Received: 15 July 2022, Accepted: 23 September 2022

Original Research

Journal of Advanced Veterinary Research
(2022) Volume 12, Issue 5, 535-539

Detection of Virulence Genes in *Aeromonas hydrophila* Isolated from Poultry Meat Using PCR Technique

Sohaila F.H. El-Hawary*

Department of Biology, Faculty of Science, Jazan University, Jazan 45142, Saudi Arabia.

***Correspondence**
Sohaila F.H. El-Hawary
Department of Biology, Faculty of Science, Jazan University, Jazan 45142, Saudi Arabia.
E-mail address: selhawary@jazanu.edu.sa

Abstract

The present study was conducted to investigate the presence of *Aeromonas* species especially*A. hydrophila* and to detect the presence of the aerolysin (*aerA*) and haemolysin (*hly*) genes in its isolates derived from a total of 80 poultry meat samples including 20 of each of breasts, drumsticks,thighs and wings. The samples were randomly purchased from some local retail shops in Assiut city, Egypt. *Aeromonas* spp. were isolated from 48(60%) of the samples analyzed, including 11(55%) of chicken breast, 12 (60%) of chicken drumsticks, 10 (50%) of chicken thigh and 15 (75%) of chicken wing. A total of 48 strains were isolated belonging to six species: *A. hydrophila* 14 (17.5%), *A. caviae* 5 (6.25%), *A. jandaei* 7(8.75%), *A. trota* 1 (1.25%), *A. schubertii* 6 (7.5%) and *A. allosacharophila* 15 (18.75%). All the recovered *A. hydrophila* organisms were confirmed by PCR assay for the presence of 16S rRNA gene and 9 strains of the tested isolates harboured this gene. Whereas the *aerA* and *hly* virulence genes were present in eight and five of *A. hydrophila* strains out of nine isolates tested, respectively. Strict hygiene measure should be taken to minimize the contamination of poultry meat with *Aeromonas* spp.

KEYWORDS
Aeromonas spp., chicken meat, aerolysin and haemolysin genes.

INTRODUCTION

Chickens are hosts to many microorganisms found on their skin, feathers, and digestive tract. These microorganisms can possibly contaminate meat during processing chain, such as slaughtering, defeathering, evisceration, and storage (Bhaisare *et al*., 2014). Moreover, when processed in unhygienic conditions, other microorganisms present in the processing environment, equipment, and processors hands/apron can contaminate the final meat product (Gideon *et al*., 2017).

Aeromonas bacteria are considered major important pathogen and opportunistic pathogens in both immune competent and immune depressed persons (Janda and Abbott, 2010). In human *Aeromonas* spp. are the causes of both intestinal and extra-intestinal infections (Khajanchi *et al*., 2010). Five *Aeromonas* spp. represented as *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas veronii*, *Aeromonas jandaei*, and *Aeromonas schubertii* are commonly associated with human intestinal infections (Janda and Abbott, 2010).

It was reviewed that aerolysin is a virulence factor take part in the pathogenesis of *A. hydrophila* (Praveen *et al*., 2016). In addition, there is good proof that *Aeromonas* species are able to produce several virulence factors at both maximum growth temperature and at refrigerated temperatures (Merino *et al*., 1995). Which may be important to raw food products which are stored at refrigeration and have a long validity period at this temperature. Accordingly, *Aeromonas* species should be continuously monitored in food products as they may be a source of food borne infection (Dallal *et al*., 2012). Chicken products have an important role in the transmission of this pathogen to humans (Praveen *et al*., 2016).

Aeromonas spp. can grow and produce toxins in refrigerated conditions indicating that refrigeration can not be effective enough to control the pathogens. As *Aeromonas* spp. are frequently isolated from food due to their psycrotrophy and the existence of the pathogens in water, faeces of humans and animals, the risks of foodborne infections are increased (Kirov, 1993).

Uma *et al*. (2010) mentioned that *Aeromonas hydrophila* is an opportunistic and primary bacterial pathogen of a variety of aquatic, terrestrial animals, and humans. Its pathogenicity has been associated with virulence factors such as aerolysin and hemolysin. The detection of the presence of such virulence factors is a better indicator of the potential health risk.

Dallal *et al*. (2012) isolated Aeromonads from 80 (32%) of the samples analyzed, including 53 (57.6%) of chicken and 27 (17%) of minced meat. The isolation rate in chicken was significantly higher than minced meat ($p<0.001$). The highest contamination was found in chicken with *Aeromonas caviae*.

There is scarce of the published information and limited studies about the prevalence of *Aeromonas* spp. especially *A. hy-*

ISSN: 2090-6277/2090-6269/

drophila and their virulence in poultry meat in Assiut city, Egypt.

Keeping in view the importance of these pathogens, therefore, the main objective of this study was to genetically re-identify previously biochemically identified *A. hydrophila* isolated from poultry meat intended for human consumption and its capability of producing virulence genes of aerolysin and haemolysin were also studied which play a key role in their pathogenicity.

MATERIALS AND METHODS

Collection of samples

In this study, a total of 80 samples of chicken parts represented by 20 from each of breasts, drumsticks, thighs, and wings were purchased from randomly selected local retail shops in Assiut province, Egypt during the period from June to September of 2018. Samples were transferred immediately in the purchased consumer bags to be examined in the Animal Health Research Institute, Assiut laboratory.

Isolation of Aeromonas spp. (Agarwal et al., 2003)

Enrichment

Twenty five grams of each piece of chicken samples were transferred aseptically and homogenized with 225 ml of 0.1 % alkaline peptone water (pH 8.4 – 8.6; Oxoid CM 9), in stomacher for 2 min. and incubated at 28ºC for 24 h.

Selective plating

After the incubation, enrichment fluid streak plated onto *Aeromonas* Selective Agar Base (Biolife, CN0801) which contains 10 mg/l ampicillin (cat. N° 4240012) and plates were incubated at 30ºC for 24 h. Following the incubation, *Aeromonas* colonies showed a visible yellow color were accepted as suspected. From the typical colonies at least 5 were chosen, subcultured onto Tryptone Soy Agar plates (TSA, Oxoid CM 131) and incubated at 30ºC for 24 h. The colonies which grew on TSA were tested for; Gram stain, oxidase, catalase, motility, growth in Nutrient broth whether containing 5 % of NaCl or none.

Identification of *Aeromonas* spp.

From the colonies detected as *Aeromonas*, esculin hydrolysis, gas formation from d-glycose, acid formation from arabinose, d-mannitol and salisin fermentation, methyle red, voges proskauer and indol tests were done for the identification as described by Carahan and Joseph (2005).

PCR assay for identification of 16S rRNA, aerolysin (aerA) and haemolysin (hly) virulence genes of A. hydrophila

The isolated *A. hydrophila* strains were sent to the Reference laboratory for veterinary Quality Control on poultry production in Animal Health Research Institute, Dokki, Giza, Egypt, for identification of 16S rRNA, aerolysin (a*erA*) and haemolysin (*hly*) virulence genes of *Aeromonas hydrophila* as follow:

DNA extraction

DNA extraction from pure isolates was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer

Primers were supplied from Metabion (Germany) and listed in Table 1.

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15µl of the products was loaded in each gel slot. 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS AND DISCUSSION

A total of 80 chicken parts were examined during this study and 48 (60%) of the chicken parts were positive for *Aeromonas* spp. According to the data in Table 2, it was determined that the highest isolation rate was from the chicken wings (75%), followed

Table 1. Oligonucleotide sequences used for detection of different genes in *Aeromonas hydrophila* and cyclic condition.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
16S rRNA	F-GAAAGGTTGATGCCTAATACGTA R-CGTGCTGGCAACAAAGGACAG	685	94˚C 10 min.	94˚C 45 sec.	55˚C 45 sec.	72˚C 45sec.	72˚C 20 min.	Nielsen *et al.* (2001)
Aerolysin (*aerA)*	F-CACAGCCAATATGTCGGTGAAG R-GTCACCTTCTCGCTCAGGC	326	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 30 sec..	72˚C 7 min.	Singh *et al.* (2008)
Hemolysin (*hly*)	F-CTATGAAAAAACTAAAAATAACTG R-CAGTATAAGTGGGGAAATGGAAAG	1500	94˚C 15 min.	94˚C 1 min.	55˚C 1 min.	72˚C 1.5 min.	72˚C 15 min.	Yousr *et al*. (2007)

Table 2. Isolation rate of *Aeromonas* spp. from chicken meat samples.

Type of samples	No. of samples	Positive samples	
		No.	%
Chicken breast	20	11	55
Chicken drumsticks	20	12	60
Chicken thigh	20	10	50
Chicken wing	20	15	75
Total	80	48	60

by drumsticks and breasts and the lowest rate was from the thigh samples. The incidence of aeromonads was detected with varying percentage in the range of 8 to 55% in previous reports. Kumar *et al.* (2000) isolated *Aeromonas* spp. from 16.7% of examined poultry meat samples. Shinde *et al.* (2005) observed moderate incidence of 24% in poultry meat, however, very high prevalence of 55% was found in the studies of Bhong and Brahmbhatt (2010). Literatures from other part of the world revealed widespread distribution of *Aeromonas* spp. in poultry meat. Fricker and Tompsett (1989) and Barnhart *et al.* (1989) found that 79.3% and 98% of poultry meat were positive for *Aeromonas*, respectively. Okrend *et al.* (1987) found that 100% of chicken meat was positive with levels of *Aeromonas* ranging from 44 to > 4.44 x 10^3 /g. In another study, Akan *et al.* (1998) isolated motile aeromonads in 90.5% of the chicken carcasses. The finding of this study was nearly agreed with Dallal *et al.* (2012) who isolated the bacterium from 53(57.6%) of examined chicken meat samples.

The current study showed that, the predominant species in chicken breast was *A. hydrophila* and *A. allosacharophila* (20%), followed by *A. caviae* (10%) and *A. schubertii* (5%). While the predominant species in chicken drumsticks and chicken wing was *A. allosacharophila* 25% and 20%, respectively. Moreover, *A. hydrophila* isolated from 25% of chicken thigh samples. These results agree with Sarker *et al.* (2020) who isolated *A. hydrophila* from skin (59.26 %) followed by leg (22.22 %) and breast samples (18.52 %). Hygiene at retail chicken shop where samples were collected, was heavily compromised and the water used for carcass washing may be an important critical point in carcass contamination from wide number of gastrointestinal food-borne zoonotic pathogens.

The isolated *A. hydrophila* organism from chicken parts (14 strains), in this study, were confirmed by PCR assay for detection of 16S rRNA gene and only 9 strains of the tested isolate were harboring this gene (Table 4 and Fig.1). The 16S rRNA gene was included as an internal control and has become the gold standard method for definitive species identification (Wang *et al.*, 2003 and Geetha and Michael, 2015).

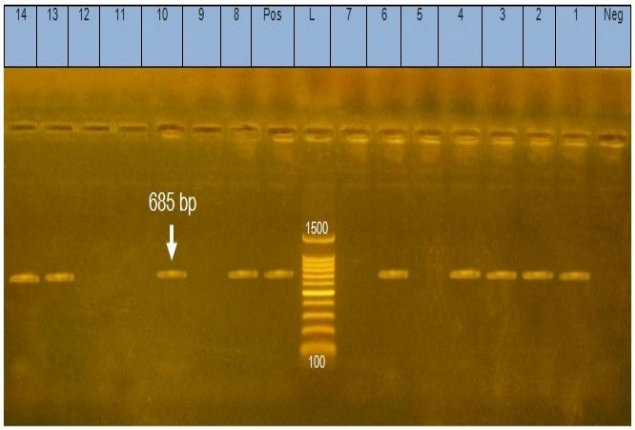

Figure 1. Agarose gel electrophoresis of PCR products of 16S rRNA for characterization of *A. hydrophila*. Lane (L): 100 bp ladder as molecular size DNA marker; Lane (pos): Control positive *A. hydrophila* for 16S rRNA; Lane (Neg): Control negative; Lanes (1-3): 16S rRNA gene-positive strains isolated from Chicken wing; Lane 4: 16S rRNA gene-positive strains isolated from Chicken drumsticks Lanes 6, 8 and10: 16S rRNA gene-positive strains isolated from Chicken thigh; Lane 13and14: 16S rRNA gene-positive strains isolated from Chicken breast.

Table 3. Distribution of *Aeromonas* spp. in the examined chicken meat samples.

Type of samples	No. of samples	*A. hydrophila*		*A. caviae*		*A. jandaei*		*A. trota*		*A. schubertii*		*A. allosacharophila*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Chicken breast	20	4	20	2	10	-	-	-	-	1	5	4	20
Chicken drumsticks	20	2	10	-	-	3	15	1	5	1	5	5	25
Chicken thigh	20	5	25	1	5	2	10	-	-	-	-	2	10
Chicken wing	20	3	15	2	10	2	10	-	-	4	20	4	20
Total	80	14	17.5	5	6.25	7	8.75	1	1.25	6	7.5	15	18.75

Table 4. Relationship among the conventional biochemical and molecular method for identification of *A. hydrophila* isolates.

Type of samples	No. of samples	Conventional method		Molecular method *16S* rRNA	
		No.	%	No.	%
Chicken breast	20	4	20	2	10
Chicken drumsticks	20	2	10	1	5
Chicken thigh	20	5	25	3	15
Chicken wing	20	3	15	3	15
Total	80	14	17.5	9	11.25

Table 5. Characterization of *A. hydrophila* virulence genes Aerolysin (*aerA*) and Haemolysin (*hly*) by PCR from different samples.

Type of samples	No. of *A. hydrophila* detected by *16S* rRNA	Aerolysin (*aerA*) gene		Haemolysin (*hly*) gene	
		No.	%	No.	%
Chicken breast	2	2	100	2	100
Chicken drumsticks	1	1	100	0	0
Chicken thigh	3	3	100	1	33.3
Chicken wing	3	2	66.7	2	66.7
Total	9	8	88.9	5	55.6

PCR was done for detecting aerolysin (*aerA*) as well as haemolysin (*hly*) genes as genetic markers for virulence determinants. The illustrated results in Table (5) and Figure (2) revealed that, eight of nine *A. hydrophila* stranis tested harbor aerolysin gene, the presence and frequency of the aerolysin gene in this study agreed with an earlier PCR study of Yogananth *et al.* (2009) and Enany *et al.* (2013) who detect this gene in all examined strains with a percentage of 100%. The results were nearly higher than that reported by Oliveira-Samira *et al.* (2012) and Yousr *et al.* (2007) who detect aerolysin gene with a percentage of 78.95%, 50.5% respectively, this gene indicate presence of virulent strains because aerolysin gene is one of the most important virulence factors for *A. hydrophila* bacteria. This agrees with Heuzenroeder *et al.* (1999) who stated that the presence of aerolysin is a strong indication of virulence in pathogenic isolates of *Aeromonas* spp.

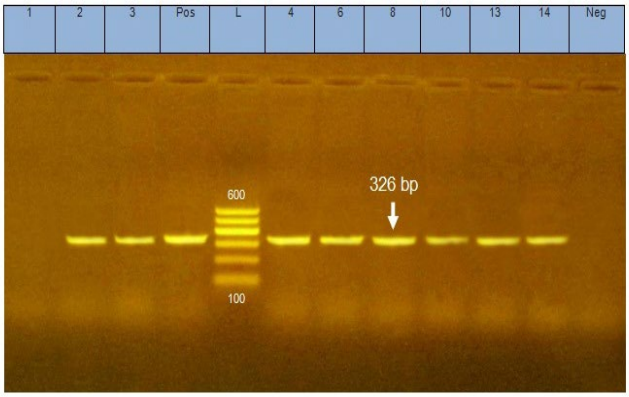

Figure 2. Representatives of the detection of aerolysin (*aerA*) gene in *A. hydrophila* on 1.5% agarose gel. Lane (L): 100 bp ladder as molecular size DNA marker; Lane (pos): Control positive *A. hydrophila* for aerolysin (*aerA*) gene; Lane (Neg): Control negative; Lane 2 and 3: Aerolysin (*aerA*) gene -positive strains isolated from Chicken wing; Lane 4: Aerolysin (*aerA*) gene-positive strains isolated from Chicken drumsticks; Lanes 6, 8 and10: Aerolysin (*aerA*) gene-positive strains isolated from Chicken thigh; Lane 13 and14: Aerolysin (*aerA*) gene-positive strains isolated from Chicken breast.

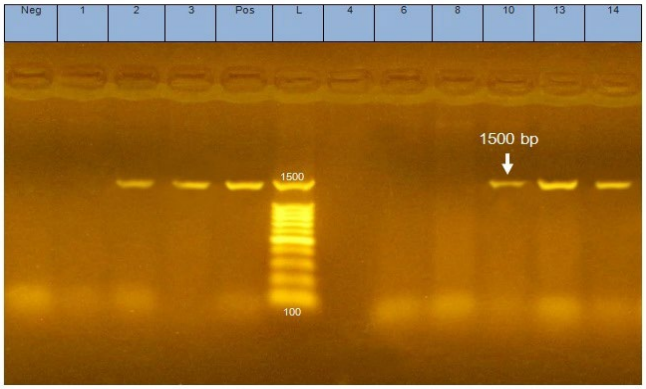

Figure 3. Representatives of the detection of hemolysin (*hly*) gene in *A. hydrophila* on 1.5% agarose gel. Lane (L): 100 bp ladder as molecular size DNA marker; Lane (pos): Control positive *A. hydrophila* for hemolysin (*hly*) gene; Lane (Neg): Control negative; Lane 2 and 3: Hemolysin (*hly*) gene -positive strains isolated from Chicken wing; Lane 10: Hemolysin (*hly*) gene-positive strains isolated from Chicken thigh; Lane 13 and 14: Hemolysin (*hly*) gene-positive strains isolated from Chicken breast.

Results in Table (5) and Figure (3) revealed that, five of nine *A. hydrophila* stranis tested harbor haemolysin gene. Nagar *et al.* (2011) also revealed prevalence of *hly* and *aerA* positive aeromonads isolated from chicken, fish and ready to eat sprouts from Mumbai. Hassan *et al.* (2020) revealed that 12 isolates of *A. hydrophila* were specific for 16S rRNA gene of which 9 isolates were positive for aerolysin (*aerA*) and 10 of isolates were positive for haemolysin (ahhl), with incidence of 75% and 83.3%, respectively. The 16S rRNA gene is an excellent and rapid way to assess the identity of *A. hydrophila*. It has been used for molecular identification of species by restriction fragment length polymorphism or direct gene sequencing (Kupfer *et al*., 2006). Hemolysin is a group of multi-functional enzymes, which play important role in the pathogenicity of *A. hydrophila*. Hemolysins include *aerA*, *ahh1*, *ahyA*, and *asa1*; *ahh1* is the most widely distributed extracellular heat labile hemolysin, the synergistic combination of *aerA* and ahh1is the most cytotoxic genotype (Wang *et al*., 2003).

CONCLUSION

The results achieved in the current study indicated the contamination of chicken products by *Aeromonas* spp. which may play a major role as a source of the transmission of Aeromonads from animals to human. The isolated *A. hydrophila* exhibited virulence properties on the bases of *aerA* and *hly* genes. It is important to give more attention to Aeromonads because they are able to produce toxin, grow under low temperatures and broad spectrum of environments so hygienic measures should be adopted to control microbial contamination.

CONFLICT OF INTEREST

The author declare that no conflict of interest exists.

REFERENCES

Agarwal, R.K., Bhilegaonkar, K.N., Singh, D.K., Kumar, A., Rathore, R.S., 2003. Laboratory manual for the isolation and identification of food borne pathogens. 1st Ed, Indian Veterinary Research Institute Izatnagar. pp. 30-31.

Akan, M., Eyigor, A., Diker K.S., 1998. Motile aeromonads in the faeces and carcasses of broiler chickens in Turkey. J. Food Protection 61, 113-115.

Bhaisare, D.B., Thyagarajan, D., Churchil, R.R., Punniamurthy, N., 2014. Bacterial pathogens in chicken meat: review. Int. J. Life Sci. Res. 2, 1-7.

Barnhart, H.M., Pancorba, O.C., Dreesen, D.W., Shotts, J.R., 1989. Recovery of *Aeromonas hydrophila* from carcasses and processing water in a broiler processing operation. J. Food Protection 52, 636-639.

Bhong, C.D., Brahmbhatt, M.N., 2010. Selective culture media for the recovery of *Aeromonas* species from poultry meat. Ind. J. Vet. Res. 20, 66-68.

Carahan, M.A., Joseph, S.W., 2005. GenusI *Aeromonas* Stanier 1943, 213 In: Bergeys Manual of Systemic Bacteriology. 2nd ed., Garnity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T., Eds, Vol. 2. Berlin pathogens causing disease in cultured Mugil Springer: 2005. pp. 557-558.

Dallal, M.M.S., Yazdi, M.K.S., Avadisians, S., 2012. Study of prevalence and antibiotic resistance in *Aeromonas* species isolated from minced meat and chicken samples in Iran. African J. of Microbiol. Res. 6, 460-464.

Enany, M.E., Shalaby, A.M., Shabanaa, I.I., EL-Gammal, A.M., Hassan, Marwa E., 2013. Characterization of *Aeromonas hydrophila* complex isolated from foods of animal origin. Suez Canal Vet. Med. J. 18, 165-176.

Fricker, C.R., Tompsett, S., 1989. *Aeromonas* spp. in foods: a significant cause of food poisoning? Int. J. Food Microbiol. 9, 17-23.

Geetha, V., Michael, A., 2015. Molecular identification and characterization of *Aeromonas hydrophila* isolated from infected fish rohu. Indian J. Appl. Res. 5, 657-659.

Gideon, I.O., Inamu, H.M., ude C.I., Judith, C.O., 2017. Mycological Quality of Fresh and Frozen Chicken Meat Retailed within Warri Metropolis, Delta State, Nigeria. Jordan Journal of Biological Sciences 10, 303 – 308.

Hassan, M.A., Ibrahim, H.M., Nahla, A.S., Suzan, H.S., 2020. Prevalence of *Aeromonas* species and their virulence factors isolated from frozen chicken meat products. Benha Veterinary Medical Journal 39, 47-51

Heuzenroeder, M.W., Wong, C.Y., Flower, R.L., 1999. Distribution of two haemolytic toxin genes in clinical and environmental isolates of *Aeromonas* spp. Correlation with virulence in a suckling mouse model. FEMS Microbiol. Lett. 174, 131–136.

Janda, J.M., Abbott, S.L., 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev. 23, 35–73.

Khajanchi, B.K., Fadl, A.A., Borchardt, M.A., Berg, R.L., Horneman, A.J., Stemper, M.E., Joseph, S.W., Moyer, N.P., Sha, J., Chopra, A.K., 2010. Distribution of virulence factors and molecular fingerprinting of *Aeromonas* species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. Appl. Environ. Microbiol. 76, 13–25.

Kirov, S.M., 1993. The public health significance of *Aeromonas* spp. in foods: a review. J. Food Microbiol. 20, 179-198.

Kumar, A., Bachhil, V.N., Bhilegaonakar, K.N., Agarwal, R.K., 2000. Occurrence of enterotoxigenic *Aeromonas* species in foods. J. Commun. Dis. 32, 169- 174.

Kupfer, M., Kuhnert, P., Korczak, B.M., Peduzzi, R., Demarta, A., 2006. Genetic relationships of *Aeromonas* strains inferred from 16S rRNA, gyrB and rpoB gene sequences. Int. J. Syst. Evol. Microbiol. 56, 2743–2751.

Merino, S., Rubires, X., Knochel, S., Tomas, J.M., 1995. Emerging pathogens: *Aeromonas* spp. Int. J. Food Microbiol. 28, 157-168.

Nagar, V., Shashidhar, R., Bandekar, J.R., 2011. Prevalence, characterization, and antimicrobial resistance of *Aeromonas* strains from various retail food products in Mumbai, India. J. Food Sci. 76, M486-M492.

Nielsen, M.E., Høi, L., Schmidt, A.S., Qian, D., Shimada, T. Shen, J.Y., Larsen, J.L., 2001. Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China? Dis. Aquat. Org. 46, 23–29.

Okrend, A.J.G., Rose, B.E., Bennett, B., 1987. Incidence and toxigenicity of *Aeromonas* species in retail poultry, beef and pork. J. Food Protection 50, 509-513.

Oliveira-Samira, T.L., Gisele, V., Mateus, M., 2012. Molecular characterization of virulence factors in *Aeromonas hydrophila* obtained from fish. Pesq. Vet. Bras. 32,701-706.

Praveen, P.K., Debnath, C., Shekhar, S., Dalai, N., Ganguly, S., 2016. Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazards: A review. Veterinary World 9, 6-11.

Sarker, B., Arif, M., Eashmen, N., Akter, M.R., Lutful Kabir, S.M., 2020. Isolation, identification and antibiogram profile of *Aeromonas hydrophila* from broiler chickens in Mymensingh Sadar, Bangladesh. Asian Australas J. Food Saf. Secur. 4, 22-30.

Shinde, S.V., Zade, N.N., Kolhe, R.P., Karpe, A.G., 2005. Prevalence of multiple drug resistance *Aeromonas* species from chicken. J. Bombay Vet. Coll. 13, 36-39.

Singh, V., Rathore, G., Kapoor, D., Mishra, B.N., Lakra, W.S., 2008. Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pond water. Indian J. Microbiol. 48, 453–458.

Uma, A., Rebecca, G., Meena, S., Saravanabava, K., 2010. PCR detection of putative aerolysin and hemolysin genes in an *Aeromonas hydrophila* isolate from infected Koi Carp (*Cyprinus Carpio*). Tamilnadu J. Veterinary and Animal Science 6, 31-33.

Wang, G.,Clark, C., Liu, C., Pucknell, K., Munro, C., Kruk, T., Caldeira, R., Woodward, D., Rodgers, F., 2003. Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas* sobria by multiplex PCR. J. Clin. Microbiol. 41, 1048–1054.

Yogananth, N., Bhakyaraj, R., Chanthuru, A., Anbalagan T., Mullai K., 2009. Detection of virulence gene in *Aeromonas hydrophila* isolated from fish samples using PCR technique. Global Journal of Biotechnology and Biochemistry 4, 51-53.

Yousr, A.H., Napis, S., Rusul, G.R.A., Son, R., 2007. Detection of Aerolysin and Hemolysin Genes in *Aeromonas* spp. Isolated from Environmental and Shellfish Sources by Polymerase Chain Reaction. ASEAN Food Journal 14, 115-122.