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Characterization of Hemolysins Genes in *Aeromonas* Species Isolates from Surface Water in Mexico

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Abstract:- ***Aeromonas*** **belongs to the family Aeromonadaceae, which are commonly found in aquatic environments. They are opportunist pathogens and causes infections due to the possession of various virulence gene. We isolated and molecularly characterized *Aeromonas* spp. from different water samples (surface water, waste water, and portable water) in the North-East of Tamaulipas. The isolates were molecularly identified by the sequencing of the gyrB gene. The potential pathogenicity of the isolates was investigated by the PCR amplification of the hemolysin virulence genes (aerA and hlyA) commonly found in *Aeromonas* species.**

Three *Aeromonas* species consisting of 123 strains were identified in this study. The species include *A. veronii* (43.75%), *A. hydrophila* (43.75%) and *A. caviae* (12.50%). The amplification of the virulent genes in the isolates revealed that 89 strains possess the aerA hemolytic gene while 31 strains possess the hlyA gene. The strains which possess aerA gene include, 39 from surfac, 49 wastewater and 1 from portable water. On the other hand, none from portable water possess the hylA gene while 12 from surface water and 19 from wastewater has the hylA genes. The recovery of *Aeromonas* spp. from different water samples particularly portable water supply make portable water potential source through which *Aeromonas* infection can be transferred and thus must be properly treated before public distribution. This observation also emphasizes the need for adequate public hygiene.

Keywords:- *Aeromonas, Virulence, Infections, Genes, Water.*

I. INTRODUCTION

Aeromonas is a genus of facultative anaerobic bacteria which are commonly found in aquatic ecosystems. Bacteria in this genus are usually oxidase and catalase positive (1). Bacteria in this genus can be mobile moving by means of a polar flagellum, or non-motile. Aeromonas are either mesophilic or psychrophilic in nature. *Aeromonas* have been isolated from water, food, fecal samples and extraintestinal sources (2). Some species are human pathogens (3) and can be acquired, mainly, by the consumption of unhygienic food of aquatic origin, such as fish, seafood and water (4). Another medium through which aeromonas infection can be transferred is the aquatic environment. Human contact with *aeromonas* laden water sample can result in the acquisition of *aeromonas* infections (1). *Aeromonas* infections are most commonly reported in children and immunocompromised individual, the latter being the most affected by infections associated with *Aeromonas* (5).

World Health Organization (WHO) listed *Aeromonas* as one of the bacteria that causes waterborne infection. In the United States, the Environmental Protection Agency (EPA) considered *Aeromonas hydrophila* as a potential waterborne pathogen.

In addition to the *A. hydrophila*, there are two other *Aeromonas* species which can also cause pathogenic infection. They are *A. caviae*, and *A. veronii* (1).

Aeromonas' pathogenicity is associated with the presence of several virulence factors in their genome. The virulence factors in *Aeromonas* include surface polysaccharides such as capsule, lipopolysaccharide, and glucan), S layer, exotoxins and extracellular enzymes, pili and flagella that confer on them, the ability to damage host tissue (6). Other virulent factors in *Aeromonas* include α and β hemolysins and cytotoxic enterotoxins. The hemolytic and cytotoxic enterotoxin are capable of causing extensive damage to the epithelium on the affected persons (7). Although, studies have shown. Enterotoxicity in some strains of *Aeromonas*, their role as etiological agent have not been fully understood due to the lack of adequate epidemiological data on associated outbreaks and animal model to reproduce gastroenteritis conditions commonly associated with *Aeromonas* infectious outbreak. (8).

More epidemiological studies are therefore important in establishing the role of *Aeromonas* species in various water borne infections. In the study we isolated *Aeromonas* species from the different water sources with the aim of understanding the possibilities role of this water sources as a means of transferring virulent *Aeromonas* species.

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II. METHODOLOGY

➢ *Sample collection*

In this work, we collected 150 water samples in sterile 50ml falcon tube from different part of Reynosa Northeast of Tamaulipas. The samples were aseptically transferred into the lab for further analysis.

➢ *Isolation of Aeromonas*

Water sample were serially diluted up to the power of 10^8 and then spread on *Aeromonas* agar (Sigma Aldrich®), plate supplemented with 0.01 mg / ml of ampicillin. The inoculated plates were incubated at 37° C for 24 hours, 136 suspected *Aeromonas* isolates were obtained, from the inoculated selective medium.

➢ *Identification of Isolates*

The isolates were identified using both biochemical characteristics described in our previous article (9) and the amplification of the gyrB gene fragment for *Aeromonas.* The biochemical characteristics tested include cytochrome oxidase test (N, N-Dimethyl-phenylenediamine) SIGMA ALDRICH®, catalase, Gram stain, glucose fermentation, and hemolysis (BD Bioxon®). Biochemical tests which were carried out on agar plates were incubated at 37 ° C for 24 to 48 hours.

➢ *Extraction and purification of genomic DNA*

The extraction of genomic DNA was carried out using the Wizard Promega Genomic cat kit. A1120, for Gram-negative bacteria according to the manufacturer's instruction. The quality and concentration of the DNA were determined by NanoDrop ™ 2000 from ThermoScientific. Subsequently, electrophoresis was performed in 1% agarose gel, in 0.5% Tris-Borate EDTA buffer at pH 8.

➢ *Amplification of gyrB region in Isolates*

A. hydrophila subsp hydrophila ATCC 7966, was used as positive control while sterile milli-Q water act as a negative control in the amplification of the gyrB gene fragments of the suspected *Aeromonas* isolates. The reaction was carried out at a final volume of 25 µl; 2.5 µl of 1X buffer, 0.75 µl of MgCl2 (1.5 mM), 0.5 µl dNTPs (0.05 mM), 0.5 µl of each of the primers (0.1 µM) gyrB-F and gyrB-R (Ortega-Balleza, 2017), 1.25 U / µl of Taq polymerase and 19 µl of sterile milli-Q water. Under the following temperatures: 95 ° C for 2 min, then 30 cycles at 95 ° C for 30 sec, 55 ° C for 30 sec, 72 ° C for 1:50 min, 72 ° C for 10 minutes. The reaction was performed in a Veriti® thermocycler from Applied Biosystems and the products were verified by electrophoresis in 1.5% agarose gel and with molecular weight marker HyperLadder ™ 100bp.

➢ *Detection of the virulence gene aerA / hlyA*

We performed PCR to amplify the aerA/hlyA regions in the isolates using the primers reported by Soler, 2002. The amplification of hlyA with the primer pair described by Abdullah *et al* 2003 was adopted for the detection of the virulence gene hlyA. The products obtained were analyzed by electrophoresis in a 1.5% agarose gel in Tris Borate EDTA buffer solution, pH 8 (TBE 1X) for 40 min at 90 V and using molecular weight marker HyperLadder ™ 100 bp. The gel was then visualized in a Kodak® photodocument with a Gel Logic 112 camera.

III. RESULTS AND DISCUSSION

The PCR amplification and analysis showed that 123 (90.44%) strains belong to the genus *Aeromonas*. The primers used amplify a partial sequence of the gene coding for the β subunit of the DNA gyrase, of about 967 bp fragment (Figure 1).

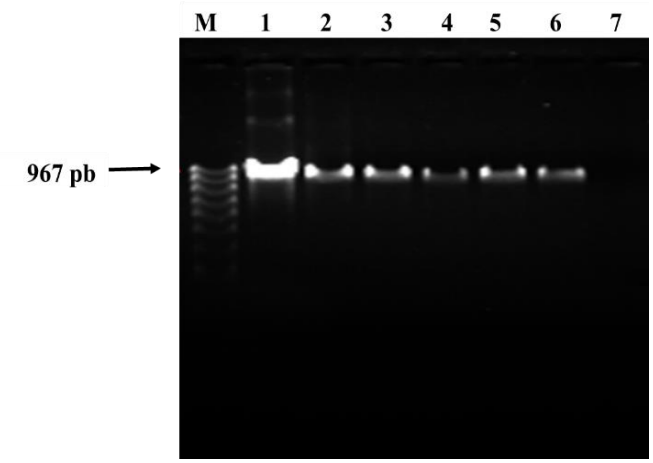

Fig 1:- PCR product of the gyrB gene, representative. 1.5% agarose gel with SYBR® Gold, 90 V for 45 min. (M), HyperLadder ™ 100bp molecular weight marker; (1), A. hydrophila subsp. hydrophila ATCC 7966; (2) 02-07a; (3) 02-07c; (4) 05-07a; (5) 0016-22a; (6) 0016-22b; (7) Negative control.

Different analyzes based on unique maintenance genes have provided coherent *Aeromonas* phylogenies (10). Different works have proposed gyrB as an ideal marker that allows the identification of species of the genus *Aeromonas* (11). In this study, 53 (43.08%) strains from surface water, 65 (52.84%) from wastewater and 5 (4.06%) from drinking water were identified as *Aeromonas* spp. These findings conform to previous works. In a study in Turkey, 50% (n = 30) of *Aeromonas* were recovered from surface water (12). Other studies also reported the recovery of 83.6% (n = 102) *Aeromonas* spp from aquatic environments (13); Similarly, González-González *et al*., (2004) that 28.2% of the isolates obtained from 40 samples in Cuba, were *Aeromonas* spp.

The hemolysins hlyA and aerA, belong to the group of cytotoxic enterotoxins, which cause hemolysis and production of mediators inflammation, thus potentiating the virulence of this bacterium (7). aerA was found in 89 (72.35%) of the strains. 39 (31.70%) strains with aerA gene

were identified in strains from surface water, 49 (39.83%) strains with aerA were from waste water, while only 1 (0.81%) strains from drinking water possesses the aerA gene.

The protein product encoded by this gene (AerA) has been reported in more than 75% of *A. hydrophila*, and in few *A. veronii, A. caviae,* and *A. trota* (Janda and Abbott, 2010). In South Africa, Igbinosa and Okoh, (2013), detected aerA in 43% of the isolates they recovered from waste water and in 21% of the strains they isolated from residual water. Similarly, Ghenghesh *et al*, (2014), (14) reported the detection aerA genes in 81.8% of the isolates recovered from water sources in Libya. The hlyA gene was found in 31 (25.20%) strains of which 12 (9.75%) were detected in surface water, 19 (15.44%) in wastewater, and none from drinking water, of about 1079 bp fragment (Figure 2).

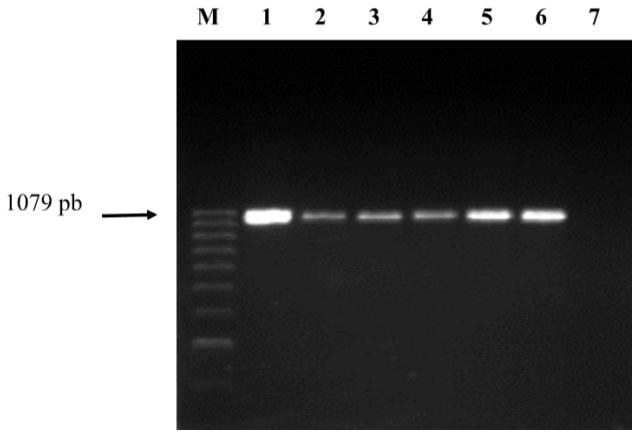

Fig 2:- PCR product of the hlyA gene, representative. 1.5% agarose gel with SYBR® Gold, 90 V for 45 min. (M), HyperLadder ™ 100bp molecular weight marker; (1), A. hydrophila subsp. hydrophila ATCC 7966; (2) 02-07a; (3) 02-07c; (4) 05-07a; (5) 0016-22a; (6) 0016-22b; (7) Negative control.

In South Africa, in 2013, 86% of hlyA was reported in surface water and 88% in wastewater (15). However, in Malaysia, these gene were not detected in the strains recovered from surface water (13). The hlyA gene codes for HlyA hemolysin, common in *Aeromonas* species. The gene is commonly detected in *A. hydrophila*, although it is also found in *A. caviae* (35%), *A. veronii* (12%), *A. trota* and *A. jandaei* (1). Only six strains (4.87%), possess both hlyA and aerA encoding genes. The possession of this pair may enhance the haemolytic activity of the strains in which they were found. It is known that these toxins can act synergistically, inducing a severe and watery diarrhea in humans (6). However, some studies suggested that only a fraction of *Aeromonas* strains are invasive, and the relative degree of invasion is lower than that observed for classical enteropathogens, such as enteroinvasive *E. coli*, *Shigella* or *Yersinia enterocolitica*. In some studies, they observed that *A. hydrophila* possesses at least four different toxins (Hly, Act, Alt and Ast) with enterotoxigenic capacities in vitro (1). The presence of the two enteroinvasive genes in some isolates from difference water samples suggest that they could be potential threat to human health. Public hygienic behavior is thus an important process to prevent the spread of infection associated with *Aeromonas*.

IV. CONCLUSION

The recovery of *Aeromonas* spp from environmental sample as reported in this study is of public importance. Of notable importance is the possession of virulent genes as *hlyA* by many of the isolates. The possession of two different genes encoding different virulent factors by some *Aeromonas* species recovered from this study is an indication of the possible risk of virulent infection that one can acquire from the indiscriminate use of water. The finding from this study therefore buttress the importance of good public hygiene in North-East Tamaulipas.

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REFERENCES

[1]. Janda JM, Abbott SL. The genus Aeromonas: Taxonomy, pathogenicity, and infection. Vol. 23, Clinical Microbiology Reviews. 2010. p. 35–73.
[2]. Castro-Escarpulli G, Figueras MJ, Aguilera-Arreola G, Soler L, Fernández-Rendón E, Aparicio GO, et al. Characterisation of Aeromonas spp. isolated from frozen fish intended for human consumption in Mexico. Int J Food Microbiol. 2003;84(1):41–9.
[3]. Martin-Carnahan A, Joseph SW. Aeromonadales ord. nov. In: Bergey's Manual® of Systematic Bacteriology [Internet]. 2007. p. 556–87. Available from: http://link.springer.com/chapter/10.1007%2F0-387-28022-7_12
[4]. William Suárez Q, Fanny Herrera A. Determinación de factores de virulencia en cepas de Aeromonas spp., aisladas a partir de pescado. Rev MVZ Cordoba. 2012;17(1):2846–51.
[5]. Beaz-Hidalgo R, Figueras MJ. Aeromonas spp. whole genomes and virulence factors implicated in fish disease. Vol. 36, Journal of Fish Diseases. 2013. p. 371–88.
[6]. Von Graevenitz A. The role of Aeromonas in diarrhea: A review. Infection. 2007;35(2):59–64.
[7]. Chopra AK, Houston CW. Enterotoxins in Aeromonas-associated gastroenteritis. Microbes Infect [Internet]. 1999 Nov;1(13):1129–37. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1286457999002026

[8]. Imhoff JF, Rodriguez-Valera F. Betaine is the main compatible solute of halophilic eubacteria. J Bacteriol. 1984;160(1):478–9.
[9]. Ortega Balleza JL, Sánchez-Varela A, Rodríguez-Luna IC, Guo X. Genes de virulencia en Aeromonas spp. (Aeromonadales: Aeromonadaceae) aisladas de Oreochromis spp. (Perciformes: Cichlidae) para consumo humano en México. Rev Biol Trop. 2018;66(4).
[10]. Martinez-Murcia AJ, Monera A, Saavedra MJ, Oncina R, Lopez-Alvarez M, Lara E, et al. Multilocus phylogenetic analysis of the genus Aeromonas. Syst Appl Microbiol. 2011;34(3):189–99.
[11]. Yáñez MA, Catalán V, Apráiz D, Figueras MJ, Martínez-Murcia AJ. Phylogenetic analysis of members of the genus Aeromonas based on gyrB gene sequences. Int J Syst Evol Microbiol. 2003;53(3):875–83.
[12]. Onuk EE, Ciftci A, Findik A, Durmaz Y. Development and evaluation of a multiplex PCR assay for simultaneous detection of Flavobacterium psychrophilum, Yersinia ruckeri and Aeromonas salmonicida subsp. salmonicida in culture fisheries. J Vet Sci. 2010;11(3):235–41.
[13]. Khor WC, Puah SM, Tan JAMA, Puthucheary SD, Chua KH. Phenotypic and genetic diversity of Aeromonas species isolated from fresh water lakes in Malaysia. PLoS One. 2015;10(12).
[14]. Ghenghesh KS, Ahmed SF, Cappuccinelli P, Klena JD. Genospecies and virulence factors of aeromonas species in different sources in a north african country. Libyan J Med. 2014;9.
[15]. Igbinosa IH, Okoh AI. Detection and distribution of putative virulence associated genes in Aeromonas species from freshwater and wastewater treatment plant. J Basic Microbiol. 2013;53(11):895–901.