Phenotypic and Genotypic Characters on Aeromonas Species Causing Mortality in Fishes

[1]Ashraf, A. Abd El-Tawab, [2]Osama, A. Saleh, [2]Somayah, M. Awad and [3]Heba, M. Absi

[1]*Bacteriology, Immunology and Mycology Dept., Fac. Vet. Med., Benha Univ.*

[2]*El-Abbassa central laboratory of Aquaculture Research, Sharkia Governorate, Egypt.*

[3]*Vet. Researcher*

A B S T R A C T

This study was carried out to identify aeromonas species causing mortalities in some fishes. A total number of 102 Nile tilapia fish (*Oreochromis niloticus*) and 45 common carp (*Cyprinus carpio*) all had clinical signs and collected from El-Abbassa fish farms and subjected to full clinical, post mortem and bacteriological examinations. The present study revealed, The most prevailing bacterial diseases affecting Nile tilapia and Common carp were motile *Aeromonas* (*A.sobria, A. veronei, A. jandae, A. hydrophila* and *A.cavae*) The pathogenicity test revealed that mortality rate was 20 – 100 % in Nile tilapia fish due to *A. sobria* experimental infection. Some isolates of *A. veronei* and *A. hydrophila* were pathogenic to Nile tilapia. *Aerolysin* and *Ahcytoen* genes were detected in all isolates of *A. sobria* subjected to PCR. Resistance genes (*qnrs and blaTEM*) were also detected in all isolates of *A. sobria* subjected to PCR. While *ermB* was negative for all isolates. *A. sobria* was sensitive to ciprofloxacin, norfloxacin and trimethoprim and resistant to amoxicillin, erythromycin, gentamycin, rifamycin, bacitracin and cephradine.

Keywords: Fish, *Aeromonas sobria*, virulence gene, resistant gene,PCR.

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1. INTRODUCTION:

Tilapia fish are likely to be the most important of all cultured fish in the 21st century. They are farmed in every manner from semi extensive to super extensive farms (Fitzsimmons, 2000). Egypt has been the second largest producer of tilapia in the world for many years and is the source of the most important strains and species used in commercial aquaculture around the world. The Nile tilapia, *Oreochromis niloticus*, account for about 80% global production and several Egyptian strains have been used in the most sophisticated breeding programs (Fitzismmons, 2008) and carps are the most largely cultivated species throughout the world (Awad, 2011).

Ahcytoen gene is a cytolytic enterotoxin gene and there are many other extracellular proteins produced by *Aeromonas* species which associated with pathogenicity and environmental adaptability such as adhesion, mucinase production, cytotoxicity casinase, collagenase, protease, lipase or other factors combine with *Aerolysin* and *Ahcytoen*

genes for pathogenic effects (Afza *et al.*, 2007), and the rapid detection of cytolytic enterotoxin (*Ahcytoen*) gene and their characterization has proven importance so that proper and rapid preventive control measures could be taken up to reduce mortality and loss in fish culture (Sarkar *et al*, 2013). Plasmid - borne quinolone resistance genes were detected in five *A. hydrophila* (4.7%), two of them carried *qnrs2,* two cefotaxine resistant *A. hydrophila* were positive for *blaTEM-1* (DengYu-Ting *et al,* 2014).

Plasmid- mediated quinolone resistance was defined among Aeromonas species isolated from water, and reported that most aeromonas strains sensitive to fluoroquinolones, doxycycline, cefotaxime and amikacin and resistant to ampicillin, rifampin, streptomycin and nalidixic acid and reported that all – positive *Aeromonas* species isolates were ciprofloxacin susceptible, while five of them were resistant to nalidixic acid (Onuk *et al* ,2015). The resistance gene more commonly detected in *streptococcus agalactia* was *ermB* (Margrita, 2013). (Somayah Awad, 2011) mentioned that eight isolates of *A. sobria* were sensitive to ciprofloxacin, streptomycin, trimethoprim + sulphame-thoxazol and tetracycline and resistant to ampicillin, erythromycin, kanamycin and vancomycin, also Gehan *et al* (2015) found that Aeromonas species, Pseudomonas species, Vibrio species and Streptococcus species all were sensitive to norofloxacin and ciprofloxacin ,also all isolates were resistant to amoxicillin and ampicillin. amikacin was effective on all isolates species except Streptococcus species. trimethoprim-sulphamethoxazol was resistant to all isolates except *Aeromonas hydrophila* and *A. sobria.*

The current study aimed to isolate and identify *Aeromonas* species causing mortality in Nile tilapia and common carp.

2. MATERIAL AND METHODS:

Samples:

A total of 147 diseased fish were represented as 102 Nile tilapia, 45 common carp were collected randomly from El-Abassa Fish Farm, Sharkia Governorate. The fishes were transferred alive in tanks to the lab in the Fish Diseases Department, Central Lab for aquaculture Research in Abbassa and subjected to clinical, postmortem and bacteriological examinations.

Experimental Fish:-

One hundred and seventy Nile tilapia clinically healthy with an average body weight 20 to 25 g were used for experimental infection.

Bacterial examinations:

Isolation and Identification:

Under complete aseptic condition, fish specimens (skin lesions, ascetic fluid and internal organs, liver, kidneys, and spleen) were inoculated over TSA(tryptic soya agar) and incubated at 25˚C for 24 hrs. Re-inoculation of cultured bacteria occurred until separated colonies appeared. The suspected purified colonies were picked up and inoculated into TSA slant for further identification according to Frerich and Hendrie, (1985) and Austin and Austin (2007).

Biochemical identification of bacteria:

- Sugar medium: 1% of (glucose, sucrose, lactose, arabinose, salicin, sorbitol, inositol, maltose, mannitol and glycrol) in peptone water with bromothymol blue indicator.
- Oxidase Discs (HIMEDIA) for Cytochrome oxidase test.

- Kovac's reagent for indol: P-dimethylaminobenzaldhyde (5 g), Amyl alcohol (75 ml) and Conc. HCl (25 ml).
- KOH 3%
- Voges-Proskaur reagent (5% alpha-naphthol in absolute ethanol and KOH 40%).
- Methyl red reagent (methyl red, 0.04 g, Ethanol, 40 ml and distilled water to 100ml).
- Hydrogen peroxide: H_2O_2 3% aq. sol.
- Lugoal's iodine solution.

Experimental infections:

One hundred seventy Nile tilapia were collected to study the experimental infection of some bacterial isolates, the fish were acclimated for 2 weeks under lab. Conditions, the experimented fish were maintained in clean well equipped glass aquaria, fish were divided into 17 equal groups, each group of 10 fish each.

(1) groups (1-12) were injected by isolates of *Aeromonas sobria*.

(2) groups (13,14) were injected by isolates of *Aeromonas veronei*.

(3) group 15 injected by *Aeromonas jandae*.

(4) group 16 injected by *Aeromonas hydrophila*.

(5) group 17 injected by 0.2 ml sterile saline (Nacl 0.85%) and kept as control .

Bacterial isolates were inoculated into TSA and incubated at 25 ˚C for 18 hrs.

Bacterial culture was collected in sterile saline (0.85% Nacl) until each ml of saline contained (10^{10} cells /ml) by using spectrophotometer the aquaria were checked for mortality, clinical signs, abnormalities daily and specimens were seeded for bacterial re- isolation as determined by Miles and Misra (1983). PCR was performed on six *Aeromonas sobria* isolates four of them from Nile tilapia and two from Common carp.

Antibiotic discs for antibiotic sensitivity test table (6)

Determination of virulence and resistance genes using PCR:

PCR was performed on six *Aeromonas sobria* isolates four of them from Nile tilapia and two from Common carp.

PCR made to 2 virulence genes *Aerolysin* and *Ahcytoen* genes ; and 3 resistant genes *qnrs* , *blaTEM* and *ermB* genes.

Oligonucleotide primers used in cPCR:

They have specific sequence and amplify a specific product as shown in

1-Extraction of DNA:

According to QIA amp DNA mini kit instructions

2-Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara) code No. RR310A kit

Oligonucleotide primers used in c PCR:

They have specific sequence and amplify a specific product as shown in table(1)

3-Cycling conditions of the primers during PCR

Temperature and time conditions of the two primers during PCR according to specific authors and Emerald Amp GT PCR master mix(Takara)kit

DNA Molecular weight marker

The ladder was mixed gently by pipetting up and down .6 ml of the required ladder were directly loaded.

4-Agarose gel electrophoreses (Sambrook *et al.*,1989):

Electrophoresis grade agarose (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask , it was heated in a microwave to dissolve all granules with agitation, and allowed to cool at 70 c , then 0.5 mg /ml ethedium bromide was added and mixed thoroughly .

The warm agarose was poured directly in gel casting apparatus was desired com in apposition and left at room temperature for polymerization .

The comb was then removed and the electrophoresis tank was filled with TBE buffer.

20 ml of each PCR product samples, negative control and positive control were loaded to the gel. the power supply was 1-5 volts/cm of the tank length . the run was stopped after about 30 min and the gel was transferrwd to UV cabinet.

The gel was photographed by a gel documentation system and the data was analyzed through computer software.

3. RESULTS

4.1. bacterial identification:

The isolated bacteria from naturally infected fishes were identified as Aeromonadaceae (*Aeromonas sobria, Aeromonas veronei, Aeomonas jandae, Aeromonas hydrophila* and *Aeromonas cavae)*
*morphological and biochemical characters of *A.sobria:*

Gram negative rods, fermentative, catalase, indole and oxidase but not H2S. casein, gelatin and starch were degraded, but not aesculin and urea.

*morphological and biochemical characters of *A. Jandae, A. veronei , A. hydrophila* and *A. Cavae: A. veronei* cultures were Gm –ve rods ,motile fermentative which produce catalase, oxidase and cytochrome oxidase but not H2S and positive for voges proskauer reaction and citrate. Acid production from glucose, sucrose, salicin, mannitol and maltose and not from inositol and arabinose.

On the other hand , cultures of *A. jandae* were Gm-ve rods ,motile fermentative which produce catalase ,oxidase , cytochrome oxidase and positive for voges proskauer, indole. Acid production from glucose, maltose and mannitol and not from arabinose, sucrose ,salicin and inositol.

While *A. hydrophila* was circular yellow-coloured colonies on R-S media, gm –ve straight rods, Catalase, indole, cytochrome oxidase are produced, but not H2S, nitrates were reduced to nitrites without the production of gas, voges proskaeur reaction was positive, but not the methyl red test. Fermentation of sucrose, arabinose and salicin.
While cultures of *A.cavae* were motile ,Gm-ve rods, produced indole, but not H2S . ferment glucose, sucrose ,arabinose, mannitol and sorbitol but not inositol and salicin. Degrade gelatin but not urea. not reduce nitrates .

*occurence of Aeromonadacea infection in tilapia fish was 49.2% . the isolated aeromonadacea were suspected as *A. sobria, A. hydrophila, A. veronei , A. Jandae* and *A. cavae. while in common carp* fish was 56.6% and suspected as *A.sobria* and *A.veronei.*

*Pathogenicity test:

1- Experimental infection of different isolates of *A. sobria* among well examined fish for bacterial infection Nile tilapia table (3,4):

The experimentally infected fish died without clinical signs except darkness in colour. In some cases while the post mortem findings was septicemic lesions of the internal organs.

A. sobria was re-isolated from (liver, kidney and spleen) of recent dead fish.

2- Experimental infection of different isolates of *A. hydrophila*, *A. veronei* & *A. jandae*. among the apparent healthy Nile tilapia table (5)

*sensitivity of different isolates of *A. sobria* to antibiograms revealed that *A. sobria* was sensitive to ciprofloxacin , norfloxacin ,trimethoprim. while resistant to amoxicillin, erythromycin, gentamycin, rifamycin, bacitracin & cephradine table(6).

*Polymerase chain reaction (PCR):

Amplification of Aerolysin and *Ahcytoen* genes from extracted DNA of *A. sobria:*

The results observed in figures (1, 2) revealed that PCR with the *A. sobria aerolysin* and *ahcytoen* primer set , amplicons were produced by all 6 isolates of extracted DNA, this represent 100% of examined samples ; this means that all the six isolates were carried the *aerolysin* and *ahcytoen* genes.

Amplification of *qnrs* and *blaTEM* genes from extracted DNA of *A.sobria*:

The results observed in figures (3, 4) revealed that by PCR with the primer set of *qnrs* and *blaTEM* genes .amplicons were produced to the isolates of extracted DNA, this represent 100% of examined samples; this means that all the six isolates carried the two resistant genes.

Amplification of *ermB* gene from extracted DNA of A. *sobria*: This done The results observed in figure (5) revealed that by PCR with *ermB* primer set, all the six isolates were negative to this resistant gene (not carry the *ermB* resistant gene) this represent 100% of examined samples.

Table (1): oligonucleotide primers sequences Source: Metabion (Germany)

Primer	Sequence	Amplified Product	Reference
Ahcytoen	GAGAAGGTGACCACCAAGAACAA AACTGACATCGGCCTTGAACTC	232 bp	Cagatay and Sen 2014
Aerolysin (Aero)	CACAGCCAATATGTCGGTGAAG GTCACCTTCTCGCTCAGGC	326bp	Singh et al. 2008
qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417bp	Robicsek et al. 2006
blaTEM	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516bp	Colom et al. 2003
ermB	GAAAAAGTACTCAACCAAATA AATTTAAGTACCGTTACT	639bp	Nguyen et al. 2009

Table (2) Aeromonas spp. isolated bacteria from examined fishes:

	Tilapia		Common Carp		Total	
	N0.	%	No.	%	No.	%
Aeromonas spp.:						
A . sobria	45	46.87	39	76.47	84	29.47
A . veronei	30	31.25	12	23.52	42	14.73
A.jandae	12	12.5	0	0	12	4.2
A. hydrophila	6	6.25	0	0	6	2.1
A. cavae	3	3.125	0	0	3	1.05
Total	96		51		147	

Table (3) pathogenicity of different isolates of *A. Sobria* in Nile tilapia

	No. of fish	0 day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
1	10	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10
2	10	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9
3	10	0	0	0	1	0	0	0	0	0	2	0	1	1	2	0	7
4	10	0	0	3	0	1	0	0	0	0	0	1	0	0	0	0	5
5	10	0	0	1	0	0	0	0	0	0	2	2	3	1	1	0	10
6	10	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	3
7	10	0	2	1	1	0	0	1	0	0	0	1	0	1	0	0	7
8	10	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2
9	10	0	0	0	0	1	0	1	0	0	0	0	1	2	2	0	7
10	10	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2
11	10	0	4	5	1	0	0	0	0	0	0	0	0	0	0	0	10
12	10	0	2	4	0	1	1	0	1	0	0	0	0	0	0	0	9
13	10	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table (4) indicate the mortality rate as differed from 20 - 100% between the different isolates of *A. sobria*.

Group no.	No. of fish	Origin of isolates	Mortality%
1	10	Liver (N.t)	100
2	10	Tail (N.t)	90
3	10	Tail (N.t)	70
4	10	Tail (N.t)	50
5	10	Liver (C.C)	100
6	10	Kidney (C.C)	30
7	10	Spleen (C.C)	80
8	10	Liver (C.C)	20
9	10	Kidney (C.C)	80
10	10	Kidney (N.t)	20
11	10	Liver (N.t)	100
12	10	Kidney (N.t)	90
13	10	Sterile Saline	0

N.t: Nile tilapia, C.C: Common carp.

Table (5) Pathogenicity of different isolates of *A. hydrophila*, *A. veronei* & *A.jandae*. among the apparent healthy Nile tilapia:

	No.of fish	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Hydrophila 1	10	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
Hydrophila 2	10	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2
Veronei 1	10	0	8	0	0	0	2	0	0	0	0	0	0	0	0	0	10
Veronei 2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jandae 1	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jandae 2	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sterile Saline	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table (6) The result of in vitro sensitivity test of 11 isolates of *A. sobria*:

		Res.	Int.	Sus.	1	2	3	4	5	6	7	8	9	10	11	12
Ciprofloxacin 5mcg	Cip 5	15	16-20	21	32(s)	32(s)	32	30(s)	23(s)	26(s)	30(s)	24(s)	22(s)	39(s)	47(s)	32(s)
Norfloxacin 10mcg	Nor 10	12	13-16	17	31(s)	30(s)	30(s)	20(s)	15(I)	32(s)	28(s)	21(s)	19(s)	33(s)	42(s)	33(s)
Cephradine	Ce 30	11	12-13	14	11(R)	16(s)	15(s)	14(s)	7(R)	14(s)	16(s)	13(I)	15(s)	15(s)	16(s)	15(s)
Amikacin	Ak 30	14	15-16	17	16(I)	17(s)	17(s)	15(I)	15(I)	13(R)	16(I)	13(R)	16(I)	18(s)	16(I)	15(I)
Bacitracin	B 10	8	9-12	13	7(R)	7(R)	7(R)	7(R)	7(R)	7(R)	7(R)	7(R)	7(R)	7(R)	10(I)	13(s)
Rifamycin	Rf 30	16	17-19	20	10(R)	9(R)	9(R)	9(R)	8(R)	8(R)	7(R)	9(R)	10(R)	9(R)	8(R)	7(R)
Fusidic	Fa 10	17	18-21	22	9(R)	10(R)	7(R)	7(R)	9(R)	9(R)	11(R)	7(R)	11(R)	10(R)	10(R)	7(R)
Erythromycin	E 15	13	14-22	23	7(R)	12(R)	9(R)	7(R)	7(R)	12(R)	10(R)	8(R)	9(R)	8(R)	10(R)	10(R)
Trimethprim	Sxt 25	10	11-15	16	15(I)	22(s)	24(s)	12(I)	12(I)	14(I)	21(s)	15(I)	7(R)	12(I)	22(s)	20(s)
Gentamycin	Cn 10	12	13-14	15	15(s)	15(s)	14(I)	11(R)	15(s)	10(R)	12(I)	12(I)	14(I)	12(I)	26(s)	12(I)
Amoxicillin	Ax 25	11	12-13	14	7(R)	7(R)	7(R)	7(R)	12(I)	7(R)	7(R)	7(R)	7(R)	8(R)	7(R)	6(R)

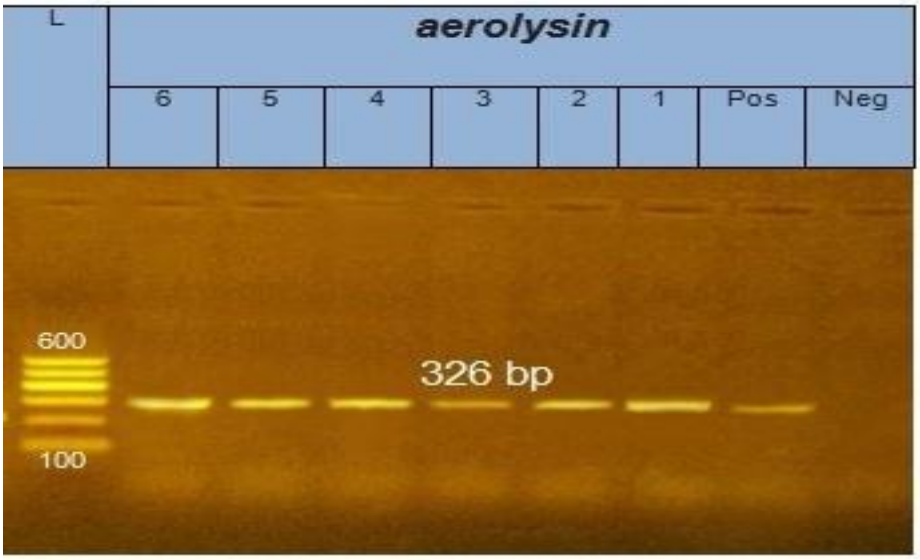

Fig. 1: Gel electrophoresis for *Aerolysin* gene of *A. Sobria*: L: Molecular Weight Marker (100-600bp). Pos: Positive control (at 326 bp). Neg: Negative control. Lane1, 2, 3, 4, 5, 6,: *A. Sobria.*

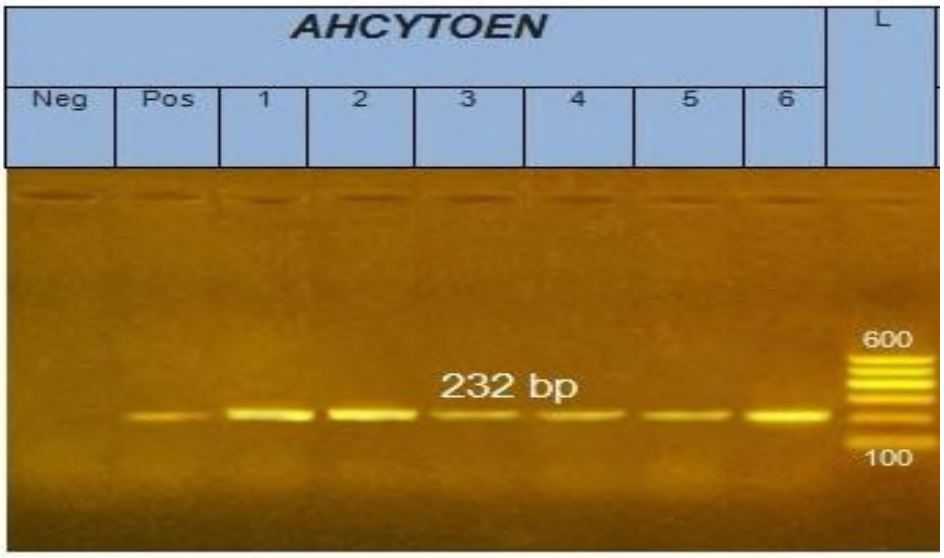

Fig. 2: Gel electrophoresis for *Ahcytoen* gene of *A. Sobria*: L: Molecular Weight Marker (100-600bp). Pos: Positive control (at 232 bp). Neg: Negative control. Lane1, 2, 3, 4, 5, 6,: *A. Sobria.*

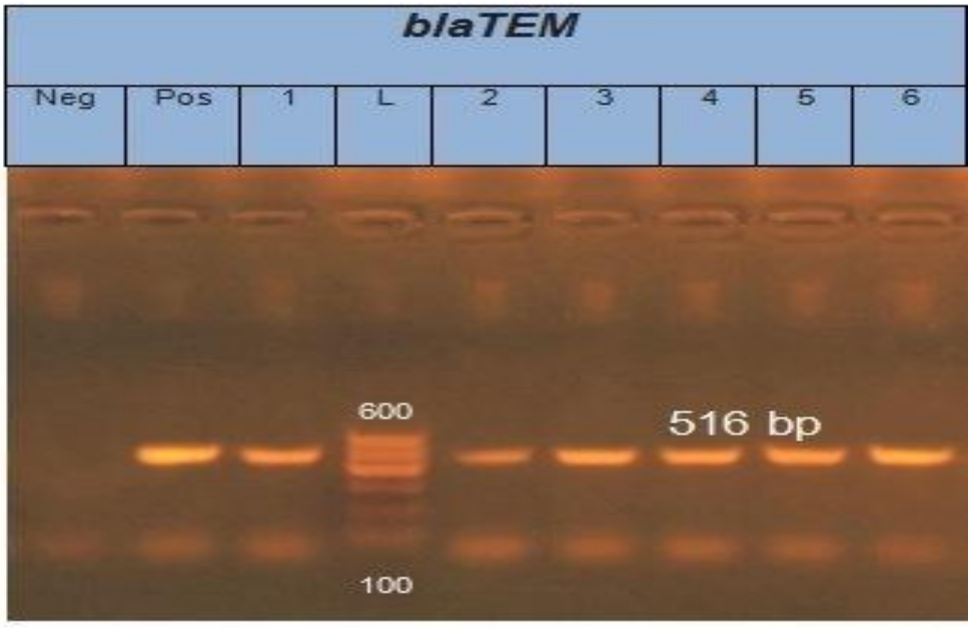

Fig 3: Gel electrophoresis for *blaTEM* gene of *A. Sobria*: L: Molecular Weight Marker (100- 600bp). Pos: Positive control (at 516 bp). Neg: Negative control. Lane1, 2, 3, 4, 5, 6,: *A.Sobria.*

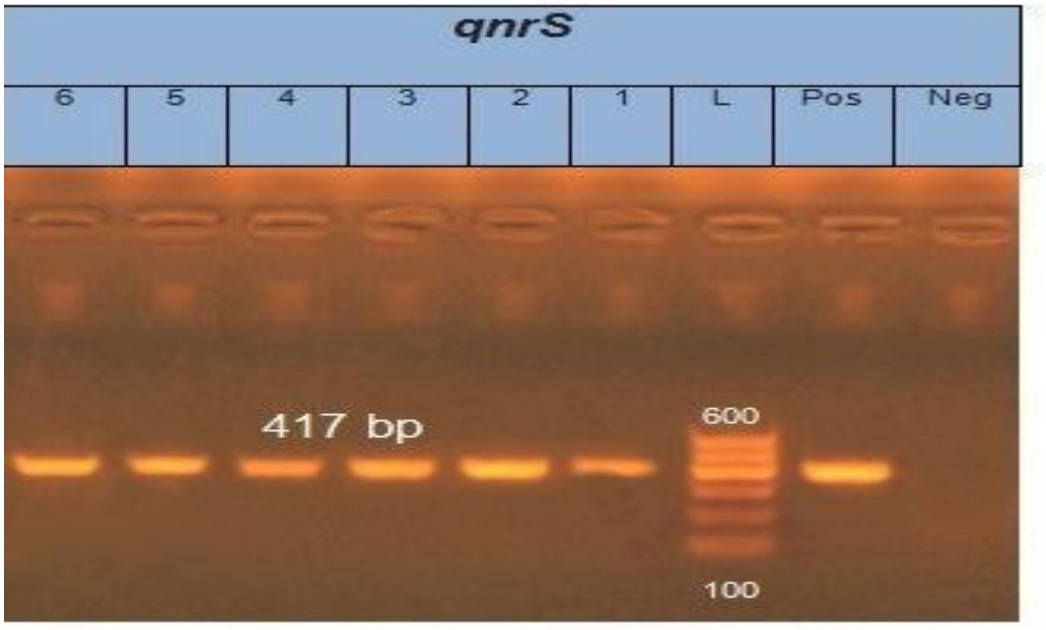

Fig4: Gel electrophoresis for *qnrs* gene of *A. Sobria*: L: Molecular Weight Marker (100- 600bp). Pos: Positive control (at 417 bp). Neg: Negative control. Lane1, 2, 3, 4, 5, 6,: *A. Sobria.*

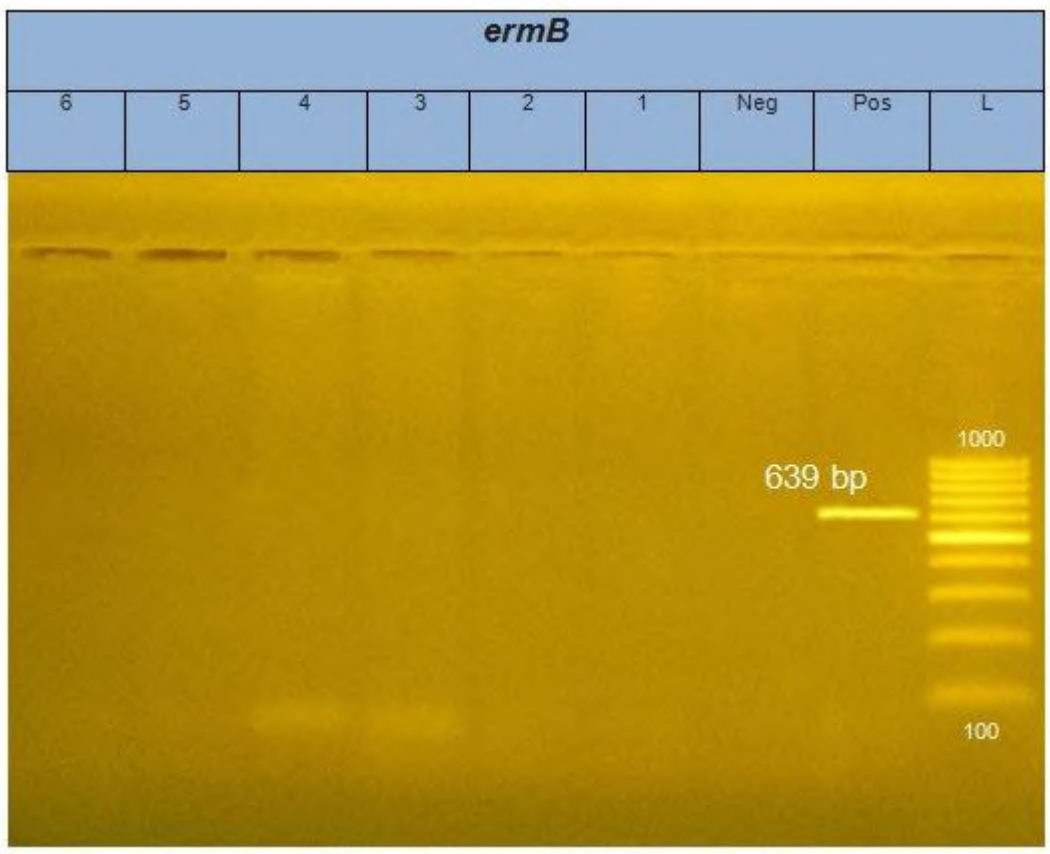

Fig 5: Gel electrophoresis for *ermB* gene of *A. Sobria*: L: Molecular Weight Marker (100- 1000bp). Pos: Positive control (at 639 bp). Neg: Negative control. Lane1, 2, 3, 4, 5, 6,: Negative.

4. DISCUSSION:

Bacterial fish diseases is a serious threat to aquaculture systems that causes severe damage and mortality in Egypt; So the outcome of the present study is to isolate and identify the most common bacterial causes of mortalities in fish and using the benefit of PCR in detection of virulence and resistant genes of bacterial fish pathogens.

In this study, the isolated bacteria from naturally infected fishes were identified as *Aeromonadacea* (*Aeromonas sobria, Aeromonas veronei, Aeromonas jandae, Aeromonas hydrophila and Aeromonas caviae*).These species identified by routine biochemical properties as described by Austin and Austin (1993 and 2007).

The biochemical properties as growth at 5 c and on 6% Nacl , acid production from arabinose ,sucrose and salicin were used to differentiate between species of *Aeromonas (A.sobria, A.veronei, A.jandae , A.hydrophila and A.caviae*) as described by Rahman *et al* (2002); Huys *et al* (2002); Austin and Austin (1993 and 2007) and Awad (2011).

In this study, the occurrence of *Aeromonade* infection in tilapia fish was 49.23% and in common carp 56.66% .

A. sobria was the most prevalent strain of *Aeromonade* in relation to other strains in tilapia and Common carp 46.8% and76.4% respectively. These results were in agreement with Wahli *et al. (*2005) who found that the pathogenic *A. sobria* had identified as a

causative agent in diseased *perca fluviatilis*. Kozinska (2007) isolated *A. sobria* from common carp. Austin and Austin (2007) reported that *A. sobria* was the main pathogen from different fish species. Awad (2011) recorded that a higher occurrence of *A. sobria* (34%) isolated from carp fish was recorded in relation to *Aeromonadacea* group.

The occurrence of *A. veronei* infection in relation to other *Aeromonade* strains in tilapia and common carp was 31.25% and 23.5% respectively that comes behind *A. sobria* directly, these results comes in agreement with Awad (2011) who recorded that *A.veronei* was the most prevalent aeromonade strain comes behind *A.sobria* in Carp fish in El-Abbassa fish farms and Awad (2015) isolated *A.veronei* from *Tilapia zilli* with average 6.25% from Timsah lake .

The occurrence of *A. jandae, A. hydrophila* and *A. caviae* in relation to other *Aeromonade* strains in tilapia fish was 12.5 ,6.25 and 3.125 % respectively. While common carp was free from these strains, while Awad (2013) isolated *A. jandae* and *A .hydrophila* from Nile tilapia with percent of 37% and 31% respectively also Abd El Ghany *et al* (2009) recorded that *A. hydrophila* was relatively the most common aeromonas species in the aquaculture environment and also Essmat (2009) mentioned that A. hydrophila was the most prevalent bacteria in Nile tilapia in El-Abbassa fish farms.

Aeromonas spp. were important pathogens to different fish species causing motile *Aeromonas* septicemia and sometimes lead to severe damage in the aquaculture.

In this study *A. sobria* was the most prevalent strain from Nile tilapia and common carp. Our results revealed that all examined isolates of *Aeromonas sobria* were pathogenic to Nile tilapia (20-100%) as Wahli *et al* (2005), Min and Ying (2005) and Somayah (2011). This study revealed that one isolate from two *A. veronei* was non pathogenic.

Two isolates of *Aeromonas hydrophila* were pathogenic with 20% mortality. Two isolates of *Aeromonas jandae* were nonpathogenic to Nile tilapia this variation of mortality rate was due to variation in the virulence among individual strains, these results were in agreement with Kozinska *et al.* (2002) and Kozinska (2005) who recorded that *A. veronei* was strongly virulent to carp and trout leading to 100% mortalities and Sahoo *et al*. (2004) ;Wang- Kai-Yu (2004); Rajeswari-Shome *et al* (2005); Pan-Houjun *et al.* (2005) and Jun *et al* (2010) and Awad (2011) recorded that *A. hydrophila* produced mortality from 0 to 100%.

Polymerase chain reaction (PCR) was known to be a technique analogous to detect in hay stick, because it is able to amplify a single target DNA molecule specifically amongst a large concentration from non target as mentioned by Sharif *et al*. (2000)

PCR- based method was sensitive, rapid and specific for detection of virulence and resistant genes of *Aeromonas sobria*. It overcomes the disadvantages of time consuming biochemical based methods.

In this study detection to two virulence genes to *Aeromonas sobria* by PCR (*Aerolysin* and *Ahcytoen* genes) were done. The six isolates of *A. sobria* which were examined by PCR technique carried these two virulence genes; and the experimental infection showed mortalities in all isolates.

Aerolysin gene is a haemolytic gene which is extracellular, soluble hydrophilic protein exhibiting both haemolytic and cytolytic properties as recorded by (Rabaan , *et al* 2001 and Yousr, *et al* . 2007)

Ahcytoen gene is acytolytic enterotoxin gene there are many other extracellular proteins produced by *Aeromonas* species which associated with pathogenicity and

environmental adaptability such as adhesion, Mucinase production, Cytotoxicity casinase, Collagenase, Protease , Lipase or other factors combine with *Aerolysin* and *Ahcytoen* genes for pathogenic effects as mentioned by (Wahli *et al*.2005; Dskalov.2006;Afza *et al* .2007; and Kozinska 2007).

Several extracellular enzymes and toxins including hemolysins, proteases and cytotoxins have been detected as virulence factors of motile *Aeromonas* but the role of each single factor in relation to pathogenesis varies as recorded by Allan &Stevenson ,1981 ; Thune *et al*. ,1982; Rodringuez *et al*. 1992 and Kozinska ,2007.

Rabid detection of cytolytic enterotoxin (*Ahcytoen*) gene and their characterization has proven importance so that proper and rapid preventive and control measures could be taken up to reduce mortality and loss in fish culture as recorded by (Sarkar *et al.*, 2013)

The results indicated that *Aerolysin* gene was detected at 326 bp site of gene, this agrees with (Singh *et al*., 2008) and disagree with Awad (2011) who revealed that the gene detected at 462 bp for *A. sobria*.

While the results indicated that *Ahcytoen* gene give band at 232 bp. this agree with Cagatay and Sen (2014) .

For resistant genes, the six isolates possessed *qnrs* (quinolones) and *bla TEM* gene (betalactamases) But negative for *erm B* (macrolides) gene.

Six isolates of *Aeromonas sobria* give band at 417 bp for *qnrS* gene this agree with Robicsek *et al*.,2006, while the gene of *blaTEM* give band at 516 bp as Colom *et al* .,2003, and the six isolates were negative to *ermB* gene, the results were in agreement with Deng Yu –Ting *et al* (2014) who recorded that plasmid –borne quinolone resistance genes were detected in five *A. hydrophila* (4.7%) two of them carried *qnrS*2 , two cefotaxime resistant *A. hydrophila* were positive for *blaTEM*-1 and Onuk. E.E *et al* (2015) who recorded that plasmid – mediated quinolone resistance was defined among Aeromonas species isolated from water, and reported that most aeromonas strains sensitive to fluoroquinolones, doxycycline, cefotaxime and amikacin and resistant to ampicillin , rifampin, streptomycin and nalidixic acid and reported that all *qnrS* –positive aeromonas spp. isolates were ciprofloxacin susceptible, while five of them were resistant to nalidixic acid.

In this study sensitivity test of *A. sobria* revealed that it is sensitive to ciprofloxacin, norfloxacin and trimethoprim and resistant to amoxicillin, erythromycin, gentamycin, rifamycin, bacitramycin and cephradine.

These results agree with Guz and Kozinska (2004); Min and Ying (2005); Durma and Turk (2009); Somayah (2011) and Shalaby (2017).

5.CONCLUSION

It could be concluded that bacterial fish pathogens were the main cause of heavy mortalities. MAS was the most prevalent disease; Aeromonas sobria was the most prevalent isolate. ciprofloxacin and norfloxacin were the drug of choice as indicated by sensitivity tests. PCR was the most specific method for detection of Aerolysin and Ahcytoen genes in Aeromonas sobria; and also for detection of qnrS, blaTEM and ermB genes.

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