Prevalence and antimicrobial resistance patterns of *Aeromonas hydrophila* strains isolated from diarrheic cattle

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Abstract

In this study, Out of 110 examined diarrheic cows, 27 cases were harbored *A. hydrophila* (24.6 %), by culturing on direct plating and enrichment in alkaline peptone water. The enrichment technique gave the highest isolation rate (92.6 %). Seasonal variation was recorded, the highest rate was in summer (40.7 %) followed by spring (29.6 %), autumn (18.5 %) then the winter (11.1 %). Virulence factors of *A. hydrophila* were studied. All isolates gave Congo red positive but with different degrees in red colour. But only 18 isolates were positive for crystal violet. On growth at 43^{o}C, 27 isolates can grow. Haemolysin production was produced by 26 isolates (96.3 %), also enterotoxin production was assessed by infant mouse assay, which gave 92.6% positive for enterotoxin. Experimental infection in mice was done to detect the pathogenicity. The number of dead mice in successive days was 57. The deaths of inoculated mice occurred within 1-4 days post inoculation. Antimicrobial resistance patterns were done by disc diffusion technique. All *A. hydrophila* isolates were resistance to penicillin G, erythromycin, amoxicillin and cephalothin (100 %), but resistance to other antimicrobials varied in degrees.

Key words: *A. hydrophila, diarrheic cows, Congo red, Antimicrobial resistance.*

Introduction

Aeromonas hydrophila have been recognized as enteric pathogen that has been implicated in human and animals (**Lee and Puthucheory, 2002 , Aziz and Amany 2005** and **Del Castille *et al*., 2012**). Also considered as food born and water born microorganism (**Hardly *et al*., 1986 and Borchard *et al*. , 2003**).

Aeromonas hydrophila constitute hazardous cause of infection to the immuno - compromised patients as well as animals under stress or in poor hygienic condition (**Gracey *et al*., 1982; Krovacek *et al*., 1989**).

Aeromonas has its characteristic frequency of isolation during different seasons; the highest frequency was recorded during summer followed by spring, less during autumn and the least frequency during winter (**Abu Leila, 2005**).

Many Virulent factors were known in *Aeromonas* species including haemolysins, enterotoxins, and cytotoxins, (**Vitovec *et al*., 2001, Gonzalez *et al*., 2002 and Thayumanavan *et al*., 2003).** *A. hydrophila* was the predominant species with pronounced different extra cellular products such as hemolysin, cytotoxins, and enterotoxins (**Lallier and Higgins, 1988**). Several toxic phenomena associated with *A. hydrophila* culture filtrate including haemolysis, cytotoxicity and enterotoxicity (**Rose *et al*., 1989**).

Aeromonas hydrophila haemolysin, enterotoxin, and cytotoxin are due to three different proteins resulting from the expression of three distinct genes (**Chakraborty *et al*., 1987**). Expression of virulence factors of *A.hydrophila* is affected by the temperature incubation and not always related to the presence of genes, also *Aeromonas* strains could be considered virulent if possessing at least two or three characters as haemolysin, cytotoxin and enterotoxin (**Gonzalez *et al.,* 2002**). The growth temperature affected the expression of some virulence factors (**Ottaviani *et al*., 2011**).

Aeromonas become highly resistant to antibiotic due to low level of administration

(**Moreira and Moraes, 2002**). Antibiotic resistance rates of *A. hydrophila* were usually higher than those of other Aeromonas strains (**Koksal *et al*., 2007**). Aeromonas were multidrug resistant (**Scoaris *et al*., 2008**).
The aim of this study was to throw some light on virulence factors of *A. hydrophila* and their antimicrobial resistance patterns.

Materials and Methods
Faecal samples of diarrheic cows were taken under aseptic conditions and in a separate polyethylene bag, labeled and send to the lab as quick as possible.
Direct plating
A loopful of examined faeces was streaked directly onto plates of blood, MacConkey agar and Aeromonas base medium supplemented with ampicillin and incubate aerobically at 37° C for 18-24 hrs. **(Gray 1984)**.
Enrichment technique
Approximately 2 gm. faeces inoculated into 25 ml sterile alkaline peptone water PH 8.6 (APW) and incubated at 20°C for 48 hrs. then subcultured onto blood agar, macConkey agar and Aeromonas base medium supplemented with ampicillin and incubated at 37°C for 18-24 hrs. **(Gray 1984)**.
Identification of Aeromonas:
Purified Colonies were subjected to morphological, cultural and biochemical identification according to **Bailey and Scott (1986)** and **Koneman *et al*., (1994).**

Detection of Virulence Factors
Congo red binding test (Gray *et al*., 1990).
All isolates were tested for its growth on Congo red medium at 25°C for 24 hrs. then left for additional 4 days CR+ orange red colonies CR- not bind to the dye give white colonies so orange or red colonies were recorded as positive Congo red.
Crystal violet binding test (Panigua *et al*., 1990).
All isolates were inoculated on trypticase soya agar for 24 hrs. at 28 °C then flooded with crystal violet. Violet colonies were recorded as positive.
Growth at 43°C (Kirov *et al*., 1990).
All isolates were tested for their ability to grow onto blood agar at 43 °C for 24-48 hr.
Haemolysin Production: according to **Koneman *et al*., (1994).**
Isolates were tested for hemolytic activity on 5 -7 % sheep blood agar.
Enterotoxin assay: by infant mouse test according to **Kirov *et al*., 1986 and Robins-Brown *et al*., 1993.**
A. hydrophila isolates were inoculated into media containing 2% casamino acid, 1% yeast extract and 0.4% glucose. The culture was incubated on a rotatory Shaker (200 r.p.m.) at room temperature for 48 hrs, then centrifuged at 12.000 xg for 10 minutes. The Supernatants were filtered through Millipore membrane filter (0.45 um) and stored at - 20°C to be used up to 10 days. 0.1 ml of culture filtrate injected through the abdominal wall into the milk filled stomach of 3 infant mice (2-4 days old). Other 3 infant mice injected with 0.1 ml saline as a negative control. All the mice were kept for 3 hours then were killed. Intestines weight related to the remaining body weight was determined. Ratio greater than 0.083 was recorded as positive test for enterotoxins.
Pathogenicity test:
According to **Brook *et al*., 1985** and **Palumbo *et al*., 1989.**
Aeromonas hydrophila strains were grown over night in BH1 broth at 28°C. The cells were washed 3 times in sterile saline. Dilutions of cultures were prepared in sterile saline for injection into groups of three mice (20 to 25 gm.), and 100 ul broth culture of each strain was injected intraperitoneal.
The mice were observed at 24 hrs. interval for 96 hrs.
Antemicrobial Susceptability test:
The disc diffusion technique was applied to all isolates of *A. hydrophila* according to **Fine gold and Martin (1982).**

Results
The results showed that out of 110 samples collected from diarrheic cows, 27 (24.6%) cows were positive for *A.hydrophila* as in table

(1). Also the results in Table (1) showed that enrichment in alkaline peptone water and subculture on the plating media gave the highest isolation rate (92.6%). On the other hand, plating samples directly on plating media gave only the percentage of 7.4%.

Seasonal incidence variations of *A. hydrophila* isolated from diarrheic cows in table (2), showed that the highest incidence rate was in summer (40.7%), followed by spring (29.6%) and Autumn (18.5%) then water (11.1%).

The results of different virulence factors of *A. hydrophila* were registered in table (3). The degree of Congo red and crystal violet binding activity, growth at 43°C, hemolysin production activity and enterotoxin production were illustrated in table (3). The results showed that all isolates of *A. hydrophila* gave Congo red positive but with different degrees in colour CR++ (10 isolates) and CR+ (17 isolates).

The incidence of crystal violet binding activity was 66.7%. Out of 27 isolates , 24 isolates were grown at 43°C. Hemolysin production was produced by 26 isolates (96.3%) out of 27 isolates. Also enterotoxin production gave 92.6% enterotoxigenic strains which cause accumulation of fluid in intestinal tract of inflected mice (T.3).

Experimental infections of mice with *A. hydrophila* intraperitonial route lead to death in percentage of 70.4 % (57/81). The deaths of inoculated mice occurred within 1-4 days post inoculation as shown in table (4).

Antimicrobial resistance patterns for 27 strains of *A. hydrophila* strains were carried out as shown in Table (5). All *A. hydrophila* strains were resistance to penicillin G, erythromycin, amoxicillin and cephalothin with 100 % while ampicillin with (96.3%) and resistance to other antimicrobial agents with different percentages. Most strains were sensitive to chloramphenicol (96.3%), ciprofloxacin and norfloxacin (92.6%) followed by gentamicin (88.9%) and kanamycin (81.5%) while neomycin (70.4%) nalidixic acid (66.7%) and streptomycin (55.6%) had moderate effect.

Table (1). Prevalence rate of *Aeromonas hydrophila* in diarrheic cows and comparison of direct plating and enrichment technique.

Type of animals	No. of examined Faecal samples	Positive No.	percentage	Comparison technique for isolation	
				Direct plating	Enrichment technique
Diarrheic cows	110	27	24.6 %	2 (7.4 %)	25 (92.6 %)

Table (2). Seasonal variation of *A. hydrophila* from diarrheic cows.

Season	*A. hydrophila* (27)	
	No.	Percentage
Autumn	5	18.5 %
Winter	3	11.1 %
Spring	8	29.6 %
Summer	11	40.7 %

Table (3). Different virulence factors of *A. hydrophila* isolated from diarrheic cows.

Name of test	*A. hydrophila* (27strains)	
	No.	Percentage
1-Degree of Congo red:		
CR++	10	37.03 %
CR+	17	62.96 %
2-Crystal violet binding:		
CV+	18	66.7 %
CV-	9	33.3 %
3-Growth at 43°C:		
Growth (+)	24	88.9 %
No growth (-)	3	11.1 %
4- Hemolysin production:		
Positive	26	96.3 %
Negative	1	3.7 %
5-Ententoxin production suckling mice:		
Positive	25	92.6 %
Negative	2	7.4 %

Table (4). Experimental infection of mice with *A. hydrophila* isolates.

Type of strains	No. of isolates	No. of tested mice	No. of dead mice per day				No. of dead	No. of positive isolates
			1	2	3	4		
A. hydrophila	27	81	9	27	14	7	57	19

* 3 mice considered as control and injected with sterile saline.
**The reisolation of *A. hydrophila* from infected dead mice mainly from intestinal content, spleen and liver.

Table (5). Antimicrobial resistance patterns of *A. hydrophila* isolated from diarrheic cows (27 isolates).

Antimicrobial agents	Sensitive		Resistance	
	No.	percent	No.	Percent
Penicillin G	00	00	27	100 %
Erythromycin	00	00	27	100 %
Amoxicillin	00	00	27	100 %
Cephalothin	00	00	27	100 %
Ampicillin	1	3.7	26	96.3 %
chloramphenicol	26	96.3 %	1	3.7%
Ciprofloxacin	25	92.59%	2	7.41%
Norfloxacin	25	92.59%	2	7.41%
Gentamicin	24	88.89	3	11.11
Kanamycin	22	81.5	5	18.5
Neomycin	19	70.37	9	33.33
Nalidixic acid	18	66.66	11	40.74%
Streptomycin	15	55.55	12	44.44%

Discussion

Aeromonas hydrophila have been recognized as enteric pathogen that has been implicated in human and animals. Also many virulent factors were known.

Isolation of *A. hydrophila* in diarrheic cows was examined bacteriologically by direct plating and pre-enrichment on alkaline peptone water then plating. Results had been demonstrated as shown in table (1). Our results showed that the prevalence rate of *A. hydrophila* was 24.6 % which is nearly similar to that recorded by **Jindal *et al*.,(1993)** who found that 27.3% of examined cows were infected with Aeromonas. On the other hand**, Ceylan *et al*., (2009)** identified Aeromonas in faecal samples of cattle with a percentage of 8.2 %.

The difference in isolation of *A.hydrophila* may be related to the methodology in isolation and climatic differences. In this concern, **Shread *et al*., (1981) and Vongraevenitz and Bucher (1983)** reported that the difference in isolation rates were probably due to different isolation techniques as the use of alkaline peptone water as enrichment media before plating on the specific media. As shown in table (1), most isolates 25 (92.6 %) were found by enrichment in alkaline peptone water then plating on specific media. Several investigators have used the enrichment technique **(Ceylan *et al*., 2009).**

The difference in isolation may be related to climatic differences, in this study the highest incidence of isolation rate was in summer (40.7 %) followed by spring (29.6 %) and autumn (18.5 %) then winter (11.1 %) (table 2). The much greater frequency of isolation in hot and warm seasons confirms the idea of **Nishikawa and Kishi (1987)** who observed that the isolation of Aeromonas increased during summer months. Also **Abu Leila (2005)** found that the infection was higher in summer followed by spring, autumn and winter. On the other hand **Razzolini *et al*., (2008)** found that isolation was similar in summer, spring and autumn and dropped in winter.

In this study all the strains were tested for virulence and pathogenicity tests (ability to bind with simple dyes, growth at 43^{o}C, production of enterotoxin in suckling mouse and pathogenicity to mice) as shown in table (3).

The Congo red and crystal violet are simple dyes that can be incorporated into agar media and uptake of dye has been proved to be a virulence marker for several pathogenic bacteria **(Statner and Georg, 1987).** In this study all *A. hydrophila* strains gave Congo red positive with different degree in red colour 10 (37.03%) gave CR++ and 17 (62.9%) gave CR+ (table 3). **Ishiguro *et al*., (1985)** described the Congo red uptake as a good virulence marker in Aeromonas. **Panigua *et al*., (1990)** reported that all Aeromonas take up Congo red dye with different degree of colour.

The ability of *A. hydrophila* to bind with crystal violet was 66.7 % (table 3). The results agree with **Mona (2003)** who found the ability *A. hydrophila* to bind with crystal violet was 64.6 % and almost agree with **Panigua *et al*., (1990)** who recorded that the percentage of *A. hydrophila* which bind crystal violet were 59.5 %.

The growth at 43^{o}C of *A. hydrophila* strains showed that 24 (88.9%) had the ability to grow (table 3). This result agrees with **Zaki *et al*., (2001)** who recorded that 85.7% of *A. hydrophila* has ability to grow at 43^{o}C.

*A.hydrophil*a produce haemolysin and enterotoxin as exotoxins **(Lallier and Higgins, 1988).**

Haemolysin production of *A. hydrophila* revealed that 26 isolates with an incidence of 96.3 % were able to produce haemolysin. This result is higher than that reported by **Thayumanovan *et al*.., (2003)** who detected that 78.4 % of *A. hydrophila* produced haemolysin, and agree with that recorded by **Santos *et al.* (1987)** as 96.6%. Also **Panigue *et al*. (1990)** and **Haque *et al.* (1996)** recorded that all the strains of *A. hydrophila* produced haemolysin (100%).

The detection of enterotoxins was considered as an indicator of enteropathogencity. In this study a total of 25 out of 27 isolates were positive for ability to produce enterotoxin using infant mice assay with an incidence of 92.6%.

These results agree with that recorded by **Goshing (1986)** who found that the incidence of enterotoxins production in *A. hydrophila* is 91 %. The result is higher than the incidence recorded by **Agarwal *et al*., (1999)** that 74.07% of *A. hydrophila* isolates were enterotoxigenic.

On the other hand lower enterotoxin production were 71.88%, 70.8% and 70.59% reported by **Cumberbatch *et al*. (1979), Gantam *et al*. (1992)** and **Kumar *et al*. (2000)** respectively. It is worthy to mention that there is no relation between the presence of putative virulence genes (haemolysin, LT, ST enterotoxin) and the clinical manifestation of *Aeromonas* infection (**Wu *et al*., 2007**).

The experimental infection of the mice with *A. hydrophila* leads to death of infected mice in 70.4 %. The deaths of inoculated mice occurred within 1-4 days (table 4). Results agree with that of **Mittal *et al*., (1980) and Brook *et al*., (1985)** who found that the main pathogenic strains were *A. hydrophila*. Also **Susana *et al*., (1992)** found that all the deaths occurred within 1 – 4 days in the infected mice with *A. hydrophila.* The results showed that 19 out of 27 strains lead to death of infected mice with the percentage of 70.4 %. This result nearly agrees with the result of **Mateos *et al*., (1992)** who found that 11 out of 15 *A, hydrophila* strains were pathogenic and cause death to mice.

Antimicrobial resistance patterns of 27 strains of *A. hydrophila* strains were done to guide the drug of choice to produce a high therapeutic effect as shown in Table (5). All *A. hydrophila* strains were resistance to penicillin G, erythromycin, amoxicillin and cephalothin with 100 % while ampicillin with (96.3%) and resistance to other antimicrobial agents with different percentages. Most strains were sensitive to chloramphenicol (96.3%), ciprofloxacin and norfloxacin (92.6%) followed by gentamicin (88.9%) and kanamycin (81.5%) while neomycin (70.4%) nalidixic acid (66.7%) and streptomycin (55.6%) had moderate effect. This result nearly similar to the reports of **Gado (1988)** who reported that gentamicin and chloramphenicol were the most antibiotics used**. Kampfer *et al*., (1999)** recorded that the *A. hydrophila* were highly susceptible to ciprofloxacin and chloramphenicol. **Thayumanavan *et al*., (2003)** found that the chloramphenicol was highly sensitive. **Shannon *et al*., (1986)** found that the resistance of Aeromonas to penicillin were due to the ability of microorganism to produce B. lactamases.

Conclusion

Aeromonas hydrophila has been recognized as enteric pathogen in cows. Enrichment in alkaline peptone water and subculture on the plating media gave the highest isolation rate. Many virulence factors of *A. hydrophila* were registered as congo red and crystal violet binding activity, growth at 43^{o} C, hemolysin and enterotoxin production. Most strains were sensitive to chloramphenicol, ciprofloxacin and norfloxacin followed by gentamicin and kanamycin.

References

Abu Leila,R. H. M. (2005). Some studies on septicemic diseases of cultured freshwater fish. M.V.Sc. Thesis, moshtoher, Benha Univ., Egypt.

Agarwal,R. K.; Kapoor, K. N.; Kalicharan, A. K.Bhilegaonkar, K.N. and Kumar. A. (1999). Paw oedema test a sensitive bioassay model for detection of enterotoxigenic potential of *Aeromonas* toxins . Indian J. Toxicol., 6 (1). 33 -38

Aziz, H.M. and Amany N.D (2005). Bacteriological studies of Faecal and water samples from different sources with special references to some Gram negative bacteria. Benha Vet. Med.J., 16 (2). 248 – 261.

Baily ,W. R. and Scott, E. G.(1986). Diagnostic Microbiology. 7th Ed. The C.V. Mosby Co., USA 241-245.

Borchard, M. A.; Stemper, M. E. and Stendridge J.H. (2003). *Aeromonas* isolates from human diarrheic stool and ground water compared by Plused field gel electrophoresis. Emerg. Infect. Dis., 9 (2). 224-228.

Brook, I.; Rogers, J.; Rollins, D. M.; Coolbaugh, J.C. and Walker, R. I. (1985). Pathogenicity of Aeromonas. J. Infect., 10(1).

32-37.

Ceylan, E.; Berktas, M. and Agaoglu, Z. (2009). The occurrence and antibiotic resistance of motile Aeromonas in livestock. Trop. Anim. Health prod., 41(2).199-204.

Chakra Borty, T.; Huhle, B.; Hof, H.; Bergbauer, H. and Goebel, W. (1987). Maker exchange mutagenesis of the aerolysin determinate *Aeromomas hydrophila* demonstrates the role of aerolysin in *A. hydrophila* associated systemic infections. Inf. Immun., 55 : 2274 – 2280

Cumberbatch, N.; Gurwith, M. J. Langston, C.; Sack, R.B. and Brunton, J. L. (1979). Cytotoxic enterotoxin produced by *Aeromonas hydrophila* relationship of toxigenic isolates to diarrheal disease. Infect. Immun., 23 (3); 829- 837.

Del Castillo CS; Hikima JI; Janq HB; Nho SW; Junq TS; Wongtavatchi J; Kondo H; Hirono I; Takeyma H and Aokki T. (2012). Comparative sequence analysis of a multidrug resistant plasmid from *A. hydrophila.* Antimicrob Agents Chemother. 2012 oct 15.

Finegold, S, M. and Martin, W. T. (1982). Diagnostic Microbiology. 6th Ed. The C.V. Mosby Co., USA 241-245.

Gado, M. S. M. (1988). Studies on some bacterial diseases of cultured freshwater fish in Kafr El-Sheikh Governorate. M. V. Sc. Thesis, Cairo University 45-55.

Gautam, A.R.; Pathak, S.P.; Ramteke, P.W. and Bhattacharjee, J.W. (1992). Virulence factors in environmental isolates of *Aeromomas spp.* J. Gen. Appl. Microbiol., 38 : 185 – 189.

Gray, S. J. (1984). *Aeromonas hydrophila* in live stock: incidence, biochemical characteristics and antibiotic susceptibility. J. Hyg., 92 (3).365-375.

Gonzalez, S.C.J.; Santos, J.A.; Garcia, L.M.L. and Atero, A. (2002). Virulence markers in *Aeromonas hydrophila* and *Aeromonas veronii biovar sorbia* isolates from freshwater fish and from diarrhea case. J. Appl. Microbiol., 93 (3) : 414 – 419.

Goshing, P. J. (1986). Biochemical characteristics enterotxigenicity and susceptibility to antimicrobial agents of clinical isolates of *Aeromonas* species encountered in the western region of Saudia Arabia. J. Med. Microbiol., 22 (1) : 51 – 55.

Gracey, M.; Burke, V. and Robinson, J. (1982). *Aeromonas* associated gastroenteritis Lancet, 10: 1304 – 1306.

Gray, S. J.; Stickler, D. J. and Bryant, T. N. (1990). The incidence of virulence factors in mesophilic *Aeromonas* species from animals and environment. Epidemicl. Infect., 105: 277 -294.

Ishiguro, E. E.; Ainsworth, T.; Trust, T.J. and Kay, W.W. (1985). Congo red agar, a differential medium for Aeromonas salmonicida, detects the presence of the cell surface protein array involved in virulence. J. Bacteriol. 164:1233-1237.

Haque, O. M.; Sugiyama, A.; Lawd, Y.; Mirorikawa, H.; Hawsar, U.; Shimada, I. and Yamzauchi (1996). Characterization of *Aeromonas hydrophila* comparative study of strains isolated from diarrheal feaces and the environment. South East Africa Asian J. Trop. Med. Public Helth., 27 (1) : 132 – 138

Hardy, T. C.; Todd, L. s. and Stringer, M.F. (1986). Toxin production by *Aeromonas hydrophila* in bacteriological media and foods. Zent. Bakteriol. Mikrobiol. Hyg., 15 : 165 – 176.

Ishiguro, E. E.; Ainsworth, T.; Trust, T.J. and Kay, W.W. (1985). Congo red agar, a differential medium for Aeromonas salmonicida, detects the presence of the cell surface protein array involved in virulence. J. Bacteriol. 164:1233-1237.

Jindal, N.; Garg, S. and Kumar, A. (1993). Comparison of Aeromonas spp. isolated from human livestock and poultry faeces. Israel J. Vet. Med., 48(2).80-83.

Kampfer, P.; Christmann, C.; Swing, J. and Huys, G. (1999). In vitro susceptibilities and Aeromonas geno species to 69 antimicrobial agents. Syst. Appl. Microbiol., 2294: 662-669.

Kirov, S.M.; Anderson,M.J. and Mcmeekin,T.A.(1990). A note on Aeromonas spp. from chickens as possible food borne pathogens. J. Appl. Bacteriol.,68:327-334.

Kirov, S.M.; Wellock, R.C. : Goldssmid, J. M. and Vangalen, A.D. (1986).
Virulence charcteristics of *Aeromonas Spp*. In relation to source and biotype.
J. Clin. Microbiol., 24 (5) : 827 -834.

Koneman, E. W.; Allen, S.D.; Dowel, V.R.; Schrechen,w.m.; Berrjer, P.C. and Winn, W.C.(1994). Color Atlas and text book of diagnostic microbiology. J.B. Lippincott Co. Philadelphia, USA.

Koksal, F.; Oguzkurt, N.; Samasti, M. and Altas, K.(2007). Prevalence and antimicrobial resistance patterns of Aeromonas strains isolated from drinking water samples in Istanbul, Turkey. Chemotherapy, 53 (1)30-35.

Krovacek, K.; Peterz, M.; Faris, A. and Mansson, I. (1989). Enterotoxigenicity and drug sensitivity of *Aeromonas hydrophila* isolated from well water in Sweden a case study. Int. J. Food Microbiol., 8 (2) : 149 – 154.

Kumar, A.; Bachhil, V. N.; Bhilegaonakar , K. N. and Agarwal, R.K. (2000). Occurrence of enterotoxigenic *Aeromonas species* in foods. J. Commun. Dis., 32 (3) : 169 – 174.

Lallier, R. and Higgins, R. (1988). Biochemical and toxigenic characteristics of *Aeromonas* spp. Isolated from diseased mammals, moribund and healthy fish. Vet. Microbiol., 18 (1) : 63 – 71.

Lee, W.S. and Puthucheary, S. D. (2002). Bacterial enteropathogens isolated in childhood diarrhea in Kuala Lumpur the chang trend. Med. J. Malaysia,57 (1).1-2

Mateos, D.; Arigulta, J.; Naharro, G. and Paniagua, C. (1992). Influence of growth temperature on the production of extracellular virulence factors and pathogencity of environmental and human strains of *Aeromonas hydrophila*. J. Appl. Bacteriol.,4(2).111-118.

Mona, M.H. (2003). Studies on motile Aeromonads in fresh water fishes. Egypt. J. Agric. Res.,81(1). 193-208.

Mittal, K. R.; Tatande, G.; Leblanc, D.; Olivier,G. and R. Tallier.(1980). *Aeromonas hydrophila* in rainbow trout: relation between virulence and surface characteristics. Can. J. Microbiol. 26:1501-1503.

Moreira, M.A.S. and Moraes,C. A. (2002). Resistance to antibiotics in Gram negative bacteria isolated from broiler carcasses. Arch. Brasil. Med. Vet. Zoot., 54(1)1-7.

Nishikawa, Y. and Kishi, T. (1987). Amodification of bile salts brilliant green agar for isolation of motile Aeromonas from foof and environmental specimens. Epidemiol. Infect.,98: 331-336.

Ottaviani D, Parlani C, Citterio B, Masini L, Leoni F, Canonico C, Sabatini L, Bruscolini F and Pianetti A (2011). Putative virulence properties of Aeromonas strains isolated from food, environmental and clinical sources in Italy : a comparative study. Inf. J. Food Microbiol. 5; 144 (3).538-45.

Palumbo, S.A.; Bencivengo,M.M.; Delcorral,F.;William, A.C. and Buchaman, R.L.(1989). Characterization of the Aeromonas hydrophila isolated from retail foods of animal origin. J. Clin. Microbial., 27 (5).854-859.

Panigua, C.; Rivero, O.; Anguita, J. and Vaharro, C. (1990). Pathogenicity factors and virulence for rainbow trout motile Aeromonas species isolated from a river. J. Clin. Microbiol., 28 (2) : 350 – 355.

Razzolini, M. T.; Di. Bari, M.; Sanchez, P.S. and Sato, M.L.(2008). Aeromonas detection and their toxins from drinking water from reservoirs and drinking fountains. Water health.,6(1).117-123.

Robins-Browne, R.M.; Takeda, T. and Fasano, K.L. (1993). Assessment of enterotoxin production by *Y. enterocolitica* and identification of a novel heat stable enterotoxin strain isolated strain isolatedfrom clinical material. Infect. Immu.,61(2).764-767.

Rose, JM; Hauston, CW;Coppenhaver, HD; Dixon, JD and Kurosky A. (1989). Purification and chemical characterization of a cholera toxin-cross – reactive cytolytic enterotoxin produced by a human isolates of *Aeromonas hydrophila.* Infect Immun.; 57 (4). 1165-1169.

Santos, Y.; Toranzo, A.E.; Dopazo, C.P.; Nieto, T.P. and Barja, J.T. (1987). Relationship among virulence for fish entetotoxigenicity and phenotypic characteristics of motile *Aeromonas.* Aquaculture, 67 (1-2) :29 -39.

Scoaris, Dde. O.; Colacite, J. Nakamura, C.V.; Ueda-Nakamura, T. de. Abreu Filho, B. A. and Dias Filho, B.P. (2008). Virulence and antibiotic susceptibility of Aeromonas spp. isolated from drinking water. Antonie. Van. Leeuwenhoek., 93(1-2).111-122.

Shannon, K., King, A. and Phillips, I.` (1986). B-lactamases with high activity against *A. hydrophila*. J. antimicrob. Chemother., 17:45-50.

Shread, P.: Donovan, T. J. and Lee, J. V. (1981). A survey of the incidence of Aeromonas in human faeces. Society for General Microbiology Quarterly, 8:184-185.

Statner, B., and W. L. George. (1987). Congo red uptake by motile Aeromonas species. J. Clin. Microbiol. 25:876-878.

Susana, M.; Silvia, C. and Juan, M. T. (1992). Effect of growth temperature on outer membrane components and virulence of *Aeromonas hydrophila* strains of serotype O: 34. Infect. Immune., 60 (10) 4343-4349.

Thayumanvan, T.; Vivekanandhan, G.; Savithamanl, K.;Subashkumar, R. and Lashmanaperumalsamy, P.(2003). Incidence of haemolysin positive and drug resistant *Aeromonas hydrophila* in freshly caught fin fish and prawn collected from major commercial fishes of Coastal south India. FEMS. Immunol. Med. Microbiol., 36(1-2).41-45.

Vitovec, J.; Adova, E.; Vladik, P. and Krovacek, K. (2001).` Enteropathogenicity of *Plesiomonas Shigelloides* and *Aeromonas* spp. In experimental mono and co infection with Cryptosporidium parvum with intestine of neonatal Balb/C mice. Comp. Immunol. Microbiol. Infect. Dis., 24 (1) : 39 -55.

Vongraevenitz, A. and Bucher, C. (1983). Evaluation of differential and selective media for isolation of Aeromonas and Plesiomonas spp. from human faeces. J. Clin. Microbiol.,17:16-21.

Zaki, H.; Sahar, R.; Ansam, M. Salman and Seleim R.S. (2001). Exotoxingenicity of *Aeromonas species* isolated from different sources. Inhibitory effect of essential oils on their growth. Veterinary Medical J., 49 (1). 111-112.

تواجد ميكروب الايروموناس هيدروفيلا فى اسهال الابقار وانماط مقاومته للمضادات الميكروبية

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الملخص العربى

تم فحص 110عينة من ابقار لميكروب الايروموناس هيدروفيلا و وجد ان 27 حالة مصابة بالميكروب بنسبة 24.6% وذلك بطريقة الزرع على الاوساط البيئة مباشرة. وبالزرع على شوربة البيبتون القلوية اللتى اعطت اعلى نسبة (92.6%) . كما تم دراسة التباين الموسمى للاصابة وجد انها 40.7% بالصيف, 29.6% فى الربيع, 18.5% بالخريف واقلهم 11.1% فى الشتاء.

كما تم دراسة عوامل الضراوة على المعزولات ووجد ان كل المعزولات قد اتحدت بصبغة الكونجو الحمراء ولكن بتفاوت فى درجة الاتحاد بالصبغة. وكانت فقط 18 عترة ايجابية لصبغة الكريستال ولكن النمو عند درجة 43 درجة مئوية اعطت ايجابية بنسبة 100%. وكانت 26 عترة مذيبة للدم بنسبة 96.3% وتم دراسة افراز السموم المعوية وكانت ايجابية بنسبة 92.6%. وايضا تم عمل عدوى صناعية للفئران وكذا عمل اختبار الحساسية للمضادات البكتيرية المختلفة ووجد ان جميع المعزولات مقاومة للبنيسيلين ج وكذا الايروثرومايسين والاموكساسيلين والسيفالوسين بنسبة 100%.