriology, Immunology and Mycology)

Submitted to

Faculty of Veterinary Medicine

Suez Canal University

(2019)

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

7.Summary

Aeromonas spp. is a major bacterial pathogen affecting a wide variety of fresh and marine water fish. Even though they have been recognized as primary fish pathogens for a long time, their status as primary human pathogens was also clear.

Lake Manzala is a brackish lake in northeastern of Egypt. It is located on the northeastern edge of the Nile Delta. Economically, Manzala Lake is considered as one of the most valuable fish sources in Egypt by about 36-50% of the total country fish production.

The present work was planned out to throw the lights on the prevalence, phenotypic, genotypic character and antibiotic resistance of *Aeromonas* spp. isolated from *Oreochromis niloticus*, *Mugil cephalus* fish and water from Manzala Lake referring to the virulent and resistant gene and pathogenicity of *Aeromonas* strains.

This study was carried out during the period from June 2018 to November 2018. A total of 200 fish samples comprising 100 *Oreochromis niloticus* fish, 100 *Mugil cephalus* fish were collected from el Gamil region at Lake Manzala. Also, a total number of 50 water samples were collected from water where fish samples harvested. The samples were taken from water and different tissues of *Oreochromis niloticus* and *Mugil cephalus* fish including muscle, kidney and gills. *Aeromonas* spp. was identified based on the morphological, conventional, and biochemical analysis.

Bacteriological examination of fish and water samples from Manzala lake showed that 137 (54.8%) of the 250 investigated samples were found positive for *Aeromonas* species, among the samples investigated, 62%, 46%,

and 58% samples of *Oreochromis niloticus*, *Mugil cephalus* and water were found positive by the cultural method, respectively.

A total of 258 isolates belonging to *Aeromonas* spp. were recovered from fish and water samples. The isolates were biochemically identified as *A. hydrophila* which was found to be the dominant with total prevalence of 48.45% (125/258) followed by *A. sobria* 28.29% (73/258), *A. caviae* 19.38% (50/258) and *A. schubertii* 3.88% (10/258) respectively and the distribution of *Aeromonas* spp. in different organs of *Oreochromis niloticus* fish were 46.8%, 32.5% and 20.6% in gill, kidney and muscle, respectively. The distribution of *Aeromonas* spp. isolates in different organs of *Mugil cephalus* fish were 43.4%, 38.5% and 18.1% from gill, kidney and muscle, respectively.

Antimicrobial susceptibilities were determined by the disk diffusion method to 70 represented isolates against 14 different antibiotic disks. Results of antibiogram showed that antibiotic resistance pattern varied between strains. In general, all isolates showed (100%) sensitivity to norfloxacin and most strains were showed high sensitivity to cefotaxime, gentamycin, nalidixic acid and amikacin. However, the highest resistance percentage (100%) of the isolates was observed with ampicillin, erythromycin and penicillin G. High incidence of multiple antibiotic resistance amongst *Aeromonas* species was observed suggesting presence of wastewater which act as a reservoir of antibiotic resistance determinants.

As all isolates were phenotypically identical, 15 representative isolates were chosen for 16S rRNA gene sequence analysis. PCR assay of 15 representatives, biochemically confirmed *Aeromonas* spp. isolates were genetically confirmed belonging to genus *Aeromonas* based on specific16S rRNA gene sequence. The representative isolates identified by sequencing of the 16S rRNA were subjected to conventional PCR to detect some virulence

genes to confirm their pathogenicity. Virulence properties of 15 representative strains showed that the majority of the strains examined carried one or more virulence genes. A significant 5 virulence gene have been found in the tested *Aeromonas* isolates. The frequencies distribution of this genes were *aero* (100%), *act* (20%), *ahcytoen* (13.33%), *lip* (13.33%), *alt* (6.67%). Meanwhile, Haemolysin (*hly*) gene could not be detected in any of examined *Aeromonas* isolates.

Furthermore, the gene encoding for antibiotic resistance, (bla_{TEM}) gene was detected in 100% of tested isolates, while *tet*A (A) gene was present in 13.33% of *Aeromonas* isolates.

Virulence study in vitro (Congo red binding assay) of 15 isolates of *Aeromonas spp.* revealed that all the isolates were virulent as they shown positive reaction to Congo red binding assay and two isolates (13.3%) were found biofilm producer assumed to be more virulent as compared to other strains. Regarding to the pathogenicity in vivo study, there were 2 groups, control group and experimentally infected group with *Aeromonas hydrophila*, each contain 10 *Oreochromis niloticus*. The tested isolate was virulent and capable of producing clinical lesions in healthy fish similar to typical sign of the disease. The mortality rate in the experimentally infected *Oreochromis niloticus* group was representing 80% of the total fish.