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Genotypic Identification and Evaluation of Several Selective Media for Recovery of *Aeromonas* spp. from Different Sources

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ABSTRACT

A total number of 250 samples were collected from Suez canal area(50) samples from *Tilapia niloticus* fish, (50)*Mugil cephalus* fish, (50) drinking water (25 tap water in addition to 25 bottled mineral water), (50) pond water and (50) childhood diarrheal samples and these samples were cultured on several selective media. The isolation rate of Aeromonas spp. from all samples using enrichment technique on starch ampicillin agar (SAA), Rimler-shotts medium (RS), Blood ampicillin agar (BAA) and MacConkey ampicillin agar (MAA) were (51.2%), (45.2%), (38.8%) and (31.6%) respectively. The incidence of Aeromonads from different sources (*Tilapia niloticus* fish, *Mugil cephalus* fish, drinking tap water, bottled mineral water, pond water and childhood diarrhea were 44 (88%), 33 (66%), 4 (16)%, 0 (0%), 42 (84%) and 5 (10%) respectively. The total number of Aeromonas isolatesfrom 250 examined samples were 260 isolatesthat were biochemically identified into 4 biotypes as 136 (52.31%) *A.hydrophila*, 81 (31.15%) *A. sobria*, 34 (13.08%) *A. caviae* and 9 (3.46%) *A. schubertii*. Results of antibiogram of isolated Aeromonas spp. demonstrated that all tested Aeromonas isolates were resistant to Erythromycin, Sulphamethoxazol-Trimethoprim beside Ampicillin, and while the highest degree of sensitivity towards Ciprofloxacin,Norfloxacin, Amikacin and Gentamicin. Molecular identification of Aeromonas isolates by Polymerase chain reaction technique using *16Sr*RNA gene revealed that all examined Aeromonas isolates were positive, and also two virulence genes (aerolysin and hemolysin genes were identified by a specific primers and they present by a percentage of (83.3%) and (8.3%) respectively in examined Aeromonas isolates.The present study highlightsthe optimum recovery of Aeromonas spp. from mixed population require enrichment in alkaline peptone water and consequtive plating on more than one media and PCRtechnique provide rapid, sensitive and confirmatory identification of Aeromonas spp. and some virulence genes. Aeromonas spp. may use as an indicator for water quality and *A.hydrophila*& *A.sobria* are predominant, emerging and enteric pathogens.

INTRODUCTION

Aeromonas infections represent a serious problem to fresh water fish production, causing a significant economic loss to fish industry Saad *et al.,* (2014).

Aeromonas species are facultative anaerobic Gram negative bacteria that is amember of the family Aeromonadaceae that are widespread in sea, river, fresh and ground water Hassan *et al.,* (2012).

Moreover Aeromonas species can grow at refrigerator temperatures and replicate at high salt concenteration Janda and Abbott (2010). Aeromonas species cause several human diseases that vary in severity from a self-limiting gastroenteritis to potentially fatal septicemia, in addition to extra intestinal symptoms such as meningitis, endocarditis and osteomyelitis with a high mortality rate specially in immune compromised person Tsai*et al.,* (2006). A large range of selective and differential isolation media have been evolved for the isolation of Aeromonas species from the environment, foods, and clinical samples Villari *et al.,* (1999). Recovery of aeromonads from the contaminated samples like faeces may require usage of selective and differential media such as MacConkey media, cefsulodin irgasan novobiocin (CIN) media beside blood ampicillin agar (10 mg/L ampicillin) USEPA (2001), moreover Sarkar*et al.,* (2012) who useda selective medium, Rimler-shotts agar for isolation of *Aeromonas hydrophila* from different sources like fish, pond water, river water and Starch ampicillin agar (SAA), bile salts inositol briliant green agar (BIBG) and Aeromonas Medium (Ryan's Medium) which were recommended Igbinosa *et al.,* (2012). Numerous extracellular enzymes and toxins including the haemolysins, proteases, lipases, DNases, and cytotoxins that have been mentioned as virulence factors of motile Aeromonads Erdem *et al.,* (2010) and Cai *et al.,* (2012),however the role of each single factor regarding its pathogenesis varies John and Hatha (2014). The aim of this study wasthe isolation of Aeromonas on four selective media Starch ampicillin agar (SAA), Rimler-Shotts media (RS), Blood ampicillin agar (BAA) and MacConkey (MAA), evaluation of bacterial growth on different media, determination the incidence of Aeromonas spp. isolated from fish, water and childhood diarrheal samples in suez canal area, identification of isolated strains biochemically, antibiogram of such isolates and detetection of some virulent genes using polymerase chain reaction PCR (aerolysin and hemolysin gene) beside *16Sr* RNA gene.

MATERIALS AND METHODS

Samples:

A total of 250 samples were collected randomly from different fish farms in Suez canal area of *Tilapia niloticus*&*Mugil cephlus* fishes(50 samples for each), drinking tap water, bottled mineral water(25 samples for each), pond water (50 samples) and childhood diarrheal stool samples(50 samples). All samples were collected under aseptic condition and transferred immediately to microbiological lab.

Bacteriological examination:

a-Isolation and identification of Aeromonas: A loopful was taken aseptically from internal organs, gills and skin inoculated into alkaline peptone water (APW) for enrichment then incubated at 30 ^{0}C for 24 hrs Villari *et al.,* (2000), 25 ml of each water samples was thoroughly mixed with 225 ml of alkaline peptone water Cruickshank *et al.,* (1980), stool samples were directly inoculated into alkaline peptone water then was inoculated aerobically at 28ºC for 24 hrs. Aloopful from alkaline peptone water was subsequently streaked onto Starch ampicillin agar (SAA), Rimler-Shotts media (RS), Blood ampicillin agar (BAA), MacConkey ampicillin agar (MAA) aerobically incubated at 37ºC for 18-24 hrs. A film from typical colony of Aeromonas spp. were stained with gram stain Varnam and Evans (1991) and confirmed on the basis of the following test:Oxidase test, resistant to vibriostatic agent O/129, esculin hydrolysis, glucose fermentation in TSI, sugar fermentation and gas production, indole production and voges-proskauer test. Identification and biotyping of the isolates was carried out according to Aerokey II of Carnahan *et al.,* (1991a).

b- Antibiotic sensitivity test for the isolated Aeromonas from fishes, water & childhood diarrheal samples was done by disc diffusion technique Ericsson and Sherris (1971).

c- Molecular typing of isolated Aeromonas was done via PCR technique:

was used for the detection of *16Sr* RNA gene besides 2 virulence genes (aerolysin and hemolysin genes), Sambrook *et al.,* (1989).

RESULTS AND DISCUSSION

The present results in Table (1) and Figure (1) indicate that some selective media originally designed for isolation of Aeromonas species from different sources enrichement technique is used on several selective media such as Starch ampicillin agar, Rimler-Shotts medium, Blood ampicillin agar and MacConkey ampicillin agar was 51.2%, 45.2%, 38.8% and 31.6% respectively. These results agree with Villari *et al.,* (1999) who stated that SAA is the most sensitive culture media and is recommended to use it in isolation of Aeromonas species. and nearly similar to results obtained by Handfield *et al.,* (1996) in which recovery of *A.hydrophila* from drinking water samples on SAA was 71.4% which was higher than RS that was 50%, In addition to Thenmozhi *et al.,* (2013) used the Starch-Ampicillin agar as a selective presumptive isolation medium for the isolation of Aeromonas isolates from the drinking water samples that grow on Starch ampicillin agar after 24 hr incubation at 37°C. These colonies were Circular, Convex, Opaque, raised, smooth and entire edges colonies, with Yellow to honey colored and amylase positive colonies (clear zone surrounding the colony).Moreover, Pin *et al.*,(1994) reported that Starch ampicillin agar was the most adequate media for the isolation *A. hydrophila* but not adequate for recovery of *A. sobria*. From other hand, the low selectivity of SAA for Aeromonas has been pointed out by Ribas *et al*., (1991).

These finding results agree with Shotts and Rimler (1973) who stated that RS medium was commonly used in fish diagnostic laboratories for cultivation of Aeromonas spp. because it contains inhibitory substances such as sodium deoxycholate, novobiocin that were added to eliminate the chance of Gram positive organisms and vibrio spp., in addition to its high sensitivity of this media which enables this media not only for the recovery of *A.hydrophila* from specific sources but also for the enumeration of this organism in the environment. Also, Samal *et al.,* (2014) usedRimler-Shotts (RS) medium for isolation of Aeromonas from different freshwater diseased fish and 59 isolates grown and produced yellow, round, small to medium, convex, elevated and transparent colonies. However, these results disagreed with Robinson*et al*., (1984) who considered that medium of RS was unsuitable for isolation of fecal Aeromonas spp. Also Rippey and Cabelli (1979) stated that inefficiency of RS agar as an optimum *A.hydrophila* recovery medium due to novobiocin contained in the medium, which suppressed the growth of sensitive environmental *A. hydrophila* this effect pointed out by Kaper *et al*., (1981) who found that *A. hydrophila* lysine decarboxylase positive strains from the aquatic environment were not detected in RS agar.

The present results revealed that SAA (51.2%) is better than BAA (38.8%) for isolation of Aeromonas and these results were similar to Konchel (1989) who observed a satisfactory recovery and good differential properities which make SAA with (10 μg/ml & 30 μg/ml) better than blood agar as SAA can differentiate Aeromonas from the background microflora. Also, he revealed that SAA medium was highly selective and yielded consistently higher recoveries, in addition to produce 85% Aeromonas colonies, compared with 36-40% on blood agar which means that SAA was better than BAA, Furthermore, these present results agree with Millership *et al.,* (1983) who reported that blood agar with ampicillin was used for isolation of Aeromonas species based on beta hemolysis and oxidase test could be directly performed

on the plate, BAA was the most widely used media for isolation of Aeromonas from stool and BAA should be used in combination with another media for optimal detection of Aeromonas strains, On the other hand Andelova *et al.,* (2006) reported that BAA is useful only for recovery of Aeromonas if screening is based on hemolysis, but approximately 10% of Aeromonas isolates would be missed because they are no hemolytic.

however, BAA (38.8%) was better than MAA (31.6%) for isolation of Aeromonas and these results agree with Fricker &Tompsett (1989) who examined 563 samples of various food samples to compare plating media MacConkey and Blood ampicillin agar (BAA). They showed that (BAA) gave 43.3% positive samples while MacConkey gave only 31.2%, on the other hand, Daku *et al.,* (2004) isolated Aeromonas species from enteric samples and found that blood agar was the most sensitive media (86.5%), followed by MacConkey agar (70.3%) and this means that the isolation rate on BAA was higher than MA and the recovery rate of Aeromonas spp. On MacConkey ampicillin agar was lower, and Ifeanyichukwu *et al.,* (2015) who used MacConkey agar and Aeromonas selective medium supplemented with ampicillin for isolation of Aeromonas species from both chlorinated and non-chlorinated water samples and yielded 60% positively, In addition to Jepessen (1995) who reported that MA was not suitable to select Aeromonas since this genus includes lactose non fermenting besides lactose fermenting strains of the same sugar.

In the current study all the media except for Rimler-Shotts medium contain ampicillin as the selective agent and some Aeromonas spp. such as *Aeromonas trota*, are generally thought to be sensitive to ampicillin (Carnahan *et al.,* 1991b) also *Aeromonas jandaei* which has been shown to occasionally be ampicillin susceptible (Overman and Janda 1999). In addition to Huddleston *et al.,* (2007) who also suggested that ampicillin as a selective agent which hinder the growth of a significant portion of Aeromonas spp. and this lead to bias, misleading information and they postulate an underestimation of diversity Aeromonas spp. and its density where ampicillin was used in the isolation media.

Table1: Sensitivity of solid specific media for isolation of Aeromonas species from different samples:

Media used	Total No. of examined samples	Positive samples	
		No.	%
Starch ampicillin agar	250	128	51.2
Rimler-Shotts media	250	113	45.2
Blood ampicillin agar	250	97	38.8
MacConkey ampicillin agar	250	79	31.6

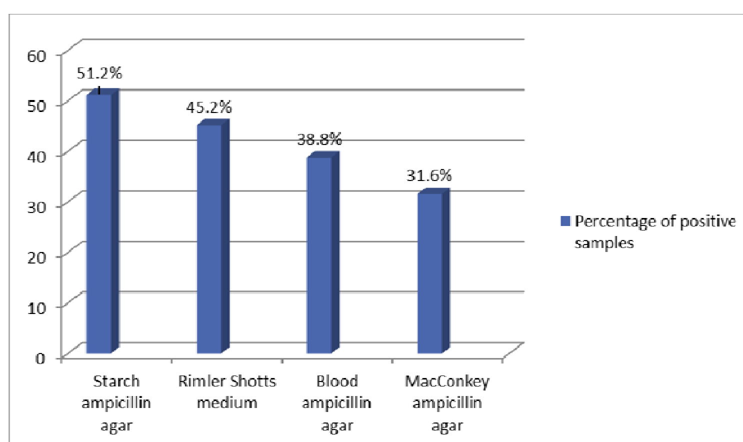

Fig. 1: Sensitivity of solid specific media for isolation of Aeromonas species from different samples

The present results as shown in Table (2) demonstrated that the frequency distribution of total Aeromonas spp. isolates recovered from all samples (*Tilapia niloticus, Mugil cephalus* fish, drinking tap water, botteled mineral water, pond water and childhood diarrheal samples) in Suez Canal area were:136 (52.31%) *A. hydrophila*, 81 (31.15%) *A. sobria*, 34 (13.08%) *A. caviae* and9 (3.45%) *A. schubertii*. These results agree with (Ghenghesh *et al.,* 2008) who stated that the most commonly isolated species from clinical samples, water and foods were *A. hydrophila*, *A. caviae* and *A. veronii biovarsobria*. And also Ottaviani *et al.,*(2011) who reported that *A. hydrophila* and *A.sobria* have been frequently isolated from food and environmental samples, which supported the present findings. The mostly commonly isolated Aeromonas spp. from environmental strains (water sources) were *A. hydrophila, A. sobria, A. caviae* and *A. schubertii*, while mostly commonly isolated Aeromonas spp. associated with clinical strains (childhood diarrheal samples) were *A. hydrophila* and *A. sobria*, as shown in Table (2). These results are similar to the data reported by Kühn *et al.,*(1997b); Ghenghesh *et al.,*(2001) and (Razzolini *et al.,* 2001) where *A. hydrophila* was the predominant species in freshwater and municipal drinkingwater supplies. Moreover, the present data also nearly agree with a study conducted in Turkey by Koksal *et al.,* (2007) who reported the isolation of Aeromonas such as *A. hydrophila* (46%), *A. sobria* (34%) and *A. caviae* (8%) and agree with John and Hatha (2013) who stated that *A. schubertii* was less than 10% and was the least predominant sp. in both water and fish samples and in contrast with the data obtained in the same study which showed that the predominant species in water samples were *A. sobria* followed by *A. caviae*, and frequency distribution of different species of Aeromonas is likely to vary with geographical locations. The finding results in Table (2) and Figure (2) showed frequency distribution of Aeromonas species isolated from different sources in fish samples (*Tilapia niloticus* & *Mugil Cephalus* fishes) that were identified biochemically into the predominant species was *A.hydrophila* and this agree with Rathore *et al.,*(2005) who reported that *A. hydrophila* was the predominant species in fish samples in India, In addition to Yadav and Kumar (2000); while in Egypt Abou El-Atta (2003) demonstrated the preponderance of *A. hydrophila* followed by *A. sobria* and *A. caviae* from fish. Similar finding observed by Sharma and Kumar (2011) In contrast with Yucel *et al.,* (2005) who affirmed that among fresh water fish spp. *A.caviae* was the prevalent species followed by *A. hydrophila* and *A. veronii biovar sobria* in Turkey. The distribution results as shown in Table (2) revealed the isolation of Aeromonas spp. recovered from *Tilapia niloticus* fish samples was 107 isolates. These are biochemically identified into *A. hydrophila* 56 (52.33%) among other Aeromonas spp. followed by *A. sobria* 33 (30.84%), *A. caviae* 14 (13.08 %) and *A. schubertii* 4 (3.73%). These results are similar to Maimona *et al*., (2015) who isolated *A. hydrophila*, *A. sobria* from tilapia fish and nearly agree with Kumar *et al*., (2000) who recorded isolation of *A. hydrophila* in fish (70.59%) followed by *A. sobria* (69.23 %) and *A. caviae* (33.33 %), but disagree with Ashiru *et al*., (2011) who recorded distribution of *A. caviae* followed by *A. hydrophila* and *A. sobria* in tilapia. On the other hand, *A. schubertii* is the least predominant spp. among Aeromonas spp. in present results, such result in agreement with John and Hatha (2013) who isolated *A. schubertii* less than (10%).

The present results as shown in Table (2) showed the recovery of Aeromonas spp. isolated from *Mugil cephalus* fish samples was 84 isolates. These are biochemically identified into *A. hydrophila* 40 (47.62%), *A. sobria* 29 (34.52%), *A. caviae* 13 (15.48%), *A. schubertii* 2 (2.38%), and this result agree with Enany *et al*., (2011) who stated the common bacterial pathogen

isolated from *Mugil cephalus* was *A. hydrophila*, In addition to the present result is nearly agreed to Salah El-Dien *et al*., (2009) who recorded isolation of Aeromonas spp. was (30 isolates) of *A. hydrophila*, (3) *A. caviae*, (1) *A. sobria* from fresh mullet samples, but disagree with Yucel *et al*., (2005) who affirmed that *A. veronii biovar sobria* was the most isolated Aeromonad in sea fish species (41.5%) followed by *A. hydrophila* (30.1%) and *A. caviae* (28.3%). In general, the present results in Table (2) showed that the predominant spp. isolated from *Tilapia niloticus* and *Mugil cephalus* was *A. hydrophila* and these results agreed with those recorded by Farid *et al*., (1978) and Shalaby (1997, 2005). The current results in Table (2) revealed that frequency distribution of Aeromonas species recovered from Drinking tap water samples and identified biochemically into *A. hydrophila* 3(75%) and *A. sobria* 1 (25%) and this results agree with Kühn *et al.,* (1997a) who reported that *A. hydrophila* was the major phenotype in drinking water samples in Sweden, while such results are higher than Di Bari *et al.,* (2007) who recorded isolation of *A. hydrophila* (48.3%) from drinking water samples. The finding results in Table (2) demonstrated that isolation of Aeromonas species are 60 isolates recovered from pond water of fish that identified biochemically into *A. hydrophila* 34 (56.66%), *A.sobria* 16 (26.66%), *A. caviae* 7 (11.66%) and *A. schubertii* 3 (5%) and this closely agree with Abd-Elall *et al*., (2014) who stated that *A. hydrophila* was more frequently isolated from pond water and John and Hatha (2013) who isolated *A. hydrophila, A. sobria* , *A. caviae* and *A. schubertii* from water samples but vary in prevalence percentages according to variation of geographical locations, In addition the less frequently isolation of *A. schubertii* is nearly in agreement with Janda and Abbott (2010) and John and Hatha (2013) who recorded isolation of *A. schubertii* in less frequent, but disagree with Evangelista-Barreto *et al*., (2010) who reported that high frequency and isolation of *A. caviae* in water. The current results in Table (2) showed the frequency distribution of Aeromonas species isolated from childhood diarrheal samples that identified biochemically into *A. hydrophila* 3 (60%) and *A.sobria* 2 (40%) are the two predominant species that isolated from stool. These results agree with Yadav and Kumar (2000) who demonstrated the same Aeromonas species (3 *A. sobria*, 2 *A. hydrophila*) from fecal samples of diarrheic children under five years of age, and these present finding agree with Pokhrel & Thapa (2004) who found that *A. hydrophila* was the most common species in stool then followed by *A. caviae* and *A. sobria* and nearly agree with Vasaikar *et al.,* (2002) who stated that *A. hydrophila* was the predominant species by 64.2 % of isolated Aeromonas from cases of gastroenteritis, then *A. sobria* 28.4 %, in addition to, Guz and Kozinska (2004) who reported that *A. hydrophila* complex and *A.sobria* complex were potential pathogens of animals and humans, characteristics of aeromonads have a public health importance, so it should be assessed, but disagree with Soltan and Moezardalan (2004) who found that *A. sobria* was the predominant species (57%) followed by *A.caviae* (36%) then *A.hydrophila* (7%) in Tehranian children presenting with diarrhea, moreover Ananthan and Alavandi (1999) who reported that the predominance of *A.caviae* in stool of children with gastroenteritis in Chennai , in addition to the frequency isolation of different species of Aeromonas can vary with the geographic allocations according to record of Sinha *et al*., (2004). While the distribution of Aeromonas species in stool samples (childhood diarrheal sample) in present study, the predominant species of Aeromonas was *A. hydrophila* followed by *A. sobria* and this result was agree with Kannan *et al*., (2010) and von Graevenitz (2007) who found *A. hydrophila* as predominant in Brazil, Thailand and India, and in contrast with previous study conducted in Europe, the United States and India, *A. caviae* was dominant followed by *A. hydrophila* and *A. veronii biovar sobria* Albert *et al*., (2000); Borchardt *et al*., (2003);

Shiinaand Iwanaga (2004). Also it may be due to other factors like the isolation and identification methods used may be of importance Abbott *et al*., (2003); Janda and Abbott (2010).The four different phenol species are observed in this present study *A. hydrophila* (52.31%), *A. sobria* (31.15%), *A. caviae* (13.08%), *A. schubertii*(3.45%) that are distributed in this suez canal geographic area, and these species composition were limited to ampicillin resistant isolates and this agree with the similar finding of Oakey *et al*., (1996) and Ormen & Ostensvik (2001) , although the similar species were reported in many previous studies but the relative isolation of these species was found to vary by John and Hatha (2013), In addition to types of Aeromonas spp. that isolated from fish (*A. hydrophila*, *A. sobria* , *A. caviae* and *A. schubertii*) are the same types of Aeromonas spp. that isolated from pond water of fish and this microbiota of pond water reflect microbiota of fish and this closely similar to Sousa and Sliva sauza (2001) who reported that Aeromonas in water medium was found represented in the internal fish organs, in Brazil. Furthermore, Awadallah and Abd-El All (2009) who stated that level of fish contamination with microorganisms was found to be directly proportional to their level in the overlying water, while types of Aeromonas spp. that isolated from drinking tap water (*A.hydrophila* and *A. sobria*) are the same types of Aeromonas spp. that isolated from childhood diarrheal samples and these findings may emphesize the findings of Holmberg *et al*., (1986) that showed acorrelation between the consumption of water and Aeromonas mediated diarrhea.

Table 2: Distribution of different Aeromonas spp. isolates from (*Tilapianiloticus&Mugil cephalus* fishes, Drinking Tap, Botteled mineral water, Pond water and Childhood diarrheal stool samples):

Samples	No. of isolates	Distribution of Aeromonas isolates							
		A. hydrophila		*A. sobria*		*A. caviae*		*A. schubertii*	
		N	%	N	%	N	%	N	%
Tilapia fish	107	56	52.33	33	30.84	14	13.08	4	3.73
Mugil fish	84	40	47.62	29	34.52	13	15.48	2	2.38
Total	191	96	50.26	62	32.46	27	14.13	6	3.14
Tap water	4	3	75	1	25	0	0	0	0
Botteled mineral water	0	0	0	0	0	0	0	0	0
Total	4	3	75	1	25	0	0	0	0
Pond water	60	34	56.66	16	26.66	7	11.66	3	5
Childhood Diarrhea	5	3	60	2	40	0	0	0	0
Total	260	136	52.31	81	31.15	34	13.08	9	3.45

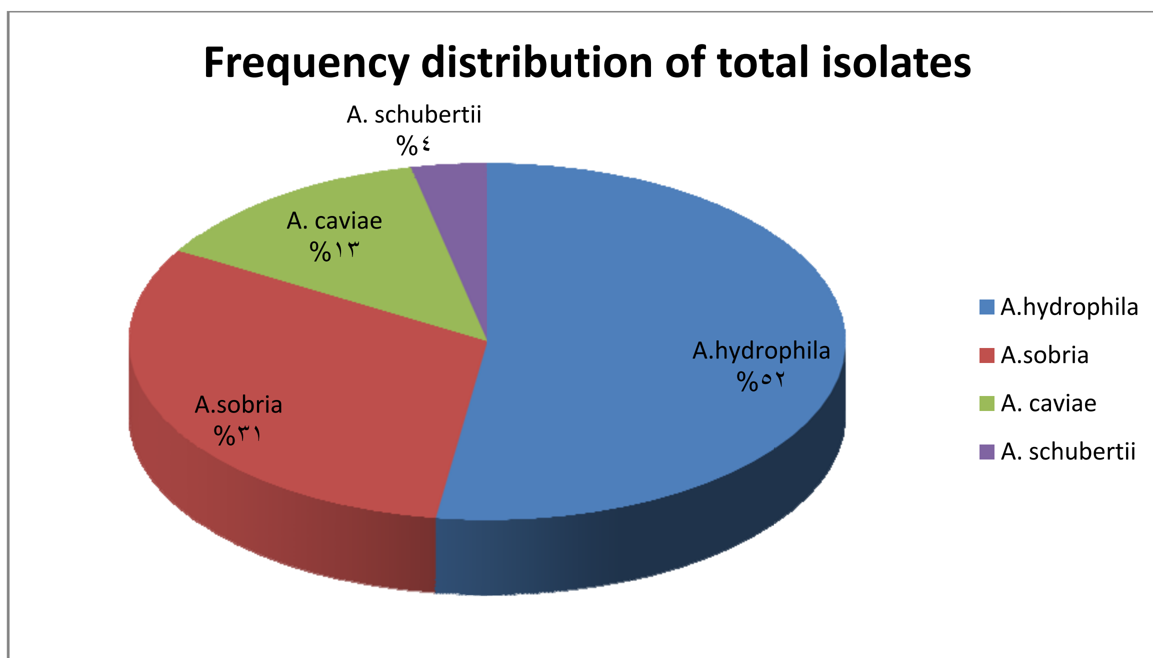

Fig. 2: Frequency distribution of total Aeromonas isolates from all samples (*Tilapia niloticus* fish, *Mugil cephalus* fish, drinking tap water, drinking botteled mineral water, pond water of fish and Childhood diarrheal samples).

The antibiotic resistance patterns against 10 antimicrobial agents were established for the48 strains from Aeromonas species isolated from the fish, water and childhood diarrheal stool samples and demonstrated in Table (3) and Figure (3). The present results revealed that all strains of *A. hydrophila*, *A. sobria*, *A. caviae* and *A. schubertii* are highly resistant to antibiotics like Ampicillin and Erythromycin as well as Sulphamethaxazole–trimethoprim. Suchresults were in concordance with Sreedharan *et al.,* (2012) and Carnahan *et al.,* (1991a). On the other hand the present results showed that *A.hydrophila* is sensitive to Gentamicin and Ciprofloxacin by a percentage (100%), Norfloxacin by a percentage (90%), Amikacin by a percentage (60%), Doxycycline and Cefotaxime by a percentage (20%), but resistant to Ampicillin and Erythromycin antibiotics as well as Sulphamethoxazol-trimethoprim and Rifampicin and such results agree with John and Hatha (2013) who stated the sensitivity of Aeromonas spp. to Gentamicin and Ciproloxacin (100%), and also agree with Enany *et al.,* (2011) who recorded that *A. hydrophila* had been resistant to Erythromycin, and nearly agree with Samal *et al.,* (2014) who stated that Norfloxacin was sensitive by (84.6%), while disagree with Awan *et al.,* (2009) that showed that Cefotaxime (90.9%), Amikacin (100%) the more sensitive. The present study revealed that *A. sobria* is sensitive to Amikacin by a percentage(100%), Ciproloxacin, Cefotaxime by a percentage (66.6%), Norfloxacin (58.33%) Rifampicin (41.66%), Doxycycline (33.3%), Gentamicin (16.66%), while is resist to Ampicillin, Erythromycin, Sulphamethoxazol-trimetoprim and this agree with Henadek (2002) that stated that *A. sobria* was sensitive to Doxycycline (33%), and agree with Awan *et al.,* (2009) who reported *A. sobria* was sensitive to Amikacin (100%) but disagree with John and Hatha (2013) who showed *A. sobria* is sensitive to Ciproloxacin and Gentamicin by a percentage (100%). The present study

showed that *A. caviae* showed sensitivity toward Amikacin, Gentamicin, Norfloxacin (100%), Ciproloxacin (87.5%), Cefotaxime (50%), Doxycycline (37.5%), while was resist to Ampicillin, Erythromycin, Sulphamethoxazol - trimethoprim and Rifampicin, and this agree with Awan *et al.,* (2009) who reported *A. caviae* was sensitive to Gentamicin (100%) and nearly similar with Amikacin (96.2%), Ciproloxacin (88%), and in contrast with them when they stated that *A. caviae* was sensitive to Cefotaxime (96%), Sulphamethoxazol-trimetoprim was sensitive by (46.2%), Erythromycin sensitive by (18.2%). In addition *A. schubertii* was sensitive to Doxycycline (100%), Cefotaxime (100%), Amikacin (100%), Norfloxacin (100%), Gentamicin (100%), Ciproloxacin (100%) beside Rifampicin (37.5%) , while was resist to antibiotics as Ampicillin and Erythromycin as well as Sulphamethoxazol-trimethoprim and this was similar to John and Hatha (2013) with that showed *A. schubertii* was sensitive to Ciproloxacin and Gentamicin (100%), and Awan *et al.,* (2009) who reported *A.schubertii* was sensitive to Ciproloxacin, Cefotaxime and Amikacin (100%), but disagree with the data obtained in the same study which revealed *A. schubertii* was sensitive to Sulphamethoxazol-trimethoprim (50%).

Table 3: Antibiogram of random isolated Aeromonas species

Antimicrobial Agent	Disccont gμ		Antibiogram according to *Aeromonas* species								Total N=48	
			A. hydrophila n=20		*A. sobria* n=12		*A. caviae* n=8		*A. schubertii* n=8			
			n	%	n	%	N	%	n	%	N	%
Ampicllin (AMP)	10	R	20	100	12	100	8	100	8	100	48	100
		S	0	0	0	0	0	0	0	0	0	0
Erytrhomycin (E)	15	R	20	100	12	100	8	100	8	100	48	100
		S	0	0	0	0	0	0	0	0	0	0
Sulphmethoxazole-Trimethoprim(SXT)	25	R	20	100	12	100	8	100	8	100	48	100
		S	0	0	0	0	0	0	0	0	0	0
Rifampicin (RD)	5	R	20	100	7	58.3	8	100	5	62.5	40	83.3
		S	0	0	5	41.6	0	0	3	37.5	8	16.6
Doxycycline (DO)	30	R	16	80	8	66.6	5	62.5	0	0	29	60.4
		S	4	20	4	33.3	3	37.5	8	100	19	39.5
Cefotaxime (CTX)	30	R	16	80	4	33.3	4	50	0	0	24	50
		S	4	20	8	66.6	4	50	8	100	24	50
Gentamicin (CN)	10	R	0	0	10	83.3	0	0	0	0	10	20.8
		S	20	100	2	16.6	8	100	8	100	38	79.1
Amikacin (AK)	10	R	8	40	0	0	0	0	0	0	8	16.6
		S	12	60	12	100	8	100	8	100	40	83.3
Norfloxacin (NOR)	10	R	2	10	5	41.6	0	0	0	0	7	14.5
		S	18	90	7	58.3	8	100	8	100	41	85.4
Ciprofloxacin (CIP)	5	R	0	0	4	33.3	1	12.5	0	0	5	10.4
		S	20	100	8	66.6	7	87.5	8	100	43	89.5

S = Sensitive R = Resistant

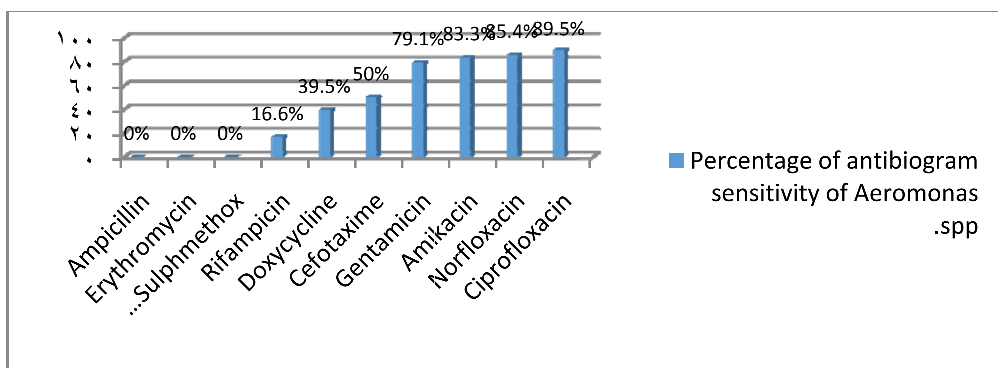

Fig. 3:Antibiogram of isolated Aeromonas species.

Conventional PCR using *16Sr*RNA gene for 12 tested Aeromonas strains which were identified biochemically as 5 strains of *A. hydrophila* and 3 *A. sobria*, 2 *A. caviae*, 2 *A.schubertii*, the present results revealed that all examined strains were positive for *16Sr*RNA gene as shown in Table (4) Figs. (4 &5) and Photo (1). These results were nearly similar with Martinez-Murcia (1999) and Wang *et al.,* (2003) who used *16Sr*RNA gene for identification of the tested strains of Aeromonas which give the same results that all isolated strains were positive for this gene presence.

PCR assay was developed with specific primers for detection of different

Aeromonas spp. virulence genes (Aerolysin and Hemolysin). The current results showed that Aerolysin gene was detected in 10 strains out of 12 (83.3%), Table (4), photo (2) and Figs. (4 &5) and this result is closely similar to Abd-ElAll *et al.,* (2014), Ottaviani *et al.,* (2011) and Singh *et al.,* (2008) who reported that total aerolysin gene detection in Aeromonas spp. in fish and pond water samples was (80%), (83.7%), (85%) respectively. They also nearly agree with Ormen and Ostensvik (2001) who used a PCR assay to detect the *aer* A gene in Aeromonas spp. environmental water isolates in Norway and reported that 79% were positive.

Table 4: Frequency distribution of *16Sr*RNA, Aerolysin and Hemolysin genes of isolated *Aeromonas* spp.:

Aeromonas strains	*16Sr*RNA gene		Aerolysin Gene		Haemolysin gene	
	No.	%	No.	%	No.	%
***A.hydrophila* n= 5**	5	100	4	80	0	0
***A.sobria* n= 3**	3	100	2	66.6	0	0
***A.caviae* n= 2**	2	100	2	100	0	0
***A.schubertii* n= 2**	2	100	2	100	1	50
Total	12	100	10	83.3	1	8.3

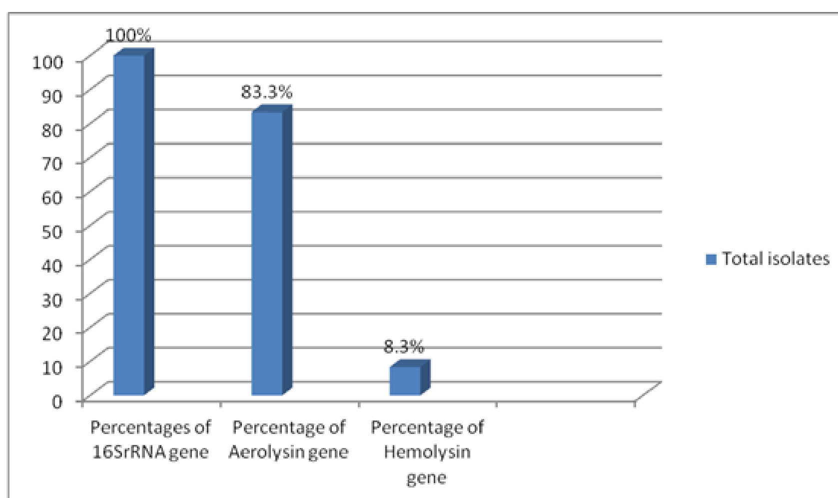

Fig. 4: Percentage of positive isolates for *16Sr*RNA gene, Aerolysin gene and Hemolysin gene.

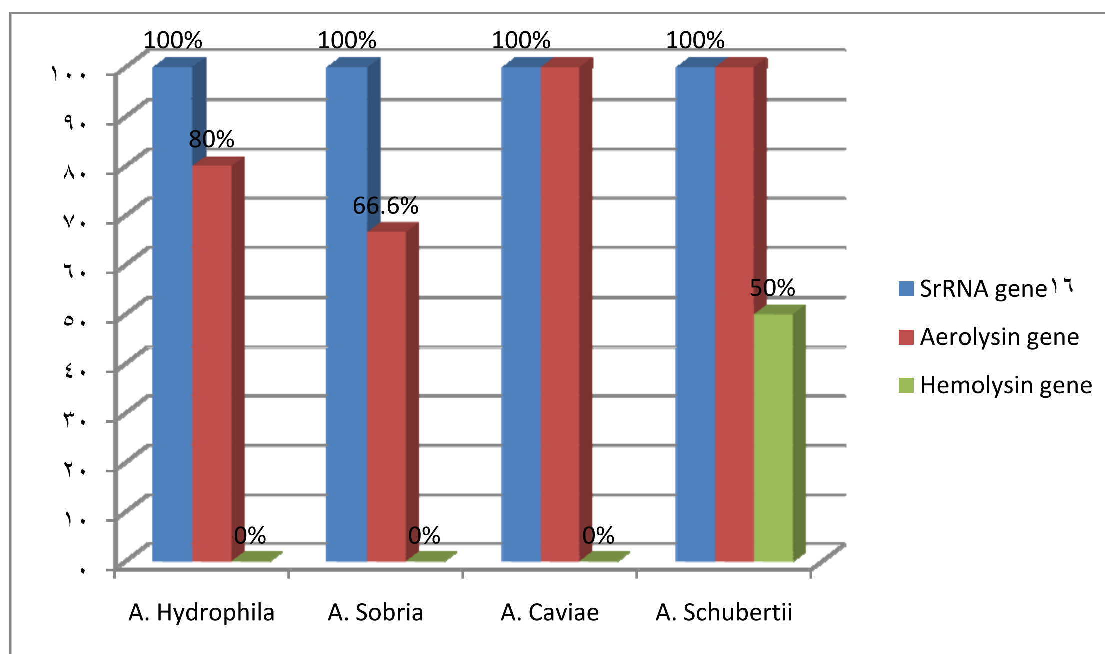

Fig. 5: Percentage of *16Sr*RNA, Aerolysin and Hemolysin genes of isolated Aeromonas species.

The current study revealed very low percentage of hemolysin gene (1 out of 12 strains) (8.3%) which belong to *A. schubertii*, while the remaining strains were not harbouring the hemolysin gene. This finding was observed also by Abdullah *et al.,* (2003). On other hand, Yucel and Citak (2003) who reported that *A. hydrophila* and *A. sobria* were been stronger producer of hemolysin but *A. caviae* was non hemolytic

In the current study some Aeromonas strains lacked both *aer* A and *hly*A genes and this was observed before in earier studies done by (Santos *et al.,* 1999 and Herrera *et al.,* 2006) who stated that aerolysin-like gene was activable under certain conditions and can be detected in apparently non haemolytic strains.

The current results revealed that aerolysin gene is 100% in *A. caviae* (2/2) and *A. schubertii* (2/2) were positive for aerolysin gene, while in only 80% of *A. hydrophila* (4/5) and 60% of *A. sobria* (2/3). This result nearly agree with Abd-El All *et al.,* (2014) and Umesha *et al.,*(2011) who detected Aerolysin gene in (100%) of *A. hydrophila* recovered from fish samples, furthermore Abdullah *et al.,* (2003) detectedaerolysin gene in all the isolates, and nearly similar to Herrera *et al.,* (2006) who mentioned that 8/9 of *A. hydrophila* were positive for aerolysin genebut differ with another study which reported a result of 2/4 of *A. caviae* and 2/2 of *A. sobria* that were positive for aerolysin gene. In contrast with Heuzenroeder *et al.,* (1999) who madea survey of clinical and environmental isolates of the Aeromonas spp. and stated that *aer*A-like sequences were found in 78%, 97% and 41% respectively, in *A. hydrophila, A. sobria* and *A. caviae* isolates, Moreover Pollard *et al*., (1990) and Lior and Johnson (1991) showed that the *aerA* gene was detected only in hemolytic, cytotoxic and enterotoxic strains of *A. hydrophila* but not in *A. sobria* and *A. caviae*.

The current results revealed that Aerolysin gene was 66.6 % positive in *A. sobria* and this result is similar to Yousr *et al.,* (2007) who detected that the same percentage of aerolysin gene of *A. sobria*, but disagree with the percentage of *A. hydrophila* and *A. caviae* where the aerolysin gene were (52.6%) and (44.7%) respectively.

The frequency and distribution of the aerolysin gene in the Aeromonas strains in this study was nearly similar with an earlier

PCR survey by Husslein *et al*., (1991) who detected the *aer* A gene in all strains belonging to *A. hydrophila* and *A. sobria* species, so the aerolysin gene seemes to be as ubiquitous like the Aeromonas spp.

In the current result, the clinical strains possesless hemolytic activity and this observation is also reported by Altwegg (1985) who stated that although, it is very likely that clinical isolates possess less number of virulence gene, it should kept in mind that Aeromonads were recognized as opportunistic microorganism that may be present in diarrheal stool as commensals rather than as primary pathogens.

Another observation , which is that one of the isolated *A. sobria* strain was lacking both aerolysin & hemolysin genes and developed multi drug resistance and another isolated *A. hydrophila* strainwas lacking hemolysin genes and developed also multi drug resistance and such results may strongly force the point of view that pathogenicity and virulence of Aeromonas spp. are multifactorial and complex Janda and Abbott(1998); Chopra *et al.,* (2000), and this agrees with Shome *et al.,* (1999) who mentioned that the production of enzymes or toxins is not reflective of biological virulence and they neigther satisfy the strain to be virulant nor avirulant in spite of; this appear to enhance the process of disease in-vivo. The whole process of pathogenesis is a complex interaction between the host, agent and environmental determinants.

Photo (1):illustrated the positive for amplification of **(685 bp)** fragment of *16Sr*RNA gene from extracted DNA of 12 Aeromonasspp. from fish, water and human stool samples.

Photo (2): illustrated **(326 bp)** fragment of (*aer*A) gene where (10) amplification Aeromonas strains were positive for aerolysin gene.

Photo (3): illustrated **(1500bp)** fragment of hemolysin gene from extracted DNA of *A. schubertii* isolated from water.

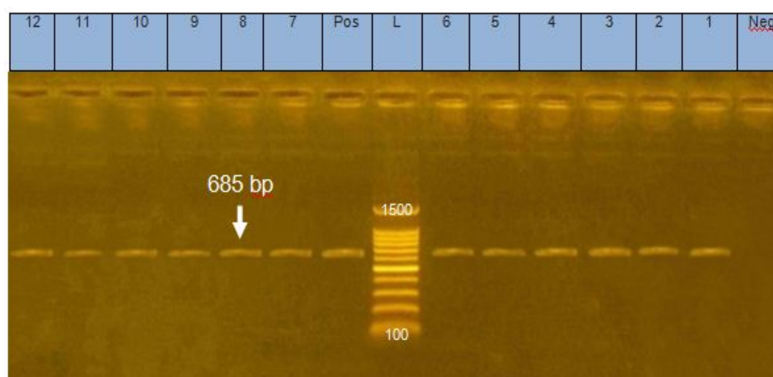

Photo 1: Electrophoretic pattern of *16Sr*RNA gene amplification of 12 Aeromonas spp. isolated from different sources.
Lanes 1-12: showed *16S* rRNA gene of **685bp** from various Aeromonas spp. of different sources positive of Aeromonas spp **from water :** *A. hydrophila* (Lane 1), *A. schubertii* (Lane 2), *A. sobria* (Lane 3) and *A. caviae* (Lane 4) ; **from fish :** *A. hydrophila* (Lane 5,6,10) , *A. caviae* (Lane 7) , *A.sobria* (Lane 8), *A. schubertii* (Lane 9) ; **from stool :** *A. sobria* (Lane 11) & *A. hydrophila* (Lane 12).

- Lane(L) for ladder (100 bp DNA ladder).

Pos. = +ve control
Neg. = -ve control

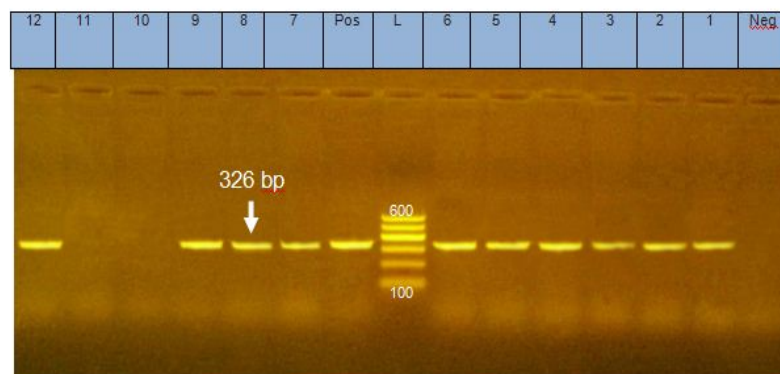

Photo 2: Electrophoretic pattern of Aerolysin gene amplification of 12 Aeromonas spp. isolated from different sources.
Lanes 1-12: showed Aerolysin gene of **326 bp** from various Aeromonas spp.of different sources positive of Aeromonas spp **from water :***A. hydrophila* (Lane 1), *A.schubertii* (Lane 2), *A. sobria* (Lane 3) and *A.caviae* (Lane 4) ; **from fish :** *A.hydrophila* (Lane 5,6,10) , *A.caviae* (Lane 7) , *A.sobria* (Lane 8), *A.schubertii* (Lane 9) ; **from stool :** *A. sobria* (Lane 11) & *A.hydrophila* (Lane 12).
Lane (L) for ladder (100 bp DNA ladder).
Pos. = +ve control
Neg. = -ve control

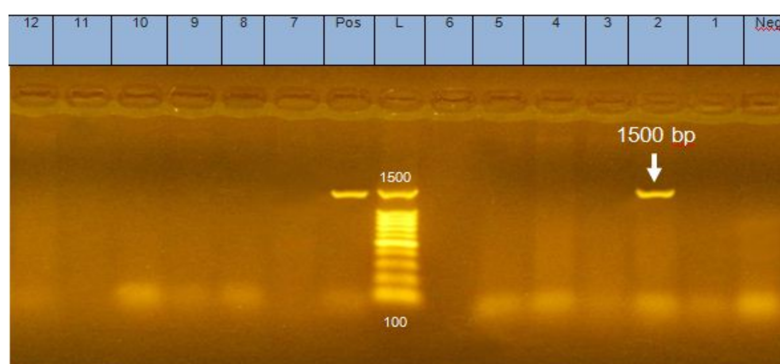

Photo 3:Electrophoretic pattern of Hemolysin gene amplification of 12 Aeromonas spp. isolated from different sources.
Lanes 1-12: showed Hemolysin gene of **1500 bp** from various Aeromonas spp. of different sources positive of Aeromonas spp **from water :***A. hydrophila* (Lane 1), *A. schubertii* (Lane 2), *A.sobria* (Lane 3) and *A.caviae* (Lane 4) ; **from fish :** *A.hydrophila* (Lane 5,6,10) , *A. caviae* (Lane 7) , *A. sobria* (Lane 8), *A. schubertii* (Lane 9) ; **from stool :** *A.sobria* (Lane 11) & *A. hydrophila* (Lane 12).
Pos. = +ve control
Neg. = -ve control
Lane (L) for ladder (100 bp DNA ladder).

CONCLUSION AND RECOMMENDATIONS

It could be concluded from the present study that the isolation of Aeromonas species from mixed population such as fishes, waters and childhood diarrhea, require enrichment in alkaline peptone water and consecutive plating on more than one media such as Starch ampicillin media and Rimler-Shotts media to avoid the missing of some Aeromonas spp. As the isolation of Aeromonas species is laborious process and biochemical identification lack specifity, so PCR technique provide rapid and sensitive method for confirmatory identification of Aeromonas species and detection of some virulence genes.

Aeromonas species seem to be prefer fresh water than brackish water and marine water, so freshwater fish (*Tilapia niloticus*) showed heavier contamination than Mugil cephilus. also the Aeromonas spp. isolated from drinking tap water and childhood diarrhea This data suggesting that the bacterial population of Aeromonas on fish and water may reflect the level of human infection .This study show that Aeromanas not only primary fish pathogen but also potentiate the evidence that Aeromonas is water born and an emerging pathogen for human. The four phenotypes species that recovered from

Suez Canal area were *A. hydrophila*, *A. sobria*, *A. caviae* and *A. schubertii*,. So routinely examination for Aeromonas spp. in Clinical laboratory of hospitals is necessary specially for *Aeromonas hydrophila* and *Aeromonas sobria* that are the predominant enteric and emerging species in Suez canal area Aeromonas species not only seems to be ubiquitous in habitats, but also Aerolysin gene and multiple resistances to antibiotics are ubiquitous.In current study Aeromonas spp. developed multiple drug resistant to Erythromycin, Sulphamethoxazol-trimethoprim, Rifampicin, Doxycycline and Cefotaxime beside the classical resistant to Ampicillin, Higher frequency of multi-drug resistance was observed for *Aeromonas sobria* than *Aeromonas hydrophila* this may be attributed to the fact that *Aeromonas sobria* is more virulent than *Aeromonas hydrophila*. so The legal restrictions is highly recommended in using antibiotics for controlling of Aeromonads infections in fishes, water and human, and recommended using of Ciprofloxacin and Norofloxacin as first line treatment followed by Gentamicin and Amikacin as 2nd line of treatment in control fish infection while in human, Amikacin or Gentamicin can used as first line treatment followed either by Norofloxacin or Ciprofloxacin as 2nd line of treatment.

Hemolytic activity could be the land mark for genus Aeromonas and in the present study Aerolysin gene is broad spread in the isolated strains of Aeromonas and Hemolytic activity of Aeromonas spp. not enhance the disease process in fish and human and not serve species specific marker so this study may enforced that pathogenicity and virulence of Aeromons spp. are multifactorial and complex so the advance investigation of other factors rather than haemolysin genes is required to understand the pathogenicity of Aeromonas.

Regular examination of pond water and their input supplies should make for prohibition their contamination with Aeromonas from sewage pollution of pond water of fish. And improving water quality may improve fish health condition In addition to apply sanitary and hygienic measurements to control biofilm formation as it may play an important role in contamination of drinking water to avoid risk of human infections.

REFERENCES

Abd-Elall, A.M.M.; Abd-El-Kader, M.A. and Atia, A.S. (2014): Occurrence, Seasonal Variations and Virulence of *Aeromonas hydrophila*and *Aeromonas caviae* in Fish Farms at East Delta, Egypt Global Veterinaria,13 (3): 328-336.

Abdullah, A.I.; Hart, C.A. and Winstanley, C.(2003): Molecular characterization and distribution of virulence associated genes amongst Aeromonas isolates from Libya. J. Appl. Microbiol., 95:1001–1007.

Abbott, S.L.; Cheung, W. K.W.; and Janda, J.M. (2003): The Genus Aeromonas: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes. J. Clin. Microbiol., 41: 2348–2357.

Abou El-Atta, M.E. (2003): Efficiency of polymerase chain Reaction (PCR) in diagnosis of some bacterial fish pathogens. Ph. D. Thesis in Vet. Sci (Bacteriology, Immunology and mycology) Suez Canal University.

Albert, M.J.; Ansaruzzman, M.; Talukder, K.A.; Chopra, A.K.; Khun, I.; Rahman, M.; Faruque, A.S.G.; Islam, M.S.; Sack, R.B. and Mollby, R., (2000): " Prevalence of enterotoxin genes in Aeromonas spp. isolated from children with diarrhea, healthy controls, and the environment. Journal of Clinical Microbiology, 38: 3785-3790.

Altwegg, M. (1985): *Aeromonas caviae*: an enteric pathogen? Infection, 13:228–230.

Ananthan S. and Alavandi S.V. (1999): Biochemical characteristics & secretory activity of Aeromonas species isolated from children with gastroenteritis in Chennai. Indian J. Med. Res. Indian J. Med. Res., 109:136.

Andelova, A., Porazilova, I. and Krejci, E. (2006): Correspondence. Aeromonas agar is useful selective medium for isolating aeromonads from faecal samples. J. Med. Microbiol., 55:1605–1606.

Ashiru, A.W.; Uabio-Egbeni, P. O.; Oguntowo, J. E.; Idika, C. N. (2011): "Isolation and antibiotic profile of Aeromonas species from Tilapia fish (Tilapia nilotica) and Catfish (Claris betrachus). Pak. J. Nutr., 10: 982-986.

Awadallah, M.A.I. and Abd-ElAll, A.M.M. (2009): Bacterial pollution indicators in aquatic environment and fish in Abu El-akhder sea with special reference to E.coli and its protien profile. J. Egypt. Vet. Med. Assoc., 69 (3):157-174.

Awan, M. B.; Maqbool, A.; Bari, A.; Krovacek, K. (2009): "Antibiotic susceptibility profile of Aeromonas spp. isolates from food in abu Dhabi, United Arab Emirates." New Microbiologica, 32: 17-23.

Borchardt, M. A.; Stemper, M. E.; Standrige, J.H., (2003): "Aeromonas isolates from human diarrheic stool and groundwater compared by pulse-field gel electrophoresis. Emerging Infectious Diseases 9: 224-228.

Cai, S.H.; Wu, Z.H.; Jian. J.C.; Lu, Y. S. and Tang, J. F. (2012): “Characterization of pathogenic *Aeromonas veronii* bv. *Veronii* associated with ulcerative syndrome from Chinese longsnout catfish (*leiocassis longirostris* günther).” Brazilian Journal of microbiology,382-388.

Carnahan, A.M.; Behram, S. and Joseph, S.W. (1991a): Aerokey II: A flexible key for identifying clinical Aeromonas species. Journal Clinical Microbiology,29: 2843-2849.

Carnahan, A.M.; Chakraborty, T.; Fanning, G.R.; Verman, D.; Ali, A.; Janda, J.M. and Joseph, S.W., (1991b): Aeromonas trot asp. Nov., an ampicillin susceptible species isolated from clinical specimens, J.Clin. Microbiol.;29: 1206-1210.

Chopra, A.K.; Xu, X.J.; Ribardo, D.; Gonzalez; M.; Kuhl, K.; Peterson, J.W. and Houston, C.W. (2000): The cytotoxic enterotoxin of *Aeromonas hydrophila* induces proinflammatory cytokine production and activatesarachidonic acid metabolism in macrophages. Infect Immun, 68:2808–2818.

Cruichshank, R.; Duguid, J.P.; Marminon, B.P.and Swain, H.A. (1980): Medical microbiology 12th ed vol. II churchil Livingstone, robert Stevenson, Edinburgh.

Daku, D.; Manickam, K.; Thomas, E.; Horsman, G.B. and Levett, P.N.(2004): Comparasion of media for isolation of aeromonas from stools in Saskatchewan. abstracts_04/fl_04_bed. htm.

Di Bari, M.; Hachich, E.M.; Melo, A.M.J. and Sato, M.I.Z. (2007): Aeromonas spp. and microbial indicators in raw drinking water sources Brazilian Journal of Microbiology, 38: 516-521.

Enany, M. EL.; Ibrahim, H.M.; Abou EL Atta, M. EL.; El-Adawy, M.M.(2011): "Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured Mugil capito fish in Ismailia Governorate." SCVMJ, XVI (1).

Erdem, B.; Karİptaş, E. and Kaya, T. (2010): “Siderophore, hemolytic, protease, and pyrazinamidase activities and antibiotic resistance in motile Aeromonas isolated from fish.” Turkish J. Biology, 34: 453-462.

Ericsson, J. M. and Sherris, J. C. (1971): Antibiotic sensitivity testing, report of an international collaborative study. Acta. pathol. Microbiol. Scand. Sect. B. (Suppl.) 217.

Evangelista-Barreto, N.S.; Carvalho, F.C.T.; de, Vieira, R.H.S. dos, F.; Reis, M.F.; dos, Macrae, A.; and Rodrigues, D. dos, p. (2010): Characterization of Aeromonas species isolated from an estuarine environment. Braz. J. Microbiol., 41 (2): 452-460.

Farid, A. H.; Amin, N. E. and Akealah, M. A. (1978): "Some studies on the bacterial flora of the genus Tilapia in Manzala Fish Farm." presented to the 14 th Arab. Vet Cong, Cairo March.

Fricker, C. R. and Tompsett, S. (1989): Aeromonas spp. In foods: asignificant cause of food poisoning? Int. J. Food Microbiol., 9: 17-23.

Ghenghesh, K. S.; El-Ghodban, A.; Dkakni, R.; Abeid, S.; Altomi, A.; Tarhuni, A.; Marialigeti, K. (2001): "Prevalence, species differentiation, haemolytic activity, and antibiotic susceptibility of Aeromonads in untreated well water." *Mem. Inst. Oswaldo Cruz*, 96(2):169-173.

Ghenghesh, K. S.; Ahmed, S. F.; El-Khalek, R. A.; Al-Gendy, A.; Klena, J. (2008): "Aeromonas-associated infections in developing countries. J. Infect Developing Countries", 2:81-98.

Guz, L. and Kozinska, A. (2004): Atibiotic susceptibility of *A. hydrophila* and *A. sobria* isolated from farmed carp (Cyprinus Carpio L.) Bull. Vet. Inst. Pulawy., 48: 391-395.

Handfield, M.; Simard, P. and Letarte, R. (1996): Differential media for quantitative recovery of waterborne *A. hydrophila*. Appl. environ. Microbiol., 62(9):3547.

Hassan, M., Bazargani-Gilani, B. Amir, T. and Hadi, E. (2012):A cytotoxicity and comparative antibacterial study on the effect of zataria multiflora Boiss, Trachyspermum copticumessential oils and Enrofloxacin on *Aeromonas hydrophila*. Avicenna j. Phytomedicine., 2: 188-195.

Henadak, A.A. (2002): Studies on *Aeromonas hydrophila* microorganisms isolated from Raw milk and milk products M. V. Sci.

Thesis (microbiology) Fac. Vet. Med. Suez Canal Univ.

Herrera, F.C.; Santos, J.A.; Otero, A.; Garcia-Lopez, M.L. (2006): Occurance of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in spain. J. Appl. Microbiol., 100 :527-536.

Heuzenroeder, M. W.; Wong, C. Y. and Flower, R. L. (1999): Distribution of two haemolytic toxin genes in clinical and environmental isolates of Aeromonas spp.: correlation with virulence in a suckling mouse model. FEMS Microbiology Letters, 174:131–136.

Holmberg, S.D.; Schell, W.L.; Fanning, G.R. *et al*., (1986): Aeromonas intestinal infections in the United States. Annals of Internal medicine., 105: 683-689.

Huddleston, J. R.; Zak, J. C. and Jeter, R. M. (2007): Sampling bias created by ampicillin in isolation media for Aeromonas. Can. J. microbiol., 53:39–44.

Husslein, V.; Bergbauer, H. and Chakraborty, T. (1991): Studies on aerolysin and serine protease from *Aeromonas trota* sp. Nov. experiential, 47: 420-421.

Ifeanyichukwu, I.; Nicodemus, M.; Chika, E.; Emmanuel, N. and Emmanuel, U. (2015): “Detection of Biofilm-Producing isolates of Aeromonas species from Drinking Water Sources in Ezza South Local Government Area of Ebonyi State, Nigeria.” J. Pharm chem Biol Sci., 3(2):316-323.

Igbinosa, I.H.; Igumbor, E.U.; Aghdasi, F.; Tom, M. and Okoh, A.I. (2012): “Emerging Aeromonas species infections and their significance in public health.” The Scientific World J., 625023.

Janda, J.M. and Abbott, S.L. (1998): Evolving concepts regarding the genus Aeromonas: an expanding Panorama of species, disease presentations, and unanswered questions. Clinical Infectious Diseases, 27(2):332-344.

Janda, J. M. and Abbott, S. L. (2010): The genus Aeromonas Taxonomy, Pathogenicity and Infection. Clin Microbiol Rev., 23: 35-73.

Jeppesen, C., (1995): "Media for Aeromonas species, Plesiomonas shigelloides and Pseudomonas species from food and environment. Inter. J. of Food Microbiol., 26: 25-41.

John, N. and Hatha, A. A. M. (2013): "Distribution, Extracellular Virulance factors and Drug Resistance of Motile Aeromonads in fresh water ornamental fishes and associated Carriage water." Inter. J. Aqua., 3(17): 92-100.

John, N. and Hatha, A. A. M. (2014): “Distribution, extracellular virulence factors and antibiogram of motile aeromonads in fresh water ornamental fishes and immune response of *Cyprinus carpio* against *Aeromonas hydrophila* infection.” Ph. D. Thesis (Microbiology) fac. Marine Sci. Cochin univ. of Science and Technology.

Kannan, S.; Chattopadhyay, UK; Pal, D.; Shimada, T.; Takeda, Y.; Bhattacharya, S.K. and Ananthanarayanan, P.H. (2010): "Isolation and identification of Aeromonas from patients with acute diarrhea in Kolkata, India. Indian Journal of Medical microbiology 19, 190-192.

Kaper, J.B.; Lockman, H.; Colwell, R.R.; and Joseph, S.W. (1981): *Aeromonas hydrophila:* ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. 50: 359-377.

Koksal, F., Oguzkurt, N., Samastı, M. and Altas, K. (2007): Prevalenceand Antimicrobial Resistance Patterns of Aeromonas Strainsisolated from Drinking Water Samples in Istanbul, Turkey. Chemotherapy., 53:30-35.

Konchel, S. (1989):The suitability of four media for enumerating Aeromonas spp. From environmental samples. Letters in Applied microbiology, 9: 67-69.

Kühn, I.; Allestam, G.; Huys, G.; Janssen, P.; Kersters, K.; Krovacek, K. and Stenstrom, T. (1997a): Diversity, persistence, andvirulence of Aeromonas strains isolated from drinking water distribution systems in Sweden. Appl. Environ. Microbiol., 63:2708-2715.

Kühn, I.; Albert, M. J.; Ansaruzzaman, M.; Bhuiyan, N.A.; Alabi, S.A.; Islam, S.M.; Neogi, K. B.; Huys, G.; Janssen, K.K.; möllby, R. (1997b): "Characterization of Aeromonas sp isolated from human with diarrhea, from healthy controls and from surface water in Bangladesh." *J. Clin. Microbiol.*, 35(2):369-373.

Kumar, A.; Bachhil, V.; Bhilegaonakar, K. and Agarwal, R. (2000): Occurrence of enterotoxigenic Aeromonas species in foods. J. commun. Dis., 32, 169-74.

Lior, H. and Johnson, W. M. (1991): Application of the polymerase chain reaction (PCR) to detection of the aerolysin gene in whole cell cultures of β- hemolytic *Aeromonas hydrophila*. Experientia, 47: 421-424.

Maimona; Samia and Sabiel, Y. A. (2015): "Detection of the causative agents of bacterial fish septicemia of Tilapia and Clarais inKhartoum state." Inter. J. recent Scientific Res., 6(6): 4374-4377.

Martínez-Murcia, A. J., (1999): Phylogenetic positions of *Aeromonas encheleia Aeromonas popoffii* Aeromonas DNA hybridization group 11 and Aeromonas group 501. International Journal of Systematic Bacteriology, 49:1403–1408.

Millership, S. E.; Curnow, S. R. and Chattopadhyay, B.(1983): Faecal carriage rate of Aeromonas hyrophila. J. Clin. Pathol., 36: 920-923.

Oakey, H.J.; Ellis, J.T. and Gibson, L.F. (1996): Abiochemical protocol for the differentiation of current genomospecies of aeromonas. Zentralblatt fur Bakteriologie, 284:32-46.

Ormen, O. and Ostensvik, O. (2001): The occurrence of aerolysin positive Aeromonas spp. and their cytotoxicity in Norwegian water sources. Journal of Applied Microbiology, 90: 797–802.

Ottaviani, D.; Parlani, C.; Citterio, B.; Masini, L.; Leoni, F.; Canonico, C.; Sabatini, L.; Bruscolini, F. and Pianetti, A., (2011): putative virulance properities of Aeromonas strains isolated from Food, environmental and clinical sources in Italy: A comparative study. Int. J. Food Microbiol., 144: 538-545.

Overman, T. L., and Janda, J. M. (1999): Antimicrobial susceptibility patterns of *Aeromonas jandaei, A. schubertii, A. trota,* and *A. veronii* biotype *veronii*. J. Clinical Microbiology, 37:706-708.

Pin, C.; Marin, M.L.; Garcia, M. L.; Tormo, J. and Cases, C. (1994): Comparison of different media for the isolation and enumeration of Aeromonas spp. In foods. Lett. Appl. Microbiol. (18): 190-192.

Pokhrel, B.M. and Thapa, N. (2004): Prevalence of Aeromonas in different clinical and water samples with special reference to gastroenteritis. Nepal Med. Coll. J., 6(2):139-143.

Pollard, D.R., Johnson, W.M., Lior, H., Tyler, S.D. and Rozee, K.R. (1990): Detection of the aerolysin gene in *Aeromonas hydrophila* bythe polymerase chain reaction. J. Clinical Microbiology, 28:2477–2481.

Rathore, G.; Swaminathan, T. R. Abidi, R.; Mahanta, P. C.; and Kapoor, D.; (2005): Isolation and characterization of motile aeromonads from aquatic environment, Indian. J. Fish., 52(2):241-248.

Razzolini M.T.P.; Bari, M.; Sanchez, P.S.; Sato, M.I.Z. (2001): "Pesquisa de *Aeromonas* e suas toxinas em águas de consumo humano provenientes de caixas d'água e bebedouros." XXI Congresso Brasileiro de Microbiologia, Foz do Iguaçu, Resumos. Anais.

Ribas, F.; Araujo, R.; Frias, J.; Haguet, J.M.; Ribas, F.R. and Lucena, F. (1991): "Comparision of different media for the identification and quantification of Aeromonas spp. in water." 59: (4): 225-228.

Rippey, S.R. and Cabelli, V.J. (1979): "Membrane filter for enumeration of *Aeromonas hydrophila* in fresh waters. Appl. Environ. microbiol. 38: 108-113.

Robinson, J.; Burke, V.; Worthy, P.J.; Beaman, J. and Wagener, L., (1984): "Media for isolation of Aeromonas species from faeces." Journal of Medical Microbiology, 18: 405-411.

Saad, T.T.; El-Gohary, M.; El-Shanat, S. and Ali, M.A. (2014): Mixed infection of ectoparasities and bacterial pathogens in *oreochromis niloticus* in El-behera governorate and its relations to economic losses. Journal of Middle East Applied Science and technology, 12: 481-488.

Salah El-Dien, W.M.; Enas, M.S.; Hanan, M.T.E. and Saleh, M. A. (2009): Prevalence of *Aeromonas hydrophila* and *Yersinia enterocolitica*in some seafood's sold in Sharkia governorate markets and the effects of heat treatments on their viabilities. Vet. Med. J., 11:121-130.

Samal, S.K.; Das, B.K. and Pal, B.B. (2014): "Isolation, biochemical character-rization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish." IW3nt. J. Curr. Microbiol. App. Sci., 3(12): 259-267.

Sambrook, J.; Fritscgh, E. F.; and Mentiates (1989): Molecular coloning. Alaboratory manual. Vol., Cold spring Harbor Laboratory press, New York.

Santos, J. A.; González, C. J.; Otero, A. and García-López, M.L. (1999): Hemolytic activity and siderophore production in different aeromonas species isolated from fish. Appl Environ Microbiol., 65:5612–5614.

Sarkar, A.; Saha, M.; Patra, A. and Roy, P. (2012): "Characterization of *Aeromonas hydrophila* through RAPD-PCR and SDS-

PAGE analysis." OpenJournal of Medical Microbiology, 2, 37-40.

Shalaby, A.M. (1997): "Studies of virulence factors of some Pseudomonadaceae and Vibrionaceae micro-organism isolated from fresh and frozen fish." Ph. D. Thesis (Microbiology) Fac. Vet. Med. Suez Canal Univ.

Shalaby, A. M. (2005): Study on prevalence of *Aeromonas hydrophila* in some foods of animal origin J. Egypt Vet. Med. Assoc., 65(6): 55-62.

Sharma, I. and Kumar, A. (2011): Occurance of enterotoxigenic Aeromonas species in foods of animal origin in North East India. eur, Rev, Med. Pharmacol. Sci., 15, 883-887.

Shiina, Y.; Ii, K. and Iwanaga, M., (2004): "An *Aeromonas veronii biovar sobria* infection with disseminated intravascular gas production. J. Infection and Chemotherapy 10:37-41.

Shome, R.; Shome, B. R. and Ram, N. (1999): "Study of virulance factors of *Aeromonash-ydrophila* isolates causing acute abdomoinal dropsy and ulcerative diseases in Indian major carps, Indian J. Fish.,46(2): 133-140.

Shotts, B. E. and Rimler, R. (1973): Medium for isolation of *Aeromonas hydrophila.* Appl. Microbiol., 26(4): 550-553.

Singh, V.; Rathore, G.; Kapoor, D.; Mishra, B.N. and Lakra, W.S. (2008): Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pondwater. Indian J. Microbiol., 48: 453-458.

Sinha, S.; Shimada, T.; Ramamurthy, T.; Bhattacharya, S. K.; Yamasaki, S.; Takeda, Y., and Nair, G. B. (2004): Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic Aeromonas species isolated from hospitalized diarrhoeal cases in Kolkata, Indian J. Med. Microbiol., 53:527-534.

Sreedharan, K.; Philip, R. and Singh, I.S.B. (2012):"Virulence potential and antibiotic susceptibility pattern of motile aeromonads associated with freshwater ornamental fish culture systems: A possible threat to public health." Brazilian J. microbial., 754-765.

Soltan Dalla, l. M. M. and Moezardalan, K. (2004): Aeromonas spp. associated with children's diarrhoea in Tehran: a case-control study. Ann. Trop. Paediatr., 24(1):45-51.

Sousa, J.A.D. and Silva Sauza, A.T. (2001): Bacterial community associated with fish and waterfrom Congonhas River, Sertaneja parana, Brazil. Brazilian Archives of Biology and Technology, 44(4): 373-381.

Thenmozhi, S.; Rajeswari, P.; Kalpana, M.; Haemalatha, M. and Vijayalakshmi, P., (2013): "Antibactericital activity of Silver nitrate on Biofilm forming Aeromonas spp. Isolated from Drinking water." Int. J. Pure App. Biosci. 1(6): 117-125.

Tsai, M.; Kuo, C.; Wang, M.; Wu, H.; Chein C., and Liu, J.(2006):Clinical features and risk factors for mortality in Aeromonas and bacteremic adult with hematologic malignancies. J. Microbiol., Immunol. Infection, 39: 150-154.

Umesha, D.; Srinivasa Rao, P.; Pani Prasad, K.; Reddy, A.K. and Srinivas, K.N. (2011): Aerolysin and haemolysin virulence genes of *Aeromonas hydrophila* isolated from diseased ornamental*s* freshwater Oscar fish and goldfish by Polymerase Chain reaction. international J. Advances in Science and Technology, 3(1): 82-89.

United States Environmental Protection Agency, (USEPA) (2001): Aeromonas in finished water by membrane filtration using ampicillin-dextrin agarwith vancomycin (ADA-V), United States Environmental Protection Agency, Washigton, DC, USA.

Varnam, A. H. and Evans, M.G. (1991): Food borne pathogen Manson Publishing Ltd, London, United Kingdom.

Vasaikar, S.; Saraswathi, K.; De, A.;Varaiya, A. and Gogate A. (2002): Aeromonas species isolated from cases of acute gastroenteritis. Indian J. Medical Microbiol., 20(2):107-109.

Villari P.; Pucino A.; Santagata N.; and Torre I.;(1999).:A comparison of Different Culture Media for the Membrane Filter quantification of Aeromonas in Water. Appl Microbiol, 29: 253–257.

Villari, P.; Crispino, M. ; Montiuori, P and Stanzione, S. (2000): Prevelance and molecular characterization of Aeromonas species in ready to eat food in Italy. J. food protection, 63: 1754-1757.

Von Graevenitz, A. (2007):" The role of Aeromonasin diarrhea: a review. Infection, 35:59-64.

Wang G; Clifford C.G.; Liu C.; Pucknell C.; Munro C.K.; Kruk T.M.; Caldeira R; Woodward, D.L. and Rodgers F.G. (2003): detection and characterization of a hemolysin gene in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. J. Clin. Microbiol. 41: 1048-54.

Yadav, A.S. and Kumar, A. (2000): Prevalence of enterotoxigenic motile aeromonads in children, fish, milk and ice-cream and their public health significance. *Southeast Asian J. Trop. Med. Public Health*, 31, 153-156.

Yousr, A. H.; Napis, 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 14(2): 115-122.

Yucel, N., and Citak, S., (2003): The occurrence, hemolytic activity and antibiotic susceptibility of motile Aeromonas spp. isolated from meat and milk samples in Turkey, J. Food Safety., 23: 189-200.

Yucel, N.; Aslim, B. and Beyatli, Y. (2005): Prevalence and resistance to antibiotics for Aeromonas species isolated from retail fish in Turkey. J. Food Quality, 28: 313-324.

ARABIC SUMMARY

التصنيف الجيني وتقييم العديد من الأوساط المختارة للكشف عنالإيروموناس من مصادر مختلفة

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تم تجميع 250 عينة عشوائية بواقع (50) عينه من أسماك البلطي النيلي و(50) عينه من أسماك البوري من المزارع السمكية بمحافظات القناة و(25) عينه من ماء الشرب و(25) عينه من المياه المعدنية و(50)عينه من مياه أحواض الأسماك بالإضافة إلى تجميع (50) عينه من براز من أطفال مصابين بالإسهال.وزرعها على الأوساط الغذائية الانتخابية والتفريقية وتقيمها فى الكشف عن هذا الميكروب وكذلك استخدام التفاعلات الحيوية الكيمائية والتحري باستعمال تقنية تفاعلا بلمرة المتسلسل لميكروبات الايروموناس.

و ذلك لإجراء الآتي عليها:-

- عزل وتصنيف ميكروب الايروموناس على أربع وسائط غذائية انتخابية والمعزولة من مصادر مختلفة .
- دراسة مقارنيه لصفات الميكروبات المعزولة فى كل من الأسماك والإنسان والماء .
- إجراء اختبار الحساسية لهذا الميكروب لتعرف على مدى إمكانية القضاء عليه والتحكم فيه.
- دراسة جينية لتحديد بعض الجينات المسببة لضراوة الميكروب ومدى تواجدها فى كل من العترات المعزولة من الأسماك والإنسان والمياه .

وقد كشفت الدراسة ان عزل ميكروبات الايروموناس من المصادر المختلفة على المستنبتالزرع (SAA)، (RS) ;(ABA) و(MAA)51,2% و45,2%و8 ,38% و31,6 % على التوالى.

وقد أسفرت النتائج ان السيادة لنوع الايروموناس هيدروفيلا52.31% يليهاالايروموناس كافي,الايروموناس سوبريا, ثم الايرموناس شبرتي بنسب 31.15 % ،13.08 % ،3.46 علي التوالى.

وفى عينات الأسماك زادت نسبة العزل في السطح الخارجي للسمكة عن الأعضاء الداخلية والخياشيم ,بينما كانت نسبة عزل ميكروبات الإيروموناس من عدد 25 عينة مياه شرب بنسبة(16%)ونسبة عزل ميكروبات الايروموناس من عدد 50 عينة من مياه أحواض الأسماك بنسبة (84%) وكانتنسبة عزل ميكروبات الإيروموناس من عدد 25 عينة من المياه المعدنية سلبية العزلبينما كانت نسبة عزل الإيروموناس من عدد 50 عينة من براز الأطفال المصابين بالإسهال 10%.

وقد تم تصنيف العترات المعزولة من سمك البلطي الى (56) عترة هيدروفيلا، (33) عترة سوبريا ،(14) عترة كافى، (4) عترى شبرتى والعترات المعزولة من السمك البوري الى (40) عترة هيدروفيلا ، (29) عترة سوبريا ، (13) عترة كافى، (2) عترى شبرتى.

وقد تم تصنيف العترات المعزولة من مياه الشرب الى (3) عترة هيدروفيلا،(1) عترة سوبريا ، وقد تم تصنيف العترات المعزولة من مياه أحواض السمك إلى (34) عترة هيدروفيلا،(16) عترة سوبريا ،(7) عترة كافى، (3) عترة شبرتى

وقد تم تصنيف العترات المعزولة من الاطفال الى (3) عترة هيدروفيلا،(2) عترة سوبريا.

وبإجراء اختبار الحساسية اتضح مدى المقاومة العالية الكاملة لميكروبات الإيروموناس للامبسلين و اريثرومايسن وسلفا ميثاكزول-تراى ميثوبريم بنسبة مقاومة (100%) وبنسبة مقاومة اكثر من 50% لكل من سيفوتاكسيم والدوكسى سيكلين وريفامبسينفى حين أظهر الميكروب حساسية لكل من السيبروفلوكاسين 89،5% و النورفلوكساسين 85،4% يليهم الإميكاسين والجنتامايسن83،3%و79،1%علي التوالى.

وبعمل مقارنة جينيه باستخدام تفاعل البلمرة المتسلسل فى محاولة الكشفوالتحريعن (*16Sr*RNA) جنس الإيروموناس وجينات الضراوة (aerolysin, hemolysin) gene فى 12 عتره من العترات المعزولة وقد وجد ان aerolysin موجود في جميع العترات ماعدا عترتين وhemolysin موجود فى عترة واحدة فقط و*16Sr*RNA موجود فى جميع العترات.