Study on *Aeromonas spp.* Isolated from raw and drinking water in Baghdad city

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Abstract

Atotal of 551 water samples drinking(five Water Treatment Plans (WTPs) and raw water(from different sites on Tigris river) were collected.According to morphological characteristics and a set of biochemical tests, one hundred twenty eight of *Aeromonas spp* isolates were obtained In this study The percentages of *Aeromonas* recovery from river water was 72.52%, from wells water was (35%).Total percentage of positive aeromonas samples of treated water(Filtration &chlorine tank, supply water of WTPs, distribution system, reserviores and other samples not related to WPTs) was 8.8%.Count of *Aeromonas* in positive aeromonas samples ranging from 1 to 175 cfu 100 ml.

The results showed that generally no significant correlation between presence of aeromonas in distribution system water and other indicators (total heterotrophic plate counts and fecal pollution), also negative correlation between residual chlorine and recovery percentage were detected.

Seasonal pattern for the incidence of isolates investigated that higher rate took place during summer months, while the lower rate occurred during winter.

The results of identification to species level were: the most predominant species is *A. hydrophla* 63 isolates(49.21%), *A. trota* 21isolates(16.41%), *A. veroni*12 isolates*(*9.37%*) A. salmoncida* 11 isolates(8.59%), and *A. schubertii*, *A. sobria*, *A. cavia* four isolates for each(3.13%), and unidentified *Aeromonas SPP* nine isolates(7.03%). *Aeromonas hydrophila* isolates revealed higher resistant for most antibiotics used in the study

Key word: *Aeromonas spp*, raw water, drinking water unidentified

دراسة لانواع *Aeromonas spp* المعزولة من المياه الخام ومياه الشرب في مدينة بغداد

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الخلاصة:

جمعت 551 عينة ماء شرب من محطات تصفية المياه وماء خام من نهر دجلة واعتمادا على الفحوصات الكيموحيوية تم الحصول على 128 عزلة *Aeromonas* بنسبة عزل72.25% من ماء النهر و35 % من الابار 8.8 % من اجمالي ماء الشرب (حوض الفلترة والكلور وماء الشرب داخل المشروع والخزانات المنزلية ونماذج ماء شرب من مناطق اخرى).تراوحت اعداد *Aeromonas* بين 1 −175 cfu/100 ml.اشرت النتائج الى عدم وجود ارتباط معنوي بين وجود *Aeromonas* ودلائل التلوث الاخرى (بكتريا التلوث البرازي وغيرها)وكذلك بين متبقي الكلور .اظهرالنمط الموسمي لوجود العزلات ارتفاع في الصيف بينما يقل في الشتاء .

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اظهرت نتائج التشخيص للانواع ان السيادة كانت لنوع *A.hydrophila* 49.21 % (63 عزلة)ثم *A. trota* 16.41% (21 عزلة) و *A.veronii* 9.37% (12 عزلة) ووجد النوع *A.salamoncda* بنسبة 8.59% (11 عزلة) ثم *A.schubrtti, A.caria, A.sobria* بنسبة3.13% (4 عزلات لكل نوع) و *Aeromonas spp* 9 عزلات7.03% .

اظهرت عزلات*A.hydrophila* مقاومة عالية لاغلب المضادات الحيوية المستعملة بالدراسة

1.Introduction

Species of *Aeromonas* are Gram-negative, non-spore-forming, and rod-shaped, facultatively anaerobic bacteria that occur ubiquitously and autochthonously in aquatic environments widely distributed in aquatic environments such as surface waters(fresh, estuarine and marine), groundwater, drinking water systems, wastewater and, soil[1].

Aeromonas species can be found in various concentrations in drinking water. The chronic exposure of immunocompromised persons to *Aeromonas* via contaminated waters could potentially lead to invasive disease, such as septicemia [2]. The World Health Organization lists *Aeromonas* in the third edition of Guidelines for Drinking-Water Quality .In 1998, the Environmental Protection Agency listed *A. hydrophila* on its "Drinking Water Contaminant Candidate List.[1]

The motile mesophilic aeromonads implicated in human illnesses include *A. hydrophila* and *A. caviae*, *A. veronii* (biotype *sobria*) . Another important aspect to take into account in relation to these bacteria is that some strains are resistant to many antibiotics, such as gentamicin, ciprofloxacin, norfloxacin, tetracycline and ceftriaxone [2].

This study was aimed to investigate *Aeromonas* especially *Aeromonas hydrophila* in raw and drinking water distribution systems that very important to ensure the good quality of public water, resulting in protection and promotion of public health .

2.Materials and Methods

2.1 Study Area

Atotal of 551 water samples (drinking and raw water)were collected during period April 2011 till February 2012

Samples included :

A- samples were collected from five Water Treatment Plans (WTPs), from the steps of stations (intake of river, sedementation tank, filteration and chlorine tank, supply drinking water of WTPs) that showed in table [1].

B- Dinking water from distribution system related to these WTPs from began, mid, and end of network of each WTPs**C**-Drinking water from collective reservoirs related to distribution system.table[2]

D- Other drinking water samples(collected from different sites).

E-Water samples from wells of different position there are: Mahmoudia, Yosufia, Karadha, Jeser Dialah and Al- Sader city.

F-Raw water samples from different sites on Tigris river, there are :Al-Doura, Abunuaas, Kadhoumia, Zaafarania, Al-Seenak, Jadyria, Abu-khreb, Al-russtumia and near vegetative oil factory.table [3]

Table 1- Five water treatment plants

WTPs	position	Regions related to WTPs
1. AL-Wahda	AL-Arasaat	AL-Wahda_Jadiriah_52 street AL-baladeyat_Karadah.
2. AL-Doura	AL-Athoreen	AL-Athoreen_AL-Jazera_Mahdy 1_Mahdy 2_Abu-Desheer
3.AL-Sader	Sader city	Orfaly _ Hi tarak _ Choader _ Gayarah _ Jameela.
4. AL- Qadisia	AL-Qadisia	AL-Mashtal _ Amen _ Ubaydy _ Zafaraniah _ Jeser Dialah.
5. AL-Rashid	AL-Rashid	Qadisia _ Yarmouk _ Harthia _ Mansour _ Askaan.

Table 2- distribution of drinking water samples

sites	No. of samples
Filteration &chlorine tanke	2 for each WTP =10
supply water of WTP	2 for each WTP =10
Distribution system	56 for each WTP =280
collective reserviors	100
other polluted regions (not related to 5 WTPs)	20
total	420

Table 3- distribution of raw water samples

Site	No. of samples
Intake	2 for each WTP =10
Sedemintation tanke	2 for each WTP =10
Different sites of Tigris	91
Wells	20

2.2 Sampling

2.2.1 Sampling of drinking water

Samples were collected according to the APHA Standard Methods [3] Prior to collection, water was allowed to run to waste at a uniform rate for 2–3 min. Water samples were collected in sterile bottles containing 1 mL of sterilized 3% sodium thiosulfate stock per L of sample to sample bottles, sodium thiosulfate were edded to neutralize any free or combined residual chlorine.Chilled for transportation and examined within a 24-hour period. Residual chlorine was measured in samples from the collective reservoirs by colorimetric method using DpD Free- combined chlorine test kit(Lamotte). Several ecological parameters were taken included temperature, pH, turbidity and total dissolved solid (TDS).

2.2.2 Sampling of river water

Samples were collected by Van Dorn apparatus at margins and in the middle of the river in different depth (surface, 2 meters and 4 meters), water samples were collected into sterile screw- capped glass bottles chilled at time of collection and during transfer to the laboratory in according with [4].All samples were processed within 5 hr of collection, also temperature, pH were taken .

2.3 Isolation

2.3.1 Isolation of *Aeromonas spp* from Drinking water samples

EPA Method 1605(filtration methods) was validated recently for detection and enumeration of *Aeromonas spp* from drinking water.The volumes, and dilutions, of samples should be chosen so that the number of colonies to be counted on the membrane filter lies, if possible, between 20 and 80, for treated waters.

Aeromonas determination was qualitative using ampicilin – dextrin agar (ADA). Volumes of 100 ml were concentrated by filtration through a 47mm diameter membrane of 0.45mm porosity. The membranes were then transfered carefully to a Petri dish of Ampicillin Dextrin Agar(ADA). Ensure that no air bubbles are trapped between the membrane filter and the medium. . The Petri dishes incubate at 37 °C for 24 hours.

The number of presumptive *Aeromonas* bacteria is generally expressed as the number of colonies per 100 ml. Calculate the presumptive count as follows:

resumptive count/100 ml = Number of colonies counted on membrane filter x 100 x DF Where DF is dilution factor, if appropriate.

2.3.2 Isolation of *Aeromonas spp* from Raw water samples

Aloop full of river water samples were cultured directly on Ampicillin Dextrine Agar(ADA) and incubate at 37 °C for 24 h.

Membrane filteration method previously described was used to the isolation of aeromonads from wells samples.

2.4 Identification of *Aeromonas*

Typical colonies (yellow on ADA agar) and submitted to biochemical screening (according to 3, 5) :Oxidase and Catalase test H2S and gas production fermentation of (glucose, sucrose, arabinose, maltose), indole production, lysine, argenin and ornithine decarboxylation motility, triple sugar iron agar test, string test, esculin hydrolysis test, DNase test and detection of haemolysin .

For further identification of the *Aeromonas* strains two API strips, namely API 20 E, ID32 E, were used

2.5 Antibiogram Test (Disk Diffusion Test)

Kirby-Bauer method was used as described by[6] to carry out the antibiogram test. Turbidity of suspension bacterial culture (for 18-24 h) was adjusted by using the McFarland device. (densimat biomerixe) equivalent to McFarland No. 0.5, .this approximately equals to ($1.5x10^8$) cfu/ml. A 0.1 ml of the culture was spreaded on the surface of Mueller-Hinton agar plates, left to dry for 15 minutes at room temperature. Inhibition zones around the discs were measured according to National Committee for Clinical Laboratory Standards (NCCLS)(7). Studied antibiotics are Cloxacillin(CX), Penicillin (P), Ampicillin-cloxacillin(APX), metronidazole(MET), bacitracin(B), Erythromycin(E), Streptomycin(S), cephalothin(KF), Tetracycline(TE), Amoxicillin(AMX), Lincomycin(L), Rifampicin(RA), . Nalidixic acid(NA) Norfloxacin(NX), Tobramycin(TOB), Nitrofurantion(FT), Chloramphenicol(C), Neomycin(N).

2.6 Statistical Analysis

The Statistical Analysis System- SAS was used to effect of difference factors in study parameters. The Chi-square test (χ^2) and least significant difference (LSD) test at the comparative between percentage and means respectively.

The usual methods, which used in order to analysis and assess the results, they include:

-Descriptive statistics:

a- Statistical tables.

b- Graphic presentation.

3.Results and Discussion:

3.1.Isolation, distribution and prevalence of *Aeromonas spp*.

Between April 2011 and February 2012, 551 samples were collected . The percentages of *Aeromonas* recovery in this study were listed in table[4].

Table 4- Number of aeromonads found in raw water, treated water, and tap water in 5 water treatment Plants (WTPs)and different sites on Tigris river

type of water		no. of samples	avarage of positive *aeromonas* samples	percentage of isolation
raw water	intak	2 for each WTP=10	10	100
	Sedimentation tank	2 for each WTP=10	8	80
	Different sites on Tigris	91	66	72.52
	wells	20	7	35
Treated water	Filtration &chlorine tank	2 for each WTP=10	0	0
	Supply water of WTPs	2 for each WTP=10	0	0
	Distribution system	56 for each WTP=280	22	7.85
	reserviores	20 for each WTP=100	5	5
	*miscellaneous samples	20	10	50
total		551	128	

* Al-Husiania, Rashdia, Aewareeg and Sab-Albour

Up to our knowledge, till now, fewer studies had performed in Iraq concerning the isolation of pathogenic bacteria from Water Treatment Plants especially *Aeromonas* and investigates of their pathogenecity.

In this study The percentages of *Aeromonas* recovery from river water was 72.52 was found to be higher than that of other local studies, occurrence of *Aeromonas* in surface water was reported by Muslim [8] identified *Aeromonas* was 4 of 20 surface water samples(20%) and by Al-Khalidi [9] was 52.94% . The isolation percentage was 20% reported by Muhammad [10] of the river samples and 10% by Al-Majamae [11].

Groundbreaking studies by Hazen, [12] and associates identified *Aeromonas* in 135 of 147 (91.8%) natural aquatic habitats sampled in the United States, in Brazil was 63% in the estuary of the River Cocó, Ceara, [13], and 40% in Italy [14**]** .

In our study rate of isolation of *Aeromonas* spp. in wells water was (35%), in contrast to Legnani [14] identified *Aeromonas* in 28.6% of wells water .

Ghegehnsh in Lybia analyzed 1000 samples of water collected from wells (980) and miscellaneous sources (120) Aeromonas species were isolated in 48.7%of them[15]

Also rate of isolation of *Aeromonas* spp. in wells water was (21.9%) by Razzolini, in Brazil.*[16]*.

In recent study the percentage of *Aeromonas* recovery from WTPs stages are: 100% from intake, 80%f rom sedimentation tank, 0% from Filtration &chlorine tank, and 0% supply water of WTPs.

Total percentage of positive aeromonas samples of treated water(Filtration &chlorine tank, supply water of WTPs, distribution system, reserviores and other samples not related to WPTs) was 8.8% (37 in 420 samples). Count of *Aeromonas* in positive aeromonas samples ranging from 1 to 175 cfu 100 ml, that was found to be higher than of other study [17]was 10 cfu /100 ml and 3 to 43 cfu /100 ml in study in Lebanon [18] and lower than mentioned by Kuhn [19].

The percentage of *Aeromonas* recovery was found to be higher than that of other local studies (only from tap water), -Al-Hashimy [20] identified *Aeromonas* in 13 of 1567(0.82%), no aeromonads were isolated from ten tap water samples by Muhammad [10] .

Relatively, our results similar to results of Razzolini [21] in Brazil found *Aeromonas* in 12 of the 200 drinking water samples examined (6%)and Figueras [17] found *Aeromonas* in 6.9% of the drinking water samples, but was found to be lower than other results were reported earlier by Lechevalier, [22] in chlorinated treated water in Oregon State, USA (27%) and by Araujo et al.[23] in tap water in Spain (70%), but in Brazil, Fuzihara found that 4.6% of drinking water samples positive for *Aeromonas*.[24]

Potable drinking water samples were collected from distal ends of the distribution system appeared relatively high recovery percentage, that due to the feeding of distribution system by multiple sources with varying water quality, the release of biofilms, scales, or sediments may occur at the interface between the sources, like, Karadah city wich fed from Al-Wahda and Al-Rashid WTPs, this observation supported by other studies .[25, 26].

Other investigation conducted by Villarruel-Lopez, in drinking water plants treatment plants and wastewater-treatment plants inMexico City

reported the presence of *Aeromonas* in 31% of the samples from both kinds of sources[27].
In contrast other study showed that 90% of domestic water supplies in areas of Cairo contained *Aeromonas*.[28].

It is worthy to mention that generally no significant correlation between presence of aeromonas in distribution system water and other indicators (total heterotrophic plate counts and fecal pollution) were detected except several samples belonged to Al-Rashid WTP, Al-Husiania and Sab-Albou were fecal polluted, this observation supported by other study [14] they found that presence of aeromonads in distribution system water indicates neither fecal pollution nor treatment failure however, a large number of aeromonads present in distribution water suggests that water conditions support growth and *Aeromonads* resistance to disinfection in biofilm which present in inner surface of pipeline .

Chao, conclude that the use of the commonly employed indicators for assessing water quality is questionable[29]. The presence of *Aeromonas spp*. does not correlate with the presence of coliform bacteria in drinking water supplies [30], they proposed using *Aeromonas spp*. as an additional indicator of drinking water quality related to presence of biofilm.

In Iraq and many countries, the governing of the microbiological quality of potable water include aerobic bacteria count, coliform bacteria enumeration *E. coli*, and *Pseudomonas aeruginosa* but *Aeromonas spp* is not specially considered.

According to our results**,** using of *Aeromonas spp* as an additional indicator were applied for the first time in ministry of Environment, Central Environmental Laboratory(microbiology lab) in Baghdad and other environment offices in Iraq to determine the microbiological quality of potable, wells, and bottled water by using filtration method and Ampicillin Dextrin Agar also enumeration of these bacteria in surface water .

The results which showed in table [5]indicated that the prevalence of these bacteria from river has a seasonal pattern with higher numbers occurring in the summer than in the winter, *Aeromonas* were recovered throughout the year, with greater numbers between July and September when water temperatures were highest. Similar observations appeared earlier by Holmes**,** [31].

Table 5- Percentage of *Aeromonas* in difference seasons (River water)

Season	No. of sample	+ve *Aeromonas* sample	Percentage (%)
Spring	30	21	70.00
Summer	56	41	73.21
Autumn	25	17	68.00
Winter	20	12	60.00
Total	131	91	---

Table 6 shows the seasonal occurrence of *Aeromonas* in drinking water during the study. The percentage of isolation was similar in Spring, Summer and Autumn and dropping in Winter, these results agreed with previous study [21] found a remarkable seasonal pattern in the occurrence of these bacteria in drinking water samples.

Table 6- Percentage of *Aeromonas SPP*. In difference seasons (Drinking water)

Season	No. of sample	+ve *Aeromonas* sample	Percentage (%)
Spring	140	13	9.28
Summer	170	16	9.41
Autumn	60	5	8.33
Winter	50	3	6.00
Total	420	37	---

Table 7 shows the *Aeromonas* frequency, average of residual chlorine concentration and some ecological parameter in drinking water of five WTPs**.**

Table 7- Positive *Aeromonas SPP* samples and some ecological parameters in drinking water

WTP	Mean ± SE					+ ve A. SPP.
	TDS	Turbidity	PH	Chlorine residue	Temp.	
1	467.60 ± 17.84	2.38 ± 0.07	7.42 ± 0.56	1.18 ± 0.04	22.00 ± 1.24	5.40 %
2	447.80 ± 21.05	1.56 ± 0.03	7.32 ± 0.49	0.66 ± 0.02	25.00 ± 1.61	8.90 %
3	481.20 ± 19.37	1.82 ± 0.05	7.40 ± 0.71	1.64 ± 0.04	25.00 ± 1.61	1.80 %
4	511.80 ± 26.73	1.88 ± 0.04	7.26 ± 0.33	0.32 ± 0.01	24.00 ± 1.54	10.80 %
5	491.00 ± 17.69	13.40 ± 0.84	7.40 ± 0.71	0.40 ± 0.01	27.00 ± 2.04	12.50 %
LSD Value	28.64 *	3.52 *	0.853 NS	0.372 *	4.107 *	4.739 *
* ($P<0.05$).						

The result showed free chlorine residuals in potable water samples ranged between 0 and 2.5 ppm.

It is important to note that there was negative correlation between residual chlorine and recovery percentage, that also was described by Burke [32], in other word certain samples with poor chlorination profiles yielded very few isolates, whereas some highly chlorinated sites liberated *Aeromonas* frequently and in relatively high numbers, for example, these bacteria found in water samples belongs to Al-Doura and Al-Qadisia WTP with residual chlorine reached to 2.5 mg/L, that agreed with Figueras etal 2005 who found free chlorine residuals in samples containing *Aeromonas* spp were between 0.05 and 1.5 mg/L.

Only in case of Al-Sader and Al-Rashid WTPs recovery percentage were 10.80 % 12.50 % and mean value of residual chlorine were 0.32, 0.40 mg/L respectively.

Aeromonads persisted in 10% of pipe lengths after disinfection with 1 mg/L chlorine[31]. Freely suspended cells are susceptible to this level, but elevated levels are required to destroy *Aeromonas* in biofilm.

Water of domestic use should be free of these organisms[32], the presence of *Aeromonas spp*. in water distribution systems suggests inadequate chlorine residuals or the potential for biofilms [30] that lead to regrowth within pipes . Our results indicated that no *Aeromonas* were identified in most of samples that collect from beginning of water distribution system that may due to high chlorine residual and there is no accumulation of biofilm and sediment, this came in agreement with Havelaar [26].

Turbidity showed highest values in samples appeared high recovery rate of *Aeromonas* such as samples collected from regions belonged to Al-Rashid WTP.

Current work indicated to negative correlation were detected between other ecological parameters(TDS, temprtute, and PH) and *Aeromonas* recovery rate .

3.2 Identification of *Aeromonas spp*

In this study, ampicillin-dextrin agar was used as selective and differentiation of *Aeromona spp*. Colonies appeared yellow or yellow with greenish –yellow periphery **(**figure1)

Havelaar conclude that ampicillin-dextrin agar performed the best for the recovery of *Aeromonas spp.* in drinking water and the differentiation by simple criteria of that genus from other common waterborne bacteria [33].The isolates that were obtained from the selective media were identified according to [3, 5] Identification to the species level was carried out by biochemical test and Api systems(figure 2) .

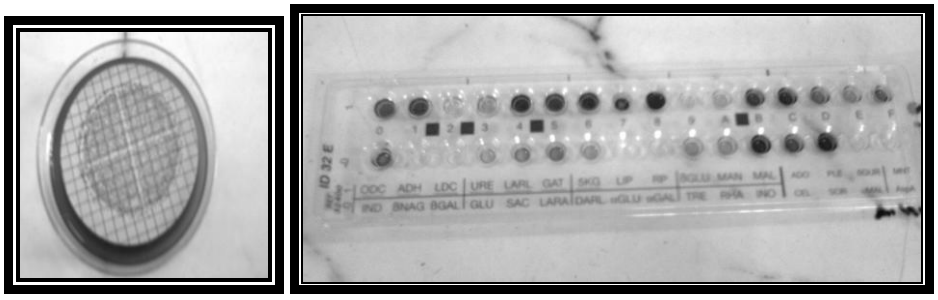
Few local studies that identified *Aeromonas spp* (that had been isolated from surface and drinking water) to species level.

The species were identified in the current study were presented in table [8].

Table 8- Frequency of *Aeromonas* species isolated from raw and drinking water

Aeromonas SPP	No.	Percentage (%)
A. hydrophila	63	49.21
A. trota	21	16.41
A. veroni	12	9.37
A. salmoncida	11	8.59
A. schubertii	4	3.13
A. sobria	4	3.13
A. cavia	4	3.13
Aeromonas SPP	9	7.03
Tota l	128	100 %

The most predominant species is *A. hydrophla* with percentage 49.21%.(table 9).The recovery percentage of all species found to be higher in RW than in DW except *A. veronii* (8 strianes isolated from DW while 4 strians from Rw) that consider as threat for public health because of *A. veronii* responsible for a variety of humn illnesses.



A B

Figure 1- (*A) Aeromona spp* on ampicillin-dextrin agar(filtration method)
(B) Results of Mini API 32 system biochemical tests for diagnosis of *Aeromona sp*s

In Iraq, Al-Khalidi [9] reported that the most frequent species which had been isolated from river water was *A. hydrophila*(58.8%). Evangelista-Barreto1 reported that *A.cavia* were the predominant species[13].

The second predominant species in our study was *A. trota* with recovery rate 16.41%, this species were identified in Iraq for the first time . Earlier studies have indicated that *A. trota* identical (synonym)to *A. A. enteropleogene* [34*]* they found that the type strains *A.trota* and *A. trota* displayed 100% 16s rRNA gene sequence identity, this observation supported by Janda, [35].

Abbott *[36*] reported that 16 (8.29%) strian of 193 *Aeromonas* strains were identified as *A. trota*.(*A. enteropleogene*).

Results showed that *A. veronii* was third predominant species**(**9.37**%).**

Earlier studies have indicated that three *Aeromonas* species (*A. hydrophila*, *A. caviae*, and *A. veronii*) are responsible for the vast majority (85%) of human infections [35] .

Table 9- Frequency of *Aeromonas* species between raw and drinking water.

Aeromonas SPP	Total No.	D.W No. (%)	R.W No. (%)	Chi-square value (χ^2)
A. hydrophil	63	18 (28.57 %)	45 (71.43 %)	9.563 **
A. trotas	21	7(33.33%)	14 (66.67 %)	9.214**
A. veroni	12	8 (66.67 %)	4 (33.33 %)	9.216 **
A. salmoncida	11	4 (36.36 %)	7 (63.64 %)	9.208 **
A. schubertii	4	0 (0.00 %)	4 (100 %)	14.50 **
A. sobria	4	0 (0.00 %)	4 (100 %)	14.50 **
A. cavia	4	0 (0.00 %)	4 (100 %)	14.50 **
Aeromonas SPP	9	0 (0.00 %)	9 (100 %)	14.50 **
Total	128	37 (28.91 %)	91 (71.09 %)	9.551 **
** (P<0.01).				

A.*salmoncida* other species that had been identified for the first time in Iraq with recovery rate (8.59%). Chauret *[37]* isolated *A. salmonicida* from biofelm in model distribution systems with percentage 1%.The species *A. salmonicida* is better known as the causa agent of furunculosis in salmonid fishes, a debilitating and lethal disease encountered in aquaculture.

In some studies, less frequently encountered species have been found in water samples, such as *A. schubertii* [35]**,** but in recent study *A. schubertii* had been identified with percentage (3.13%).. *A. schubertii* had been isolated from human infections [35].

A.sobria and *Acavia* were identified with frequency rate (3.13%), thes two species were isolated only from river (Tigris) water.

Al-Khalidi[9) reported that prevelance rate of *A.sobria* and *A.cavia* from river water was 11.7%, 5.8% respectively ..

In addition several researchers studied prevalence of these bacteria in drinking water Razzolini *[21*] reported the most frequent species were *A. caviae* (41.7%), followed by *A. hydrophila* (15.6%), *A. allosacharophila* (10.4%), *A. schubertii* (1.0%), and *Aeromonas spp*. (31.2%). *A. hydrophila, A. caviae* and *A. sobria* have also been commonly reported in others studies conducted in drinking water[38].

3.3 Antibiotic susceptibility of *Aeromonas hydrophila*

Antimicrobial resistance is a fact which is increasingly worrying health authorities, due to its increasing occurrence each year because the rapid dissemination of antibiotic resistance genes in bacterial populations as a consequence of the intensive use of antibiotics in medicine and agriculture.

Eighteen different antibiotics discs were used to perform this test, along with all *Aeromonas hydrophila* isolates. Results are shown in figure2 .The methods described in NCCLS (2003) were followed to determine whether the isolates were resistant or not.

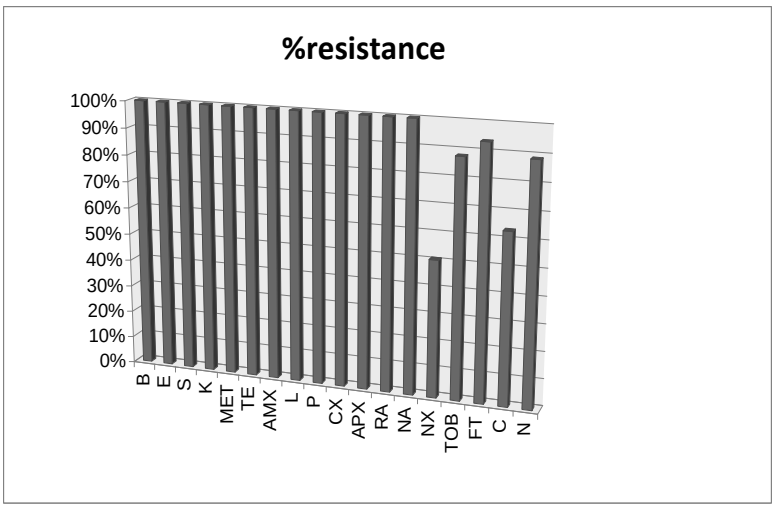


Figure 2- Percentages of.*A hydrophil*a resistance to antibiotics

The results showed that all *Aeromonas hydrophila* isolates were resistant to each of cloxacillin, penicillin, ampicillin- cloxacillin (CX), metronidazole (MET), bacitracin (B), erythromycin (E), streptomycin (S), cephalothin (KF), tetracycline (TE), amoxicillin (AMX), lincomycin (L), rifampicin (RA), . nalidixic acid (NA) in ratio (100%).

In contrast Muslim**,** 2005 reported that *Aeromonas hydrophila* isolates were resistant only to five antibiotics in ratio 100%.

Our results showed that thirty two isolates 50.79% were resistant to norfloxacin(NX), , 88.8%(56 isolates)to tobramycin(TOB), 93.6%(59 isolates) to nitrofurantion(FT), 63.4%(40 isolates) to chloramphenicol(C) and 88.8%(56 isolates) to neomycin(N), none of the isolates was susceptible to all antibiotics tested in ratio 100%, This observation supported by Abulhamd[39].

This may suggest that there are more than one mechanism of antibiotic resistance exhibited by *Aeromonas hydrophila* local isolates.They may include low permeability of *Aeromonas hydrophila* cells membrane to many antibiotics[40] or the presence of plasmids that confer resistance to many antibiotics [41].

The aeromonads have been regarded as universally resistant to penicillins. du**e** to the production of multiple inducible, chromosomally encoded B-lactamases [42]

In contrast, the local study, high susceptibility of environmental isolates of *Aeromonas hydrophila* to Chloramphenicol and tetracyclin, streptomycin, and neomycin were observed[11]. Other local study conducted by Muhammad who found that environmental isolates were completely susceptible (100%) to, Neomycin, Norfluxacilin, tetracyclin, Chloramphenicol, Rifampicin and, Nalidixic acid [10].

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