Benha University
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BACTERIOLOGICAL AND MOLECULAR STUDIES ON SOME BACTERIA CAUSING MORTALITY IN FISHES

A Thesis Submitted
By

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Abbreviation	Meaning
aadA1	Streptomycin resistant gene.
***Aer*A**	Aerolysin gene
Act	cytotoxic heat- labile enterotoxin
alt	cytotonic heat-labile enterotoxin
AML	amoxicillin
ARGs	Antimicrobial resistance genes
Asp	Alkaline serine protease
Ast	cytotonic heat- stable enterotoxin
Ahcytoen	*A. hydrophila* cytolytic enterotoxin gene.
***bla*TEM**	β-lactamase ampicillin resistance gene
BSIBG	Bile salts Irgasan brilliant green agar
CIP	ciprofloxacin
CT	Colistin sulphate
CTX	cefotaxime
***ctx*A**	Cholera toxin A- subunit
EUS	epizootic ulcerative syndrome
GEN	gentamycin
***gyr*A**	DNA gyrase, subunit A gene
***hly*A**	Haemolysin toxin
MAS	Motile *Aeromonas* Septicemia
***Mcr*-1**	Polymyxin resistant gene
omp	outer membrane proteins
PCR	Polymerase Chain Reaction
***rec*A**	Recombinase gene
RFLP	restriction fragment length polymorphism
***rpo*A**	RNA Polymerase alpha gene
R–S media	Rimler – Shotts agar medium
S	streptomycin

***sul*1**	Sulphonamides resistant gene.
TE	tetracycline
***tet*A**	Tetracycline resistant A gene.
T.C.B.S	Thiosulphate –citrate –bile salts –sucrose agar
tdh	Thermostable direct hemolysin gene
***tox*R**	Cholera toxin transcriptional activator gene
trh	Tdh –related hemolysin
TSA	Tripticase Soy agar
TSI	Triple sugar iron agar
vvha	Cytolysin hemolysin

7. SUMMARY

Vibrio and *Aeromonas* species are responsible for wide range spectrum of diseases among fish, leading to high mortalities and high economic losses, beside their role in gastrointestinal and extra intestinal infections in humans. Therefore, the present study was performed on 100 diseased fishes, 50 Nile tilapia (*O. niloticus*) and 50 mullet fish (*M. cephalus*), of various sizes were collected from different fish farms at Kafr El-sheikh Governorate during the period from January to October (2019) for determination the prevalence of *Vibrio* and *Aeromonas* infection and phenotypic characterization and detection of some virulence genes in some isolated strains. Samples were taken from apparently path-gnomic lesions in liver, kidneys, spleen, heart, anterior intestine, and gills of these fishes after clinical and postmortem examination.

The results of bacteriological examination revealed that, the prevalence of *Vibrio* infection with *Vibrio* species isolation were, 65 out of 100examined fishes (65%). A total of 36 *V. parahaemolyticus* strains (55.4%) were isolated and identified, 20 (30.76%) from *O. niloticus*, and 16 (24.6%) from *M. cephalus*. Meanwhile, 22 *V. alginolyticus* strains (33.8%) were isolated, 13 (20.0%) from *O. niloticus*, and 9 (13.8%) from *M. cephalus* fish. Besides 7 *V. cholera* strains (10.8%) were isolated, 5 (7.7%) from *O. niloticus*, and 2 (3.1%) from *M. cephalus*. *Vibrio parahaemolyticus* was isolated from liver, kidneys, spleen, heart, intestine, and gills with a prevalence of 30.5(11/36), 16.7(6/36), 11.1(4/36), (3/36) 8.3, 2.8(1/36) and 30.5% (11/36), respectively.

A total of 72 *Aeromonas* species were isolated and identified. Sixty five *A. hydrophila* strains (90.3 %) were isolated, 26 (36.1%) from *O. niloticus* and 39(54.2%) from *M. cephalus*. Meanwhile, 7 *A. caviae* strains (9.7 %) were isolated, 1 (1.4%) from *O. niloticus* and 6 (8.3%)

from *M. cephalus*. *Aeromonas* species detected in the liver, kidney, spleen, heart, intestine, and gills with a prevalence of 32.3(21/65), 27.7(18/65), 6.2(4/65), 17.0(11/65), 6.2(4/65) and 10.8% (7/65), respectively.

The in-vitro sensitivity tests for the isolated *V. parahaemolyticus* strains (n=36) showed high resistance for amoxicillin 91.7% and colistin 63.9% followed by cefotaxime 58.3% and streptomycin 52.7% . The isolated *Vibrio alginolyticus* were highly resistant to streptomycin (86.4%) and amoxicillin (72.8%) followed by colistin (68.2%) and cefotaxime (59.1%). The isolated *Vibrio cholerae* were highly resistant for amoxicillin (85.7%), colistin, and streptomycin (71.4% for each) followed by cefotaxime (57.1%).

The sensitivity tests for the isolated *A. hydrophila* revealed that the isolated *A. hydrophila* (n= 65) were highly resistant for amoxicillin 100.0% and tetracycline 87.7% followed by streptomycin 63.1%, cefotaxime 57.0% and colistin sulfate 54.0%. The isolated *A. caviae* were highly resistant for amoxicillin (100.0%) and tetracycline (85.7%) followed by cefotaxime (71.4%), streptomycin, and colistin (57.1% for each).

The molecular screening of 5 *Vibrio* species isolates using species-specific PCR (for *V. parahaemolyticus* (***tox*R** gene) and for *V. alginolyticus* (**collagenase** gene)), all five isolates were identified as *V. parahaemolyticus*. On the other hand, no *V. alginolyticus* isolates were identified. In addition, PCR results showed the ***rec*A** virulence gene was detected in three out of five random isolated *V. parahaemolyticus*. Meanwhile, the ***trh*** virulence gene was not detected in any of the tested 5 *V. parahaemolyticus* isolates. Also, five randomly selected *A. hydrophila* isolates were submitted for the screening of virulence genes by PCR.

Results revealed that ***aer*A** and ***hly*A** virulence genes were detected in all five random isolated *A. hydrophila*. Meanwhile, ***Ahcytoen*** was detected in 4 out of 5 *A. hydrophila* studied strains and ***fla*** virulence gene was detected in 1 out of 5 *A. hydrophila* isolates. Five random isolates from each *V. parahaemolyticus* and *A. hydrophila* were subjected to PCR amplification targeting the antimicrobial resistance determinants β-lactamase **(*bla*TEM)**, tetracycline resistance **(*tet*A (A))**, and aminoglycosides **(*aad*a1)** and polymyxin resistant **(*mcr*1)** genes which were amplified in all five tested *A. hydrophila* and all five *V. parahaemolyticus* studied strains.