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Heavy Metal Resistance of *Aeromonas hydrophila* Isolated from Raw and Drinking Water in Baghdad City

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Abstract
A total of 551 water samples (drinking and raw water) were collected In this study, A*eromonas.hydrophila,* were detected by biochemical tests and PCR (16s rRNA gene). The results of identification showed that *A.hydrophila* had recovery rate 63 isolates (49.21%). The results revealed that all *A.hydrophila* isolates were PCR positive or the 16S rRNA gene and the results of sequencing showed that two isolates of *A.hydrophila*(local isolates) had percentage similarities 100% with *A. hydrophila* ATCC 7966 in GenBank database .All strains had a minimal Inhibitory Concentration(MIC) distribution pattern for lead cetate rranged (900-1200 µg/ml), and mercury chloride ranged (40-80 µg /ml).

Keywords: Heavy Metal, *Aeromonas hydrophila,* Drinking Water. Resistance.

مقاومة المعادن الثقيلة لبكتريا *Aeromonas hydrophila* المعزولة من المياة الخام ومياه الشرب في مدينة بغداد

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الخلاصة
جمعت 551 عينة ماء خام وماء شرب وتم عزل بكتريا *Aeromonas hydrophila* وتم تشخيصها بوساطة الفحوصات الكيموحيوية وتقنية تفاعل البلمرة وتتابع النيوكليوتيدات .اظهرت نتائج التشخيص ان نسبة العزل كانت 49.21% (63 عزلة بكتيرية)وكذلك اعطت جميع عزلات البكتريا نتيجة موجبة لتفاعل البلمرة المتسلسل (تتابع النيوكليوتيدات) وكانت عزلتان محليتان ذات نسبة تشابة 100% مع العزلات القياسية في بنك الجينات .اعطت جميع العزلات تراكيز المثبطة الدنيا التي تراوحت بين 900–1200 مايكروغرام/ مل لخلات الرصاص و40–80 مايكروغرام/ مل لكلوريد الزئبق.

Introduction

Aeromonas hydrophila is a Gram negative bacteria widely distributed in freshwater environments [1]. It is a well-known fish [2, 3] and human pathogen [4]. Since some strains of *Aeromonas* are enteropathogens possessing a range of virulence factors (enterotoxins, cytotoxins, haemolysins, and invasive ability), infected fish may be vehicles of human infection [5-7] some are heavy metal resistant [8].

The presence of toxic heavy metals in the environment has resulted in the development or acquisition by bacteria of genetic systems that counteract their effects. Many bacterial heavy metal resistance systems are based on efflux, and two groups of efflux systems have been identified. These can be either P-type ATPases, e.g., the Cu(II), Cd(II), and Zn(II) ATPases of gram-negative bacteria [9].

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Material and methods

One hundred and thirty one raw water samples (from Tigris river) and four hundred and twenty drinking water samples from five water treatment plants (WTPs) in different area in Baghdad) were collected. Prior to collection of drinking water was allowed to run at a uniform rate for 2–3 min. in a sterile bottles containing sodium thiosulphate to a final concentration of 0.01% (W/V) to neutralize any free or combined residual chlorine. One liter of water sample was collected in each bottle, the samples were carried out to the laboratory special aspectic cool box. All the samples were transported to the laboratory on ice and analyzed within 24 h [10].

Drinking water were filtered by filtration apparatus then the membranes were then transfered carefully to a Petri dish of Ampicillin Dextrin Agar(ADA),then the Petri dishes were incubated at 37 °C for 24 hours.Aloop full of river water samples was cultured directly on Ampicillin Dextrine Agar(ADA) and incubated at 37 °C for 24 h[11].

Identification of bacterial isolates

Bacterial isolates were identified by the procedures described in Bergey's Manual of Systematic Bacteriology [12].

For further identification of the *Aeromonas spp.* isolates, two API strips: API 20 E, ID32 E, also these isolats were identified by PCR *16s r*RNA gene,by using Primers: 5-GGGAGTGCCTTCGGGAATCAGA-3(Forward primer) and 5-TCACCGCAACATTCTGATTTG-3(Reverse primer) [13].

Determination of Minimal Inhibitory Concentration (MIC) of heavy metals resistance

The minimal inhibitory concentration (MIC) of metals (Mercury chloride and Lead acetate) was determined by an agar dilution procedure, as previously described [8, 14-16]. *A.hydrophila* isolates grown on heavy metals incorporated media (nutrient agar pH 7), Heavy metals concentrations used in this test were :900,1000,1100, and 1200 µg/ml for lead acetate and 40,50,60,70, and 80 µg/ml for mercury chloride.

MIC was observed when the isolates failed to grow on plates after incubation period at 37 °C for 24 hr , MIC breakpoints (µg/ml) for considering a bacterial isolate as susceptible (S) or resistant (R) to lead and mercury as reported by [14] : lead S <800, R >800, mercury S <54, R >54.(S:sensitive ,R:resistance).

Results and Discussion

The results of identification showed that *A. hydrophila* had recovery rate 63 isolates(49.21%),forty five *A. hydrophila* isolates from raw water (Tigris river) and eighteen isolates from drinking water were obtained .

The results revealed that all *A. hydrophila* isolates were PCR positive for the 16S rRNA gene Figure-1,-2, and the results of sequencing showed that two isolates of *A. hydrophila*(local isolates) had percentage similarities 100% with *A. hydrophila* ATCC 7966 in GenBank database(according to sequencing were carried out in Korea using Sequencer applied biosystem (3730XL)USA (genetic analyzer 3730XL). Figure-3.

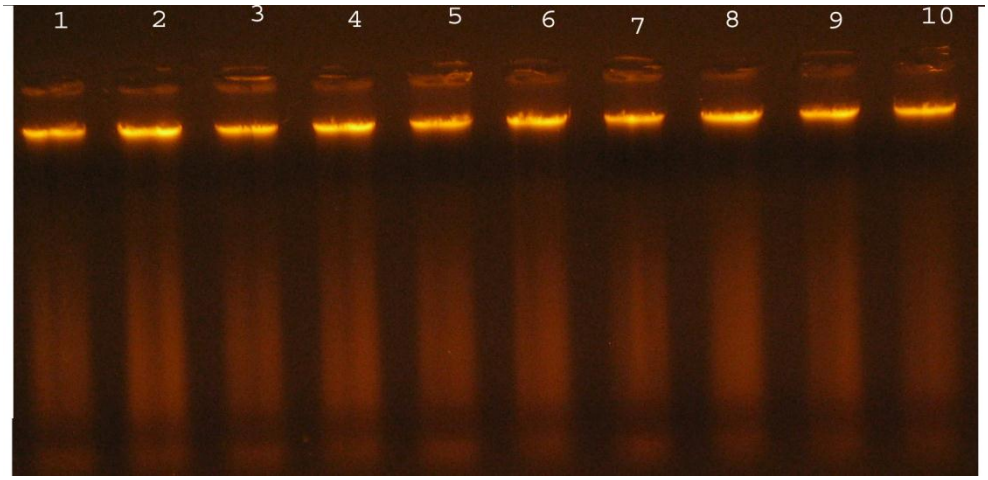

Figure 1-Agaros gel electrophoresis (1%) of genomic DNA of *A. hydrophila* isolates ,1.5 hour at 5 V/cm, stained with ethidum bromide and visualized on a UV transilluminator. Lane 1-10: Genomic DNA extracted from different *A. hydrophila* . isolates. Purity between (1.8- 1.9)

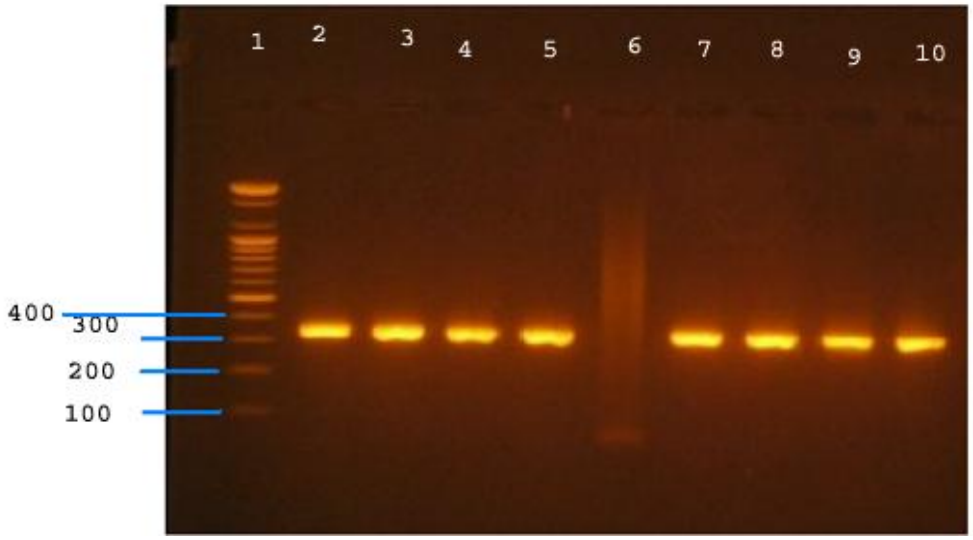

Figure 2-Agaros gel electrophoresis (1.5%) of amplified 16S rRNA gene (356 bp) of *A. hydrophila* for 1.5 hour at 5 V/cm, stained with ethidum bromide and visualized on a UV transilluminator.Lanes 1: 100 bp DNA ladder (2Kb). Lane 6: negative control. (had all PCR mixture except of DNAtemplate). Lane 2-5 and 7-10*: A. hydrophila* .

Alignment statistics for match #1

Score	**Expect**	**Identities**	**Gaps**	**Strand**
460 bits(249)	2e-126	249/249(100%)	0/249(0%)	Plus/Minus

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Query  1       CTAGCTTGCAGCCCTCTGTACGCGCCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGC  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216174  CTAGCTTGCAGCCCTCTGTACGCGCCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGC  216115

Query  61      CATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTTGAG  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216114  CATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCCTTGAG  216055

Query  121     TTCCCACCATTACGTGCTGGCAACAAAGGACAGGGGTTGCGCTCGTTGCGGGACTTAACC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216054  TTCCCACCATTACGTGCTGGCAACAAAGGACAGGGGTTGCGCTCGTTGCGGGACTTAACC  215995

Query  181     CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCTGATTCCCGAAG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  215994  CAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTCTGATTCCCGAAG  215935

Query  241     GCACTCCCA  249
               |||||||||
Sbjct  215934  GCACTCCCA  215926
```

Figure 3- PCR analysis of *A. hydrophila* isolates

Heavy metal resistance of *A. hydrophila*

All isolates had a (MIC) distribution pattern for lead acetate ranged (900-1200 µg/ml), and mercury chloride ranged (40-80 µg/ml). The results of MIC of heavy-metals (lead and mercury) are shown in the Table -1.

Table 1- MIC distribution patterns of heavy metals for *A.hydrophila*

Heavy metal	MIC (µg/ml)	% no. of inhibited isolates
Lead acetate (PbCH3COO)	900	30.1%(19 isolates)
	1000	60.3% (38 isolates)
	1100	87.3% (55 isolates)
	1200	100%
Mercury chlorid (HgCl2)	40	9.5% (6 isolates)
	50	19.1%(12 isolates)
	60	60.3%(38 isolates)
	70	80.9%(51 isolates)
	80	100%

MIC breakpoints (µg/ml) for considering a bacterial isolate as susceptible (S) or resistant (R) were those reported in [14] for lead S < 800, R >800;for mercury S <54, R >54.

All isolates (100%) of *A. hydrophila* were resistant to lead acetate that came in accordance with the finding of [15] and forty five isolates (71.42%) were resistant to mercury chloride that disagreement with results of Paniagua [15] , who found that all isolates of *A.hydrophila* were susceptible to mercury chloride ,that may due to different between isolates in current study and Paniagua. In study conducted by Miranda and Castillo **(**1998) [8] found that resistant aeromonads to heavy metals are easily recovered from water sources, posing a potential public health risk,that due to avariety of detoxifying mechanism developed by resistant microorganisms such as binding with bacterial cell envelopes, metal reduction, metal efflux [15].

Bacterial heavy metal resistance determinants commonly reside on plasmids, and selective pressure for one resistance indirectly leads to selection for the others, as the responsible genes are linked. Since many low-statuses farmer people consume these fish and usually swim in this freshwater pond, contact with the heavy metal resistant *Aeromonas* posess an important human health risk. In addition, passage of these bacteria from fish to humans would permit the horizontal gene transfer from *Aeromonas* to other bacteria harbored by the exposed persons[15, 16].

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