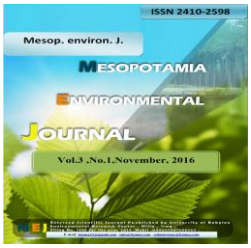
Mesopo. Environ. j., Special Issue C :121-129, 2017
ISSN 2410-2598
proceeding of 1st National conference of science and Art
University of Babylon

Mesopotemia Environmental journal
journal homepage:www.bumej.com



The study characters of *Aeromonas hydrophila* on some media

Lubna Abdul MuttalibAl-shalah

Environmental research center / Babylon university , Iraq .

Corresponding Author: lubnamsa77 @gmail.com.

To cite this article:

Al-shalah. L. AM, The study characters of *Aeromonas hydrophila* on some media. Mesop. environ. j., 2017, Special Issue A.;121-129 .

Abstract

The aim of this study was to investigate the study characters of *Aeromonas hydrophila* on some media +isolated from clinical sources in Hilla city, Iraq. A total of 822 samples were collected from fecal specimens from patients. Samples were collected from rectal swab and from those who suffering from diarrhea. The period of the research was from October 2013 to February 2014 at public health lab, Hilla city. Results of this study revealed that out of 822 fecal samples, 13 isolates (1.58%) were belonged to Aeromonas spp. However, other bacterial isolates belonged to other genera similar to *Aeromonas* were also recovered. Out of 13 Aeromonas spp., eight *A. hydrophila* isolates (61.53%) were obtained, while the other isolates were distributed as: four isolates of*A. salmoncidia* (30.76%), and one of *A. sobria*. Isolation and detection of *A. salmoncidia* species was first recorde in Iraq. The efficacy of routine enteric agars for supporting the growth of *Aeromonas hydrophila*, MacConkey, and xylose lysinedeoxycholate appeared to be the most satisfactory routine agars for Aeromonas spp. recovery when used inconjunction with blood agar.bacterial isolation showed agood growth of *A.hydrophila* on TCBS medium .

Mesopotamia Environmental Journal **ISSN 2410-2598**
Mesop. environ. j. 2017, Special Issue C.;121-129
(proceeding of 1st National conference of science and Art –University of Babylon)

Introduction

Diarrhea disease is an important cause of morbidity and mortality indeveloping countries, particularly in infants and elders [1].

Diarrheas are produced by viral, bacterial, and parasitic infections, as well asfood intolerances, reaction to medicines, and other physiological andimmunological disorders. Bacterial infections are responsible for 20-40% ofdiarrhea illness, and several bacterial species have been frequently ascribed todiarrhea episodes, including *Campylobacter jejuni E. coli*, *Salmonella* spp.,*Vibrio cholera*, *Yersinia enter Aeromonas* spp., and *Plesiomonas* spp.[2].

In the last decades, *Aeromonas* have been increasingly recognized as relevantetiological agents in gastrointestinal infections [3].*Aeromonas* species are pathogens that cause foodborne gastroenteritis in humanand extraintestinal symptoms such as septicemia, meningitis, endocarditis [4,5].Among *Aeromonas* species, *Aeromonas hydrophila* is the mostcommonly associated with human infections, leading to intestinal and non-intestinaldiseases. Furthermore, increased resistance of this organism toantibiotics and chlorination in water presents a significant threat to public health. [6,7].Isolation of *A. hydrophila* from contaminated samples such as feces requirethe use of selective and differential plating media such as MacConkey agar,cefsulodinirgasannovobiocin (CIN) agar, or blood ampicillin agar (10 mg/lampicillin). To facilitate recovery of *A. hydrophila* from heavily contaminatedspecimens such as feces, used different media for the isolation,and differential this bacteria from other bacteria.

Material and methods

Collection of fecal samples

This cross sectional study was designed to assess the occurrence of *A. hydrophila* in patients with diarrhea. A total of 822 fecal samples were collected. They were collected from rectal swab (routine work) and from patients suffering from diarrhea who attending public health lab, Hilla city, Iraq.

Specimen collection and analysis was carried out from October 2013 to February 2014.

Isolation and identification of bacterial isolates

All specimens were cultured on alkaline peptone water, then transfer to TCBS and MacConkey agerby swabbing and incubated at 37ºC for 24 hr. Each primary positive culture identified depending on the

morphological properties such as (Shape, swarming, odor and lactose or non-lactose fermentation on MacConkey) [9].Different Biochemical tests were used for identification of bacterial isolates according to standard methods [9,10] The Vitek 2 system was used to confirm the biochemical test according to the manufacturer's instructions.

Results and discussion

Isolation of *Aeromonas hydrophila*

Results of this study revealed that out of 822 clinical sample 13 isolates (1.58%) were belonged to *Aeromonas* spp., however other bacterial isolates belong to other genera similar to *Aeromonas* were also recovered (Table 1).

Table 1: The percentages of *Aeromonas* spp. recovered from fecal specimens

Sample	Bacteria type	NO. of isolates	%
Positive	*Aeromonas* spp.	13	1.58%
Negative	*Pseudomonas* Spp., *Pantoea spp*. and *Proteus* Spp. and E*nterobacter cloacae*	809	98.4%
Total		822	100 %

The low isolation rate of this bacteria 1.58% may be attributed to the fact that most cases of diseases caused by this bacteria are occurred usually in warm months , while the collection of samples in this study was in cold months. Agge*et al.* 1985 [11] reported that *A. hydrophila* similar to other enteric pathogens was seen more often in hot months. the frequency of *A. hydrophila* cases during warm months was 1.25 case per month, whereas during cold months it was 0.83 case per month[12] .

The other reason of this result may be due to fact that *A.hydrophila* infects mainly children, elderly, and immunocompromised persons, while this study was focused on subjects of youth age group. Most studies are

focusing isolating bacteria from feces of children only, and the fact that bacteria *Aeromonas* occur in children under two years at high rates, because of lack immune system completely, and abase infant formula of milk plays role in promoting the growth and reproduction of bacteria.

In a local study, Obaid (2013)[12] reported that 2.7% of *A. hydrophila* isolates were recovered from 479 patients from different ages and sexes. Naji (2013)[13] isolated this bacteria from children, the isolation rate of *A. hydrophila* was 4.08%. However, several authors found higher isolation rate of *Aeromonas* from clinical cases. AL-Fathlawy (2012) [14]obtained 20.17% of *A. hydrophila*from clinical and environmental sample. On contrast, Borchardt*et al* (2003)[15] showed low isolation rate (0.66%) of *A.hydrophila*among 2565 diarrheic stool specimens submitted to a Wisconcin clinical reference laboratory.

Results showed that (8) isolates were diagnosed as *A. hydrophila* (61.53%), while the other isolates were distributed as (4, 30.7%) *A. salmoncidia* and (1, 7.6%) *A. sobria*.

Result of isolation rate in the present study was similar to many studies conducted worldwide,Kannan*et al.*,(2001) [16] which found that the isolation rates of *A. hydrophila* were 60%, and 58.8% respectively, also he found that several species of *Aeromonas* were detected from acute diarrhea which were *A. caviae* (20%), *A. veronii* (10%), *A. schubertii* (4%), *A. jandaei* (3%), and *A. trota* (3%).

A. hydrophila and *A. sobria* tended to cause acute infection in human [17], while *A. salmonicida* cannot grow at 37°C, it is not pathogenic to humans [18].

Authors also referred to isolate *Aeromonas* spp. from different clinical specimens like blood (63%), wounds (11%), ascites (9%), feces (8%), and bile (3%). In addition to different unknown body sites [19]

3.2 Identification of *A. hydrophila*

Members of the genus *Aeromonas* are not difficult to isolate from clinical specimens in the diagnostic laboratory, but are often misidentified as belonging to the genus *Vibrio* or *Plesiomonas* [19]. Results of the phenotypic characteristics of the colonies *Aeromonas* has shown conformity with what was reported by[20,21 and,22].The microscopic examination of the bacteria stained by gram stain showed that the cells were gram negative, rod shaped, and the cells appeared singly to pairs, or as short chains [23].

Results ofbiochemical tests carried out for identification of isolates were compared with standard methods [11,9]. All isolates were positive for oxidase, and catalase .Oxidase test is used for differential of *A. hydrophila* from other enteric bacteria. Results also found that *A. hydrophila* isolates had the ability to ferment glucose on Kligler iron ager (Alk/acid). They appeared positive to heamolysis test, motility test, and

utilization of citrate, but they were negative to string test and urease test. String test is used to differentiate between *A.hydrophila* and *V. cholera* isolates[24]. Identification of *A. hydrophila* was confirmed using Vitek 2 system. Out of 13 *A.hydrophila* isolates (identified using biochemical tests), only 8 isolates was identified as *A.hydrophila.* The other 5 isolates were identified as *A. soberi* (1 isolate) and *A. salamoncidia* (4 isolates) had showed 85- 99% identification percentage probability. This study appeared that most of this media have high efficient to support growth of *A. hydrophila* .bacterial isolation showed a good growth of *A. hydrophila* on TCBS medium and isolates produced yellow colonies /green color due to sucrose fermentation, with diameter of colonies ranged from 2-3 mm, while on blood agar, colonies appeared dark grey color beta- hemolytic (Figure 1). On the MacConkeyagar formed relatively small pale colonies is non- lactose fermenter table (2). also *A. hydrophila* showed good growth in anaerobic condition because, *Aeromonas hydrophila*facultative anaerobic, that is known to be pathogenic in humans [25].on SS agar the colonies of A. hydrophila appear clear ,colorless and transparent , and XLD agar colonies appear yellow to yellow -red .Mathur,etal (2003) showed in his research out of 71 *Aeromonas* isolates, 10 were sensitive toampicillin and all these grew on MacConkey agar. The61 isolates growing on ASBA were haemolytic.

Table 2: Characters of *A. hydrophila* isolates on different culture media

Medium	**Characters of colonies**
TCBS agar	yellow shin with diameter ranged from (2-3)mm.
MacConkey agar	As pale like shaped indicated that *A.hydrophila* is unable to ferment lactose sugar
Blood agar	smooth, convex, rounded and β-hemolytic colonies and pale white to grey color
XLD ager (Xyloz	colonies yellow toyellow-red
SS ager(Salmonella – shigella)	Clear, colorless and transparent

Mesopotamia Environmental Journal **ISSN 2410-2598**
Mesop. environ. j. 2017, Special Issue C.;121-129
(proceeding of 1st National conference of science and Art –University of Babylon)

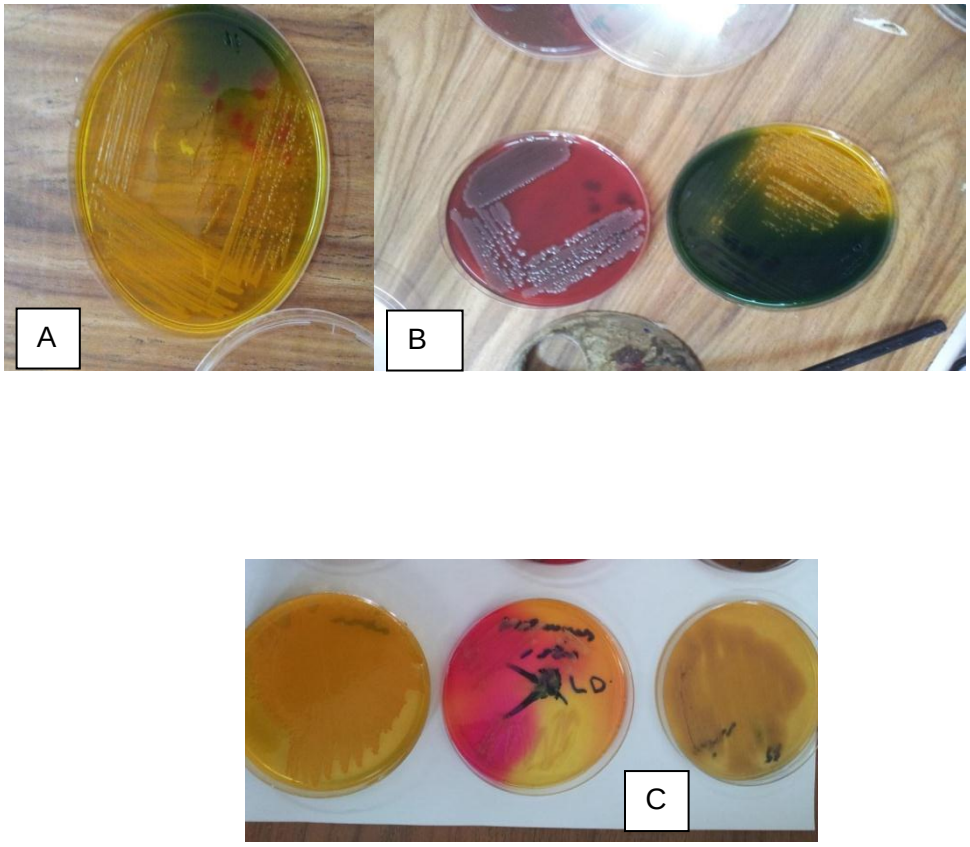


Figure 1: Characters of *Aeromonas hydrophila* on (A) TCBS agar, (B) Blood agar,(C)SS agar,XLD agar .

Reference

[1] Mansour A.M., Abd-Elkhalek R., Shaheen H., El-Mohammady H., Hassan K.,Riddle M.; Sanders J. W., Sebeny P.J., Refaey S.; Sylvia Y.N. Burden of*Aeromonas hydrophila* associated diarrhea among children younger than 2 years in rural Egyptian community. J Infect Dev Ctries., 6 (12):842-846, 2012.

[2] **Vila O., Ruiz J., Gallardo F., Vargas M.S. L., Figueras M. J., and Gascon J.** *Aeromonas* spp. and Traveler's Diarrhea: clinical features andantimicrobial resistance. Emerg. Infect. Dis., 9 (5): 552-555, 2003.

[3] Guerra, I.M. F., Fadanelli-Raquel F.M. , Schreiner F. D. ; Anapaula L. ;WollheimC.C.; Sergio O. P. *Aeromonas* associated Diarrheal disease in south Brazilprevalence virulence factors and antimicrobial resistance. Braz. J. Microbiol., 38:638-643, 2007.

[4] Mansour A.M., Abd-Elkhalek R., Shaheen H., El-Mohammady H., Hassan K.,Riddle M.; Sanders J. W., Sebeny P.J., Refaey S.; Sylvia Y.N. Burden of *Aeromonas hydrophila* associated diarrhea among children younger than 2 years inruralEgyptian community. J Infect Dev Ctries., 6 (12):842-846, 2012.

[5] Ramalivhana N.J. Molecular characterization of *Aeromonas hydrophila*and antimicrobial activities of selected medicinal plants against pathogenic isolatesfrom water and stool samples in era of HIV/AIDS in Limpopo province, southAfrica. University of south Africa .Ph.D thesis, 2010.

[6] Ashira A.W., Uaboi –Egbeni P.O., Oguntowo J.E., and Idika C. N. (2011).Isolation and antibiotic profile of *Aeromonas* species from Tilapia fish (*Tilapianilotica*) and cat fish (*Clariasbetrachus*). Pak. J. Nut.,10 (10): 982 -986, 2011.

[7] Ferwana N.I. Occurrence of *Yersinia enterocolitica* and *Aeromonashydrophila* in clinical, food and environmental samples in Gaza strip. M.Sc thesis, College of the Islamic University-Gaza, Deanship of Graduate Studies, Faculty ofScience ,Biological Sciences Master Program, Microbiology, 2007.

[8] Das A., Vinayasree V., Santhosh C.R., and Sree Hari S. Surveillance of *Aeromonas sobria* and *Aeromonas hydrophila* from commercial food stuffs andenvironmental sources. J. Experimental Sci., 3(9): 36-42, 2012.

[9] ErdemB., Kariptas E., Cil E., and Isik K. Biochemical identification and numerical taxonomy of *Aeromonas* spp. isolated from food samples in Turkey. A thesis of Science, Artsand Science Faculty, Evraw Univ., 35: 463 -472, 2011 .

[10] Sarkar A., Saha M. and Roy P. R. Identification and typing of *Aeromonas hydrophila* through 16 s DNA – PCR finger printing. J. Aquacult. Res. Der 3:6, 2012.

[11] Abbott S.L., Cheung W.K.W., and Janda J.M. .The Genus *Aeromonas*: Biochemical characteristics, Atpyical Reactions ,and phenotypic Identification Schemes. J. Clin. Microbiol., 41 (6): 2348-2357, 2011.

[12] Igbinosy I.H., Tom M., Ignmbor E.U., Okoh A. ; and Ighdasi F. Emerging *Aeromonas* species infections and their significance in public Health.The Scientific world J. doi :10.1100 /2012/ 625023, 2012.

[13] Markov G., Kirov G., Lyutskanoy V., and Kondarer M. NocrotizingFascitis and Myonecrosis due to *Aeromonas hydrophila*. Wounds,19(8): 223-226, 2007.

[14] Aminov, R.I., Garrigues-Jeanjean, N. and Mackie, R.I. (2001).Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Applied and Environmental Microbiology 67, 22–32, 2001.

[15] Brenner D.J., Krieg , N.R. and Staley ,J.S. .Bergeys manual of systematic bacteriology .vol ,2 ,2nd edition . partB, pp.556-578, 2005.

[16] Forbes,B.A., Daniel , F.S. and Alice ,S. W. Bailey and Scotts diagnostic microbiology .12 th ed. , Mosby Elsevier company ,USA, 2007.

[17] MacFaddin, J.K. Biochemical test for identification of medical bacteria. 3rd ed. Lippincott Williams and Winkins. A Wolter Kluwer Company. Philadelphia Baltimor. New York, 2000.

[18] Clinical and Laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty third informational supplement, 2005.

[19] Aggeri W.A., McCormick J.D ., and Gurwith M.J. Clinical and Micro-biological feature of *Aeromonas hydrophila* associated diarrhea.J. Clin. Microbiol., : 909-913, 1985.

[20] Wong H. Enteropathogenic Aeromonads. Department of Microbiology, Soochow University, 2007.

[21] Obaid ,A.J S-Layer role of *Aeromonas hydrophila* isolated from diarrhea specimens . . M.Sc thesis, College of Science, University of Babylon, 2013.

[22] Naji,N.Q. Dissemination and Immunological properities of *Aeromonas hydrophila* isolates from children with diarrhea in AL-Muthana province. . M.Sc thesis, College of Science, University of AL-Muthanna, 2013.

[23] AL-Fatlawy H.N.K. Bacteriological and Molecular study of *Aeromonas hydrophila* isolated from clinical and Environmental samples. M.Sc thesis, College of Science, University of Kufa, 2012.

[24] Borchardt M. A., Stenper M. E., and Standridge J.H. *Aeromonas* isolates from Human Diarrheic stool and Groundwater compared by pulsed –field gel electrophoresis. Emerging infectious Diseases, 9 (9), 2003.

[25] Moyer N.P. Clinical significance of *Aeromonas* species isolated from patients with diarrhea. J. Clin. Microbiol., 2044-2048, 1987.

[26] MathurM., Saraswathi A De,K., Varaiya A., Athalye S .VIBRIONACEAE FROM CASES OF ACUTE DIARRHOEA AND THEIRANTIMICROBIAL SENSITIVITY PATTERN - A FIVE YEAR PROSPECTIVE STUDY .Indian Journal of Medical Microbiology, *21 (3):199-201*, 2003.