CONTENT

LIST OF ABBREVIATION

A. caviae	: *Aeromonas caviae*
A. hydrophila	: *Aeromonas hydrophila*
A. jandaei	: *Aeromonas jandaei*
A. sobria	: *Aeromonas sobria*
Aero	: Aerolysin gene
As	: Ampicillin/Sulbactam
B.H.I.A	: Brain heart infusion agar
bp	: Base pair
C. columnaris	: *Cytophaga columnaris*
Ce	: Cephotaxime
Cf	: Ciprofloxacin
D.W.	: Distilled water
dATP	: Deoxy adenosine triphosphate
dCTP	: Deoxy cytosine triphosphate
dd H_2O	: Deionized-distilled water
dGTP	: Deoxy guanine triphosphate
dNTPs	: Deoxyribonucleotides triphosphatase

dTTP	: Deoxy thiamine triphosphate
E. ictaluri	: *Edwardsiella ictaluri*
E. tarda	: *Edwardsiella tarda*
EDTA	: Ethylene diamine tetra acetic acid
F. columnare	: *Flavobacterium columnare*
F. columnaris	: *Flexibacter columnaris*
G	: Gentamycin
hr.	: hours
I	: Intermediate
I/P	: Intraperitoneal
M.R	: Methyl red
mg	: Milligram
ml	: Milliliter
Nv	: Novobiocin
O. niloticus	: *Oreochromis niloticus*
O/F	: Oxidation-Fermentation
Ox	: Oxacillin
PCR	: Polymerase Chain Reaction.

Ps. fluorescens	: *Pseudomonas fluorescens*
Ps. aeruginosa	: *Pseudomonas aeruginosa*
R	: Rifampicin
R	: Resistant
R.S medium	: Rimler- Shotts medium
S	: Sensitive
T.S.A	: Tryptic soya agar
T.S.I agar	: Triple Sugar Iron Agar medium
TAE	: Tris-base glacial Acetic Acid
TBE	: Tris-base boric acid
V.P	: Voges-Proskauer
- ve	: Negative
+ ve	: Positive
µg	: Microgram
µl	: Microliter

6. SUMMARY

The main objective of the present study was to isolate and identify the most common bacterial causes of mortalities and low productivity among cultured fish and assessed the value of using multiplex PCR in rapid detection of bacterial fish pathogens. This investigation was carried out in Abbassa Central Laboratory of Aquaculture Research, Sharkia Governorate, Egypt. A total number of 150 clinically and grossly diseased Nile tilapia (*Oreochromis niloticus*) were collected from different fish farms and transferred alive or recently dead and subjected to full clinical, postmortem and bacteriological examinations.

The results of this study could be summarized as follows:

1- Naturally infected *O. niloticus* showed that the most common clinical signs noticed due to bacterial pathogens infections were decrease of feed intake, swam near the surface of water, increased mucus secretion and congested gills. Hemorrhages at the base of fins, abdominal part, gill cover and necrotic area on the gill cover were also noticed. Abdominal distention and intestinal prolepses were observed. The postmortem findings were congested liver, in other cases congestion at the margins with necrosis and in others pale colour and yellowish white patches were observed. Spleen was dark in colour. Kidneys and gonads were congested and watery ascitic fluid was recorded.

2- Biotyping results of bacterial isolates revealed the following:- **102/150 (68%)** were *Aeromomas hydrophila,* **18/150 (12%)** were *Aeromonas jandaei*, **15/150 (10%)** were *Aeromonas sobria*, **9/150 (6%)** were *Aeromonas caviea*, **18/150 (48%)** were *Pseudomonas fluorecens*, **72/150 (12%)** were *Pseudomonas aeruginosa,* **21/150 (14%)** were *Edwardesiella tarda,* **15/150 (10%)** were *Edwardesiella ictulari* and **27/150 (18%)** were *Flavobacterium columnare*.

3- *A. hydrophila* was circular yellow-colored colonies on R-S media. Gram negative, straight, rods, motile by single polar flagella, Catalase, indole, cytochrome oxidase and phosphatase are produced, but not H_2S, Nitrates were reduced to nitrates without the production of gas, Voges-Proskauer reaction was positive, but not so the methyl red test.

4- *A. sobria* showed motile, fermentative, Gram-negative rods, which produced catalase, indole, and oxidase, but not H_2S. Acid was produced from glucose (with gas), mannitol and sucrose, but not from arabinose, inositol, or sorbitol. Nitrates were reduced, and the Voges-Proskauer reaction was positive. While, *A. jandaei* were motile Gram-negative rods, that produced indole, degrade gelatin, and starch, Acid produce from galactose, glycogen, mannose, sucrose and trehalose, but not arabinose or lactose. While *A. caviae* showed motile, fermentative, Gram-negative rods, cultures produce indole, but not H_2S, degrade gelatin, but not urea, ferment arabinose, glucose, mannitol, sorbitol and sucrose, but not inositol, melibiose or rhamnose, grow in 0% (w/v) sodium chloride, not utilise citrate as a sole carbon source and do not reduce nitrates.

5- *Ps. fluorescens* was Gram-negative short bacilli and motile, circulated, convex, entire edge, glistening, creamy colour and 1-2 mm in diameter. Some isolates gave fluorescence pigment. *Ps. flurescens* isolates revealed positive oxidase test. Isolates were oxidative in O/F, positive for catalase. Isolates were negative for citrate, H_2S, indole and gelatinase production. Some isolates utilized arabinose and glucose oxidatively. On the other hand, *Pseudomonas aeruginosa* was gram negative bacteria, motile, with short rods and non spore former. It gave positive reactions for oxidase and catalase tests and negative reactions with starch. *Ps. Aeruginosa* gave positive reactions for liquefactions of gelatin, production of H_2S and indole, reduction of nitrate, utilization of citrate.

6- *Edwardsiella tarda* was Gram-negative, short rods and motile. Cytochrome oxidase was negative. While indole and methyl red were positive. H_2S production was positive. Acid production was produced from mannose and maltose. On the other hand, *Edwardsiella ictaluri* cultures comprised Gram-negative, rod-shaped, fermentative organisms, which were motile by peritrichous flagella. Catalase was produced, but not H_2S, indole and oxidase. The methyl red test was positive, but not so the Voges-Proskauer reaction. Nitrates were reduced. Acid was produced from fructose, galactose, glycerol, maltose, mannose and ribose, but not from arabinose, inositol, lactose, sorbitol, starch or sucrose.

7- *F. columnare* was a Gram-negative, long bacilli forming typical “hay stacks” or “columns” in wet-mount preparations and motile. These bacteria had a characteristic rhizoid pattern of growth on a low

nutrient agar medium. The suspected colonies on cytophaga agar at 25°C for 24hr. were yellow in color, rhizoid edge, flat and 5mm in diameter.

8- Ciprofloxacin was the drug of choice against *A. hydrophila*. Also, *A. hydrophila* was sensitive to Gentamycin and Cephotaxime and to lesser extent against Rifampicin. While it was resistance to Oxocillin, Ampicillin and Novobiocin.

9- It was noticed that *O. niloticus* artificially injected intraperitoneally with pure culture of *A. hydrophila* isolates showed a higher mortality rates. The clinical signs and postmortem changes associated with experimental infection were similar to the naturally infected fish. *A. hydrophila* isolates were re-isolated from freshly dead experimentally infected *O. niloticus.* On the contrary, the control groups showed neither clinical signs nor lesions. Moreover, no bacteria were isolated from organs of control group.

10- PCR primers were selected for the aerolysin gene and 16S rDNA. Multiplex PCR was proved to be a reliable and sensitive protocol for genotyping of *Aeromonas hydrophila* isolates recovered from naturally infected fish.

11- The results of amplification of aerolysin gene in ٩ isolates of *A. hydrophila* by specific PCR analysis revealed that amplification was done in ٤ out of ٩ isolates; this indicated that the pathogenic aerolysin gene weren't present in all pathogenic *A. hydrophila* strains.

12- The results of amplification of 16S rDNA gene in the same isolates of *A. hydrophila* by specific PCR analysis revealed that amplification was done in all isolates; this indicated that all tested strains were confirmed as *A. hydrophila*.

13- The results of amplification of both aerolysin and 16S rDNA genes together in the same isolates by the multiplex PCR revealed that amplification was done in all isolates for the 16S rDNA gene and 4 isolates for the aerolysin gene.

14- By comparing between antibiotic sensitivity test, pathogenicity test and mPCR in selected isolates, the results revealed that the strains which were resistant to many antibiotics and had aerolysin gene gave the high mortality percent than the others sensitive to different antibiotics and had no aerolysin gene, this indicate that the aerolysin gene considered one of the most important genes responsible for pathogenicity of *A. hydrophila* strains.

7. Conclusion

- It could be concluded that bacterial pathogens was the main causes of heavy mortalities and low productivity among cultured fish.

- *Aeromomas hydrophila* was the most prevalent bacterial pathogens.

- Ciprofloxacin was the drug of choice for the treatment of infected fish.

- PCR is the most sensitive, rapid and specific method for the detection of bacterial fish pathogens. Multiplex PCR was proved to be a reliable and sensitive protocol for genotyping of *Aeromonas hydrophila*.

- By comparing between antibiotic sensitivity test, pathogenicity test and mPCR in selected isolates, our results revealed that the presence of aerolysin gene in *A. hydrophila* strains were correlated to resistance to many antibiotics and higher mortality percent among the infected *O. niloticus*.