Isolation and Characterization of A. hydrophila from the Al-Jadryia River in Baghdad (Iraq)

Hawraa Natiq. Kabroot Al-Fatlawy1,*, Hazim. Aziz. AL-Hadrawy2

1Department of Biology, College of Sciences, Kufa University, Najaf, Iraq
2Department Ecology, College of Sciences, Kufa University, Najaf, Iraq
*Corresponding author: hawraank1@yahoo.com

Received June 25, 2014; Revised July 25, 2014; Accepted August 06, 2014

Abstract In this paper, we described detection of Aeromonas hydrophila in water of AL-Jadryia river (Baghdad city, Iraq), during the period from July, 2012 to April, 2013. The samples collected from water of Jaderia river and transfer to Bacteriology laboratory for diagnosis. A. hydrophila isolates which were diagnosed by three methods (Culture method, biochemical tests, Api20E system), in Culture method was isolated (71) isolate on blood agar and TCBS media and (44) isolates by biochemical tests while Api20E kit was the important method for diagnosis, which has led to isolate and diagnosis of (36) isolate of A. hydrophila. Also which used the PCR method of gene Tetracycline gene (tetA-E) was the best methods for diagnosis, which has led to isolate and diagnosis as (27) isolates of A. hydrophila have tetA-E sequence gene of all samples. The virulence factors of bacteria were detected and showed that all isolates produced of hemolysin, protease, lipase enzymes and also detection of tetracycline resistant gene of these samples.

Keywords: Aeromonas hydrophila of water, tetracycline gene (tetA-E), lipase, hemolysin, protease

Cite This Article: Hawraa Natiq, Kabroot Al-Fatlawy, and Hazim.Aziz. AL-Hadrawy, “Isolation and Characterization of A. hydrophila from the Al-Jadryia River in Baghdad (Iraq).” American Journal of Educational Research, vol. 2, no. 8 (2014): 658-662. doi: 10.12691/education-2-8-14.

1. Introduction

The genus Aeromonas is a member of the family Aeromonadaceae that are primarily aquatic organisms found in water [1]. Aeromonas infections are one of the most common bacterial diseases diagnosed in marine and cultured freshwater fish. Aeromonas hydrophila is found in diverse habitats, including soil, widely in fresh and salt water also frequently found in chlorinated and non-chlorinated drinking water, and is pathogenic to warm and cold-blooded animals [2]. Aquatic environment along with water and seafood is thus important potential source for the transmission of A. hydrophila resulting in human infections. Aeromonas spp. have been involved in wound infections, sepsis, outbreaks of water, and food-borne gastroenteritis [3]. Direct contact with contaminated water and soil is the most frequent cause of gastrointestinal and wound infections in humans [4].

There has been an increasing incidence of antimicrobial resistance among Aeromonas sp. isolated from aquaculture environments, five classes of genetically distinguishable tetracycline resistance determinants designated Athrough E, have been described among aerobic enteric gram-negative bacteria and Several studies have shown tetE to be the predominant determinant among the different classes of tetracyclineresistant genes (4,15).

The pathogenicity of A. hydrophila infection have been implicated in the cause of numerous human infections, such as gastroenteritis, cellulitis, meningitis, bacteremia, soft-tissue infections, peritonitis, and broncho pulmonary infections [5]. Various putative virulence factors (aerolysin/hemolysin, proteases, lipases, DNases, enterotoxins) that may play an important role in the development of diseases, either in humans or in fish, have been described in several species of the genus [6]. Some authors reported that the production of cytotoxins and hemolysin is related to A. hydrophila and A. veronii bv, and hemolytic molecules seem to be related to enterotoxigenicity [7]. Virulence in Aeromonas hydrophila is multifactorial which these enzymes that play significant role in pathogenesis. A. hydrophila has been an increasing incidence of antimicrobial resistance among Aeromonas sp. isolated from aquaculture environments [8]. Broad-spectrum antibiotics, such as tetracycline, are prescribed clinically for the treatment of such infections. Several studies have shown tetE to be the predominant determinant among the different classes of tetracycline resistant genes of A. hydrophila [6,9].

Molecular studies on Aeromonas species have received a little attention in Iraq and this study was considering the first molecular study in Iraq. The present study is carried out to achieve the following objectives:

1- Isolation of A. hydrophila isolates among the environmental samples and identification by API20E system and used the PCR technique, with specific primer (tetA-E) sequence gene.
2. Detection the virulence factors of *A. hydrophila* isolates such as hemolysin, lipase, protease and Phospholipase.

2. Methods

A total of samples collection (440) samples of water were obtained from Al- Jadyria bridge water in Baghdad who attended to Bacteriology laboratory in Sciences faculty during the period from August,2012 to the April,2013. They were swabbed onto thiosulfate citrate bile salts sucrose (TCBS) agar and MacConkey agar (MC) the plates were incubated overnight.

2.1. Identification of *A. hydrophila*

Morphological colonies characteristics were recorded on the media that are used (MacConkey agar, blood agar and TCBS agar) for primary identification of *A. hydrophila* and microscopic properties by Gram's stain was used to examine the isolated bacteria for studying the microscopic properties such as gram reaction, shape and motility [10]. While Biochemical tests used Oxidase test, Catalase test (Hydrogen Peroxide 3%), Simmon's Citrate test, Indole Production test, Motility test were all these tests and urease test result according to [11]. Also, API20E system was carried out according to the procedure of (Biomerix company, France).

2.2. Detection Virulence Factors of *A. hydrophila*

Heamolysin was detection according to [12] tested for β-hemolytic activity on base agar (Himedia, India) supplemented with 7% sheep erythrocytes. A loopful of an overnight growth from nutrient agar were cultured on blood agar by streaking method, incubated at 37°C for 24 h. Protease was detected by [13] was tested on 2% agar-agar (Himedia, India) containing 10% (w/v) skimmed milk (Himedia, India). A loopful of an overnight growth from nutrient agar were cultured on agar by streaking method, incubated at 37°C for 24 h. While Lipase detection was performed on Tween 20 agar [12].

2.3. Molecular Identification Tetracycline Resistant Gene of *A. hydrophila*.

PCR Assay:

The wizard genomic DNA purification kit is designed for isolation of DNA from G- bacteria by a universal extraction kit of (Promega company). Gel electrophoresis was used for detection of DNA by UV transilluminator [14]. Concentration of DNA was determined spectrophotometrically by measuring its optical density at 260 nm (Extinction coefficient of dsDNA is 50 μg/ml at 260 nm) the purity of DNA solution is indicated by ratio of OD260/OD280 which is in the range of 1.8± 0.2 for pure DNA [16]. PCR program that apply in the thermocycler. The PCR products and the ladder marker are resolved by electrophoresis on 1.2% agarose gel. [15].

3. Results

3.1. Isolation and Identification of *A. hydrophila*

A total of (440) samples were collected from water of AL-Jadyria river (Baghdad city, Iraq), and was observed (71) isolate on media. [Figure 1] reveals that *A. hydrophila* isolates are characterized by their ability to ferment glucose with gas formed on kligler iron agar (Alk/Acid), it produces (Alkaline) red color top and bottom (acidic) yellow color with gas formed but not H2S; it gives a positive result to, catalase, Indole, simmone citrate tests. API 20E system is used to confirm identification of *A. hydrophila* included in this study. The results demonstrate that (36) isolate were positive in identification by API20E, as shown in [Figure 2]. Using the analytical profile index of this system the identification percentage is (id% = 93.8).
The ability of *A. hydrophila* (36) isolates to produce some virulence factors. All *A. hydrophila* showed positive result for hemolysin production (100%), type beta (β-hemolysin), when cultured on blood agar medium. The isolates also showed the ability to produce protease to hydrolyze the protein (100%), when inoculated on skim milk agar for 24 hours at 37°C. Also the ability to hydrolyze fats by lipase enzyme when inoculated on 1% Tween agar for (3-5) days at 37°C. While all isolates of *A. hydrophila* have no ability to produced urease enzyme as shown in [Figure 3].

While, molecular identification by PCR technique has been used to amplify gene of the (tet-E) gene from genomic DNA of all *A. hydrophila* isolates. DNA is extracted from all isolates. The results of isolates diagnosis using the PCR technique for (tet-E) detection clarify that isolates of *A. hydrophila*, producers carrying (tet-E) gene by 26/36(72.22). Specific primers of *aerA* gene with genomic DNA of *A. hydrophila* isolates were used in this study as shown in [Figure 4]. The PCR assay was performed to detect the(tetE) gene of *A. hydrophila*, tetA-E – Forward Primer (F) 5'-ATGAACCGCACTGTGATGATG–3' and tetA-E – Reverse Primer (R) 5'-ACCGACCATTACGCCATCC-3' with size (744 bp), these primers synthesized by Alpha DNA company, Canada[15]. The thermocycler for this primer by following: Initial denaturation 94°C for 3 min and (94°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec for 30 cycles) and 72°C for 10 min final elongation. The amount (concentrations) of the PCR components were included final volume 25 µl in tube PCR by DNA template (5µl), Forward primers (2.5µl), Reverse primers (2.5µl), Gotaq master mix (12.5 µl) and distilled water (2.5 µl).

4. Discussion

The present study is conducted to isolation and identification (36) isolate of *A. hydrophila* bacteria from water samples of AL-Jadryia river (Baghdad city, Iraq). *Aeromonas hydrophila* causes a wide range of human illnesses possible routes of transmission include contaminated water, food and exposure of wounds to environments that contain the pathogen. In general, the
genus Aeromonas are facultative anaerobic, oxidase positive, Gram negative bacteria whose natural habitat is in the aquatic environment. Some species are pathogenic for animals and human.

Identification of A. hydrophila depends on the colonial morphology, biochemical tests, Api20E system and molecular identification for (tet-E) gene. The colonies of A. hydrophila are yellow shin /green color on TCBS agar due to sucrose ferment, with diameter ranged from (2-3) mm. In addition, those colonies appear as pale like shaped on the MacConkey agar that indicated A. hydrophila is unable to ferment lactose sugar. But it is grow on the blood agar with produces smooth, convex, rounded and _-hemolytic colonies and pale white to grey color, [Figure 1], these typical characteristics being described by referential studies [17]. These results are agree with [18,19,20]. API20E system is characterizes by fast detection of bacteria without the need for many of culture media as well as reduce cultural contamination, and it is used to confirm identification of A. hydrophila, [21]. The isolation rate of Aeromonas hydrophila in many developing countries. In environmental isolates, Aeromonas hydrophila has been isolated in rates as high as 82% in Senegal [22] and in 77% of food samples in Kenya [23]. In the Venda region of South Africa, Aeromonas species are isolated from clinical and environmental samples [24], while the studies in Iraq refer that the isolation rate of A. hydrophila by 6.6% from stool cases in Baghdad[25] who study of Ecological and physiological on A. hydrophila and role enterotoxin in pathogenicity, and 8.9% in Baghdad [26] which studied, some characteristics and immunological effects of A. hydrophila enterotoxin. Whereas [27] refer that the isolation rate reach to 9% from stool samples and 20% from Tigris water, when studied the crude growth extract effect A. hydrophila on normal and Cancer cell lines.

A. hydrophila bacteria have produced a variety of biologically active extracellular products similar to the virulence factors of enteropathogenic bacteria and these virulence factors associated with health effects in humans [28] and causes many diseases as significant human pathogens causing a variety of extra-intestinal infections. Extra-intestinal and gastrointestinal infections were known to occur in previously healthy hosts as well as immunocompromised or otherwise susceptible populations [29]. Virulence factors were present in two forms, cell-associated structures, and extracellular products. The cell-associated structures involved pili, flagella, outer membrane proteins, lipopolysaccharide, and capsules. The major extracellular products include cytoytic, cytolytic, hemolytic, and enterotoxic proteins. Aeromonas produce an array of filamentous structures, including short rigid, and long wavy pili, as well as polar and lateral flagella. Polar flagella and lateral flagella are described by[30,31]. Polar flagellins function as adhesions, while lateral flagellum’s serve as colonization factors [32].

The present study identifies some virulence factors associated with pathogenicity of A. hydrophila, where it is observed that all environmental isolates had ability to produce β-hemolysin (100%) which cause complete hydrolysis of RBCs on blood agar, this result similar to [33,34]. As shown to be cytoytic for the erythrocytes and mammalian cells in culture. However, the hemolytic activity of A. hydrophila has been used as an indicator of enterotoxicity and may be responsible for outbreaks of diarrhea [35]. As researchers indicate that isolates had high pathogenicity and also environmental are responsible for diseases occurs in human because they secrete different toxins [36]. Some reports were indicated that β-hemolysin which is produced from A. hydrophila has a close relationship to the production of toxins in the cell producing enzyme, and toxin called Cytotoxic factors. Hemolysin is made in the logarithmic phase of cell growth [37], and it is virulence factor important for A. hydrophila bacteria. On other hand, in this study was reveal that A. hydrophila were able to hydrolyze the protein by protease enzyme (100%) when tested on skim milk agar, and these results were agree with [38,39] who indicates that A. hydrophila is producing protease enzyme. Protease enzyme secreted outside of the cell through a process of growth as they accumulate significantly in the phase stability of the bacteria, and it is one of virulence factors important for A. hydrophila bacteria [40].

A variety of antibiotics have been used to treat infection caused by A. hydrophila and have proved useful in many cases, but multiple antibiotic resistances are common among A. hydrophila [28]. Many strains of A. hydrophila are known to harbour mobile elements that encode antibiotic resistance and can be transferred among themselves or to other bacterial species’ to establish multiple antibiotic resistances [41]. The widespread use of antibiotics has been identified as a major factor responsible for the increased incidence of antibiotics resistance [1]. Molecular identification of (tet-E) gene by PCR used to amplify a precise fragment of DNA from a complex mixture of starting material usually template genomic DNA. A number of reports are available for PCR amplification of conserved (tet-E) gene of A. hydrophila [42]. The present study showed that most of isolates of A. hydrophila produces carrying (tet-E) gene by 26/36(72.22). Specific primers of aerA gene with genomic DNA of A. hydrophila isolates were used in this study as shown in [Figure 4]. The results of this study agree with several studies [37,43]. This results leading to higher level of resistance to is recorded for these antibiotic may be caused by initial mutation located in a single site on the A. hydrophila chromosome [43].

5. Conclusions

The following conclusions are extracted from the present study:
1- The frequency of A. hydrophila isolates in water of AL-Jadryia river (Baghdad city) was higher in environmental isolates.
2- The molecular study provides definite identification of antibiotics gene such as tetracycline gene.
3- Most environmental isolates produced many virulence factors involve heamolysin, lipase and protease.

References

[1] Andrea. B; Cyrino,J.E.P.(2006). Antibiotic Resistance of Aeromonas hydrophila isolated from Pirarucus mesopotamicus (Holmberg, 1887) and Oreochromis niloticus (Linnaeus, 1758). UFAM - Centro de Apoio Multidisciplinar - Divisão de Biotecnologia, Av. Rodrigo Otávio, 3000 - 69077-900 - Manaus, AM - Brasil.
American Journal of Educational Research

662

[2] Pulu AP, Gomesb LM, Miguel MAL, Balassianoa IT, Queirozb MLP, Freitas-Almeida AC, de Oliveiraa SS., 2006. Antimicrobial resistance in food and clinical Aeromonas isolates. Food Microbiol 23: 504-509.

[3] Youassouf, S., Rassul,G.R.A. and 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 (2): 115-122.

[4] Janda, J. M., R. P. Kokka, and L. S. Guttertz. (1994). The susceptibility of S-layer-positive and S-layer-negative Aeromonas strains to complement mediated lysis. Microbiology 140: 2899-2905.

[5] Brouqui P. and D.Raoult. (2001). Endocarditis due to rare and fastidious bacteria. Clin. Microbiol. Rev. 14:177-207.

[6] Schmidt A.S, Bruun M.S, Dalsgaard I and Larsen J.L. (2001). Incidence, distribution, and spread of tetracycline resistance determinants and integromonsocassociated antibiotic resistance genes among motile aeromonads from a fish farming area. Journal of Applied Environmental Microbiology 67: 5675-5682.

[7] Lee, W.S., and Puthucheary, S. D.(2001). Retrospective study of Aeromonas infection in a Malaysian urban area: a 10-year experience. Singapore Med. J. 42:957-960.

[8] Rhodes G, Johns G, Swings J, Megan P, Hiney M, Smith P, Pickup RW (2000). Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: Implication of Tn1721 in dissemination of the tetracycline resistance determinant, Tet A. Appl. Environ. Microbiol. 66: 3883-3890.

[9] Miranda, C.D; Kohrangen C, Ulep C, Schwarz S, Roberts MC (2003). Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. Antimicrob. Agents Chemother., 47: 883-888.

[10] Jawetz, E.; Melnick, J.I. and Adelberg, E.A. (2007). Medical Microbiology. (24th ed). Appleton and Lange U.S.A.

[11] MacFaddin, J.K. (2000). Biochemical test for identification of medical bacteria. (3rd ed). Lippincott Williams and Winkins.

[12] Mullai Nila, K. (2009). Detection of virulence gene in Aeromonas. Microbid. 290(4-5): 363-367.

[13] Benson, H.J. (2002). Microbiological Applications: Laboratory Manual in General Microbiology. (8th ed). Complete version. McGraw-Hill U.S.A.

[14] Sambrook, J., and Russell, R.W.(2001). Molecular cloning: A laboratory manual, 3rd ed. Cold spring harbor, N.Y.

[15] Jun, J.W.; Kim, I.H.; Oh, I.K.; Cho, I.H.; Shin, Y.M. (1998). Occurrence of tetracycline-resistant Aeromonas hydrophila infection in Korean cyprinid loach (Misgurnus anguillicaudatus). African Journal of Microbiology Research Vol. 4(9), pp. 35-73.

[16] Collee, J.G.; Fraser, A.G.; Marmino, B.P.; and Simons, A. (1996). Manual in General Microbiology. (8th ed). Complete version. McGraw-Hill U.S.A.

[17] Al-Taee, R. K.I.(2002). Study of some virulence factors of the Aeromonas hydrophila enterotoxin. M.Sc. Thesis, College of science, University of Baghdad. In Arabic.

[18] Ali, H.S.A.(2008). Studying the Cruise growth effect of Aeromonas hydrophila. M.Sc. Thesis, College of science, University of Kufa. In Arabic.

[19] Ahani, E.; Dromigny, J.A.; Tall, F.; Ndiaye, M.; Koné, M.; Naharro, G.; Riano, J.; de Castro, L.; Alvarez, S.; Luengo, J.M. (2003). Isolation of enterotoxigenic, hemolytic and antibiotic-resistant Aeromonas strains from infected fish in Saudi Arabia. Food Microbiology. 20: 223-230.

[20] Ali, S.; Goussain, D.; Raoult, D.(2004). Virulence and cytotoxicity of seafood borne Aeromonas hydrophila. Microbiol. Res. 159: 251-254.

[21] Trower, C.J. Abo, S.; Majeed, K.N. and Itzstein, M.V.(2000). Motility and the polar flagellum are required for Aeromonas caviae adherence to HEp-2 cells. Journal of Infection and Immunity 69(7): 4257-4267.

[22] Rahban, A. A.; Gyllios, I.;Tomas, J.M., and Shaw,J.G.(2001). Motility and the polar flagellum are required for Aeromonas caviae adherence to HEp-2 cells. Journal of Infection and Immunity 69(7): 4257-4267.

[23] Rahban, A.A. and Shaw, J.G.(2002). Lateral flagella and swarming motility in Aeromonas species. J. Bacteriol. 184: 547–555.

[24] Kirov, S.M.; Tassell,B.C.; Smenn, A.B.T.; O’Donovan, L.A.; Rahban,A.A. and Shaw, J.G.(2002). Lateral flagella and swarming motility in Aeromonas species. J. Bacteriol. 184: 547–555.

[25] Rahban, A.A. and Shaw, J.G.(2002). Lateral flagella and swarming motility in Aeromonas species. J. Bacteriol. 184: 547–555.

[26] Burgos, S.; Castrisios, M.and Shaw, J.G(2004). Aeromonas flagella (polar and lateral) are enteroctye adhesins that contribute to biofilm formation on surfaces. Infect. Immun. 72:1939-1945.

[27] Kadhim, S.A.(2009). Detection of some bacterial causes of watery diarrhea in the province of Baghdad and some northern governorates, with a study of pathological effects. M.Sc.Thesis, College of Science, University of Baghdad. In Arabic.

[28] Jayarranan, S.; Illanchezian, S.; Manoharan, M.S. and Valsalam, S.(2010).Virulence and cytotoxicity of seafood borne Aeromonas hydrophila. Food Microbiology. 27, pp. 35-73.

[29] Vaz, R. R.; Gomesb LM, Miguel MAL, Balassianoa IT, Queirozb MLP, Freitas-Almeida AC, de Oliveiraa SS., 2006. Antimicrobial resistance in food and clinical Aeromonas isolates. Food Microbiol 23: 504-509.

[30] Rabaan, A.A. and Shaw, J.G.(2002). Lateral flagella and swarming motility in Aeromonas species. J. Bacteriol. 184: 547–555.

[31] Rahim Z, Sanyal SC, Aziz KMS, Huq MI, Chowdhury AA (1984).Isolation of enterotoxogenic, hemolytic and antibiotic-resistant Aeromonas hydrophila strains from infected fish in Bangladesh. Appl.Environ. Microbiol., 48: 865-867.

[32] Garg, N.; Yoganathan, N.; Bhakaryaj, R.; Chantthuru, A.; Anbalagun, T.; Mullai Nila, K. (2009). Detection of virulence gene in Aeromonas hydrophila isolated from fish samples using PCR technique. Global J. Biotechnol. Biochem., 4, 51–53.

[33] Abrami, J.; Firaz, M. and Good, V.F.G.(2000). Surface dynamics of aerolysin on the plasma membrane of living cells. Int.J. Med. Microbiol. 290(4-5): 363-367.

[34] Al-Taee, R. K.I.(2002). Study of some virulence factors of the Aeromonas hydrophila bacteria isolated from clinical source. M.Sc. Thesis, College of science, University of Baghdad. In Arabic.

[35] Pandey,A.; Naik,M.; Dubey,S.K.(2010). Hemolysin, Protease, and EPS Producing Pathogenic Aeromonas hydrophila Strain An4 Shows Antibacterial Activity againstMarine Bacterial Fish Pathogens. Laboratory of Bacterial Genetics and Environmental Biotechnology, Department of Microbiology, Goa University, Taleigao Plateau, Goa, 403206, India.

[36] Trower, C.J. Abo, S.; Majeed, K.N. and Itzstein, M.V.(2000). Production an enterotoxin by a gastro- enteritis associated Aeromonas strain. J. Med. Microbiol. 49:121-126.

[37] Janda, J. M.(2001). Chapter 59. Aeromonas and Plesiomonas, p. 1237-1270. In M. Sussman (ed.), Molecular medical microbiology, vol. 2. Academic Press, London, United Kingdom.

[38] Wang, G.; Clifford, C.G.; Liu, C.; Pucknell, C.; Munro, C.K.; Kruk, T.M.; Caldeira, R.; Woodward, D.J., and Rodgers, F.G.(2003). Detection and characterization of a hemolysin gene in Aeromonas hydrophila and Aeromonas sobria by multiplex PCR. J CLin Microbiol 41:1048-1054.

[39] Wilcox, M.H.; Jones, B.L. (1995). Prevalence, pathogenesis antibiotic susceptibility profiles, and in vitro activity of selected medicinal plants against Aeromonas isolates from stool samples of patients in the Venda region of South Africa. Journal of health population nutrition. South Africa. Journal of health population nutrition. 662