WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 163-171. Research Article ISSN 2277– 7105

PUTATIVE VIRULENCE FACTOR AND ANTIMICROBIAL SUSCEPTIBILITY OF LOCALLY BACTERIA *AEROMONAS HYDROPHILA* ISOLATED FROM SURFACE TIGRES RIVER IN BAGHDAD, IRAQ

***Rasha Mohamed Sajet AL-Oqaili, **Sura Mohammed Sachit and ***Ahmed Mawlood Shieer**

*Department of Biology, College of Science, Al-Mustansiriya University, P. O. box 14022, Baghdad, Iraq.

**The Ministry of Higher Education and Scientific Research /Unit of R&D.

***The head of Al-Mustansiriya University.

Article Received on
23 Jan 2016,
Revised on 13 Feb 2016,
Accepted on 07 Mar 2016
DOI: 10.20959/wjpr20164-5762

***Correspondence for Author**
Rasha Mohamed Sajet AL-Oqaili
Department of Biology, College of Science, Al-Mustansiriya University, P. O. box 14022, Baghdad, Iraq.

ABSTRACT

The genus *Aeromonas* (family *Aeromonadaceae*) are medically important, Gram-negative, rod-shaped and are ubiquitous in aquatic environments.25 environmental isolates positive tested for,biochemical including, oxidase, negative string test,growth in 0 and 1% NaCl, , growth in 7% NaCl. All isolates of environmental *A. hydrophila* were a positive result Lecithinase , DNase, protease produces and hemolysin . The results showed that all isolates were sensitive to chloramphenicol, tetracycline. Resistance to streptomycin, cephalothin erythromycin and nalidixic acid,92%,80%,36% and 20% respectively.*A. hydrophila* caused, villi atrophy and fibrosis with inflammatory cells reached to mucosa , in liver , the effect hepatocytes in apoptosis with pyknotic nuclei and wide spread necrosis of hepatocytes with mononuclear leucocytes infiltration of the area.

KEYWORDS: Isolates locally Iraq, Antimicrobial, Histopathology, virulence factor, *Aeromonas hydrophila.*

INTRODUCTION

Aeromonas species are micro-organisms It is found in aquatic enviroments such as ground water, drinking water (mineral and tap water), marine water, saline water, clean rivers, lakes,

storage reservoirs and waste water.[1,2,3,4,5] *Aeromonas hydrophila* has been recognized as normal gut flora of aquatic and terrestrial organisms.[6,7] In addition, it is an important opportunistic pathogen for fish[8,9]and associated with a wide from environmental, *Aeromonas hydrophila* is isolated from various foods such as sea food, raw milk, beef, pork, lamb and poultry.[10,11] Oxidase and catalase positive ,urease negative.[12,13] *Aeromonas* species produce a variety of virulence factors, including cytotoxic and cytotonic enterotoxins, haemolysins (aerolysins) , proteases, haemagglutinins and lipases.[12,14] *Aeromonas* species, including *A. hydrophila* , *A.caviae* and *Aeromonas sobria.*[13,15] They have been documented in a variety of human illnesses, including septicaemia, Gastroenteritis , Bacteremia , meningitis, wound infections and lung infections.[16,17] *Aeromonas*-associated diarrhoea is sporadic, similar to infections caused by *Vibrio cholerae* non-O1 and non-O139 serogroups.[11]

METHODS

sample collection.

Ten different sites located in Bagdad and sample collection from Tigres river. Water samples were collected in sterile 250 ml glass bottle. Inoculations into selective media were conducted within 24 h after collection of the water samples.[13]

Isolation and identification of bacteria.

100 ml of water sample was inoculated into 100 ml double-strength alkaline peptone-water enrichment media and incubated at 35± 2°C for 24 h. A sample from this enrichment culture was streaked with a loop on Ampicillin dextrin agar (ADA) Supplement with Ampicillin dextrin (1ml/1litre) incubated for 24 h at 35± 2°C.[13] Shape and motility were determined by microscopy. Isolates were tested for the string reaction and Oxidase[13,18], and sensitivity to the vibriostatic agent (O/129) [19,20], by using conventional biochemical tests.[13,20,21] And identified by system Api 20 E and Mini Api 32 E, Salt tolerance was determined by growth of the isolates at 35°C ± 2°C in peptone broth without NaCl and supplemented with (1,2,3,4,6,7% NaCl).[13]

Detection of virulence factor

All isolates were cultured on human blood agar plates to detection haemolysin and incubated for24 h at 35± 2°C. Haemolytic activity of the isolates with human erythrocytes was determined as described previously.[22,23] The amount of released haemoglobin in the supernatant was measured spectrophotometrically at 540 nm ,and blood cells lysis (100%) .

Proteolytic protease activities were examined by the plate assay method use skim milk agar.[24] The DNase agar plates were inoculated with bacterial culture and incubated at 35º± 2C for 24 h. The bacterial colonies were flooded with 5% of toludine blue solution to detection haemolysin.[25] It was performed on it's specific media which prepared (NaCl 1%, Nutrient agar 100 ml sterilize by autocalving after cold at 45 °C and added one egg yolk) as mentioned by.[26] As following: the specific media for detection Lecithinase was inoculated with a singel colony of overnight growth from nutrient agar incubated for (24-48)hr at 37°C.Appearance of white to brown color elongated precipitated zone around colonies which considered a positive result to produces Lecithinase.

Enteropathogenicity and Histopathology

Using the sealed-adult-mouse model using Swiss albino mice weighing about 15–20 g. infections were allowed to proceed for 24 h. The mice were killed and their intestines and liver were aseptically removed. For histopathology was as described by.[27,28] After 24 h post-inoculation, mice were euthanized and sections of small intestine were immediately fixed in 10% neutral buffer formalin. Following fixation, tissue samples were embedded in paraffin, sectioned at 5 μm and stained with haematoxylin-eosin for light microscopic examination.

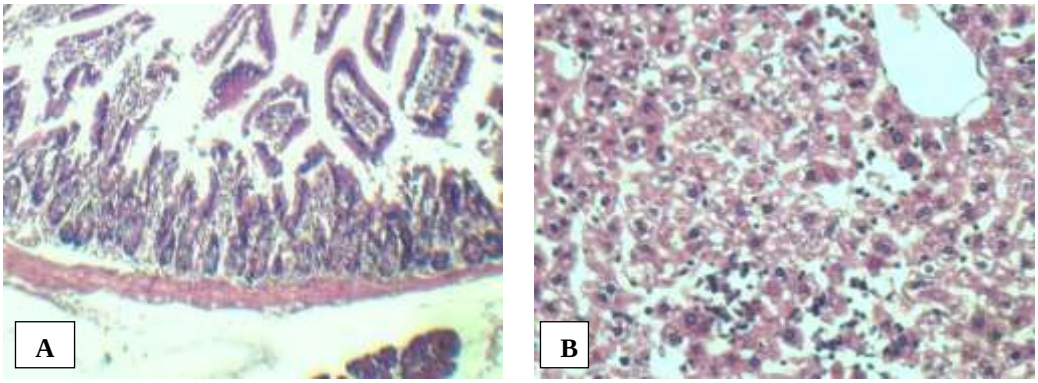
Antimicrobial susceptibility test.

Antimicrobial susceptibility testing was performed by the disk diffusion method[29] with commercially available disks (HiMedia) of 6 antimicrobial drugs: Erythromycin **E(10μg)**,Chloramphenicol **C(10 μg)**,Tetracyclin **TE(30 μg)**, Streptomycin **S (10 μg)**, Nalidixic acid **NA(30 μg)**, Cephalothine **KF (30 μg)** .Bacterial culture for 24 h was compared with the standard turbidily solution (McFarland), this approximately equals to ($1.5x10^8$) cfu/ml. A 0.1 ml of the culture was spreaded on the surface of Mueller-Hinton agar(HiMedia) plates, left to dry for 15 minutes at room temperature. The antibiotic discs were placed on the surface of the medium and incubated at 35º± 2C for 24 h. Isolates were considered susceptible, reduced susceptible or resistant to a particular antimicrobial agent on the basis of the diameters of the inhibitory zones that matched the criteria of the manufacturer's interpretive table, which followed the recommendations of the National Committee for Clinical Laboratory Standards.[30] *E. coli* ATCC 25922 was used for quality control.

RESULTS and DISCUSSION

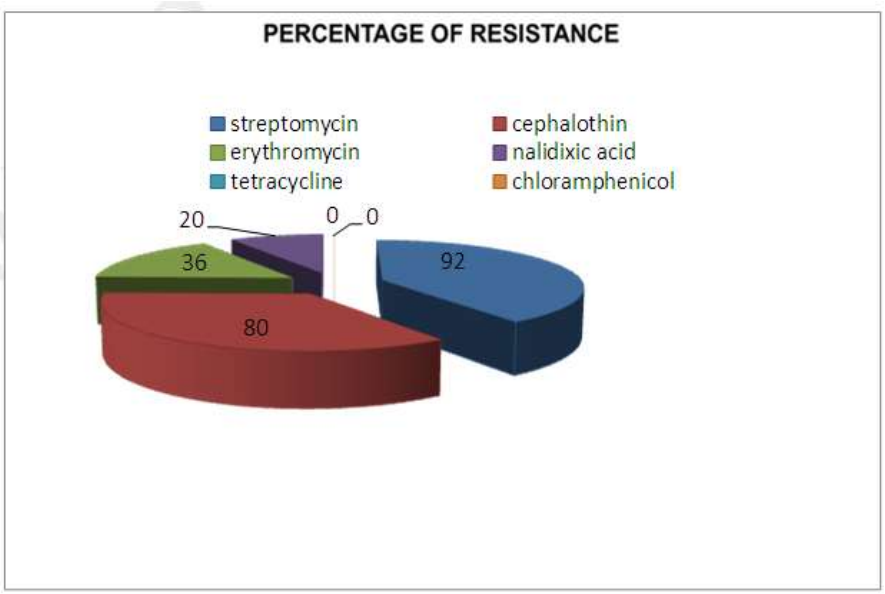
150 samples of natural surface water were collected from 10 sites different , *A. hydrophila* isolated from 25 samples, All the isolates were Yellow or green colonies were picked from the (ADA) agar,Gram-negative, rod-shaped.[13,31,32] identified *A. hydrophila* isolates were positive for biochemical including TSI reactions (acid butt, alkaline slant, H2S negative, and with or without gas production), oxidase, growth in 0 and 1% NaCl , growth in 7% NaCl.[13,19] All isolates were also identified by Api 20 and Api-32 ID system , which included 32 biochemical tests. The obtained results are shown in All of the environmental isolates showed a negative string reaction.[18] This reaction together with resistance to 0/129 disk were used to differentiate Aeromonas from Vibrio which gives a negative result to a was susceptible to 0/129 disk.[13] The ability of the isolates to adhere to host cells was evaluated by agglutination assay using human erythrocytes. All environmental *Aeromonas hydrophila* isolates were tested for their ability to agglutinate human erythrocytes. All isolates of environmental *A. hydrophila* showed agglutination with human erythrocytes .The hemolysis zone (β-hemolysin) appeared around colonies it's meant that colonies have a ability to hemolyzed red blood cells[22,24,30,33] From these results it can be concluded that no particular type of erythrocytes was significantly more sensitive to hemagglutination than the other.[22] After incubated at 24 h flooded of toludine blue solution,colonies showed a rose-pink color surrounding the areas of bacterial growth indicated a positive result that lysis DNA. Colonies showed a brown color surrounding the areas of bacterial growth indicated a positive result Lecithinase and positive result to prodact protease,Colonies showed a halo transparent surrounding the areas of bacterial.[19,22] In the intestine *A. hydrophila* caused, villi atrophy in some regions , and fibrosis in the others with inflammatory cells reached to mucosa , in liver , the effect was obvious by presence of hepatocytes in apoptosis with pyknotic nuclei and wide spread necrosis of hepatocytes with mononuclear leucocytes infiltration of the area.[31] The results showed that in Figure(2).Toxins plays an important role in the pathogenicity of enteric bacteria.[34] *Aeromonas hydrophila* may cause diarrhea by producing many toxins that lead to activation of adenyl cyclase which converts ATP to cAMP, then cAMP causes hypersecretion of sodium, potassium and bicarbonate ions, results in fluids accumulation in lumen of intestine.[35,36] β-hemolysin (aerolysin) is a pore-forming toxin that plays a key role in the pathogenesis of *Aeromonas hydrophila* infections.[22,37] Lecithinase are related to the hydrolysis of lipid barrier in intestinal epithelial cells . Lecithinase causes in free Arachidonic acid from Phospholipids found in cell intestine,and plays an important role in formantation Prostaglandin (PGE2), also it can cause fluid accumulation in the lumen of the

intestine and the induction of diarrhea.[38] Proteases also play an important role in mucous barrier penetration to reach bacteria to surface of epithelial cells that lining small intestine and cause infection.[39]



Figure(2): Histopathology in the (A) tissue samples of intestine and (B) tissue samples of liver . power 200X with haematoxylin-eosin for light microscopic examination.

Six different antibiotic discs were used to perform this test, along with all *Aeromonas hydrophila* isolates. The methods described in NCCLS[30]were followed to determine whether the isolates were resistant or not.The results showed that all isolates were sensitive to chloramphenicol, tetracycline 100%. However, 20% (5 isolates) were resistant to nalidixic acid . Resistance to 80% (20 isolates) to cephalothin . 92% (23 isolates) were resistant to streptomycin. Moreover, 36% (9 isolates) were able to resist erythromycin. (Figure-3). Similar results were observed by.[22]



Figure(3): The Susceptibility of *Aeromonas hydrophila* isolates to aminoglycosides antibiotcs.

It is noted from figure (3) that the environmental isolates have multiresistance to antibiotics.This may suggest that there are more than one mechanism of antibiotic resistance exhibited by *Aeromonas hydrophila* local isolates.They may include low permeability of *Aeromonas hydrophila* cells membrane to many antibiotics[40]or the presence of plasmids that confer resistance to many antibiotic.[41]

REFERENCES

1. Gurwith, M. J. and Williams, T.W.(1977). Gastroenteritis in children: A two-year review in Manitoba. J. Infect. Dis., 136(2): 239-247.
2. Altwegg, M. (1999). *Aeromonas* and *Plesiomonas.* in. Manual of Clinical Microbiology. Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F. C. and Yolken, R. H. 7th (ed.). Vol. 1. Am. Soci. USA., 507-515
3. Brandi, G.; Sisti, M.; Giardini, F.; Schiavano, G. and Albano, A. (1999). Survival ability of cytotoxic strains of motile *Aeromonas* spp. in different type of water. Lett. Appl. Microbiol., 29(4): 211-215.
4. Kuhn, I.; Allestam, G.; Huys,G.; Janssen, P.; Kersters, K.; Krovacek, K. and Stenstrom, T. (1997). Diversity, persistence and virulence of *Aeromonas* strains isolated from drinking water distribution systems in Sweden. Appl. Environ. Microbiol., 63(7): 2708-2715.
5. Villari, P.; Crispino, M.; Montuori, P. and Boccia, S. (2003). Mole- cular typing of *Aeromonas* isolates in natural mineral waters. Appl. Environ. Microbiol., 69(1): 697-701.
6. Snower, D.; Ruef, C.; Kuritza, A. and Edberg, S. (1989). *Aeromonas hydrophila* infection associated with the use of medicinal leeches. J. Clin. Microbiol., 27(6): 1421-1422.
7. Santos, Y.; Toranzo, A. E.; Barja, J. L.; Nieto, T. P. and Villa, T. G. (1988). Virulence properties and enterotoxin production of *Aeromonas* strains isolated from fish. Infect. Immun., 56(12): 3285-3293.
8. Filler, G. and Ehrich, J. H. (2000). Acute renal failure in an infant associated with cytotoxic *Aeromonas sobria* isolated from patient's stool and from aquarium water as suspected source of infection. J. Clin. Microbiol., 38(1): 469-470.
9. Leung, K.Y. and Stevenson, R. M. W. (1988). Tn5-induced protease-deficient strains of *Aeromonas hydrophila* with reduced virulence for fish. Infect. Immun., 56(10): 2639-2644.

10. Kingombe, C. B.; Howald, D. and Jemmi, T.(2000). Abklaving der epidemiologie von Aeromonaden and Plesiomonaden in fleisch and fleis chwaren und ihre bedeutung als lebensmittelverjifter. Projekt., 1: 95.02
11. Sinha, S., Shimada, T., Ramamurthy, T., Bhattacharya, S. K., Yamasaki, S., Takida, Y. & Nair, G. B. (2004). Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. J Med Microbiol., 53: 527–534
12. Merino, S., Aguilar, A., Nogueras, M. M., Regue, M., Swift, S. & Tomas, J. M. (1999). Cloning, sequencing, and role of two phospholipases (A1 and C) from mesophilic *Aeromonas* sp. serogroup O:34 . Infect Immun., 67: 4008–4013
13. Eaton, A.; Greenberg, A.E.; Rice, E.W. and Clesceri, L. S. (2005) .Stander method for the examination of water and wastewater .21 ed
14. Merino, S., Aguilar, A., Nogueras, M. M., Regue, M., Swift, S. & Tomas, J. M. (1999). Cloning, sequencing, and role of two phospholipases (A1 and C) from mesophilic *Aeromonas* sp. serogroup O:34. Infect Immun., 67: 4008–4013.
15. Hiransuthikul, N., Tantisiriwat, W., Lertutsahakul, K., Vibhagool, A. & Boonma, P. (2005). Skin and soft-tissue infections among tsunami survivors in southern Thailand. Clin Infect Dis., 41: e93–e96.
16. Murray, P. R. ;Baron, E. ;PFaller, M. A.;Tenovor, F.C. and Yolke, R. H. (2000) . Manual of clinical Microbiology (7th ed .) ASM press , Washington Unite State of America., 304.
17. Janda, J. M. & Abbott, S. L. (1998). Evolving concepts regarding the genus *Aeromonas:* an expanding panorama of species, disease presentations, and unanswered questions. Clin Infect Dis., 27: 332–344
18. Smith, H. L., Jr (1970). A presumptive test for *vibrios*: the ‘string’ test. Bull World Health Organ., 42: 817–818.
19. Abbott, S. L.; Cheung, W. K. and Janda, J. M. (2003). The genus *Aeromonas*: Biochemical characteristics, atypical reaction, and phenotypic identification schemes. J. Clin. Microbiol., 41(6): 2348-2357.
20. Holt, J. G.; Krieg, N. R.; Sneath, P. H; Staley, J. T. and Williams, S. T. (1994). Bergey’s Manual of Determinative Bacteriology. 9th (ed.). Williams & Wilkins, U.S.A., 190-191.
21. Fobes, B. A.; Sahm, D. F. and Weissfeld, AS. (1998). Bailey & Scott’s Diagnostic Microbiology. 10th (ed.). Mosby, Inc. USA., 488-499.

22. Muslim ,S. N. (2005) . Biochemical and Genetic Studies on Hemolysin Produced by *Aeromonas hydrophila* Isolated from Diarrheal Patients and from Surface Water. ph.D. Thesis. Biology/Microbiology. University AL-Mustansirya, college of science.
23. Ramamurthy, T., Bag, P. K., Pal, A., Bhattacharya, S. K., Bhattacharya, M. K., Sen, D., Shimada, T., Takeda, T. & Nair, G. B. (1993**).** Virulence patterns of *V. cholerae* Non-O1 isolated from hospitalized patients with acute diarrhoea in Calcutta, India. J Med Microbiol., 39: 310–*317.*
24. Nader, M.I.; Melcnian, A. K.; Aziz, G.M. (2009).Production of Protease Enzyme from locally bacteria *Aeromonas hydrophila* GMA1 isolated from patients infected with diarrhea .Iraqi Journal of Biotechnology., 8(1): 142-155.
25. Atlas, R.; Parks, L. and Brown, A. (1995). Laboratory Manual of Experimental Microbiology. 1st (ed.). Mosby, Inc. Missouri., 87-110.
26. Baron, E. J. ;Petersond, L.R. and Finegold, S. M. (1994).In Bailey & Scotts Diagnostic Microbiology. 9th (ed.). Mosby.louis,Baltimore., 429-430.
27. Richardson, S. H., Giles, J. C. & Kruger, K. S. (1984). Sealed adult mice: new model for enterotoxin evaluation. Infect Immun., 43: 482–486.
28. Chang, C. & Miller, J. F. (2006). Campylobacter jejuni colonization of mice with limited enteric flora. Infect Immun., 74: 5261–5271.
29. Baron, E. J. and Finegold, S. M. (1990) . Diagnostic Microbiology. 8th (ed.).The C.V. Mosbycomp. USA.
30. National Committee for Clinical Laboratory Standards. /NCCLS. (2002). Performance Standard for Antimicrobial Susceptibility Test. Twelfth information supplement.
31. Bhowmik,P.; Bag, P.K.; Hajra, T.K.; De, R.; Sarkar, P.and Ramamurthy,T. .Pathogenic potential of *Aeromonas hydrophila* isolated from surface waters in Kolkata, India .J Med Microbiol., 2009; 58(12): 1549-1558
32. Kühn, I., Albert, M. J., Ansaruzzaman, M., Bhuiyan, N. A., Alabi, S. A., Huys, G., Islam, M. S., Janssen, P., Kersters, K. & other authors (1997). Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls and from surface water in Bangladesh. J Clin Microbiol 35, 369–373.
33. Elliot,E.L.;Kaysner,C.A.;Jackson,L. andTamplin ,M.L. (2001).*Vibrio cholerae* , *Vibrio parahemolyticus*, *Vibrio valnificus* and other *Vibrio spp*. In:Food and Drug a dministration : Bacteriological Analytical Manual, chapter 9 , 8th ed .Edited by Merker ,R.L. ,AOAC International ,Gaithers burg .MD.

34. Nataro, J. P., Sears, C., Fasano, A. & Bloch, R. J. (2003). Enteric microbial toxins and the intestinal epithelial cytoskeleton. In Microbial Pathogenesis and the Intestinal Epithelial Cell, 301–332. Edited by G. A. Hecht. Washington, DC: American Society for Microbiology.
35. Mahon,I. and Manuselis, K. (2000). Medical Microbiology.(Ch.17). Mosby-year book. Europe.
36. Sha, J.; Kozlova, E. V. and Chopra, A. K. (2002). Role of various enterotoxins in *Aeromonas* hydrophila-induced gastroenteritis: Generation of enterotoxin gene-deficient mutants and evaluation of their enterotoxic activity. Infect. Immun., 70(4): 1924-1935.
37. Figueroa, A. P.; Heuser, J. E.; Akopyants, N. S.; Morisaki, J. H.; Giono, C. S.; Enriquez, R. F. and Berg, D. E. (2001). Cell vacuolation caused by *Vibrio cholerae* hemolysin. Infect. Immun., 69(3): 1613-1624.
38. Oliver,J.D. and Kapper ,J.B. (1997) .Vibrio species . In:Food microbiology :fundamentals and frontiers .Edited by Doyl,M.P.;Beuchat,L.R. and Montiville,T.J. ,ASM Press , Washington DC.,USA., 228 – 260.
39. *Silva, A. J.; Leitch, G. J.; Camilli, A.; Benitez, J.A. (2006) .Contribution of hemagglutinin/protease and motility to the pathogenesis of ElTor biotype V.cholerae .Infect .Immun., 74: 2072 – 2079.*
40. *Zemelman, R.; Gonzalez, C.; Mondaca, M.; Silva, J.; Merino, C. and Dominguez, M. (1984). Resistance of Aeromonas hydrophila to beta-lactam antibiotics. J. Antimicrob. Chemother., 14(6): 575-579*
41. *Shannon, K.; King, A. and Phillips, I. (1985). In vitro antibacterial activity and susceptibility to beta- lactamases. J. Antimicrob. Chemother*., 15(Suppl C): 15-23.