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Isolation and Antimicrobial Resistance Patterns of *Aeromonas hydrophila* from Diarrheal children in Thi-Qar Province/Iraq

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Abstract:

This study was carried out to isolate *Aeromonas hydrophila* from diarrheal children; 150 fecal samples were collected from diarrheal children with age (1-13 years) of both sexes that had suffering from diarrhea in Mohammed Al -Mosawi and Bent-Al-Huda hospitals in Thi-Qar province. Twenty isolates were diagnosed as genus *Aeromonas hydrophila*. The results revealed that the rate of *Aeromonas hydrophila* isolates in fecal samples of diarrheal children was (13.3%) and the age groups of 1-5 years were the highest percentage of diarrheal cases (60%) and the highest percentage of *Aeromonas hydrophila* infection (40%). Antimicrobial susceptibility testing to 20 *Aeromonas hydrophila* isolates showed that (70%) of isolates were resistance to Amikacin, while (60%) of isolates were resistance to Ciprofloxian, Cefotaxime and Gentamycin ,(35%) of isolates were resistance to Ceflazim and only (20%) of isolates were resistance to Imipenem, and the results appeared increasing prevalence of multidrug resistant between *Aeromonas hydrophila* isolates that isolated from diarrheal children in Thi-Qar province.

Keywords: *Aeromonas hydrophila*; Diarrheal children; Antimicrobial Resistance.

1. Introduction:

Diarrhea remains a major public health problem in developing countries, it's the leading causes of childhood morbidity and mortality. An estimated one billion episodes and 2.5 million deaths occur each year among children under five years of age. About 80% of deaths due to diarrhea occur in the first two years of life (WHO, 2005). The genus *Aeromonas* belongs to the family *Aeromonadaceae* within the *Gammaproteobacteria* and comprises Gram-negative, nonspore- forming, motile bacilli or coccobacilli rods with rounded ends which measure 1-3,5 µm across ; They are facultative anaerobic, oxidase, catalase and indol-positive, able to reduce nitrate to nitrite and are, glucose-fermenting, generally resistant to the vibriostatic agent O/129 (Jawetz *et al*.,2001). Motile *Aeromonas* spp. are pathogens that cause foodborne gastroenteritis in human and extraintestinal symptoms such as; septicemia, wound infections, menengitis, endocarditis and osteomyelitis ;a high mortality rate in immunocompromised person.(Gold and Salit,1993) Recently, *Aeromonus* have been widely studied as agents of infection in human ,animals ,reptiles and fishes ;Most strains of *A.hydrophila* isolated from patients with acute diarrheal disease are enterotoxigenic (Soler *et al*.,1987) .A rapidly expanding body of literature suggests that *Aeromonas* spp. cause mild, selflimiting diarrheal disease in previously healthy adults, and a spectrum of intestinal disease ranging from acute dysentery to chronic watery diarrhea persisting for weeks or months has been reported recently (Al-atar *et al*.,1997) .Antimicrobial resistance among enteric pathogens is a serious problem in developing countries where there is a high

frequency of gastroentric illness and many antibiotic resistance fall routinely into inadequate use. Antibiotic resistance is particularly relevant in pathogenic Aeromonas species in which, besides the classical resistance to β- lactamic antibiotics, multiple- resistance has been frequently identified adhere bacteria can receive and transfere antibiotic resistance genes to other Gram negative bacteria (Ivani *et al*.,2007).The aim of this study was to isolate *Aeromonas hydrophila* from diarrheal children in Thi-Qar province, Iraq and investigate the antimicrobial susceptibility for them.

2. Materials and Methods:

1. Collection of specimens:

150 samples were collected during the period from 17 October 2016 to 17 June from people who suffer from diarrhea in Mohamed Al-Mosawy, Al-husain and Bint Al-huda hospitals in Thi-Qar province and the data such as (name, age ,sex, and address) was recorded and the sample were cultured by steaking on alkaline pepton water after that incubate in 37 ◦c for 6 hours.

2. Culture on culture media:

The sample culture on: TCBS agar, blood agar and MacConkey agar; the suspected colony culture on nutrient agar for biochemical tests and antibiotic susceptibility test.

3. Biochemical tests:

Biochemical tests have been conducted according to (Winn *et al*., 2007) including the following tests {KI test, TSI, Catalase, Oxidase, Lactose fermentation, Urease, Indole, citrate utilization and Motility}.

4. Api-20E system (Analytical profile index for Enterobacteriaceae test):

Api-20E system is used clinically for the rapid identification of the bacterial isolates this test done according to (Leboffe and Piercr, 2005).

5. Antimicrobial Susceptibility Test:

The antimicrobial susceptibility test has been done by disc diffusion method according to (Bauer *et al*. 1966).

3. Results:

1. Isolation and characterization of *Aeromonas spp.*:

Total of 150 sample which were collected 20 isolates of *Aeromonas* from stool samples obtained from patients admitted with acute diarrhea in three hospitals Mohamed Al-Mosawi , Al-Husain teaching and Bent Al-Huda hospital in Thi-Qar province as in figure (1).

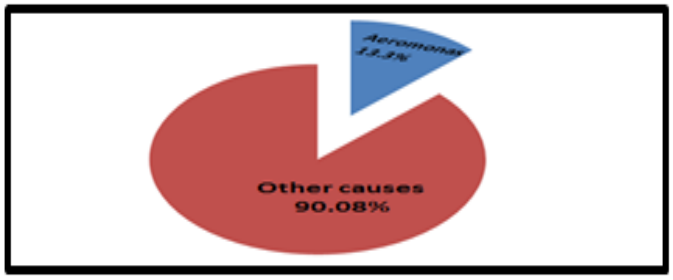

Figure (1): The occurrence of *Aeromonas* spp. isolated from 150 diarrheal patients.

2. Colony Morphology:

The results showed the different morphology characteristics of all *Aeromonas* which grow on different media as in table (1) and figure (2).

Table (1): Culture characteristics of *Aeromonas*

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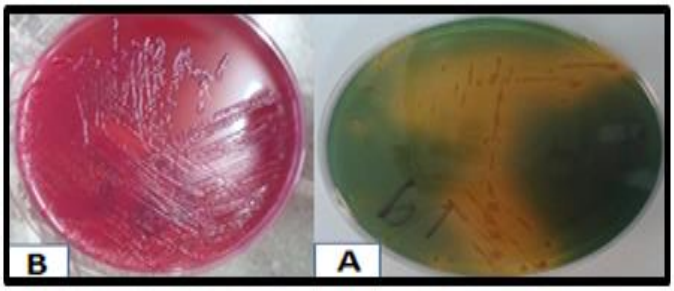
Culture Media	Morphology of colonies
TCBS	yellowish, convex, entire, smooth, moderate, opaque,
Blood agar	Large, round. Raised, opaque and β-hemolysis
MacConkey agar	Lactose fermenter



3. Conventional Biochemical results:

The results of the biochemical tests showed that all 20 isolates were positive to catalase and citrate utilization test while negative to oxidase, urease, and indole tests as shown in table (2).

Table (2): Biochemical tests of *Aeromonas*

NO.	Biochemical test	Result
1-	KI and TSI	Red/Yellow
2-	Catalase	+
3-	Oxidase	+
4-	Lactose fermentation	lactose fermenter
5-	Urease	+
6-	Indole	–
7-	citrate utilization	+
8-	Motility	+

4. Api-20E system identification:

The result of Api-20E test has revealed that only 20 isolates from 150 sample were identified *Aeromonas* as figures (3).

Figure (3): Api-20Esystem of *Aeromonas* spp.

5. Percentage of *Aeromonas* infection in diarrheic patients:

The results of isolation of *Aeromonas* from 150 fecal samples of diarrheic patients were (20) *Aeromonas* isolates (13.3%)

The important clinical signs have been appeared in patients whom suffering from diarrhea were vomiting, abdominal pain and fever. Moreover, watery diarrhea was a common.

6. Percentage of diarrheal patients and their infection with *Aeromonas* according the age:

150 Patients (1-10years) suffering from diarrhea, from both sexes were include in this study. According the age; the age groups of 1-5 years were the highest percentage of diarrheal cases (60%) and 6-10 years were (40%) as in table (3)

Table (3): Percentage of diarrheal children and their infection with *Aeromonas* according the age

Age	No. of *Aeromonas*	Percentage (%)
1-5 years	90	60%
6-10 years	60	40%

The antimicrobial susceptibility test for 20 *Aeromonas* most of the isolates were sensitive to amikacin(30%), Imipenem(20%), Ceflazim(35%) while (60%) of isolates were resistance to Ciprofloxian, Cefotaxime and Gentamycin as in table (4-4,5) and figure(4-14)

Table (4): Percentage of antibiotics resistance by *Aeromonas* against 6 types of antibiotics according to CLSI, 2006 (n=20)

NO.	IMI	CIP	CAZ	CTX	AK	GM
1	S	R	I	R	S	R
2	S	R	S	R	S	R
3	S	S	S	S	R	S
4	S	S	S	S	I	R
5	S	I	S	S	S	S
6	S	S	S	S	I	R
7	S	S	S	S	S	S
8	S	R	I	R	S	S
9	S	R	S	R	S	I
10	S	R	S	R	S	R
11	S	R	S	R	S	R
12	S	R	I	R	R	R
13	S	I	R	R	R	R
14	I	I	R	R	S	R
15	R	R	R	S	R	R
16	R	R	R	S	R	R
17	I	R	R	R	R	S
18	R	R	I	R	S	S
19	R	S	R	I	S	R
20	I	R	R	R	S	S

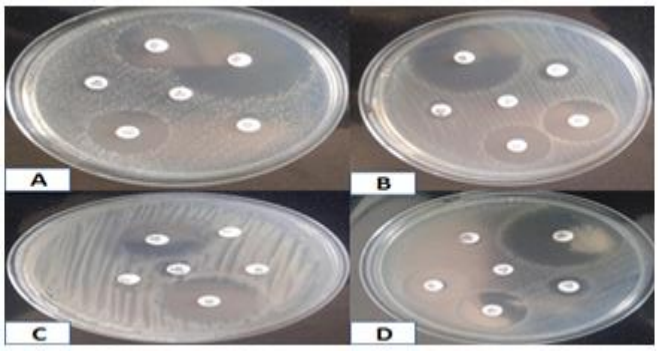


Figure (4): Antimicrobial susceptibility test of *Aeromonas* isolates

4. Discussion:

Aeromonas spp. are pathogens that cause foodborne gastroenteritis in human and extraintestinal symptoms such as; septicemia, wound infections, menengitis, endocarditis and osteomyelitis with a high mortality rate in immunocompromised person (Stelma ,1988) ; (Gold and Salit,1993). Our findings showed that (13.3%) of watery diarrheal childrin infected with *Aeromonas*; The socio-cultural and environmental circumstances including poor sanitation, massive religious gatherings and some natural disasters such as severe flooding, facilitates the spreading of the *Aeromonas* disease; Al-Hashemy (2012) reported thirteen isolates of *Aeromonas hydrophilia* were successfully recovered from drinking water in Baghdad and no isolates of *Aeromonas* from clinical samples ; these difference may be because the period of study. The present study reported *Aeromonas* isolates become resistant to commonly used antibiotics and multidrug resistance has been on a rise. We also found different patterns of antimicrobial susceptibility for *Aeromonas hydrophilia* isolates obtained from Thi-Qar province and other province and other countries patients with *Aeromonas*. The results in our study showed high levels of resistance to amikacin(70%), (60%) of isolates were resistance to Ciprofloxian, Cefotaxime and Gentamycin Ceflazim(35%) while Imipenem(20%), However ; Poobalane *et al*. (2008) who pointed out that *A. hydrophila* isolated from water, food and clinical samples was not sensitive to many antimicrobial drugs. Bhowmik *et al*. (2009) have agreed with our results work but, they also added that an additional resistant of this bacterium to Ampicillin, Tetracycline, Streptomycin. The identification of this diversity of resistant among *Aeromonas* spp. isolates may serve as reservoir of antibiotic resistance genes, which is of great public health concern in developing countries (Kivanc *et al*., 2011). The incidence of resistance among bacterial isolates to specific antibiotics has been reported by various authors world over and that associated with public health (Suhet et al., 2011).

Antimicrobial drug resistance in *A. hydrophila* can developed by efflux pumps, spontaneous mutation in chromosome, presence of the conjugative plasmids, and trimethoprim/sulfamethoxazole (SXT) elements and integrons (Jawetz *et al*.,2001). These could be partly explained by the fact that *A. hydrophila* pathogens are not able to stable to carry the resistance-causing plasmids (Sack *et al*., 1997).). In addition to the factors related to the microorganism itself and horizontal gene transfer; Antimicrobial resistance patterns in *A. hydrophila* can different greatly in depending on geographical location, patterns of antibiotic consumption among the studied people, and the period of study. the lacking or inadequate regulation of antibiotic consumption, inappropriate

implementation of existing laws, and population movements are among people and the behavioral factors that related to the emergence of multidrug-resistant microorganisms (MDR) in development countries (Ilic *et al*.,2012).

5.Conclusions :

1-The proportion of isolation *Aeromonas* from diarrheal children in Thi-Qar province is about (13.3 %).
2-Increasing prevalence of multidrug resistant between *Aeromonas* isolates**.**

6.Recommendation:

1-More study of Antibiotic susceptibility by using MIC.
2-Using the molecular techniques such as PCR for identification and characterization of *Aeromonas.*

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