1st International Conference (Central Laboratory For Aquaculture Research In Cooperation With Worldfish), Cairo, Egypt, 20-22 November, 2017, Vol. 1. 62-78

GENE SEQUENCING AND PREVALENCE OF SOME *Edwardsiella Tarda* VIRULENCE GENES

Mohammed S.M Gado[1]; Refaat M.A Elgamal [2] and Marwa F. Abd El-Kader[2]

[1]*Fish Diseases and Management Dept., Fac. Vet. Med., Kafrelsheikh Univ.*

[2]*Fish Health and Diseases Dept., Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.*

Abstract

Edwardsiella tarda is a serious bacterial pathogen especially under farm conditions; causing Edwardsiellosis disease, leading to mass mortalities in various fish species. Molecular identification provide rapid and accurate diagnosis of disease causes also, Gene sequencing have a rapid identification of the bacterial mutation and adaptation processes which closely related to the virulence divergence and niche adaptation of the pathogen resulting in reliable vaccines and useful treatments. Fifty-three bacterial isolates formerly identified as *E.tarda* on the basis of their biochemical properties, isolated from two different naturally diseased fish species (*Clarias gariepinus and Oreochromis niloticus*) at Kafr El-sheikh governorate fish farms, preserved in TSA semisolid media were succumbed PCR identification using specific primers. Four virulence genes and its distribution in *E. tarda* positive isolates were detected as well as DNA sequencing analysis to the positive virulence genes compared to the gene bank database of the National Center for Biotechnology Information (NCBI). Out of fifty-three bacterial isolates; confirmed with PCR, only fifty isolates were identified as *E. tarda*; forty-six strains from *C. gariepinus* and four strains from *O. niloticus*, and all the examined positive isolates carried one or more of the tested virulence genes. *E. tarda* strains were divided into four groups with eight genotypes (genetic profiles), depending on the frequency of the virulence genes in the positive *E. tarda* strains. Gene sequencing revealed very high similarity (98-100%) between the detected virulence genes and the published sequences of *E. tarda* virulence genes in the gene bank database of (NCBI).

Key words: Edwardsiellosis *E. tarda* virulence genes. gene bank NCBI gene sequencing

INTRODUCTION

Edwardsiella tarda is a gram-negative, motile enteric pathogen of the family *Enterobacteriaceae*; causing hemorrhagic septicemia with high economic losses, the seriousness of *E. tarda* infection in its wide spread fish host range Carlos *et al*. (2012).

E. tarda have been incriminated as a common bacterial pathogen especially under culture conditions; causing edwardsiellosis or emphysematous putrefactive disease, leading to mass mortalities in various fish species Ewing *et al*. (1965)

Virulence genes screening is essential for determining the potential pathogenicity of *E. tarda* acting as biomarkers in diagnosis of pathogenic *E.tarda* Srinivasa Rao *et al*. (2001) and Srinivasa Rao *et al*. (2003).

The gyrB gene is a single-copy gene encodes the ATPase domain of DNA gyrase an enzyme essential for DNA replication Huang (1996), the gyrB gene was regarded as a suitable phylogenetic marker for the identification and classification of bacteria Yamamoto *et al*. (1999).

The host pathogenicity of *E. tarda* had a good correlation with its hemolysin rather than with the extracellular proteases Xiong and Lu (2001).

The gadB, fimA and other five virulence genes; orfA, citC, katB, mukF and ssrB were presented only in the virulent not in a virulent strains, in other words, are specific to the pathogenic *E. tarda,* Srinivasa Rao *et al*. (2003).

Mutation in gadB gene of *E. tarda* resulted in attenuation of the mutant in vivo and acid sensitivity in vitro indicating that the mutant was unable to survive or cause infection inside the host, Srinivasa Rao *et al*. (2003). Gene sequencing provide a rapid identification of the bacterial mutation and adaptation processes which closely related to the virulence divergence and niche adaptation of the pathogen resulting in rapid reliable and useful vaccines and treatments.

Indeed, the detection and sequencing of the virulence genes of *E. tarda* could predict the virulence potentials of the organism in the fish resulting in earlier implantation of the control measures, therefore, the present work was planned to: Identify the isolates by PCR using specific primers, detection of four virulence genes and its distribution in *E. tarda* positive isolates and DNA sequencing analysis to the positive virulence genes and matched to the gene bank database of the National Center for Biotechnology Information (NCBI).

MATERIALS AND METHODS

Bacterial isolates:

Fifty-three bacterial isolates formerly identified as *E.tarda* on the basis of their biochemical properties, isolates from two different naturally diseased species (*C. gariepinus and O.niloticus*) at Kafr El-sheikh governorate fish farms, preserved in TSA semisolid media were used.

Polymerase Chain Reaction (PCR):

After DNA extraction according to Shah *et al*. (2009) Application of PCR for identification of 16S rDNA as species specific for *E. tarda*, hlyA, gyrB, fimA and gadB genes as virulent factors of such organism was performed essentially by using Primers (Pharmacia Biotech, Ireland) as shown in the Table (1).

DNA Amplification reaction for 16S rDNA gene of *E.tarda*:

The amplification was performed on a Thermal Cycler using 25 µl of PCR mixture containing 3 µl of boiled cell lysate, 200 M of desoxynucleotide triphosphate (dNTP mixture), 1.4 U of Taq DNA polymerase, buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl and 3 mM MgCl2) and 1 µl of each primer. The PCR annealing temperatures tested ranged from 45 to 55°C. Both the intensity of the amplicons for each targeted DNA and the absence of nonspecific bands were considered in the selection of optimal PCR conditions. The cycling protocol was one cycle of 94 °C for 2 min, 35 cycles of 95 °C for 2 min, 45 °C for 1 min 30 sec, and 72 °C for 2 min, and a final elongation at 72 °C for 7 min.

PCR amplified products were analyzed by 1.5% of agarose gel, stained with ethidium bromide and visualized as well as captured on UV transilluminator, then compared with the marker DNA ladder (100 bp), Castro *et al*. (2014).

Table 1. Primer sequences of *E.tarda* used for PCR identification system.

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
16S rDNA (F)	**5′** AGAGTTTGATCCTGGCTCAG **′3**	450	Castro *et al*. (2014)
16S rDNA (R)	**5′** AAGGAGGTGATCCAGCCGCA **′3**		
hylA (F)	**5′** CCTTATAAATTACTCGCT **′3**	1106	Chen and Lai (1998)
hylA (R)	**5′** TTTGTGGAGTAACAGTTT **′3**		
gyrB (F)	**5′** GCATGGAGACCTTCAGCAAT **′3**	415	Wang *et al*. (2012)
gyrB (R)	**5′** GCGGAGATTTTGCTCTTCTT **′3**		
fimA (F)	**5′** CTGTGAGTGGTCAGGCAAGC **′3**	441	Wang *et al*. (2012)
fimA (R)	**5′** TAACCGTGTTGGCGTAAGAGC **′3**		
gadB (F)	**5′** ATTTGGATTCCCGCTTTGGT **′3**	583	Wang *et al*. (2012)
gadB (R)	**5′** GCACGACGCCGATGGTGTTC **′3**		

Amplification reaction for hylA gene:

The PCR for amplification of hylA gene was performed in a total reaction volume of 25 µl using solution containing 100 pmol of each primer, 400 µM dNTP and buffer (10 mM TrisHCl pH 8.8; 1.5 mM MgCl2; 50 mM KCl; 0.1% triton-X-100) in an Eppendorff tube. PCR was started with an initial denaturation step of 5 min at 94 °C followed by 35 thermocycles with each cycle consisting of denaturing at 94 °C for 1 min, annealing at a range of 50 to 55 °C for 1 min, and extending at 72 °C for 1 min. Finally, 5 µl of each amplicon was electrophoresed in 1 % agrose gel) and visualized under UV transilluminator, Chen and Lai (1998).

Multiplex PCR for gyrB, fimA and gadB genes:

The targeted genes of E. tarda strains were gyrB (gyrase B), fimA (fimbrial operon) and gadB (glutamate decarboxylase). Specific primers were

used to amplify the three virulence genes. To amplify the genes, 25 µl of reaction mixture was made containing 20ng of template DNA, 20 pM of primers, 160 µM of dNTP mix, 1.25 U Taq polymerase, 1×Taq buffer, and 0.5 mM MgCl2. The three genes were amplified individually using the specific primers with 32 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR product was visualized on 1.5% agarose gels stained with ethidium bromide, and visualized under UV transilluminator, then compared with the marker DNA ladder (100 bp), Wang *et al*. (2012).

Gene sequences and nucleotide sequence alignment of the four virulence genes:

PCR products of four strains of *E.tarda* (presented all groups), were succumbed to the gene sequencing using the four genes namely; haemolysin gene(hlyA), glutamate decarboxylase gene(gadB), major fimbrial subunit protein(fim A) and gyrase B gene (gyrB), according to Wang *et al*. (2009)

First, the PCR products were purified using the fast gene gel PCR extraction kit to remove unused primers and nucleotides from the template DNA by adding 0.5 µL EXOSAP-IT and 4.5 µL TE buffer for each reaction. Reaction mix was incubated at 37ºC for 20 min followed by incubation at 80ºC for 20 min, Fig.(). Second, the positive virulence genes were subjected to the DNA sequencing analysis for confirmation and compared to the published sequences in the gene bank database of the national center for biotechnology information center (NCBI). Sequencing PCR reactions were performed in a 10 µL reaction volume containing Template DNA 1 µL, 5x Buffer 1.75 µL, Primer (sense and antisense) 1 µL, Master Mix 0.5 µL, and RNase free water 5.75 µL. PCR was carried out using an Ex-taq PCR amplification kit. The PCR cycling program consisted of 29 cycles of 45 sec at 95ºC, 45 sec at 60ºC, and 1 min at 72ºC, with an additional step of 2 min at 72ºC. Sequencing of the PCR products was carried out using an ABI PRISM 3100 automated sequencer. Sequencer TM Software (Version 4.2.2, Gene Codes Corporation, MI, USA) was used for sequence data analyses.

RESULTS AND DISCUSSION:

Molecular characterization of the bacterial isolates:

Out of fifty-three bacterial isolates formerly identified as *E.tarda* on the basis of their biochemical properties; only fifty isolates were identified as *E. tarda;* forty-six strains from *C. gariepinus* and four strains from *O. niloticus*, when succumbed to the molecular characterization of DNA using specific primers for *E.tarda*, revealing the presence of a common band at 450 bp, which were closely similar to that reported by Castro *et al*. (2014) denoting, the accuracy of PCR results, rather than that of the biochemical reactions , Fig. (1).

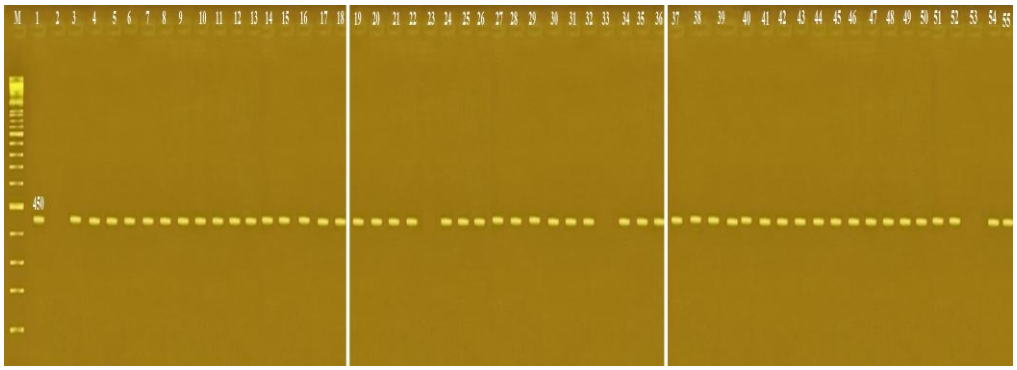


Fig.1. Agarose gel electrophoresis of PCR of 16S rDNA (450bp) for characterization of *E. tarda isolates* .

- Lane M: 100 bp ladder as molecular size DNA marker.
- Lane 1 control positive for *Edwardesiella tarda* .
- Lane 2 control negative for *Edwardesiella tard*.
- Lane 3-22, 24-32, 34-52 and 54-55 positive for *Edwardesiella tarda.*
- Lane 23,33and 53 negative for *Edwardesiella tarda*.

Molecular characterization of *E. tarda* virulence genes:

PCR revealed that all the examined positive isolates carried one or more of the tested virulence genes, Fig. (2 and 3).

The prevalence of *E.tarda* virulence genes were as follow; gyrase B gene (gyrB) was the most frequent virulence gene; detected in 43 strains, haemolysin gene(hlyA) detected in 40 strains, major fimbrial subunit protein

(fimA) detected in 26 strains and glutamate decarboxylase gene(gadB) detected in 20 strains, Fig. (4 and 5).

On the basis of PCR results; *E.tarda* strains were divided into four groups with eight genotypes (genetic profiles), depending on the frequency of the virulence genes in the positive *E.tarda* strains; one genotype (hyl A+ gad + fim + gyr +) carrying 4 virulence genes; 14 isolates (28%), three genotypes(hyl A+ gad - fim + gyr +) , (hyl A+ gad + fim - gyr +) and (hyl A- gad + fim + gyr +) carrying 3 virulence genes; 11 isolates (22%), two genotypes (hyl A+ gad - fim - gyr +) , (hyl A- gad- fim + gyr +) carrying 2 virulence genes; 15 isolates (30%) and two genotypes (hyl A+ gad - fim - gyr -) , (hyl A- gad - fim - gyr +) carrying 1 virulence gene; 10 isolates (20%), Fig. (6 and 7).

Delmas *et al.* (2006) developed a species-specific method to detect *E.tarda* on the basis of PCR amplification of the gyrB gene, indeed, Lan *et al*. (2008) used the gyrB gene as a taxonomic marker.

Ibrahem *et al.* (2011) detected *E.tarda* as early as 24 h post infection using the oligomers of hemolysin gene as primers for PCR assay. The hlyA gene is an Extracellular hole-forming and is not regulated by iron Chen et al., (1996). The haemolysin is a noted virulence element and is widely distributed in pathogenic isolates of *E. tarda*, Wang *et al*. (2009) and correlated with the mortality of *E.tarda* infected fish Shen and Chen, (2005).

The fimbriae help in adhesion of the bacteria to host cells, Duguid and Old (1980) and Mohanty and Sahoo (2007), adherence is the primary step for invading the host and causing infection, hence any mutation in genes involved in the attachment would affect the infective ability of the bacteria which led to attenuation of this mutant, Srinivasa Rao *et al*. (2003). The fimbrial proteins induced more significantly under higher sodium chloride concentrations which play an important role in the virulence of *E. tarda* in marine environment, Yasunobu *et al*. (2006).

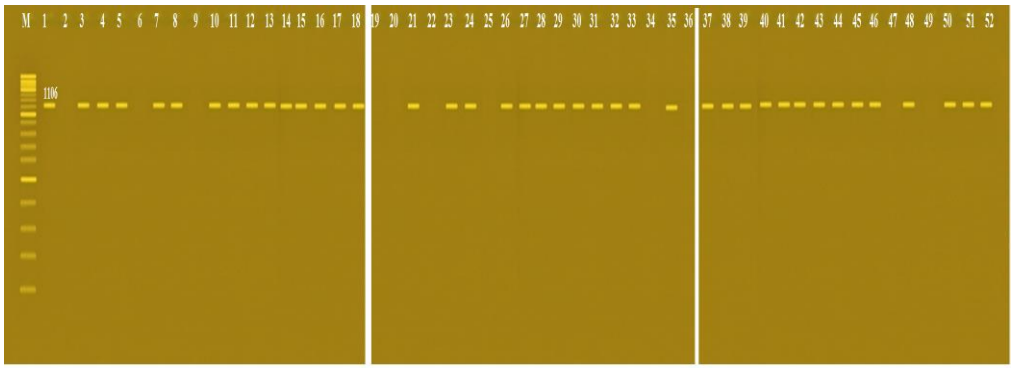


Fig 2. Agarose gel electrophoresis of PCR of hlyA gene (1106 bp) virulence factor of *E. tarda.*

- Lane M: 100 bp ladder as molecular size DNA marker.
- Lane 1 control positive for hlyA gene.
- Lane 2 control negative for hlyA gene.
- Lane 3-5,7,8,10-18,21,23-24,26-33,35,37-46,48 and 50-52 positive for hlyA gene .
- Lane 6,9, 19,20,22,25,34,36,47and 49 negative for hlyA gene .

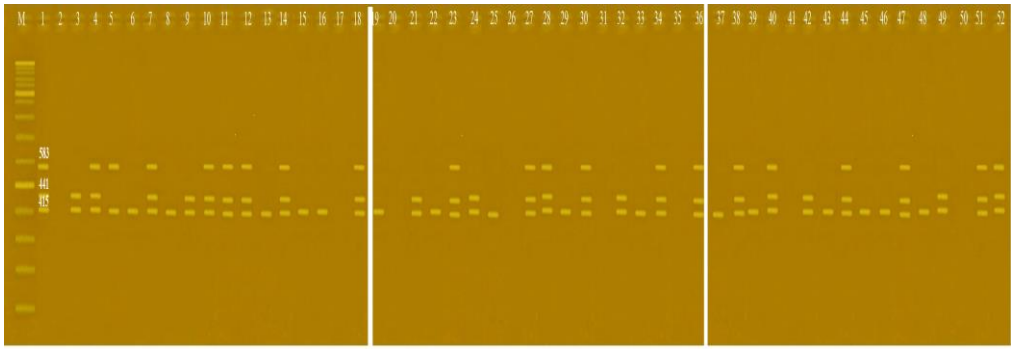


Fig 3. Agarose gel electrophoresis of multiplex PCR of gyrB (415bp) ، fimA(441) and gadB (583) virulence genes of *E. tarda.*

- Lane M: 100 bp ladder as molecular size DNA marker.
- Lane 1: Control positive of *E. tarda* for gyrB, fimA and gadB genes.
- Lane 2: Control negative for gyrB, fimA and gadB genes.
- Lanes 4, 7, 10, 11, 12, 14, 18, 23, 27, 28, 30, 34, 36, 38, 40, 44, 47, 51 and 52: Positive *E. tarda* strains for gyrB, fimA and gadB genes.
- Lanes3, 9, 21, 24, 32, 42 and 49: Positive *E. tarda* strains for gyrB and fimA genes.
- Lane 5: Positive *E. tarda* strains for gyrB and gadB genes.
- Lanes6, 8, 13, 15, 16, 19, 22, 25, 29, 33, 37, 39, 43, 45, 46 and 48: Positive *E. tarda* strains for gyrB gene.
- Lanes 17, 20, 26, 31, 35, 41 and 50: negative for gyrB, fimA and gadB genes.

The glutamate decarboxylase gene (gad B) provide resistance to bacteria towards phagocytes- mediated killing inside the host, the Gad system neutralizes acidity and enhances the survival under extreme acid conditions, the glutamate-dependant acid resistance system requires the glutamate decarboxylase gene for protection under acidic conditions. Mutation in gad B gene of *E.tarda* resulted in attenuation of the mutant in vivo and acid sensitivity in vitro indicating that the mutant was unable to survive and cause infection inside the host, Srinivasa Rao *et al*. (2003). Bacteria fight against serum- and phagocyte- mediated killing by gad B.

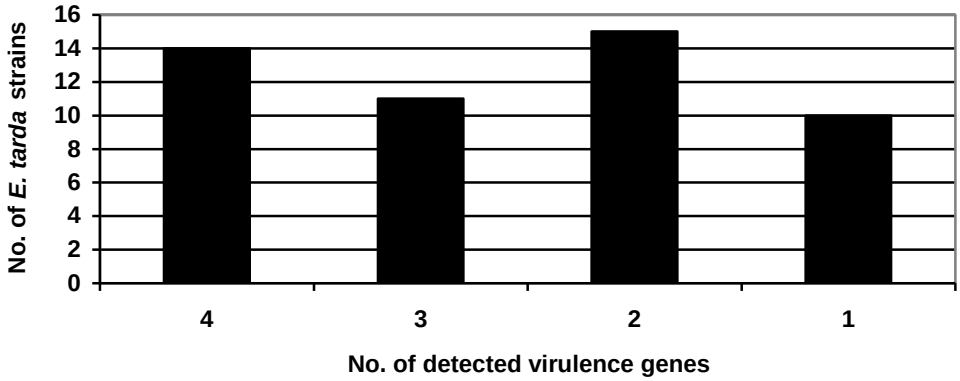


Fig. 4. Detection of four virulence genes in *E.tarda* isolates.

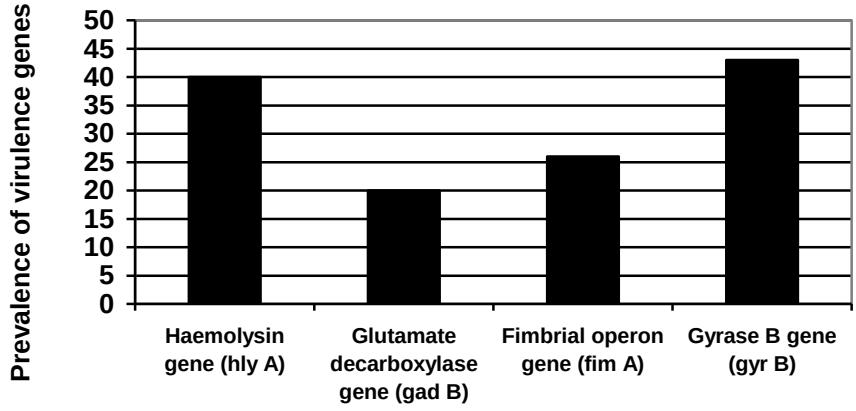


Fig. 5. Frequency of virulence genes in *E.tarda* strains.

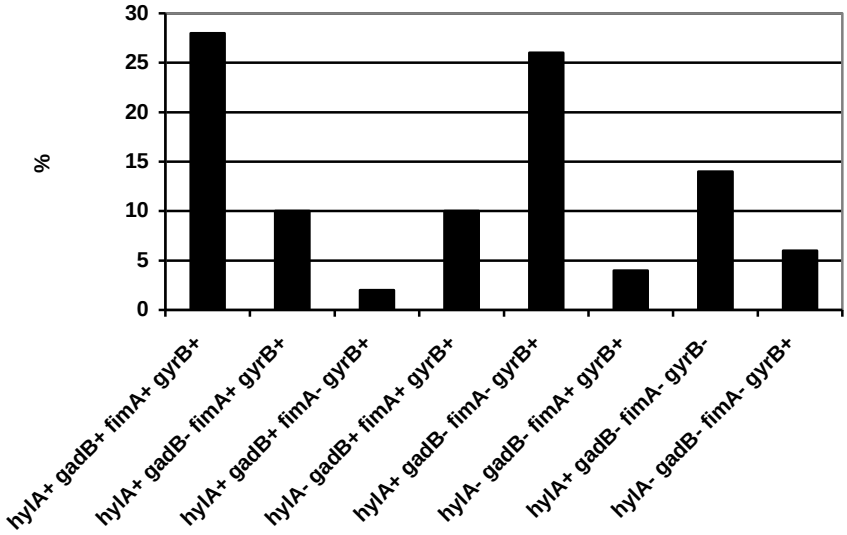


Fig. 6. virulence gene profiles of *E.tarda* strains.

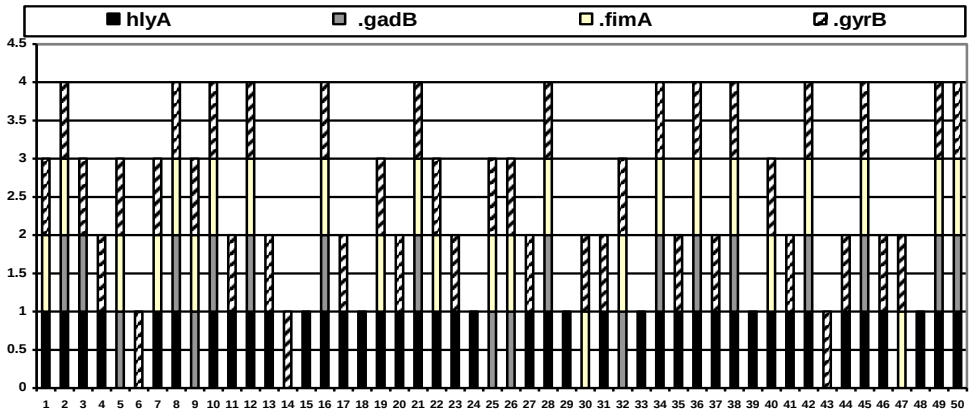


Fig. 7. Distribution of four virulence genes in fifty *E.tarda* strains.

Gene sequences and nucleotide sequence alignment of the four virulence genes:

The results revealed that; the nucleotide sequences of the four virulence genes of the four strains showed no differences and no mutations, Fig.(8, 9, 11, 13 and 15); revealed very high similarity (98-100%) between the detected

virulence genes and the published sequences of *E.tarda* virulence genes in the gene bank database of the national center for biotechnology information (NCBI).

The nucleotide sequences alignment of haemolysin gene(hlyA) showed 99% identity, Fig. (10), glutamate decarboxylase gene(gadB) showed 99% identity, Fig.(12), major fimbrial subunit protein(fim A) showed 100% identity, Fig. (14) and gyrase B gene (gyrB) showed 98% identity, Fig. (16),

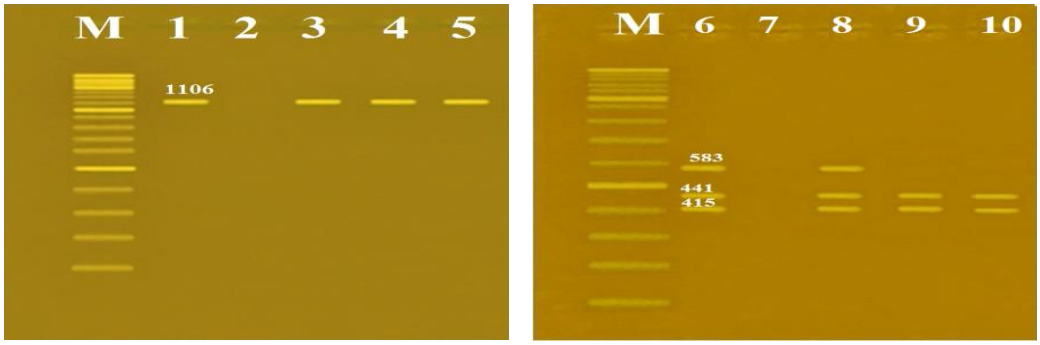


Fig. 8. Agarose gel electrophoresis of purified PCR products of different four genes of the four *E.tarda* strains.

- Lane M: 100 bp ladder as molecular size DNA marker.
- Lane 1 control positive for hlyA gene (1106bp).
- Lane 2 control negative for hlyA gene.
- Lane 3 strain No.8.of *E.tarda* ; positive for hlyA gene (1106bp).
- Lane 4: strain No.1.of *E.tarda*; positive for hlyA gene (1106bp) .
- Lane 5: strain No.15.of *E.tarda*; positive for hlyA gene (1106bp).
- Lane 6: Control positive of *E. tarda* for gyrB (415bp), fimA (441bp) and gadB (583bp) genes.
- Lane 7: Control negative for gyrB, fimA and gadB genes.
- Lane 8: strain No.8.of *E.tarda*; positive for gyrB(415bp), fimA (441bp) and gadB (583bp) genes.
- Lane 9: strain No.1.of *E.tarda*; positive for gyrB(415bp) and fimA (441bp) genes.
- Lane 10: strain No.30.of *E.tarda*; positive for gyrB(415bp) and fimA (441bp) genes.

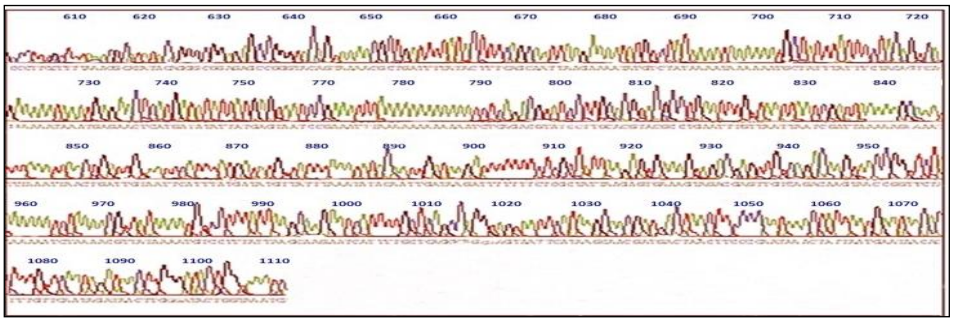


Fig. 9. Nucleotide sequence of the hlyA gene of *E. tarda.*

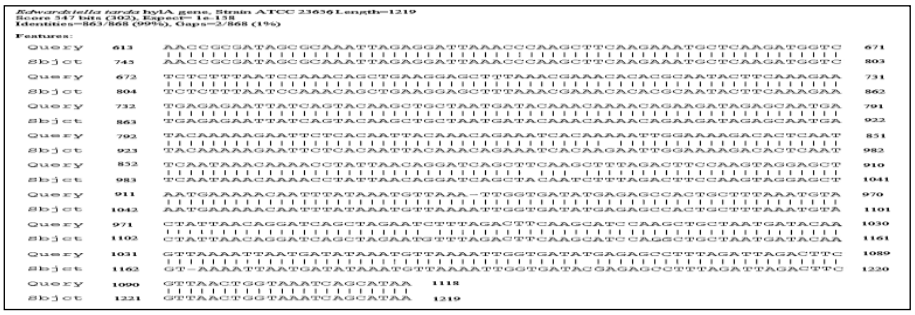


Fig. 10. Nucleotide sequence alignment of the hlyA gene of *E.tarda* showing 99% identity.

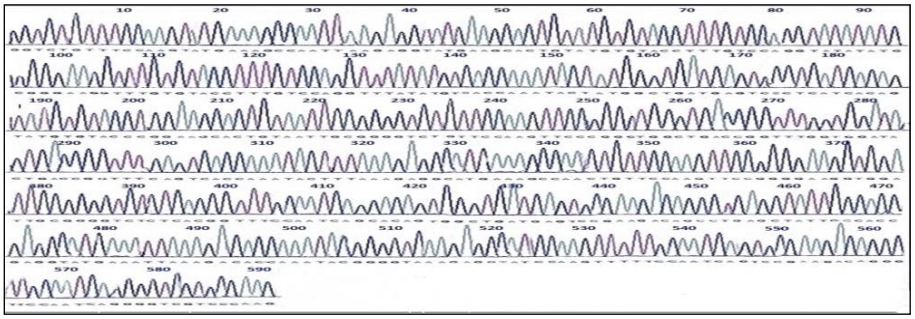


Fig. 11. Nucleotide sequence of the gad B gene of *E. tarda*.

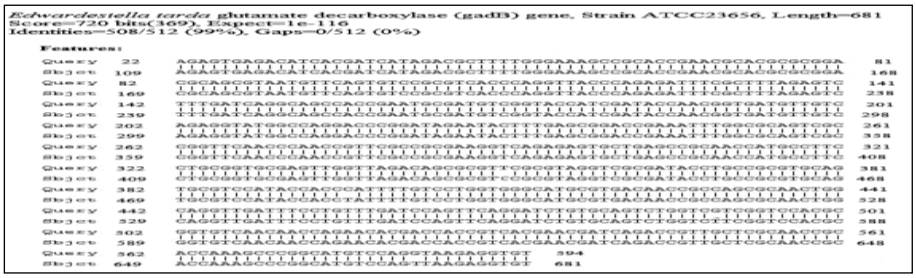


Fig. 12. Nucleotide sequence alignment of the gad B gene of *E.tarda* showing 99 % identity.

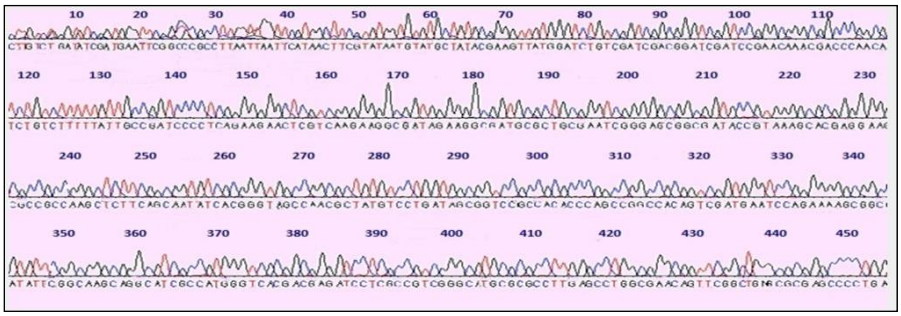


Fig. 13. Nucleotide sequence of the fim A gene of *E.tarda*.

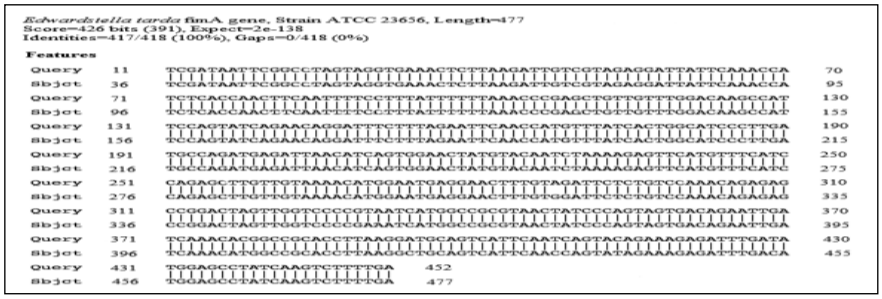


Fig. 14. Nucleotide sequence alignment of the fim A gene of *E.tarda* showing 100 % identity.

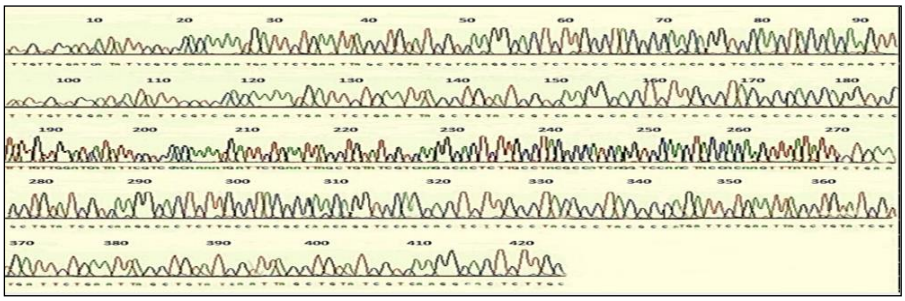
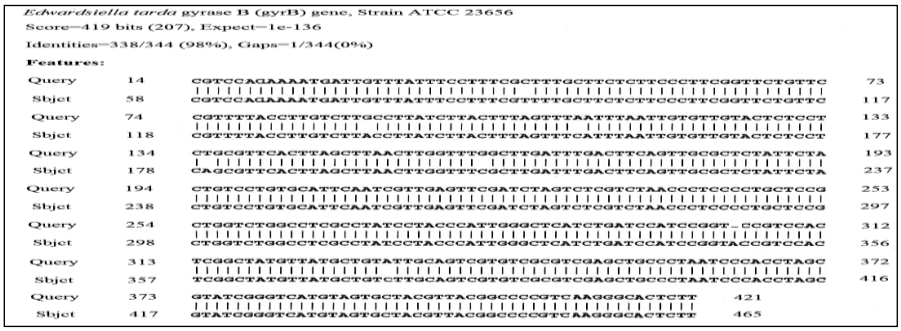


Fig. 15. Nucleotide sequence of the gyr B gene of *E. tarda.*



Edwardsiella tarda gyrase B (gyrB) gene, Strain ATCC 23656
Score=419 bits (207), Expect=1e-136
Identities=338/344 (98%), Gaps=1/344(0%)
Features:

Query	14	CGTCCAGAAAATGATTGTTTATTTCCTTTCGCTTTGCTTCTCTTCCCTTCGGTTCTGTTC	73
Sbjct	58	CGTCCAGAAAATGATTGTTTATTTCCTTTCGTTTGCTTCTCTTCCCTTCGGTTCTGTTC	117
Query	74	CGTTTTACCTTGTCTTGCCTTATCTTACTTTAGTTTAATTTAATTGTGTTTGTACTCTCCT	133
Sbjct	118	CGTTTTACCTTGTCTTACCTTATCTTTACTTTAGTTTCATTTAAATTGTGTTTGTACTCTCCT	177
Query	134	CTGCGTTCACTTAGCTTAACTTGGTTTGGCTTGATTTGACTTCAGTTGCGCTCTATTCTA	193
Sbjct	178	CAGCGTTCACTTAGCTTAACTTGGTTTCGCTTGATTTGACTTCAGTTGCGCTCTATTCTA	237
Query	194	CTGTCCTGTGCATTCAATCGTTGAGTTCGATCTAGTCTCGTCTAACCCTCCCCTGCTCCG	253
Sbjct	238	CTGTCCTGTGCATTCAATCGTTGAGTTCGATCTAGTCTCGTCTAACCCTCCCCTGCTCCG	297
Query	254	CTGGTCTGGCCTCGCCTATCCTACCCATTGGGCTCATCTGATCCATCCGGT-CCGTCCAC	312
Sbjct	298	CTGGTCTGGCCTCGCCTATCCTACCCATTGGGCTCATCTGATCCATCCGGTACCGTCCAC	356
Query	313	TCGGCTATGTTATGCTGTATTGCAGTCGTGTCGCGTCGAGCTGCCCTAATCCCACCTAGC	372
Sbjct	357	TCGGCTATGTTATGCTGTCTTGCAGTCGTGTCGCGTCGAGCTGCCCTAATCCCACCTAGC	416
Query	373	GTATCGGGTCATGTAGTGCTACGTTACGGCCCCGTCAAGGGCACTCTT 421	
Sbjct	417	GTATCGGGTCATGTAGTGCTACGTTACGGCCCCGTCAAGGGCACTCTT 465	

Fig. 16. Nucleotide sequence alignment of the gyr B gene of *E.tarda* showing 98 % identity.

CONCLUSION

PCR, simple reliable accurate techniques may be of a great help in fish disease diagnosis, which in turn anticipate in rapid interference and treatment as well, reducing the drastic economic losses in aquaculture.

There were differences in the presence or absence of a number of virulence genes among *E. tarda* strains, suggesting that the virulence profile may be useful tool for prediction of pathogenic strains.

Detection and sequencing of the virulence genes of *E.tarda* could predict the virulence potentials of the organism in the fish resulting in earlier implantation of the control measures

The identified virulence genes especially gadB and fimA, could be used as prescreening biomarkers for detection of pathogenic *E. tarda*.

REFERENCES

Carlos, A.I.; G. Marlly; M.T. Victor and W.F. Hugh, 2012. Novel brain lesions caused by *Edwardsiella tarda* in a red tilapia (*Oreochromis spp*.). J. Vet. Diag. Invest., 24 (2): 446-449.

Castro, N.; A. Toranzo and B. Magarinos, 2014. A multiplex PCR for the simultaneous detection of *Tenacibaculum maritimum and Edwardsiella tarda* in aquaculture. Inter. Microbiol., (17): 111-117.

Chen, J. and S. Lai, 1998. PCR for direct detection of *Edwardsiella tard* from infected fish and environmental water by application of the hemolysin gene. Zoological Studies., 37(3): 169-176.

Delmas, J.; F. Breysse; G. Devulder; J.P. Flandrois and M. Chomarat, 2006. Rapid identification of *Enterobacteriaceae* by sequencing DNA gyrase subunit B encoding gene. Diagn. Microbiol. Infect. Dis., 55: 263–268.

Duguid, J.P. and D.C. Old, 1980. Adhesive properities of *Enterobacteriaceae*. In E. H. Beachey (ed) Bacterial adherence receptors and recognition. Chapman and Hall, London, United Kingdom. 6: 185-217.

Ewing, W.H.; A.C. McWhorter; M.R. Escobar and A.H. Lubin, 1965. *Edwardsiella*, a new genus of *Enterobacteriaceae* based on a new species; Int. Bull. Bacteriol. Nomencl. Taxon., 15: 33–38.

Huang, W.M., 1996. Bacterial diversity based on type II DNA topoisomerase genes. Annu. Rev. Genet., 30: 79–107.

Ibrahem, M.D.; I.B. Shaheed; H. Abo El-Yazeed and H. Korani, 2011. Assessment of the susceptibility of poly culture reared African catfish and Nile tilapia to *Edwardsiella tarda*. J. American Science., 7 (3): 779-786.

Lan, J.; X.H. Zhang; Y. Wang; J. Chen and Y. Han, 2008. Isolation of an unusual strain of *Edwardsiella tarda* from turbot and establish a PCR detection technique with the gyrB gene. J. Appl. Microbiol., 105 (3): 644-651.

Mohanty, B. R. and P. K. Sahoo, 2007. Edwardsiellosis in fish: a brief review. J. Biosci., 32 (7): 1331-1344.

Shah, D.; S. Shringi; T. Besser and D. Call, 2009. Molecular detection of food borne pathogens, Boca Raton: CRC Press, In Liu, D. (ed). Taylor & Francis group, Florida, USA, Pp. 369-389.

Shen, Y-R. and J-D. Chen, 2005. Expression of virulent genes of *Edwardsiella tarda* correlated with mortality of diseased-fish infection. J. Fish. Soc. Taiwan., 32: 80–81.

Srinivasa Rao, P.S.; T.M. Lim and K.Y. Leung, 2001. Opsonized virulent *Edwardsiella tarda* strains are able to adhere to and survive and replicate within fish phagocytes but fail to stimulate reactive oxygen intermediates; Infect. Immun., 69: 5689–5697.

Srinivasa Rao, P.S.; T.M. Lim and K.Y. Leung, 2003. Functional genomics approach to the identification of virulent genes involved in *Edwardsiella tarda* pathogenesis. Infect. Immun., 71: 1343–1350.

Wang, Q.; M. Yang; J. Xiao; H. Wu; X. Wang; Y. Lv; L. Xu; H. Zheng; S. Wang; G. Zhao; Q. Liu and Y. Zhang, 2009. Genome sequence of the versatile fish pathogen *Edwardsiella tarda* provides insights into its adaptation to broad host ranges and intracellular niches. J. PloS One 4(10):e 7646.

Wang, X.; M. Yan; Q. Wang; L. Ding and F. Li, 2012. Identification of *Edwardsiella tarda* isolated from duck and virulence genes detection. African J. Microbiol. Res., 6 (23): 4970-4975.

Xiong, Q and C. Lu, 2001. Detection of pathogenic *Edwarsiella tarda*. Wei. Sheng. Wu. Xue. Bao., 41(6): 736-740.

Yamamoto, S.; P.J. Bouvet and S. Harayama, 1999. Phylogenetic structures of the genus Acinetobacter based on gyrB sequences: comparison with the grouping by DNA–DNA hybridization. Int. J. Syst. Bacteriol., 49: 87–95.

Yasunobu, H.; Y. Arikawa; K. Fumtsuka-Uozumi; M. Domo; T. Iida; M.M. Mahmoud; J. Okuda and T. Nakai, 2006. Induction of hemagglutination

activity of *Edwardsiella tarda* by sodium chloride. Fish Pathol., 41: 29–34.

التسلسل الجيني ومدى انتشار بعض جينات الضراوة في الإدواردسيلا تاردا

محمد سعيد محمد جادو*، رفعت محمد علي الجمل،**
مروة فتحى عبد الغفار عبد القادر**

** قسم امراض الأسماك ورعايتها–كلية الطب البيطري جامعة كفرالشيخ.*
***قسم بحوث أمراض وصحة الاسماك، المعمل المركزى لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعة، مصر.*

الملخص العربى

تكمن خطورة مرض الادواردسيلوزيس والذي يسببه ميكروب الادواردسيلا تاردا أنه يؤدي الى معدلات نفوق عاليه في مختلف أنواع الأسماك. تم عزل ٥٣ معزوله بكتريه من أسماك قرامبط وبلطي نيلي مريضه طبيعيا،من مزارع الأسماك في محافظة كفرالشيخ ، وتم تصنيفها حسب خواصها البيوكيميائيه الى ميكروب الادواردسيلا تاردا والتي أخضعت للتعريف بالتوصيف الجزيئي. تم تحديد أربعه جينات ضراوة وتوزيعها في معزولات الادواردسيلا تاردا، كما تم اجراء تحليل التسلسل الجيني لها، ومن ثم تمت مقارنتها بما هو مسجل في بنك الجينات. فقط خمسون من جمله ثلاثه وخمسون معزوله، صنفت على أنها ادواردسيلا تاردا، وتوزيعهم كالتالي؛ ست واربعون عتره بكتيريه من أسماك القرامبط، فقط أربع عترات بكتيريه من أسماك البلطي النيلي، وتمخض ذلك عن توفر واحد أو أكثر من جينات الضراوه في المعزولات الاجابيه. بناء على ذلك، فقد تم تقسيم عترات الادواردسيلا تاردا الى اربع مجموعات لها ثمانيه أنواع جينيه طبقا لعدد التتكرارات لجينات الضراوة في معزولات الادواردسيلا تاردا، وكذلك التسلسل الجيني أوضح أكثر من ٩٨% تماثل مابين جينات الضراوة المختبره وتلك المسجله ببنك الجينات.