Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.11, 2014

PCR Detection of Putative Hemolysin and Aerolysin Genes in An *Aeromonas Hydrophila* Isolates from Diarrhea in Babylon Province

Wejdan, R. Taj Aldeen [1] ,Abeer,F.,Al-rubaiae[2], Oruba,K.H.,Al-bermani[2] , Noor,S.Naji[1]
1Biology department ,science college ,Babylon university,2 biology department college of science for women Babylon university
Corresponding auther:1wejdan.tajeldeen@yahoo.com

Abstract
Aeromonas spp are considered as apportunistic infection and enterotoxgenic pathogen and can cause severe diarrhea . A total of one hundred and forty two stool samples were collected from patients with diarrhea at Babylon hospital for maternity and pediatrics of a period between December 2012 to June 2013.The 6(4.22%)strains of *Aeromonas hydrophil*a were isolated from 142samples of diarrhea stools .The *Aeromonas hydrophila* identified based on colony and microscopic morphology ,biochemical tests and confirmed by Api 20E .Then investigation was done on hemolysin ,All the isolates of *Aeromonas hydrophila* have the β-hemolytic activity on the blood agar .the polymerase chain reactions carried out to detect the presences of hemolysin and aerolysin genes in 6 *Aeromonas hydrophila* isolates .A100%(6 isolates)of *Aeromonas hydrophila* isolates were contained hemolysin genes (*ahh* 1a)and 50%(3 isolates) were contain aerolysin gene (*aer*A).the band appearance in amplified gene bacteria shows molecular weight of hemolysin (130 p) and the molecular weight of aerolysin (309 bp) .
Key word:*Aeromonas*, aerolysin, hemolysin, PCR

1.Introduction

Aeromonas spp are Gram negative ,short rod shape ,facultative anaerobes resistance to O/129 vibriostatic &non spore forming (Alperi *et al* .,2010).they are ubiquitous microorganisms found in both aquatic and environment al habitats such as estuary sediment ,sea water , sea grass, sea food, water used water , food and drinking water (Abbott *et al* ., 2003; Matyer *etal* ., 2007 ; Martinez Mucia *et al .,*2000). Aeromoanads are frequently isolated from different food and drinking water (Ottaviani *et al.,*2011) *Aeromonas* spp. Are considered as opportunistic infections and exterogenic pathogen, Aeromonas can cause sever diarrhea ,dysentery and bactermia (Trower *et al .,* 2000; Blair *et al.,* 1999). Virulence of *Aeromonas* spp. is multifactorial and incompletely understood. Factors contributing to virulence include toxins, proteases, hemolysins, lipases, adhesins, agglutinins, and various hydrolytic enzymes (Janda and Abbott 1996). Virulence factors are present in two forms, cell-associated structures, and extracellular products The main route of transmission for Aeromonas gastroenteritis is considered to be fecal oral. Studies on the etiology of travelers' diarrhea revealed *Aeromonas* to have a prevalence of about 3% in diarrheic patients returning from Asia and Africa (Shah *et al*.,2009). Among other clinically relevant aeromonads like *Aeromonas caviae*, *A. trota,* and *A. veronii* biovar *sobria,* the most frequently isolated pathogen *A. hydrophila* is mainly associated with diarrheal illness accompanied by abdominal pain and nausea (Adamki *et al* ., 2006). virulence factors of aeromonas spp. Including toxine , protease S , hemolysin ,lipase ,adhesin ,agglutinins and various hydrolytic enzymes (Janda and Abott,1996).
A. hydrophila is mainly associated with diarrheal illness is accompanied by abdominal pain and nausea (Adamki *et al* ., 2006).
A.hydrophila is the most commonly involved in human infection such as septicemia and gasteroenteritis(Chopra and Houston ,1999). The pathogenicity of a. hydrophila infection by producing virulence factores such as cytotoxin , protases , S- layer and aerolysin (Rahaman *et al.,* 1997).Some researcher , states the virulence factors are determinant of bacterial pathogenicity (Vadivelu *et al* ., 1995).the virulence of A.hydrophila is closely related to β-haemolysin produced . Screening of hemolysin genes the most effective way to detecting and characterizing aeromonas virulence factores (Yousre *et al.,* 2007)
Two hemolytic toxine have been described the *A.hydrophila* a hemolysin (*hyl* A(Hirono &Aoki,1991).and aerolysin (*aer* A(Howard *et al*., 1987). Aerolysin produced by some *Aeromonas* sp. And posses both hemolytic and enterotoxic activity (hemolytic enterotoxine)(Xu *et al*., 1994).
The present study was therefore carried out to document the presence of pathogenic *A. hydrophila* in diarrheal stool samples at Bayblon province /Iraq . Two hemolytic toxins , hemolysin and aerolysin have been described in *A.hydrophila* . no report is available from Babylon province., in this study a search was made for the presence of hemolysin and aerolysin genes in the genome of *A. hydrophila* isolated from diarrhea specimens .

2. Materials and methods.

2.1 Collection of samples

one hundred and forty two (142) Stool samples were collected from patients attending to Babylon hospital for maternity and pediatrics during the period from Dec 2012 to April 2013 .Samples were collected in screw capped bottles and transported to the laboratory in ice box with ice packs .Information was also obtained from patients regarding age groups . All samples were analyzed within 8 h. for collection .

2.2Isolation of Aermonas hydrophila

One gram of each sample was briefly emulsified in 3ml of sterile 0.85%(w/v) saline and vortexed for 30 sec. Organ debris was allowed to settle down for 5 min .the samples were put it in alkaline peptone water (oxoid pH 9)and sub cultured after incubation at 37C for 6 h. onto to macconkey agar and aeromonas agar at 37C for 24 h .(Nzeaka *et al*.,2002; Jatau and Yalubu, 2004). All the isolates were grown on trypticase soy agar (TSA) at 37° for 18 h .the strians first identified as *Aeromonas* spp. According to colony morphology on Macconkey and Aeomonas agar and by microscopic Morphology(gram stian)and by chemical tests (Oxidase ,Catalase,Motility ,H_2S production ,Citrate utilization ,Indole ,Methyl red &Vogas Proskure,)and by String test ,Lysine decarboxylase ,Argnine dehydrogenase, Ornithine decarboxylation for differentiated from *Vibrio cholera* and the diagnostic of these strians confirmed by Api 20E (Biomerieux,france)

2.3Hemolytic activity

The strains were tested for β-hemolytic activity on a the blood base agar (oxoid)supplement with 5% sheep erythrocytes .five micro liters of each suspension was streaked onto plates and incubated at 22 C and 37C for 24 h. the presence of clear colorless zone surrounding the colonies indicated β-hemolytic activity (Gerhardt *et al*., 1981).

2.4Oligonucleotide primers and PCR conditions

The polymerase chain reaction (PCR)was used to detect the presence of hemolysin and aerolysine in all *Aeromonas* isolates . the primer used for hemolysine gene and aerolysine gene (table 1)The *ahh1*a primer set was designed to amplify a 130-bp fragment of *A. hydrophila* extracellular hemolysin gene *ahh1* (Wang *et al* .,2003). The AH-*aer*A primer set amplified a 309-bp fragment of the *A. hydrophila* aerolysin gene *aer*A (Wang*etal*.,2003).

TABLE(1)Primer pairs used for PCR amplification

Primer pair	Sequence (5to 3)	Target gene	Size of PCR amplicon (bp)	Reference or GenBank accession no.
AHH1F AHH1R	GCCGAGCGCCCAGAAGGTGAGTT GAGCGGCTGGATGCGGTTGT	*ahh*1a	130	Wang *et al*.,2003
AH-aerAF	CAAGAACAAGTTCAAGTGGCCA ACGAAGGTGTGGTTCCAGT	*aer*A	309	Wang *et al*.,2003

PCR was carried out on cycler using the following cycle :preheating at 95 C for 5 min followed by 30 cycles at 95C for 2 min ,55 C° for 1min and 72 C for 1 min followed by 7min final extension at 72 C .PCR products were examined by electrophoresis in 1.5 %agrose gel in TBE buffer .the gel stained with EtBr and saw under U.V. ligt (Yogananth *et al.,*2009)

2.5DNA extraction

DNA extraction from gram negative bacteria was performed according to the genomic DNA purification kit supplemented by the manufacturing company(Gene aid) and it was stored in 2-8 C .

3.Results

3.1 *isolation of Aeromonas hydrophila.*

Out of the one hundred and forty two (142) diarrheic stool samples analyzed .6(4.22%) were found to be positive for *Aeromonas hydrophila*. The prevalence per age groups as shown in figure (1) showed the age groups ,<6years having height rate of 2.11%(3 isolates) from total samples analyzed .age groups 7-12years having 1.40%(2 isolates)and >13 years having 0.70%(1 isolate).

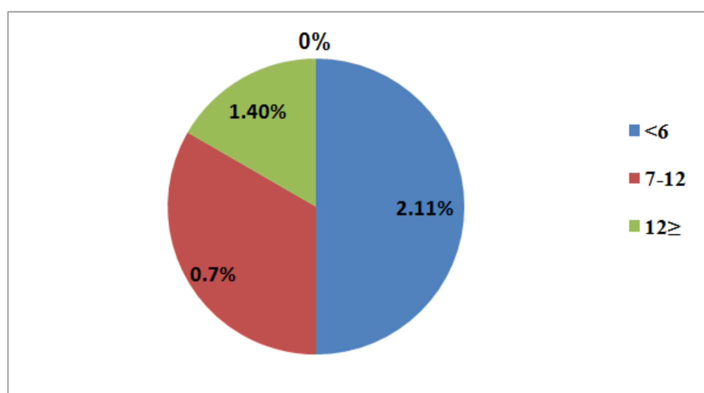

Figure (1) prevalence of the *Aeromonas hydrophila* in diarrhea samples according to age groups and percentage from total samples

3.2 Identification of *A.hydrophila*

The colonies of bacteria grown in culture media appeared 1-3 mm in diameter. *A.hydrophila* showed a yellow shine color on TCBS agar and non lactose fermenters on Macconkey agar . and it was smooth ,convex ,rounded ,β-hemolytic colonies and pale white to grey color on blood agar . this bacteria appeared gram negative, rod shaped, singly ,in pairs ,or even as short chains at the microscopic examination The biochemical tests used to confirmed the initial diagnosis of *A.hydrophila* (table 2) . *A.hydrophila* presented appositive result to each of the oxidase , catalase , indole , methyl red , simmon citrate,motility test,vogas prokauer and gelatin liquefaction this results

Table 2:- biochemical tests of *A. hydrophila* isolates

No.	Type of test	Result
1-	**Oxidase**	+
2-	**catalase**	+
3-	**Indole test**	+
4-	**MRtest**	+
5-	**Citrate test**	+
6-	**Gelatin liquefaction**	+
7-	**Motility test**	+
8-	**VP test**	+

To differentiated *A.hydrophila* from *V.cholera* by string test that the all *A.hydrophila* was gave negative results to it) and the *A.hydrophila* was gave positive results to argnine dehydrogenase and lysine decarboxylation and negative result to ornithine decarboxylation in compare to *V.cholera* was positive result to string test ,and ornithine and lysine decarboxylation and negative result to argnine dehydrogenase (table3)

Table (3):-differentiated between *A.hydrophila* and *V.cholera* by string test and amino acid utilization

Test type	***V. cholera***	***A.hydrophila***
String test	+	_
Lysine decarboxylation	+	+
Argnine dehydrogenase	_	+
Ornithine decarboxylation	+	_

In this study standard of biochemical tests by API 20Ewere used to confirm identification of *A.hydrophila*, According to the result of API 20E test, the isolates were identical to the reference of Bergey's Manual of Determinative Bacteriology. Characterization (based on their morphological and biochemical reactions using the API 20E test showed that these isolates were phenotypically identified as A. *hydrophila*

3.3 *Hemolysin activity*

The results of this study revealed that the all *A.hydrophila* positive to the β-hemolysin on blood agar .Nucleic

acid amplification methods targeting virulence genes of hemolysin and aerolysinin *A.hydrophila* isolates. The specific PCR products corresponding to the 130 bp fragment of the ahh1 gene and the 309 bp fragment of the aerA gene were detected from pure cultures (Figure 2, Figure3)

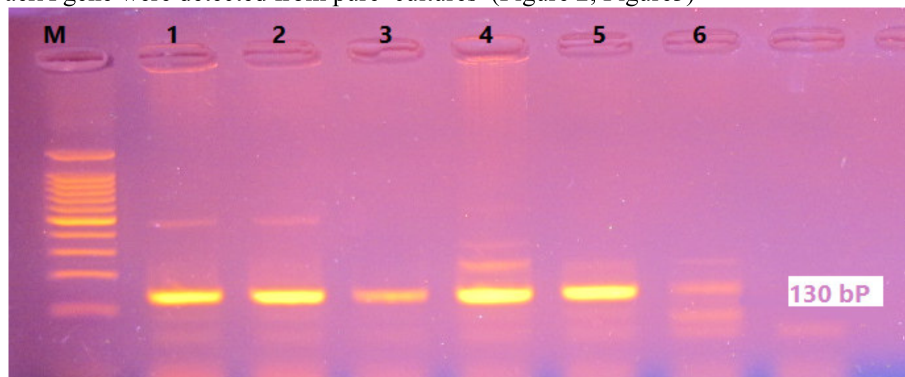

Figure (2) Electrophoresis of PCR amplification products on 2% agarose gel, Lane 1 to Lane 6 ahh1a gene of *Aeromonas hydrophila* Lane M marker DNA standard (100-1000) bp

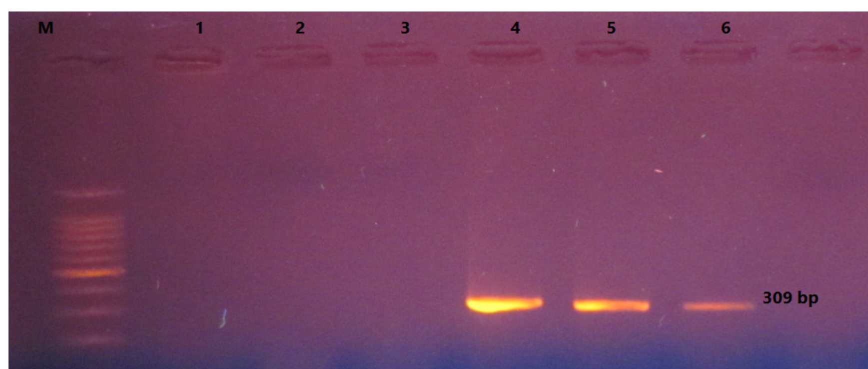

Figure (3) Electrophoresis of PCR production on 2% agarose gel, Lane 1 to Lane 6Aero gene for *Aeromonas hydrophila* Lane M marker DNA standard (100-1000) bp

4. Discussion

Among bacterial etiologies of diarrhea *A. hydrophila* is recognized increasingly as aclinically significant entric pathogen .however there are limlted data on prevalence and associated severity of diarrheal disease caused by *A. hydrophila* in many regions(Mansour *et al.*,2012).

Out of the one hundred and forty two (142) diarrheic stool samples analyzed .6(4.22%) were found to be positive for *Aeromonas hydrophila*. Our results are agree with the findings of 4.7 %incidence of *A. hydrophila* in Chennai India(Vila *et al.*,2003) ,and higher than the finding of 1.28 %(Vasaikar *et al.*,2002) and 1.4 %of *A. hydrophila* from Mumbai, India (Deodhar *et al.*,1991)and 3.12% in Nigeria (Rogo *et al.,*2009) . Alavandi and Anandhan(2003) reported Aeromonas associated diarrhea in 13 %samples in Chennai, while Kuijper *et al*(1987) and Ogunsanya *et al*(1994) reported 3.7 % in Netherlands and 1.4 %in Lagos, Nigeria respectively. However, higher prevalence of 17.7 and 28.1 %were recorded during 2000 and 2001 in Kolkata, India(Sinha et al.,2004) . the recovery of *A. hydrophila* from children <6 years represented the highest percent(2.11%) in this study compare with other age groups . It is believed that gasteroenteritis caused by *A. hydrophila* occurred more commonly in children with acute diarrhoea and adults with traveller's diarrhea (2%). self-limiting watery diarrhea but could be more severe in children(Kuijper *et al*., 1987). Recovery rates among children with diarrhea vary geographically: 0.62 to 4% in Malaysia (Lee and Puthucheary 2001; Lee and Puthucheary 2002), 0.75% in Nigeria (Kehinde *et al*., 2001), 2% in Sweden (Svenungsson *et al.,* 2000), 2.3% in Taiwan (Juan *et al*., 2000), 4.8% in Switzerland (Essers *et al*., 2000), and 6.8% in Greece (Maltezou *et al.*, 2001) The isolation rates for human fecal specimens vary widely, as geographical areas, patient populations, food habits, level of sanitation, and culture methods influence the recovery rates (Dumontet *et al.*, 2003).

The colonies of bacteria grown in culture media appeared 1-3 mm in diameter and this result agreed with Brenner *et al*.(2005). *A.hydrophila* showed a yellow shine color on TCBSagar and non lactose fermenters on macconkey agar . and it was smooth ,convex ,rounded ,β-hemolytid colonies and pale white to grey color on blood agar This is agreement with Janada &Abbott(2010)and Rogo *et al*.,(2009).this bacteria appeared gram negative, rod shaped, singly ,in pairs ,or even as short chains at the microscopic examination (Brenner *et al* .,2005).

The biochemical tests used to confirmed the initial diagnosis of *A.hydrophila* . *A.hydrophila* presented appositive result to each of the oxidase , catalase , indole , methyl red , simmon citrate,motility test,vogas prokauer and gelatin liquefaction this results are almost finding in other researchers report (Erdem *et al* .,2011;Kivanc *et al.,*2011).
To differentiated *A.hydrophila* from *V.cholera* by string test that the all *A.hydrophila* was gave negative results to it (Martin –Carnahan and Joseph ,2005) and the *A.hydrophila* was gave positive results to argnine dehydrogenase and lysine decarboxylation and negative result to ornithine decarboxylation in compare to *V.cholera* was positive result to string test ,and ornithine and lysine decarboxylation and negative result toargnine dehydrogenase (Parija,2009).
In this study standard of biochemical tests by API 20Ewere used to confirm identification of *A.hydrophila* .indeed Adel *et al* ,(2011)and Orozova *et al*,(2010)noticed that all suspected colonies were subsequently confirmed to be *A.hydrophila* using API 20E system and analytical profile index give very good identification .
β-hemolysin s as an important bacterial virulence factors which promoting channel formation leading to cell death . the results of this study revealed that the all *A.hydrophila* positive to the β-hemolysin on blood agar this results agreements with Janada and Abott(2010) and EPA (2006). the -hemolytic activity of A. hydrophila has been used as an indicator of enterotoxicity and may be responsible for outbreaks of diarrhea (Rahim *et al.,* 1984) Nucleic acid amplification methods targeting virulence genes are used for detection of pathogenic bacteria and to differentiate pathogenic from non-pathogenic strains (Chacon *et al*., 2003; Sen and Rodgers 2004). Two hemolytic toxins have been found , hemolysin and aerolysin. When the genotypes of known virulent strains as defined in Wong *et al.* (1996) were compared, it was apparent that all the *A. hydrophila* isolates with the a *hh1*a and *aer*A genotype were virulent in the suckling mouse model. These isolates also demonstrated ≤- hemolytic and cytotoxic activities. Due to the fact that the *aer*A and hemolysin genes were found in the vast majority of the diarrhoeal isolates from this species (Michelle *et al*., 1999),this results agreement with Howard *et al*., (1987)The two haemolytic toxins, haemolysin and aerolysin have been described in *A. hydrophila.* When the PCR was performed to detect aerolysin gene (*aer*A), we found that *aer*A were associated with A hydrophila(52.6%) harbored *aer*A(Yousr *et al* ,2007). The major hemolysin produced by aeromonads is called aerolysin, though it is know by several other names (cytotoxic enterotoxin, Asao toxin, and cholera toxin cross-reactive cytolytic enterotoxin). Aerolysin is produced by some strains of *A. hydrophila,* Wang *et al*. (2003) developed a multiplex PCR method for detection of hemolysin and aerolysin genes in *A. hydrophila* and *A. sobria* The range of virulence of aeromonads is thought to result from the variety of genotypes present in the environment. Both phenotypic and genotypic heterogeneity are common among aeromonads, Xia *et al.* (2004) cloned the β-hemolysin gene from A. hydrophila isolated from freshwater fish in China. The cloned β-hemolysin sequences were used in a PCR assay to survey environmental isolates to detect potential pathogenic *A. hydrophila* strains.(Alperi *et al* .,2010).

5. Conclusions

Aeromonas hydrophila recognized one of the most important factors that cause diarrhea disease especially in children under 6 years old and the *Aeromonas hydrophila* have virulence factors such as hemolysin and aerolysin, that confirm pathogenicity this bacteria.

REFERENCES

Abbott, S. L., W. K. W. Cheung, and J. M. Janda. 2003. The genus Aeromonas: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. Journal of Clinical Microbiology 41(6):2348-2357.
Adamski J, Koivuranta M, Leppa ¨nen E. Fatal case of myonecrosis and septicaemia caused by Aeromonas hydrophila in Finland. Scand J Infect Dis 2006; 38:1117–9
Adamski J,Koivuranta M.,Leppaneu E.(2006).Fatal case of myonecrosis and septicemia caused by Aeromonas hydrophila in finland ,Scand J infect Dis 38:1117-9.
Adel,M.Mansour,A.M.,Elkhalek,R.A.,Shaheen,H.I.,Mohammady,H.E.,Refaey,S.,Hassan,K.,Riddle,M.,Sanders,J .W.,Sebeny,P.J.Young,S.Y.N.&Frenk,R,M(2011).Buden of aeromonas hydrophila –associated diarrhea among children younger than 2 year in rural Egyptian community .Journal Infection Development Countries6(12):842-846.
Alavandi SV, Ananthan S. Biochemical characteristics, serogroups, and virulence factors of Aeromonas species isolated from cases of diarrhoea and domestic water samples in Chennai. Indian J Med Microbiol 2003; 21 : 233-8.
Alperi,A.,Martinez-Murcia,A.J.,Wenchien,K.O.,Arturo,M.Maria ,J.S.&Maria,J.F. (,2010).Aeromonas taiwanensis sp. Nov. and Aeromonas sanarellii sp.nov., clinical species from Taiwan . In Intertional Journal of Systematic and Evolution Microbiology ,60(2048-2055).
Blair J E ,Woo Ming Ma ,McGuire P K . (1999).Aeromonas hydrophila bactermia acquired from an infected

swimming pool .Clin infect Dis.28:1336-7.
Brenner,D.J.,Krieg,N.R.and Staley,J.S.(2005).Bergey's Mannual of systematic Bacteriology .Vol,2,2nd edition .part B,pp.556-578.New York:Spring
Chacon,M.,R., Soler,L.,Groisman,E.,A.,Guarro,J.,& FiguerasmM.,J.(2004).Type III secetion system gene in clinical Aeromonas isolates .Jouranl of clinical Microbiology 42(3):1285-1287.
Chopra,A.K. and Houston ,C.W.(1999).Entrotoxins in Aeromonas associated gasteroenteritis ,Microbes Infect.1:1129-37.
Deodhar LP, Saraswathi K, Varudkar A. Aeromonas spp. and their association with human diarrheal disease. J Clin Microbiol 1991; 29 : 853-6
Dhalla S, Flynn JT. Aeromonas hydrophila induced fulminant diarrhea. N Y State J Med 1980; 80 : 1965-6.
Dumontet, S., V. Pasquale, M. Mancino, G. Normanno, and K. Krovacek. 2003. Incidence and characterisation of Aeromonas spp. Inenvironmental and human samples in southern Italy. New Microbiologica 26(2):215-225.
EPA,Enviromental Protection Agency.(2006).Aeromonas :Human Health Criteria Document .EPA, Washington ,DC.USA.
Erdem,B.,Kariptas,E., Cil,E.,Isik,K.,(2011).Biochemical identification and numerical taxonomy of Aeromonas spp.isolated from food samples in Turkey ,Turkey Journal Biology 3:463-272.
Essers, B., A. P. Burnens, F. M. Lanfranchini, S. G. Somaruga, R. O. von Vigier, U. B. Schaad, C. Aebi, and M. G. Bianchetti. 2000. Acute community-acquired diarrhea requiring hospital admission in Swiss children. Clinical Infectious Diseases 31(1):192-196..
Gerhardt,P.,Murray ,R.G.E.,Castilow,R.N.,Nester E.W.,Wood,W.A.Keieg,N.R.Phillips,G.B. (1981).Manual Methods for General Bacteriology American Sociaty for microbiology ,Washington DC.
Hirono, I., and T. Aoki. 1991. Nucleotide sequence and expression of an extracellular hemolysin gene of Aeromonas hydrophila. Microb. Pathog. 11: 189–197.
Hirono,I &Aoki, T.(1991).Nucleotide sequence &expression of an extracellular hemolysin gene of Aeromonas hydrophila .Microb .Pathog.11:189-197.
Howard S.P.Garland W.J. Green,M.J. &Buckley ,J.T.(1987).Nucleotide sequence of the gene for the hole forming toxin aerolysin of A. hyrophila .J.Bacteriol.196:2869-2871.
Janada,J.M. and Abott,S.L.(2010).The genusAeromonas,taxonomy,pathogenicity , and infection .Clinical Microbiology Review.23:35-37.
Janda, J. M., and S. L. Abbott. 1998. Evolving concepts regarding the genus Aeromonas: an expanding Panorama of species, disease presentations, and unanswered questions. Clinical Infectious Diseases 27(2):332-344
JandaJ.M.andAbbott,S.L.(1996).Human pathogen in B.Austin M.Altwegg.P.Gosling and S.W.Josseph(eds)Thegenus eromonas John Wiley and Sons New York,Ny:39-76.
Jatau, E.D., and Yakubu, S.E., 2004. Incidence of Aeromonas hydrophila in tilapia obtained from Ahmadu Bello University Dam Zaria. Nigeria Journal of Scientific Research 4, 86-91.
Juan, H.J., R.B. Tang, T.C. Wu, and K.W. Yu. 2000. Isolation of Aeromonas hydrophila in children with diarrhea. Journal of Microbiology, Immunology & Infection 33(2):115-117..
Kehinde, A. O., R. A. Bakare, A. A. Oni, and A. O. Okesola. 2001. Childhood gastroenteritis due to Aeromonas hydrophila in Ibadan, Nigeria. African Journal of Medicine & Medical Sciences 30(4):345- 346.
Kivanc,M.,Yilmaz,M.,&Demir,F.,(2011).The occurrence of Aeromonas in drinking water and Porsuk river.Brazilian Journal of Microbiology 42:126-131.
Kuijper EJ, Zanen HC, Peeters MF. Aeromonas-associated diarrhea in the Netherlands. Ann Intern Med 1987; 106 : 640-1
Lee, W. S., and S. D. Puthucheary. 2001. Retrospective study of Aeromonas infection in a Malaysian urban area: a 10-year experience. Singapore Medical Journal 42(2):057-060 .
Lee, W. S., and S. D. Puthucheary. 2002. Bacterial enteropathogens isolated in childhood diarrhoea in Kuala Lumpur--the changing trend.[see comment]. Medical Journal of Malaysia 57(1):24-30.
Maltezou, H. C., A. Zafiropoulou, M. Mavrikou, E. Bozavoutoglou, G. Liapi, M. Foustoukou, and D. A. Kafetzis. 2001. Acute diarrhoea in children treated in an outpatient setting in Athens, Greece. Journal of Infection 43(2):122-127
Mansour,A.,M.,Elkhalek,R.A.,Shaheen,H.I.,Mohammody ,H.E.,Refaey,S.,Hassan,K.,Riddle,M.,Sanders,J.W.,Sebeny,P.J.,Young,S.Y.N.&Frenk,R.(2012).Burden of Aeromonas hydrophila –associated diarrhea among children younger than 2 years in rural Egyptian community .Journal Infection Dev Ctries 6(12):842-846.
Martin –Carnahan,A., and Joseph ,S.,W.,(2005).OrderXII.Aeromonadales ord. nov.In Bergey'sMannul of systematic Bacteriology ,2 nd ed .,Edited by D.J. Brenner ,N.R.Krieg,J.T.Staley.New York :Springer.vol.,part B, PP.556-578.
Martinez-Murcia, A. J., N. Borrell, and M. J. Figueras. 2000. Typing of clinical and environmental Aeromonas veronii strains based on the 16S-23S rDNA spacers. FEMS Immunology & Medical Microbiology 28(3):225-

232
Matyer,F.,Kaya,A&Dincer,S.(2007).Distribution and antibacterial drug resistance of Aeromonas spp.from fish and brackish waters in southern Turkey.Annalsof Microbiology. 57(443-447).
Michelle, C., Callegan, B.D., Jett, L.E. and Michael, S.G. 1999. Role of Hemolysin BL in the pathogenesis of extraintestinal Bacillus cereus– infection assessed in an endophthalmitis model. Infection and Immunity, 67: 3357– 3366.
Nzeako B.C., Okafor, N., and Azikiwe, N., 2002. Prevalence of Aeromonas hydrophila in seasonal episodes of gastroenteritis in Nsukka Nigeria. Kuwait Medical Journal 34 (1), 16-19
Ogunsanya TI, Rotimi VO, Adenuga A. A study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria. J Med Microbiol 1994; 40 : 10-4.
Orozova,P.,Chikova,V.,&Najdenski,H.,(2010).Antibiotic resistance of pathogenic for pathogenic of thogenic for fish isolates of Aeromonas spp. Bulgarian Journal of Agricultural science .16(3):376-386.
Ottaviani,D,MParlanimC.,Citterio,B.,Masini,L.,Leoni,F.,Canonico,C.,Sabatini
Bruscolini,F.,&Piantti,A.(2011).Putative virulence properities of Aeromonas strains isolated from food ,environmental and clinical sourcesin Italy :Acomparative study .International journal of Food Microbiology 144:538-545.
Rahim Z, Sanyal SC, Aziz KMS, Huq MI, Chowdhury AA (1984). Isolation of enterotoxigenic, hemolytic and antibiotic-resistant Aeromonas hydrophila strains from infected fish in Bangladesh. Appl. Environ. Microbiol., 48: 865-867.
Rahman,M.H.,Kawai,K.,Kusuda,R.(1997).Virulence of starved Aeromonas hydrophila to Cuprinid fish.Fish Pathol.32m163-168.
Rogo,L.D.,Attah,A.,Bawa,E.,Aliyu,A.M.Aliyu,M.S. &GaiyamA.Z.(2009).Antimicrobial susceptibility pattern of Aeromonas hydrophila among patients presented with diarrheal attending two teaching hospitals in northen Nigeria .Bayero Journal of Pure and Applied Sciences2(1):91-95.
Sen,K. and Rodgers,M.(2004).Distribution of six virulence factors in Aeromonas species isolated from US drinking water utilities :a PCR identification Journal Applied Microbiologica 97:1077-1086.
Shah N, DuPont HL, Ramsey DJ. (2009). Global etiology of travelers' diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg 80:609–14.
Sinha S, Shimada T, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y, et al. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic Aeromonas species isolated from hospitalized diarrhoeal cases in Kolkata, India. J Med Microbiol 2004; 53 : 527-34.
Trower C J,Abo S,Majeed K N,Von Itzstein M.(2000)Production of an enterotoxin by gastero-enteritis associated Aeromonas strain,J Med Microbiol 49:121-6.
Vadivelu,J.,Puthucheary ,S.D.,Phipps,M.Chee,Y.W.(1995).Possible virulence factors involved in bactermia caused by Aeromonas hydrophila .In Journal of Med .Microbiology 42(171-174).
Vasaikar S, Saraswathi K, De A, Varaiya A, Gogate A. Aeromonas species isolated from cases of acute gastroenteritis. Indian J Med Microbiol 2002; 20 : 107-9
Vila J, Ruiz J, Gallardo F, Vargas M, Soler L, Figueras MJ, et al. Aeromonas spp. and traveler's diarrhea: clinical features and antimicrobial resistance. Emerg Infect Dis 2003; 9 : 552-5
Wang G, Clark CG, Liu C, Pucknell C, Munro CK, Kruk TMAC, Caldeira R, Woodward DL, Rodgers FG (2003). Detection and charac- terization of the hemolysin genes in Aeromonas hydrophila and Aeromonas sobria by multiplex PCR. J. Clin. Microbiol., 41(3): 1048- 1054.
Wong, C. Y., M. W. Heuzenroeder, and R. L. Flower. 1998. Inactivation of two haemolytic toxin genes in Aeromonas hydrophila attenuates virulence in a suckling mouse model. Microbiology 144(2):291-298.
Wong, C.Y.F., Mayrhofer, G., Heuzenroeder, M. W., Atkinson, H. M., Quinn, D. M. and Flower, R.L.P. 1996. Measurement of virulence of aeromonads using a suckling mouse model of infection. FEMS Immunology and Medical Microbiology, 15: 233-241.
Xia, C., Ma, Z., Habibur, M.R. and Zhi-Guang, W. 2003. PCR cloning and identification of the haemolysin gene of Aeromonas hydrophila from freshwater fishes in China. Journal of Aquaculture, 229 : 45–53.
Xia, C., Z. H. Ma, M. H. Rahman, and Z. G. Wu. 2004. PCR cloning and identification of the beta- haemolysin gene of Aeromonas hydrophila from freshwater fishes in China. Aquaculture 229(1-4):45-53
Xu, Xin-J. Ferguson, M.R., Popov, V.L., Houseton C.W., Peterson,J.W., and Chopra, A.K., 1998. Role of a cytotoxic enterotoxin in Aeromonas mediated infection: development of transposon and isogenic mutants. Infectious Immunology 66 (8), 3501-3509.
Yogananth,N.Bhakyaraj,R.,Chanthuru,A.,Anbalagnaan,T.&Mulkai ,K.N.,
Yousr, A.H., 1Napis, S., Rusul, 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 Vol. 14, 115-122

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar