



Faculty of Veterinary Medicine
Department of Bacteriology, Mycology and Immunology

Beni-Suef University

Pathogenic bacterial diversity regarding the acquired antimicrobial resistance in integrated fish farm

Thesis Presented by

Maha Azmy Mahmoud Sacran

(B. V. Sc., Fac. Vet. Med., Cairo University, Beni-Suef branch 1994)

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Under Supervision of

Prof. Dr Walid Hamdy Hassan.

Head and Professor of Bacteriology, Mycology and Immunology,
Fac. Vet. Med., Beni-Suef University

Dr. Mortada Mohamed Abdel-Hamed Hussein

Head and Associate Prof. of Fish Diseases and Management,
Fac. Vet. Med., Beni-Suef University

Prof. Dr. Alaa Eldin Abdel Moaty Eissa

Head and Professor of Aquatic Animal Medicine
and Management, Fac. Vet. Med., Cairo University.

Dr. Soad Sabry Abd-El- Halem

Senior Researcher in Fish Diseases
Department, Animal Health
Research Institute in Dokki.ARC.

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Abstract

A study was conducted to investigate bacterial pathogens associated with diseases outbreaks in African catfish, *C. gariepinus*, raised in earthen pond aquaculture system with special concern with these which showed AMR. Emphasis on detection AMR on traditional and molecular levels was in concern. Additionally, pathogenicity and median lethal dose LD₅₀ of selected bacterial species was elaborated. Alternative antimicrobial agent was tested against the selected pathogens together with its biological tolerance in fish model. Two hundred fifty diseased catfish were collected from different localities at Fayoum Governorate during the period from October 2016 to October 2019. In parallel, samples from poultry droplets fertilizers and poultry carcasses and chicken slaughter house byproducts used as fish feed were collected. After clinical and postmortem examinations, samples from kidneys, livers and external lesions from fish were subjected to bacteriological isolation. At the same time, bacterial isolation was performed from poultry collected samples. The isolated bacteria (total 116, fish 99, poultry 17 isolates) were identified on basis on morphological, conventional biochemical tests and confirmed by, API® 20 E, API® 20 NE, API® 20 Strep. The most prevalent bacterial isolates isolated from fish were belonged to *Aeromonads* species 28 (35.7%), however *Enterococci* was the most prevalent ones isolated from poultry. Summer was the highest prevalence season associated with bacterial isolation (49.1%), while kidneys were the highest isolation site. A part from *Aeromonads* and *Enterococci*, other bacterial species including *Shewanella* spp., *Pseudomonads*, *Vibriosis* and *Staphylococcus* were also obtained. I/P injection of *A. hydrophila* BNS 0119 at dose 0.1 ml containing 3×10^8 , 1.5×10^8 , 1.5×10^7 , 1.5×10^6 , and 1.5×10^5 CFU/ml, showed cumulative mortality rates 50% with LD₅₀ of 1.5×10^7 . Conventional AntibioGram assays showed variable resistance levels of the tested pathogens to antibiotics tested, however, *A. hydrophila* BNS 0119 showed acquisition of resistance tetracycline genes on molecular level tested. Production and control of fish health safely together with living in harmony with the nature, natural substances considered as an important area for future developments in aquaculture. Regarding the obtained results, Trivir® at high doses of 1000, 500, 250 µg/ml could be used as antimicrobial disinfectant for utensils used in fish farms, however, low doses (125, 64, 32 µg/ml) could be used *in vivo* (fish) as a bacteriostatic antiseptic agent, particularly, during farm operational processes.

Key words: Integrated fish farming, Catfish (*C. gariepinus*), AMR, fish bacterial pathogen, Carvacrol.

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List of Abbreviations

<i>A. hydrophila</i>	<i>Aeromonas hydrophila</i>
ABM	<i>Aeromonas</i> base medium
<i>A. salmonicida</i>	<i>Aeromonas salmonicida</i>
API 20E	Analytical profile index 20E
BP medium	Baird-Parker medium
BHIB	Brain heart infusion broth
<i>Chry.indolegens</i>	<i>Chrysobacterium. Indolegens</i>
<i>C. gariepinus</i>	<i>Clarias gariepinus</i>
DNA	Deoxyribonucleic acid
<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
<i>E. coli</i>	<i>Escherichia coli</i>
FAO	Food and Agriculture Organization
H₂O₂	Hydrogèn peroxide
H₂S	Hydrogen sulfide
I/P	Intraperitoneal
MAC medium	MacConkey medium
LD₅₀	median lethal dose
MR	Methyl red
MR-VP	Methyl red Voges–Proskauer
MIC	minimum inhibitory concentration

Contents

<i>O. niloticus</i>	<i>Oreochromis niloticus</i>
OTC	Oxytetracycline
PBS	Phosphate Buffer Saline
PCR	Polymerase chain reaction
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
<i>P. fluorescens</i>	<i>Pseudomonas fluorescens</i>
rRNA	Ribosomal ribonucleic acid
SS agar	<i>Salmonella-Shigella</i> agar
<i>Sh.putrifaciens</i>	<i>Shewenella.putrifaciens</i>
Spp.	Species
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>TCBS medium</i>	Thiosulphate citrate bile salt sucrose medium
TSA	Tryptone soya agar
TSB	Tryptone soya broth
USA	United States of America
<i>V. anguillarum</i>	<i>Vibrio anguillarum</i>
<i>V. cholera</i>	<i>Vibrio cholera</i>
<i>V. parahaemmmolyticus</i>	<i>Vibrio parahaemolyticus</i>
VP	Voges–Proskauer
CFU	Colony forming unit
FAO	Food and Agriculture Organization