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Yersinia ruckeri infection and pathogenicity of the cultured *Oreochromis niloticus*

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ABSTRACT

Yersinia ruckeri is a highly virulent Gram-negative fish pathogen affecting many farmed and wild fish species. In the present study, *Y. ruckeri* has been isolated from 6 tilapia (*Oreochromis niloticus*) farms located in Kafrelsheikh Governorate. Studied farms suffered from increased fish mortality just after the decreased feed intake; the most common clinical signs were the erythematic appearance of lips, congestion to extensive haemorrhages all over the external body surface, and fins and tail erosions. Internally, diseased fishes showed petechial haemorrhages on the external hepatopancreas surface with engorged gall bladder filled with faint-colored bile. The spleen and posterior kidney were congested, and the haemorrhagic gastroenteritis was also dominant. Out of 150 clinically diseased fish, 96 *Y. ruckeri* were biochemically identified, and 45 were isolated from the liver. Molecular screening of selected isolates for certain virulence genes indicated that *Yrp1* was the most common virulence gene present in 60% of tested isolates, followed by *YhlA* gene in 40 %, and finally *Yrlnv* gene in 20%, while *YhlB* gene was completely absent. All the tested *Y. ruckeri* isolates were virulent for *O. niloticus*; challenge test indicated the presence of a direct relation between isolate pathogenicity and isolate virulence genes content (genotype). Isolate with 2 virulence genes was more pathogenic than that with one or without any virulence gene. The LD_{50} ranged between $3.9x10^6$ and $9.7x10^6$ for the studied isolates. In conclusion, *Y. ruckeri* was responsible for the disease condition affecting the studied Nile tilapia farms.

INTRODUCTION

Fish constitute a major source of protein, fatty acids, vitamins, minerals and essential micronutrients for the expanding world population (**Aboyadak *et al.*, 2016**). Thus, aquaculture repesents the main practical solution to meet the increasing human population being the fastest growing meat production sector. The global aquaculture production reached 122.6 million tons in 2020, with a total estimated value of about 281.5 billion USD **(FAO, 2022)**. However, intensive aquaculture production is associated with the spread of infectious diseases and outbreaks **(Abd El Tawab *et al.*, 2022)**.

Infectious bacterial diseases are considered the main problem in farmed fish resulting in heavy mortalities and subsequent huge economic loss **(Saleh *et al*., 2019)**.

Yersinia is a genus of Gram-negative, rod-shaped, facultative anaerobic bacteria; it is a member of the family Enterobacteriaceae. *Yersinia* comprises several pathogenic species, which cause diseases in humans and other animals including fish **(Imnadze *et al*., 2020)**.

Yersinia ruckeri is the causative agent of enteric redmouth disease in various salmonids species worldwide; this disease was first described from the rainbow trout in the Hagerman Valley of Idaho, USA in the 1950s **(Zorriehzahra *et al*., 2017)**. Yersiniosis is a systemic bacterial infection caused by *Y. ruckeri* and affects many fish species including the Nile tilapia, carp, catfish, sturgeon, perch and burbot **(Eissa *et al*., 2008; Tobback *et al*., 2010; Kumar *et al*., 2015; Guijarro *et al*., 2018)**.

The disease is affecting fish with different age ranges (fry, fingerlings even adult spawners) but it is more fatal in younger fish (fry and fingerlings). The early stage of the disease started with decreased feed intake with insignificant mortalities which increased over time **(Horne and Barnes, 1999)**. Disease classical signs are imbalanced swimming near the water surface, skin darkening, exophthalmia, and ulceration with subcutaneous haemorrhages particularly around the mouth **(Shaowu *et al*., 2013)**; the internal lesions are characterized by the presence of petechial haemorrhages on the gastrointestinal tract and liver and enlarged darkened spleen **(Kumar *et al*., 2015)**.

Biochemical characterization of bacteria is still an important method for the identification of bacterial pathogens **(Abd El Tawab *et al*., 2022)**. The most important biochemical characteristics of *Y. ruckeri* as mentioned in the studies of **Horne and Barnes (1999)** and **Tobback *et al.* (2007)** are the ability to ferment glucose, positivity for catalase, negativity for oxidase and the potetial to reduce nitrate. In addition, *Y. ruckeri* secretes b-galactosidase and decarboxylases for lysine and ornithine decarboxylation.

Molecular tools are not only effective in the identification of bacterial pathogens, but also very important for detecting virulence factors encoded in virulence genes **(El-Bahar *et al.*, 2019)**. The detection of virulence genes is important in understanding bacterial pathogenicity. Many virulence factors have been detected in *Y. ruckeri* as the extra-cellular products responsible for disease signs, particularly haemorrhages **(Romalde & Toranzo, 1993)**. *YhlA* gene is responsible for the production of hemolytic and cytotoxic proteins and *YhlB* gene, encoding a putative protein that is involved in secretion and/ or the activation of hemolysin **(Fernandez *et al*., 2007)**. *Yrlnv* gene is responsible for *Y. ruckeri* invasin (surface exposed adhesins) that plays a potential role in biofilm formation and bacterial virulence **(Wrobel *et al*., 2020)**. Besides, *Yrp1* gene is encoded for metalloproteases production **(Fernandez *et al*., 2003)**.

Y. ruckeri is responsible for severe damage in infected fish tissues. Degenerative changes were observed in several organs of infected fish including the liver, spleen, kidney and intestine. Histopathological changes in the fish liver included focal necrosis of hepatocytes, pyknotic nuclei, fatty degeneration, sinusoidal congestion and dilatation **(Tobback *et al*., 2009; Adel *et al*., 2020)**.

The present work aimed to study the prevalence of *Y. ruckeri* in the cultured Nile tilapia (*Oreochromis niloticus*) and determine the genotypic characterization and pathogenicity of the recovered isolates concerning their virulence genes profile.

MATERIALS AND METHODS

Statement of compliance with the ethics guidelines

Experimental fish used in the present research were handled, transported, examined and euthanized following the guidelines of the National Advisory Committee for Laboratory Animals Research **(NACLAR, 2004**; **CCAC, 2005)** for the care and use of fish in teaching and research.

Fish samples

One hundred and fifty moribund *Oreochromis niloticus* were collected from six diseased fish farms located at Trombat seven, Elriad District, Kafrelsheikh province. Twenty-five fish were collected from each farm, diseased fish weight ranged between 50 and 150g. Fish were rinsed with sterile phosphate buffer before storage in a clean plastic bag which was placed in ice pox as mentioned in the study of **Ali *et al*. (2021a)**. Fish from each farm were collected on a separate day and then immediately transported to the Fish Diseases Lab, the National Institute of Oceanography and Fisheries for bacterial isolation.

Gross clinical examination

The clinical and postmortem examination was performed as described in the work of **El-Bahar *et al*. (2019)**, in which the clinical signs and behavioral abnormalities appearing on the infected fish were recorded on the farms during fish collection and storage. In the laboratory, the external surfaces of the fish were disinfected with 70% ethyl alcohol, then dissected for observation and record of gross pathological signs. Afterward, fish were subjected to microbiological examination.

Bacterial isolation

Using a sterile cotton swab, swaps were taken separately from the liver, kidney, spleen and heart under a complete aseptic condition in a laminar airflow chamber, and then the swab from each organ was added separately to a tryptic soya broth tube Difco, USA, and tubes were incubated at 28ºC for 24h. A loopful was taken from each tube and cultured on ribose ornithine desoxycholate agar and xylose lysine deoxycholate then

characteristic of *Y. ruckeri* colonies was further cultured on sheep blood agar following the procedures of **Tobback *et al.* (2007)** and **Carson *et al.* (2019)**. Seeded plates were incubated at 28ºC for 48h. Single characteristic of *Y. ruckeri* colony (small yellow colony on ribose ornithine desoxycholate agar and bright pink colony on xylose lysine deoxycholate agar) was picked up from each plate, then incubated in tryptic soy broth and subjected to the biochemical identification.

Biochemical Identification

Biochemical identification was performed as described in the work of **Abd El Tawab *et al.* (2022)**. Briefly, each isolate was subjected to Gram staining and then examined microscopically for cell morphology. Further biochemical tests such as motility, oxidase, catalase, citrate utilization, gelatin hydrolysis, Voges Proskauer, H_2S production, tryptophan deamination, indole production, beta-galactosidase, arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase, urease, glucose oxidation-fermentation, and sugar utilization tests (mannitol, inositol and rhamnose) were performed for proper identification of the recovered isolates. The obtained results were compared to *Y. ruckeri* reference strain. Then, each biochemically identified isolate was preserved at - 80 ºC with 25% glycerol in triplicates for further use.

Molecular detection of virulence genes

Genomic DNA extraction

Y. ruckeri genomic DNA was extracted using QIAamp DNA Mini Kit, (Cat. No., 51304), QIAGEN, Germany, as described by **Ali et al. (2022)**. Briefly, 200µl of 48h-seeded broth was taken in 1.5ml Eppendorf tube. Then, 200µl of AL buffer and 20µl of protease were added, Eppendorf tube was vortexed then incubated at 56ºC for 10min. 200µl of 96% ethanol was added followed by vortex mixing, and the mixture was added to the spin column. The spin column was centrifuged at 4032g, then 500µl of AW1 buffer was added followed by centrifugation at 4032g. This step was followed by adding 500µl of AW2 buffer and succeeded by re-centrifugation at 4032 g. 100µl of elution buffer was added to the spin column membrane and then centrifuged at 4032g; the centrifugate was collected in a 200µl PCR tube and preserved at - 80ºC for virulence gene detection.

Amplification of virulence genes

Y. ruckeri samples were screened for the presence of four virulence genes *Yrp1, YrInv, YhlA* and *YhlB*; the primer sequences used in this study are presented in Table (1). PCR reaction mixture was prepared by adding 1.0µl of each primer to 5µl of templet DNA, 12.5µl of 2X master mix and 5.5µl of nuclease-free water. PCR reaction was carried out in Bio-Rad thermal cycler. PCR conditions for the amplification of each virulence gene started with an initial denaturation at 95ºC for 5min, followed by 35

cycles as presented in Table (2). Then, a final extension step was performed at 72ºC for 10min.

Tabel 1. Primer sequences used in the detection of *Y. ruckeri* virulence genes

Gene	Primer Sequence 5'-3'		Size (bp)	Reference
Yrp1	**F:**	TATTCAACTGAAAGTGTA	961	**Abdel-Latif *et al*., 2014**
	R:	ATAGCTCATAATACTGA		
YrInv	**F:**	ATTATATGATTTAGCAAGGTTTGAAAA	608	**Wrobel *et al*., 2020**
		TATTATTTTAAAAAGTTGGCGCGGGA		
	R:	AATGCCCTCTTGGGTTATCAAGAGGGT		
		CATCTGCAAGGTTTCGCTGTGGCCGT		
YhlA	**F:**	ATATCCGGGCCGAAGGC	938	**Fernandez *et al*., 2007**
	R:	ATTGTCGATCAATAAGC		
YhlB	**F:**	ATAACCGGTGGAGATCA	735	
	R:	CAGGTTATGAGTGCGGT		

Tabel 2. PCR programming for amplification of *Y. ruckeri* virulence genes

Gene	35 cycles		
	Denaturation	**Annealing**	**Extension**
Yrp1	94 ºC for 30 sec	40 ºC for 40 sec	72 ºC for 50 sec
YrInv	94 ºC for 30 sec	50 ºC for 40 sec	72 ºC for 45 sec
YhlA	94 ºC for 30 sec	50 ºC for 40 sec	72 ºC for 50 sec
YhlB	94 ºC for 30 sec	50 ºC for 40 sec	72 ºC for 45 sec

Detection of virulence gene amplicons

Virulence gene amplicons were separated by gel electrophoresis of PCR products in 1.5% w/v molecular grade agarose gel containing 0.5 µg/ml ethidium bromide in ESH3350-SYS horizontal electrophoresis, supplied with EV3000 power supply, Consort, Belgium. The power supply was adjusted at 100V for one hour; bands were visualized using the gel documentation system, Azure C200, Azure Biosystems, USA.

Experimental fish

One-hundred and five healthy *Oreochromis niloticus* with 50 ± 5g in weight were transported to the wet fish diseases laboratory, NIOF, under the optimum condition mentioned by **Ali *et al*. (2021b)**, and fish were observed for 15 days for acclimatization.

Pathogenicity test

A pathogenicity test was performed according to the method of **Saleh *et al.* (2021)** to determine the possible role of virulence genes in *Y. ruckeri* pathogenicity. The LD_{50} of isolate number 1 (*YhlA*$^+$, *Yrp1*$^+$), isolate number 3 (*Yrp1*$^+$), isolate number 5 (*Yrlnv*$^+$), and isolate 4 (negative for all virulence genes) were determined for *Oreochromis niloticus*. A single colony was picked up from the agar surface and then incubated on brain heart infusion broth at 28ºC for 28h. Bacterial growth was collected and then adjusted to (5×10^5, 5×10^6 and 5×10^7) CFU ml^{-1}. One-hundred and five fish were randomly divided into 5 equal groups in triplicates (21 fish/group & 7 fish/replicate (a, b & c). Fish in group 1 were inoculated with 0.2ml of normal saline and served as a negative control; fish in the other 4 groups were inoculated with a particular bacterial suspension containing 10^5, 10^6 or 10^7 CFU.fish $^{-1}$ as represented in Table (3). Feed was restricted at 24h before infection; fish were observed twice daily for 7 days for monitoring the clinical signs and mortality rate. Dead fish was considered only if the challenged bacterial strain was re-isolated and the lethal dose of fifty (LD_{50}) was calculated as described in the study of **Saganuwan (2011)**.

Table 3. Experimental design for pathogenicity test

***Y. ruckeri* isolate number**	**Replicate / Group**	**Inoculum concentration**		
		a	**b**	**C**
-	1	PBS	PBS	PBS
1 (*YhlA*$^+$, *Yrp1*$^+$)	2	10^5	10^6	10^7
3 (*Yrp1*$^+$)	3	10^5	10^6	10^7
5 (*Yrlnv*$^+$)	4	10^5	10^6	10^7
4	5	10^5	10^6	10^7

21 fish/group and 7 fish/replicate

RESULTS

Gross clinical examination

Naturally and experimentally infected fish showed skin darkening, corneal opacity, marked congestion to extensive haemorrhages all over the external body surface, and fins and tail erosions. The most common sign particularly in naturally diseased fish was the erythematic appearance of lips. Internally, diseased fishes showed petechial haemorrhages on the external hepatopancreas surface, with an engorged gall bladder filled with faint-coloured bile. Spleen and posterior kidney were congested and hemorrhagic gastroenteritis was also dominant (Fig. 1).

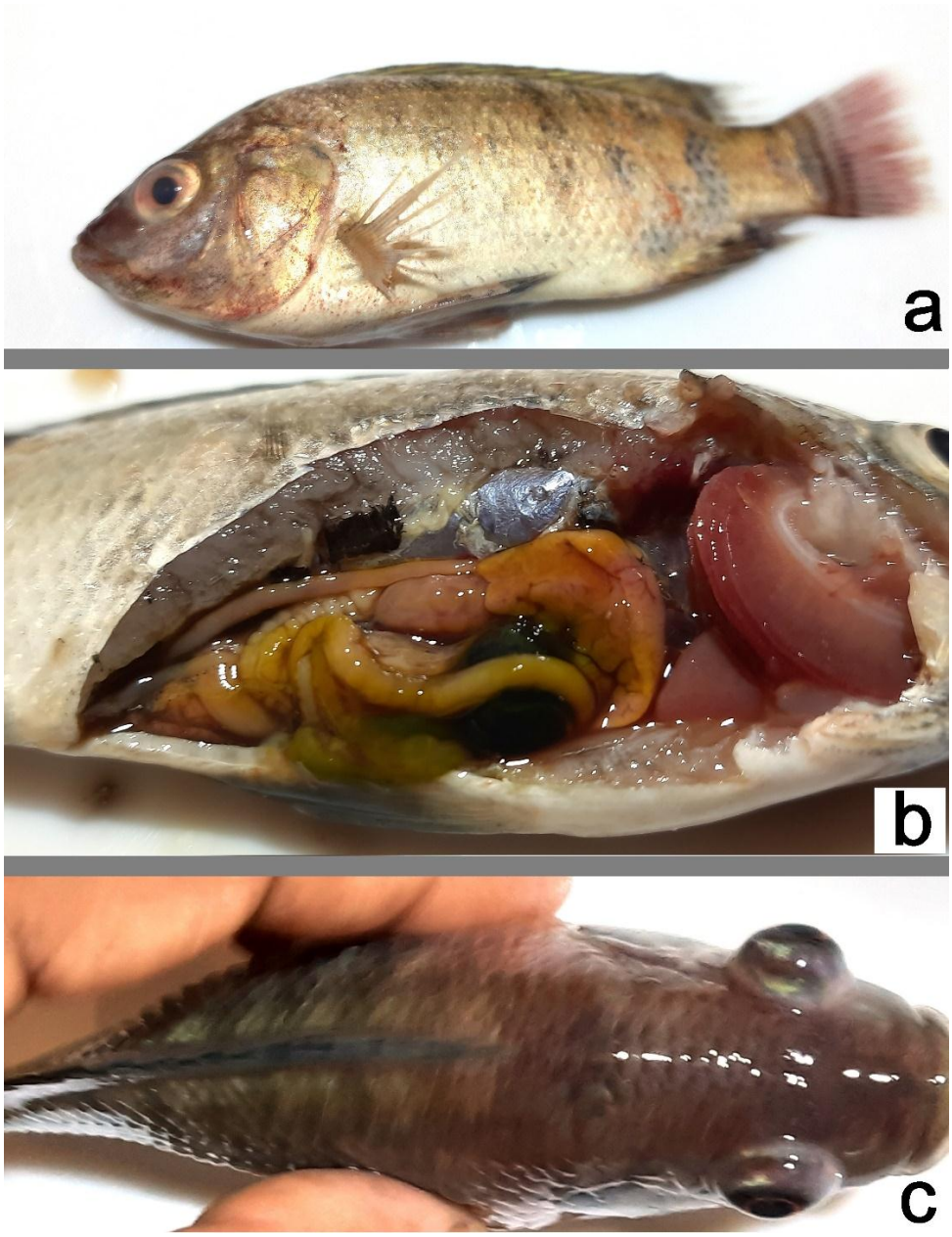

Fig. 1. Experimentally infected *O. niloticus* with *Y. ruckeri* **a)** Skin ulceration, petechial haemorrhage around the lower jaw, pectoral fin and tail erosions. **b)** Icteric liver and enlarged distended gall bladder. **c)** Bilateral exophthalmia.

Biochemical characteristics of recovered *Y. ruckeri* isolates

Y. ruckeri isolates grown on CIN agar as medium size colonies had deep pink to red centres with pale pink border after 48h of incubation at 28ºC. Microscopic examination indicated the presence of Gram-negative short bacilli. Isolates were weakly motile, catalase positive, cytochrome oxidase negative, and could not produce H_2S but could secrete beta-galactosidase and utilize glucose; the full biochemical profile of Y. *ruckeri* isolates is listed in the Table (4).

Table 4. Morphological and biochemical characters of *Y. ruckeri* isolates

Test	Result
Gram staining reaction	Gram-negative
Cell morphology	Rod-shaped bacilli
Motility	Motile
Oxidase	-
Catalase	+
Citrate Utilization	+
Gelatin hydrolysis	+
Vogus Proskauer	+
H_2S production	-
Tryptophan deamination	-
Indole production	-
Beta-galactosidase	+
Arginine decarboxylase	+
Lysine decarboxylase	+
Ornithine decarboxylase	+
Urease test	-
Glucose for O/F	+
Mannitol	+
Inositol	-
Rhamnose	-

Prevalence of *Y. ruckeri* in diseased *O. niloticus*

Isolation and biochemical identification indicated that 96 *Y. ruckeri* isolates were recovered from 150 clinically diseased *O. niloticus* with a total prevalence equal 64%. 44.49% of the recovered isolates were from hepatopancreas followed by the posterior kidney (34.37), the heart (12.5) and the spleen (8.3) as represented in Table (5).

Table 5. *Y. ruckeri* isolates retrieved from organs of the naturally infected *O. niloticus*

Organ	*Y. ruckeri* isolate	
	No.	%
Liver	43	44.79
Heart	12	12.5
Kidney	33	34.37
Spleen	8	8.3
Total	96	

Distribution of virulence genes among five randomly selected *Y. Ruckeri* isolates

Yrp1 was the most common virulence gene present in 60% of tested isolates, followed by *YhlA* which was present in 40%, then *Yrlnv* that present in 20%. Meanwhile, the *YhlB* gene was completely absent in all of the screened isolates (Table ٦ & Fig. ٢, 3).

Table 6. Genotype profiling of selected *Y. Ruckeri* isolates based on the virulence genes content

Isolate No.	*YhlA*	*YhlB*	*Yrlnv*	*Yrp1*	Genotype
1	+	-	-	+	$YhlA^{+}$, $Yrp1^{+}$
2	+	-	-	+	$YhlA^{+}$, $Yrp1^{+}$
3	-	-	-	+	$Yrp1^{+}$
4	-	-	-	-	-
5	-	-	+	-	$Yrlnv^{+}$
VG %	40	0	20	60	

VG %: Virulence genes % in isolate

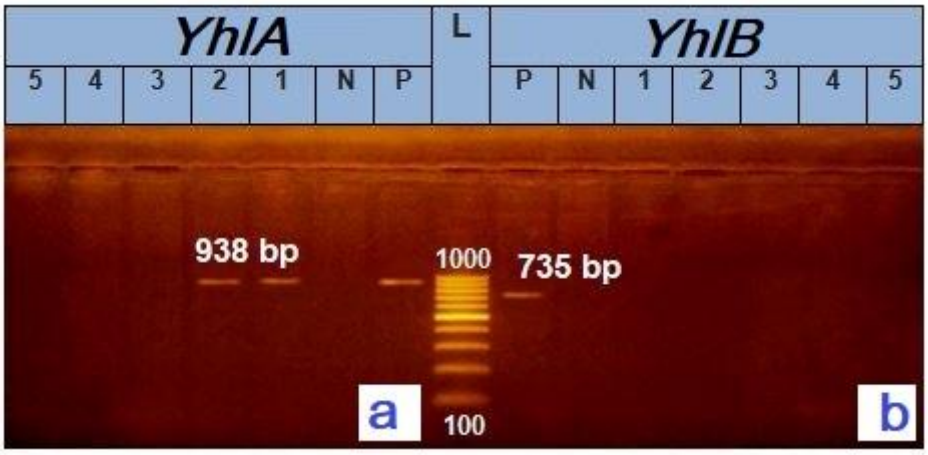

Fig. 2. a) Agarose gel electrophoresis of PCR product indicating the presence of *YhlA* gene at 938 bp in *Y. ruckeri* isolate number 1 & 2. **b)** Complete absence of *YhlB* gene in all the tested isolates. Lane L: 100 -1000 bp DNA Ladder. N: Negative control for P: Positive control *Y. ruckeri* strain have *YhlA* and *YhlB* genes.

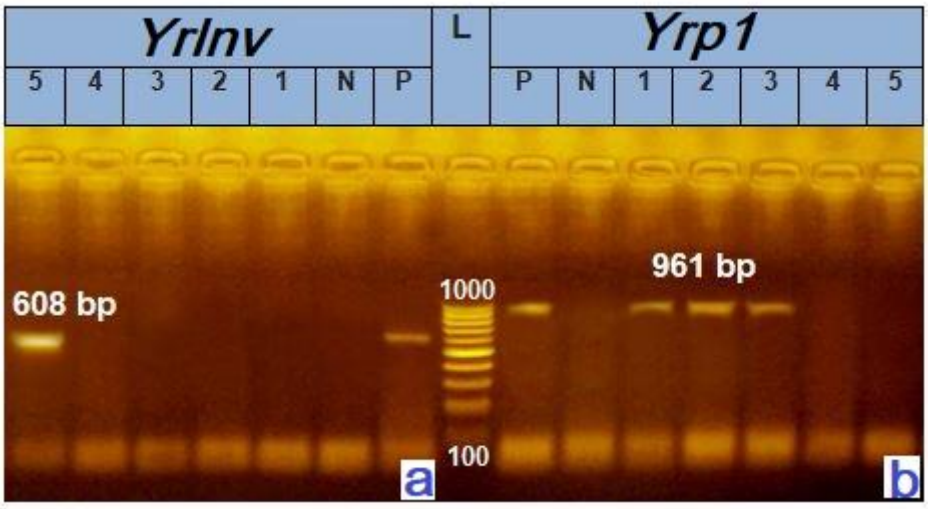

Fig. 3. a) Agarose gel electrophoresis of PCR product indicating the presence of *Yrlnv* gene at 608 bp in *Y. ruckeri* isolate number 5. **b)** Presence of *Yrp1* gene at 961 bp in *Y. ruckeri* isolate number 1 & 2 & 3. Lane L: 100 -1000 bp DNA Ladder. N: Negative control for P: Positive control *Y. ruckeri* strain has *Yrlnv* and *Yrp1* genes.

Results of pathogenicity test

Pathogenicity test indicated the presence of a direct correlation between isolate genotype (virulence gene content) and isolate pathogenicity. Isolate number (1) was the most virulent with LD_{50} equals 3.9×10^6, followed by isolate number (3 & 5) with one

virulence gene (7.77×10^6); while, isolate number (4) was the least virulent strain with LD_{50} equals 9.7×10^6; pathogenicity test results are demonstrated in the Table (7).

Table 7. Effect of virulence genes on the mortality rate of challenged *O. niloticus*

Group / Inoculum	Number of dead fish				
	١	٢	٣	٤	٥
$10^{٥}$	0	1	٠	1	٠
10^{6}	0	٣	٢	١	١
$10^{٧}$	0	٥	٣	3	٣
Total mortality	٠	9	٥	٥	٤
LD_{50}	٠	**3.9×10^6**	**7.77×10^6**	**7.77×10^6**	**9.7×10^6**

Group 1: Negative control, **Group 2:** (*YhlA*$^+$, *Yrp1*$^+$), **Group 3:** (*Yrp1*$^+$), **Group 4:** (*Yrlnv*$^+$), **Group 5:** negative for all virulence genes.

DISCUSSION

The Nile tilapia (*O. niloticus*) is considered the most important cultured fish species in Egyptian aquaculture **(Ali *et al*., 2021a)**, and the third most cultured fish worldwide **(FAO, 2021)**. The Egyptian production from cultured Nile tilapia reached about 1.1 million tons in 2019 **(GAFRD, 2021)** as the third main global producer of this fish species. This work has been conducted to identify the bacterial pathogens responsible for disease outbreaks affecting cultured tilapia in a trial to support the aquaculture sector by determining disease causes and studying bacterial pathogenicity.

In this work, 96 *Y. ruckeri* isolates have been identified from clinically diseased Nile tilapia, many researchers also have previously isolated *Y. ruckeri* from diseased *O. niloticus* **(Abd El-Ghani *et al*., (2001); Eissa *et al*., 2008; Aly *et al*., 2021)**. In this study, the total prevalence of *Y. ruckeri* was 64%, which was quite similar to **Abdel-Latif *et al*., (2014)** findings (77%) and also in the same study area (Kafrelsheikh).

Initial isolation using selective media indicated the presence of *Y. ruckeri* which grown on ribose ornithine desoxycholate agar as small yellow colonies due to ribose fermentation that was typical as mentioned by **Furones *et al*., (1993)**, it also produced a small bright pink colony surrounded by diffuse pink colouration on xylose lysine deoxycholate agar as typically reported by **Zorriehzahra *et al*., (2017)**. *Y. ruckeri* appear as bull eye shape medium size colonies as described by **Carson *et al*., (2019)**. Our results indicated that recovered isolates were motile Gram-negative rod shape bacilli as recorded by **(Ahmed, 2019)**. Biochemical identification confirmed the preliminary isolation results on selective media, *Y. ruckeri* isolates were capable of producing catalase, acetylmethyl carbinol, Beta-galactosidase, Lysine decarboxylase and Ornithine decarboxylase typically as mentioned by **Abdel-Latif *et al*., (2014)**, isolates also ferments glucose and utilize mannitol following **(Adel *et al*., 2020)** descriptions.

Pathogenicity test proved high virulence of the recovered *Y. ruckeri* isolates for *O. niloticus* and the LD_{50} was correlated to challenging strain genotype (virulence genes content) also the highest LD_{50} was recorded for *Y. ruckeri* devoid of any virulence genes. This result was logical and in harmony with **(Sha *et al*., 2002; Chacon *et al*., 2003; El-Bahar *et al*., 2019)** who reported that the virulence of Aeromonas isolates in challenged fish is directly related to the number of virulence genes content of challenging isolate. On the other side, other researchers as **Oliveira *et al*., (2012)** deny the presence of any relationship between microorganism pathogenicity and the genetic content of virulence genes. Virulence genes are responsible for the production of virulence factors including hemolytic, cytotoxic and lytic enzymes that destructs the host tissues and impair the normal physiological functions of membrane parries, facilitating the attachment, multiplication and invasion of the pathogen to the host body. In accordance to this research results, many researchers have described the presence of multiple virulence genes in *Y. ruckeri* isolates recovered from diseased fish **(Abdel-Latif *et al*., 2014; Fernandez *et al*., 2007; Wrobel *et al*., 2020)**. Protease 1 (*Yrp1*), peptidases (*YrpAB*), and pore-forming toxin (*YhlBA*) are the major virulence encoded genes in *Y. ruckeri*, these virulence factors are responsible for typical clinical symptoms of enteric redmouth disease in fish **(Wrobel *et al*., 2019)**. Protease 1 is responsible for the digestion of a wide range of cellular matrix and muscle proteins, such as myosin, actin and fibronectin **(Guijarro *et al*., 2018)**. The pore-forming toxin is responsible for cytolysis and hemolysis of fish RBCs and other cells **(Navais *et al*., 2014)**.

The clinical signs and post-mortem lesions that appeared on experimentally infected fish are nearly similar to those that appeared on naturally diseased fish mainly the extensive haemorrhages all over the external body surface and erythematic appearance of the lower lip together with congested internal organs and distended gall bladder, nearly similar gross signs were mentioned by **(Eissa *et al*., 2008; Abdel-Latif *et al*., 2014)** in infected *O. niloticus* with *Y. ruckeri*. These signs are mainly attributed to bacterial invasion with the expression of virulence genes and the production of different toxins and hydrolytic enzymes.

CONCLUSION

Y. ruckeri was the main bacterial pathogen responsible for the disease outbreak affecting the studied Nile tilapia farms at Kafrelsheikh. Three virulence genes were found in the recovered bacterial isolates, virulence genes are contributing to *Y. ruckeri* pathogenicity for *Oreochromis niloticus* as there was a direct correlation between virulence gene profile of *Y. ruckeri* isolate and isolate pathogenicity.

REFERENCES

Abd El Tawab, A.; El Hofy, F.; Ali, N.; Saad, W.; El-Mougy, E. and Mohammed, A. (2022): Antibiotic resistance genes in *Streptococcus iniae* isolated from diseased *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries, 26(2): 413-428. doi: 10.21608/ejabf.2022.230602.

Abd El-Ghani, M.; Marzouk, M. S.; Monam, H.; Abd El-Latief J. I. and Nada, H. S. (2001): *Yersinia ruckeri* as the causative agent of enteric redmouth disease (Erm) in Delta Nile fishes. Journal of Egyptian Veterinary Medicine Association, 61: 173-185.

Abdel-Latif, H. M. R.; Khalil, R. H.; Saad, T. T. and El-bably, R. H. (2014): Identification and Molecular Characterization of *Yersinia ruckeri* Isolated from mass mortalities of Cultured Nile tilapia at Kafr ElSheikh Governorate. Global Journal of Fisheries and Aquaculture Researches, 1 (2): 1 -17.

Aboyadak, I. M.; Sabry, N. M.; Ali, N. G. and El-Sayed, H. S. (2016): Isolation of *Staphylococcus epidermidis, Bacillus cereus* and *Pseudomonas stutzeri* from diseased European seabass *(Dicentrarchus Labrax)* for the first time in Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 20(4): 103-114.

Adel, M.; Ina-Salwany, M.Y.; Yeganeh S.; Karimi I. and Zafariian, A. (2020): An experimental study to evaluate pathogenicity of *Yersinia ruckeri* isolated from rainbow trout (*Oncorhynchus mykiss*) in Caspian trout (*Salmo caspius*). Iranian Journal of Fisheries Sciences, 19(5): 2409 - 2421, DOI: 10.22092/ijfs.2020.122408.

Ahmed, O. M. (2019): Bacteria Associated with Fresh-water Aquaculture Tilapia Fish (*Oreochromis niloticus*) in Suez, Egypt. Food Science and Nutrition Research, 2(1): 1 - 7.

Ali, N. G.; Ali, T. E. S.; Kamel, M. F.; Saleh, R.; Sherif, A. H. and Aboyadak, I. M. (2022): Eradication of *Livoneca redmanii* infestation in cultured Argyrosomus regius. Aquaculture, 558, 738373, https://doi.org/10.1016/j.aquaculture.2022.738373.

Ali, N. G.; Ali, T. E.; Aboyadak, I. M. and Elbakry, M. A. (2021a): Controlling *Pseudomonas aeruginosa* infection in *Oreochromis Niloticus* spawners by cefotaxime sodium. Aquaculture, 544, 737107, https://doi.org/10.1016/j.aquaculture.2021.737107.

Ali, N. G.; El-Nokrashy, A. M.; Gouda, M. Y. and Aboyadak, I. M. (2021b): Summer Mortality Syndrome affecting Cultured European seabass at Kafrelsheikh Province, Egypt. Frontiers in Marine Sciences, 8:717360, doi:10.3389/fmars.2021.717360.

Aly, S. M.; Fadel A. and Abdel-Rahman, H. G. (2021): Yersiniosis in Oreochromis niloticus; Prevalence, Antimicrobial Resistance and Immunological Response to Ascorbic Acid and Difloxacine. Egyptian Journal of Aquatic Biology and Fisheries, 25(1): 423 – 441.

Carson, J.; Wilson, T. and Douglas, M. (2019): Australian and New Zealand Standard Diagnostic Procedures (ANZSDP) for Yersiniosis in fish. https://www.agriculture.gov.au/sites/default/files/documents/anzsdp-yersiniosis_0.pdf.

CCAC (2005): Guidelines on the care and use of fish in research, teaching and testing. Canadian Council on Animal Care. 1510–130 Albert Street Ottawa on Canada, K1P 5G4, ISBN: 0–919087–43–4. https://ccac.ca/Documents/Standards/Guidelines/Fish.pdf

Chacon, M. R.; Figueras, M. J.; Castro-Escarpulli, G.; Soler, L. and Guarro, J. (2003): Distribution of virulence genes in clinical and environmental isolates of Aeromonas spp. Antonie Van Leeuwenhoek, 84: 269 – 278

Eissa, A. E.; Moustafa, M.; Abdelaziz, M. and Ezzeldeen, N. A. (2008): *Yersinia ruckeri* infection in cultured Nile tilapia, *Oreochromis niloticus*, at a semi-intensive fish farm in lower Egypt. African Journal of Aquatic Science, 33(3): 283–286.

El-Bahar, H. M.; Ali, N. G; Aboyadak, I. M.; Khalil, S. A.; and Ibrahim, M. S. (2019): Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in *Oreochromis Niloticus*. International Microbiology, 22, 479–490. doi:10.1007/s10123-019-00075-3.

FAO (2021): Fishery and aquaculture statistics 1976 – 2019, www.fao.org/fishery/statistics/software/fishstatj/en (accessed May. 02, 2022).

FAO (2022): The state of world fisheries and aquaculture 2022. https://www.fao.org/3/cc0461en/online/sofia/2022/executive-summary.html.

Fernandez, L.; Lopez, J. R.; Secades, P.; Menendez, A.; Marquez, I. and Guijarro, J. A. (2003): In vitro and in vivo studies of the *Yrp1* protease from *Yersinia ruckeri* and its role in protective immunity against enteric red mouth disease of salmonids. Applied and Environmental Microbiology, 69: 7328–7335.

Fernandez, L.; Prieto, M. and Guijarro, J. (2007): The iron and temperature regulated haemolysin *YhlA* is a virulence factor of *Yersinia ruckeri*. Microbiology, 153: 483–489.

Furones, M. D.; Gilpin, M. L. and Munn, C. B. (1993): Culture media for the differentiation of isolates of *Yersinia ruckeri*, based on detection of a virulence factor. Journal of Applied Bacteriology, 74: 360 – 366.

GAFRD (2021): Fish statistics yearbook 28th edition. General Authority for Fish Resources, Ministry of Agriculture Publications, Egypt.

Guijarro. J. A.; García-Torrico, A. I.; Cascales, D, and Méndez, J. (2018): The Infection Process of *Yersinia ruckeri*: Reviewing the Pieces of the Jigsaw Puzzle. Frontiers in Cellular and Infection Microbiology, 8:218, doi: 10.3389/fcimb.2018.00218.

Horne, M. T. and Barnes, A. C. (1999): Enteric redmouth disease (*Yersinia ruckeri*). In: Woo PTK, Bruno DW (eds) Fish diseases and disorders. Viral, bacterial and fungal infections. CABI Publishing, Wallingford, pp: 445–477

Imnadze. T.; Natradze, I.; Zhgenti, E.; Malania, L.; Abazashvili, N.; Sidamonidze, K.; Khmaladze, E.; Zakalashvili, M.; Imnadze, P.; Arner, R. J.; Motin, V, and Kosoy, M. (2020): Identification of a Novel *Yersinia enterocolitica* Strain from Bats in Association with a Bat Die-Off That Occurred in Georgia (Caucasus). Microorganisms. 2020 8(7):1000. doi: 10.3390/microorganisms8071000.

Kumar, G.; Menanteau-Ledouble, S.; Saleh, M., and El-Matbouli, M. (2015): *Yersinia ruckeri*, the causative agent of enteric redmouth disease in fish. Veterinary Microbiology, 46:103, doi: 10.1186/s13567-015-0238-4.

NACLAR (2004): National Advisory Committee for Laboratory Animals Research. 20 biopolis way #08-01 centros singapore 138668. https://www.nas.gov.sg/archivesonline/data/pdfdoc/AVA20050117001.pdf.

Navais, R.; Méndez, J.; Perez-Pascual, D.; Cascales, D. and Guijarro, J. (2014): The *yrpAB* operon of *Yersinia ruckeri*, encoding two putative U32 peptidases is involved in virulence and induced under microaerobic conditions. Virulence, *5*: 619–624.

Oliveira, S. T. L.; Veneroni-Gouveia, G. and Costa, M. M. (2012): Molecular characterization of virulence factors in *Aeromonas hydrophila* obtained from Fish. Pesquisa Veterinaria Brasileira, 32(8): 701 – 706.

Romalde, J. L. and Toranzo, A. E. (1993): Pathological activities of *Yersinia ruckeri*, the enteric redmouth (ERM) bacterium. FEMS Microbiology Letters, 112: 291–300.

Saganuwan, S. A. (2011): A modified arithmetical method of Reed and Muench for determination of median lethal dose (LD50). African Journal of Pharmacy and Pharmacology, 5(11): 1543 - 1546

Saleh, H.; Ali, N. G.; Aboyadak, I. M. and Nadia, S. A. (2019): Subcellular degenerative changes in hepatopancreas and posterior kidney of *Streptococcus iniae* infected Nile tilapia using Transmission Electron Microscope. Egyptian Journal of Aquatic Biology and Fisheries, 23(1): 305 - 316, https://ejabf.journals.ekb.eg/article_28099.html.

Saleh, N. E.; Helal, M.; Ali, N. G.; Abbas, E., and Abdel-Tawwab, M. (2021): Effects of using vital wheat gluten in practical diets on growth, intestinal histopathology, pro inflammation related gene expression, and resistance of White Seabream (*Diplodus sargus*) to *Staphylococcus epidermidis* infection. Aquaculture, 537: 736508.

Sha, J.; Kozlova, E. V. and Chopra, A. K. (2002): Role of various enterotoxins in Aeromonas hydrophila-induced gastroenteritis: generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity. Infection and Immunity, 70(4): 1924 – 1935.

Shaowu, L. Di. W.; Hongbai, L. and Tongyan, L. (2013): Isolation of *Yersinia ruckeri* strain H01 from farm-raised amur sturgeon *Acipenser schrencki* in China. Journal of Aquatic Animal Health, 25: 9–14.

Tobback, E.; Decostere, A.; Hermans, K.; Haesebrouck, F. and Chiers, K. (2007): *Yersinia ruckeri* infections in salmonid fish. Journal of Fish Diseases, 30: 257–268.

Tobback, E.; Decostere, A.; Hermans, K.; Ryckaert, J.; Duchateau, L.; Haesebrouck, F. and Chiers, K. (2009): Route of entry and tissue distribution of *Yersinia ruckeri* in experimentally infected rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 84: 219–228.

Tobback, E.; Hermans, K.; Decostere, A.; Broeck, W, V.; Haesebrouck, F.; and Chiers, K. (2010): Interactions of virulent and avirulent *Yersinia ruckeri* strains with isolated gill arches and intestinal explants of rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms, 90, 175–179, doi: 10.3354/dao02230.

Wrobel, A.; Leo, J. C. and Linke, D. (2019): Overcoming Fish Defences: The Virulence Factors of *Yersinia ruckeri*. Genes, 10, 700; doi:10.3390/genes10090700.

Wrobel, A.; Saragliadis, A.; Perez-Ortega, J.; Sittman, C.; Gottig, S.; Liskiewicz, K.; Spence, M.H.; Schneider, K.; Leo, J.C.; Arenas, J. and Linke, D. (2020): The inverse autotransporters of Yersinia ruckeri, YrInv and YrIlm, contribute to biofilm formation and virulence. Environmental Microbiology, 22(7): 2939–2955, doi:10.1111/1462-2920.15051.

Zorriehzahra, M. J.; Adel, M. and Torabi D. S. (2017): Enteric redmouth disease: Past, present and future: A review. Iranian Journal of Fisheries Sciences, 16(4):1135 -1156.