Study adhesion ability of of *Aeromonas hydrophila* strains isolated from raw and drinking water in Baghdad city

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Abstract

The ability of *A.hydrophila* isolates (63 isolates) to form biofilm, was studied and the results showed that fifty six isolates (88.8%) gave positive results in Congo red agar, while 51 isolates (80.7%) gave positive results in Christensen method, sixty isolates(95.2%) produced biofilm on Polystyrene microtiter plates. Results revealed that all drinking water isolates produced biofilm (18 isolates)and 42 raw water isolates produced biofilm (depending on Polystyrene microtiter plates test). The more two efficient isolates(one isolated from drinking water DW, and other isolated from raw watervRW) which produced biofilm was chosen to study the effect of different values of temperature and disinfectants on the ability of *A.hydrophila* to adhere to four solid surfaces (stainless steel SS, galvaze iron GI, polyvinyl chloride PVC and unplasticised polyvinyl chloride UPVC) under different factors.Our results showed that higher number of *A. .hydrophila* was adhered on uPVC followed by the PVC and SS and GI was the least in ability to attraction of bacteria at 37°C. *RW* isolate appeared higher ability to produce biofilm on all surfaces than *DW* isolate. *A. .hydrophila* (*DW, RW*) has ability to adherence on four solid surfaces at low temperature 4°C greater than 37°C. The MIC values varied according to the type of disinfectants in the range (1550-7500 µg/ml. In this experiment, dettol was found as the best antiseptic against both isolates of *A. hydrophila*(*DW, RW*) recording a minimum value of MIC (1550) µg/ml, follows by bleach with MIC(7500) µg/ml, .The bleach at MIC concentration was the most efficient disinfectants than other disinfectants in its ability to remove the adherent bacteria on solid surfaces followed by dettol .

دراسة قابلية الالتصاق لعزلات *Aeromonas hydrophila* المعزولة من الماء الخام وماء الشرب في مدينة بغداد

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الخلاصة

درست قدرة ثلاثة وستون عزلة من *A.hydrophila* لتكوين الغشاء الحيوي واظهرت النتائج ، ان 56 عزلة (88.8%)اعطت نتيجة موجبة لفحص اكار احمر الكونغو بينما اعطت 51 عزلة (80.7%) اعطت نتيجة موجبة لفحص الانابيب (كرستينسن) و 60 عزلة (95.2%)اعطت نتيجة موجبة بطريقة اطباق المعايرة الدقيقة . اظهرت الدراسة الحالية انه جميع عزلات ماء الشرب منتجة للغشاء الحيوي (18 عزلة) و (42) عزلة ماء خام منتجة للغشاء الحيوي .

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اختيرت اكثر عزلتين كفوئتين(واحدة معزولة من ماء الشرب*DW* واخرى من الماء الخام*RW*) في انتاج الغشاء الحيوي والتي تملك جينات الهيمولايسيين لدراسة تأثير قيم مختلفة من الحرارة و المطهرات على قابلية *A.hydrophila* على الالتصاق بالسطوح الصلبة مثل stainless steel (SS), , galvaze iron(GI) polyvinyl chloride (PVC), . unplasticised polyvinyl chloride(UPVC).

اظهرت النتائج ان PVC, UPVC اكثر السطوح جذبا للبكتريا *A.hydrophila* ثم SS واخيرا GI كانت اقل جذبا للبكتريا في درجة 37 مْ وبتعداد خلايا اعلى للعزلات المعزولة من الماء الخام.

اظهرت كلا العزلتين (*DW, RW*) قابلية كبيرة للالتصاق على السطوح الاربعة في درجة 5 اكثر من درجة 37،

تغايرت نتائج تراكيز المثبطة الدنيا للمطهرات (MIC) اعتمادا على نوع المطهر(7500 – 1550) µg/ml كان المطهر (ديتول) احسن المطهرات ضد كلا العزلتين (*RW, DW*) اذ كانت MIC 1550 µg/ml ثم القاصر 7500 µg/ml، في حين أظهرت النتائج بأن القاصر كان أكفأ مطهر من بين المطهرات الثلاثة في قابليته على إزالة البكتريا الملتصقة على الأسطح الصلبة الاربعة **ثم الديتول** .

1. Introduction

Biofilms occur whenever water is in contact with a solid surface, such as a distribution system pipe. Biofilms represent a build up of microorganisms attached to a surface and embedded in a matrix of various organic polymers of microbial origin**.**[1]

Earlier studies reported the presence of *Aeromonas* in pipe biofilms in drinking water supplies.Organisms forming biofilms typically produce extracellular polysaccharides and accumulate in hydrated structures on surfaces. It is estimated that 99% of all bacteria in natural environments exist in biofilms.[1, 2].

Biofilm in water distribution system cause several problems its play a key role in the contamination of drinking water and transmission of different pathogens, also in protecting pathogenic bacteria which harboring virulence factors in addition biofilm cause neutralization of chlorine that lead to the ineffectiveness of chlorine in treatment of drinking water therefore this study was aimed to investigate the ability of *Aeromonas hydrophila* isolates (which isolated from raw and drinking water to adhere to solid surfaces under different factors .

2. Material and methods

2.1 Bacterial identification

One hundred and thirty one raw water samples (Tigris)and four hundred and twenty drinking water samples(from five water treatment plants WTP) were collected during period April 2011 till February 2012. Drinking water were filtered by filtration apparatus then the membranes were then transfered carefully to a Petri dish of Ampicillin Dextrin Agar(ADA).

Incubate the Petri dishes at 37 °C for 24 hours.

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

Bacterial strains were identified by the procedures described in Bergey's Manual of Systematic Bacteriology [3].

Typical colonies (yellow on ampicillin dextrin agar) submitted to biochemical screening :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 API 20 E and, ID32 E, were used.

2.2- detection of bacterial ability to produce slime layer

2.2.1-Congo red agar

This medium was inoculated with a single colony of tested bacterial by streaking, incubated at 37º C for 24 hr, a positive result was indicated by black colonies. Non-slime producers usually remained pink [4, 5].

2.2.2 - Christensen method

Glass tubes containing 10 ml of tryptic soya broth were inoculated with single colony of test bacteria by sterile loop; negative control was made by adding 10 ml of tryptic soy broth to a glass culture tube. The tubes were incubated at 37ºC for 24-48 hr. After that the tubes content was decanted and 10 ml of 0.1% safranin stain solution was added to all tubes including negative control. Each tube was then gently rotated to ensure uniform staining of any adherent material on the inner surface and the contents was gently decanted. The tubes were then placed upside down to drain. A positive result was indicated by the presence of an adherent layer of stained material to the inner surface of the tube.[6].

2.2.3.Qualitative and quantitative estimation of biofilm formation on polystyrene microtiter plates.

Studied bacterial isolates cultured in Brain Heart Infusion (BHI) broth with glucose incubated at 37ºC for 18 hour, after that bacterial culture was diluted in BHI medium and adjusted in comparison to MacFarland tube 0. 5. Two hundred microliters of this bacterial culture were used to inoculate 96-well polystyrene microtiter plates and later incubated for 24-48 hrs at 37C^{o}. After incubation, all wells were washed with phosphate buffer saline for the elimination of unattached cells (2-3) times.

Afterward, (200) µl of 1% crystal violet was added to each well, shaking the plates three times to help the colorant to get the bottom of the well. After 10 minutes at room temperature, each well was washed with (200) µl sterile phosphate buffer saline (PBS) to remove the planktonic cells and stain which not adhered to the well. Only the adhered bacteria forming the biofilm were kept on the surface of the well. The Crystal violet bound to the biofilm was extracted later with (200) µl of ethyl alcohol, and then absorbance was determined at 492 nm in an ELISA reader for determination of the degree of biofilm formation.

Controls were performed with Crystal Violet binding to the wells exposed only to the culture medium without bacteria.[7]

All the assays were performed in triplicates. The biofilm degree was calculated as follows: Based on the O.D. produced by bacterial films, strains were classified into the following categories: no biofilm producers, weak, moderate or strong biofilm producers, as previously described (8). Strains were classified as follows: OD ≤ ODc no biofilm producer, ODc < OD ≤ 2 × ODc weak biofilm producer, 2 × ODc < OD ≤ 4 × ODc moderate biofilm producer and 4 × ODc < OD strong biofilm producer. All tests were carried out in triplicate and the results were averaged.

2.3Selection of test surfaces are used in the adhesion tests

Four types of solid surfaces :stainless steel (SS), galvanized iron(GI), polyvinyl chloride –(PVC) and unplasticised polyvinyl chlorideUPVC which are used in water distribution system were selected for the adhesion tests, then every solid materials were cut into small pieces (coupons) 1cm². These surfaces were soaked for 24 h in absolute ethanol, washed and rinsed thoroughly with deionized water, then coupons were autoclaved at 121◦C for 15min[9].

2.4dherence of bacteria to solid surface test

Bacterial suspension were prepared by inoculation bacteria in tryptic soya broth at 37°C for 24hr, bacteria were harvested by centrifugation at 3000rpm for 10 min, then washed three time with PBS(pH7) at 3000 rpm for 10 min, then the bacterial cells were resuspended in PBS 1x10^{9} cfu/ml.,), then every type of coupons were put in bacterial suspension for 24 hr at 37ºC for different time (2, 4, , 24, 48, 72hr.). Then every pieces were raised from bacterial suspension, rinsed by PBS(pH7) to remove unattached bacteria(reversible adherent cells) .They were put in sterile PBS in test tube .The adhered bacteria were released from pieces by vigorous vortex .The liquid is serially diluted and number of biofilm producing cells were enumerated by viable Plate count in 1cm² of solid surface .

2.5 Effect of temperature on adherence of *A.hydrophlia* to solid surfaces

Bacterial suspension were prepared as previous described then every types of coupons were put in bacterial suspension at (5, , 37and 40°C) for (2, 4, , 24, 48, 72 hr.) Every coupons were raised from bacterial suspension, they were rinsed by PBS(pH7) to remove unattached bacteria, They were put in sterile PBS in test tube. The adhered bacteria were released from pieces by vigorous vortex [10, 11]The liquid then serially was diluted and number of biofilm producing cells were enumerated by viable plate count in 1cm² of solid surface .

2.6 Determination of minimal inhibitory concentration (MIC)of disinfectants

- A serials of two fold dilutions of disinfectant were prepared, The disinfectants dilutions were prepared in a nutrient broth medium
- Bacterial suspension was prepared from overnight plate culture in 5ml of saline equivalent to a mcfarland 0.5 standard .
- After disinfectants dilutions were completed, 0.1ml of bacterial suspension was added to all dilutions except sterility control tube .
- MIC determine by highest dilution of disinfectant inhibit bacterial growth.

*Two sterility control tubes were prepared :first (disinfectant +broth), Second (nutrient broth).And positive control (bacterial suspension). [12]

2.7 Effect of t disinfectants on adherence of *A .hydrophila* to solid surfaces

To carry out this test test bacterial suspension were prepared as previous described then the bacterial cells were resuspended in PBS $1x10^9$ cfu/ml. Every type of coupons put in bacterial suspension at 37°Cfor 48 hr.Every pieces were raised from bacterial suspension, rinsed by PBS to remove unattached bacteria with preparation control for each pieces(untreated pieces with disinfectants) that the initial number for galvanized iron surface was($1.6x10^5$ cfu ⁄cm²), stainless steel ($8x10^5$cfu⁄cm²), PVC was($2.6x10^6$cfu⁄cm²)and UPVC was $9x10^6$. Then they were put in disinfectants'solutions at 37°C for different times (, 2, 4, 24, 48, 72 hr.). Every pieces were raised from disinfectants'solutions, rinsed by PBS and they were put in tube with sterile PBS. The adhered bacteria were released from pieces by vigorous of vortex into sterile solution PBS .The liquid then serially diluted and number of biofilm producing cells were enumerated by viable plate count in 1cm² of solid surface.

2.8 Statistical Analysis

The Statistical Analysis System was used to explain the effect of difference factors in the 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

Forty five *Aeromonas hydrophila* isolates from raw water and eighteen isolates from drinking water were obtained

3.1 Adhesion test

3.1 1.Congo red agar

Fifty six isolates (88.8%) of *A.hydrophila* gave positive results in Congo red agar method, pigmented colonies were considered as normal slime-producing strains, whereas unpigmented colonies(pink) were classified as non-slime-producing strains. As showen in Figur 1.

Percentage of isolates that appeared as very black colonies were47.6%(30 isolates), black 23.8%(15isolates), red 17.4(11 isolates) and non biofelm producer isolates(pink)were 11.1%(7 isolates) .

3.1.2 Christensen method

While 51 isolates (80.7%) gave positive results in Christensen method(adherent layer of stained material to the inner surface of the tube)but in variable degrees, 50.7%(32 isolates) gave adherent growth (slime layer)in larg amount while 30.15%(19 isolates) gave adherent growth (slime layer) in few amount. Figure. (2).

3.1.3 Polystyrene microtiter plates

The ability of *A, hydrophlia* isolates to produce biofilm were evaluated(quantitative) by using pre-sterilized 96-well polystyrene microtiter plates and then absorbance was determined at 492 nm in an ELISA reader for determination the degree of biofilm formation for studied strains that adhered on the surface of the microtiter well, absorbance were represented the degree of the biofilm thickness that formed by the studied strains.

All *A.hydrophlia* isolates assayed for the production of biofilm, and the absorbance values ranged 0.062- 1.813 .

Moreover, 50 to 60% of mesophilic aeromonads are able to produce many unsheathed peritrichous lateral flagella when grown in viscous environments or over surfaces [13].

Sixty isolates(95.2%) produced biofilm on Polystyrene microtiter plates.

Obviously, *A.hydrophlia* No.1(raw water) and No.3(drinking water) achieved the highest biofilm thickness 1.813, 1.123 respectively.

The results showed that the larger percentage of biofilm producer isolates were estimated by polystyrene microtiter plate methods than other stuied methods, figure 3-.

The results indicated that each isolate showed a different potential to form biofilm under the same conditions of experimentation.

Bacteria were classified using the scheme of [8] into four groups as shows in table -1.

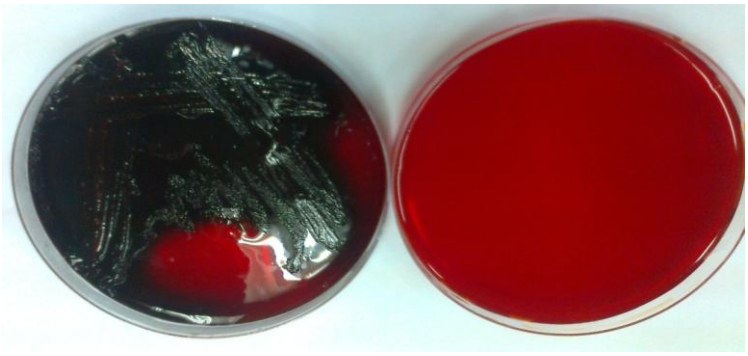

Figure 1- *A.hydrophila* isolate in Congo red method, showing the black colonies

Figure 2- slime layer formation by *A. hydrophila* on the surface of the tested glass tube (Christensen method). (A): weak slime layer (B): strong slime layer

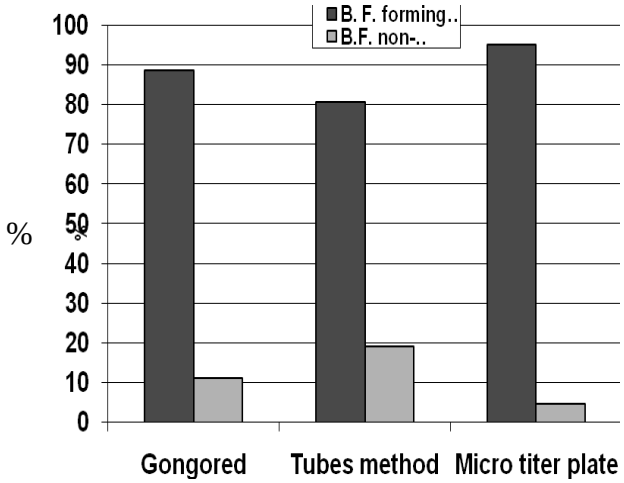

Figure 3- Percentage of biofilm forming strains by using three methods (congored, tube, microtiterplate)

Table 1- Four groups of biofelm producer isolates according to OD value by using the scheme of 8 Stepanovic´ *et al.* (2000)

OD group	No.	Mean ± SE
Non.	**3**	**0.062 ± 0.004**
Weak	**3**	**0.125 ± 0.01**
Moderate	**9**	**0.252 ± 0.03**
Strong	**48**	**1.175 ± 0.07**
LSD value	**----**	**0.108 ***

In Tunisian study reported that, only 65% of the strains produced slime On Congo Red Agar, 65% of the strains were able to form biofilm on glass tube. Most *A. hydrophila* strains (95%) were adhesive to polystyrene with high optical density values[14].

3.2.1 Study effect of several factors on on adherent ability of *A. hydrophila* (*DW, RW*) strains .

The more two efficient isolates(*DW, RW*) to produce biofilm was chosen to study effect of different values of temperature and, disinfectants on, the ability of *A.hydrophila* to adhere to solid surfaces

***3.2.2.A. hydrophila* adherence to solid surfaces test**

This test was performed to study the biofilm formation on four solid surfaces (10)-(11).Ttwo isolate of *A.hydrophlia* (*DW, RA*) that has the ability to produce slime layer was used. The result the *A.hydrophlia* shown the ability to adherence and form biofilm on the four solid surfaces (stainless steel (SS), galvanized iron(GI), polyvinyl chloride (PVC) and unplasticised polyvinyl chloride(UPVC).

Results analysis between logarithm of adherence cells number per cm² of solid surface and the time at 37°C is shown in the figure 4, that *RW* isolate appeared higher ability to produce biofilm on all surfaces than *DW* isolate, reasone may be due to the source of *DW* isolate in the pipe line of the distribution system and this type of cells considered stressed cells because of nutrient poor conditions.

Our results showed that higher number of *A. hydrophila* was found on uPVC follows by the PVC and SS and GI was the least in ability to attraction of bacteria.

Statistical evidence showed that the generic type of pipe materials has agreater influence the density of the bacteria in the water system [15].

It is important to note that the factor time also has greater influence the density of bacteria on tested surfaces figure 4-.the maximum higher count of both isolates was obtained after 48hr, [15].

This pattern of adhesion can be explained by the composition of the materials that were tested .The characteristics of the material composing pipes may greatly influence the on densities of bacteria fixed in a distribution system. Studies have pointed out that the roughness of the material used for the distribution of potable water contributes to bacterial attachment [16, 17].

Also Manuel, (1)reported that the roughness also affected biofilm accumulation on polymeric materials like PVC.[1]

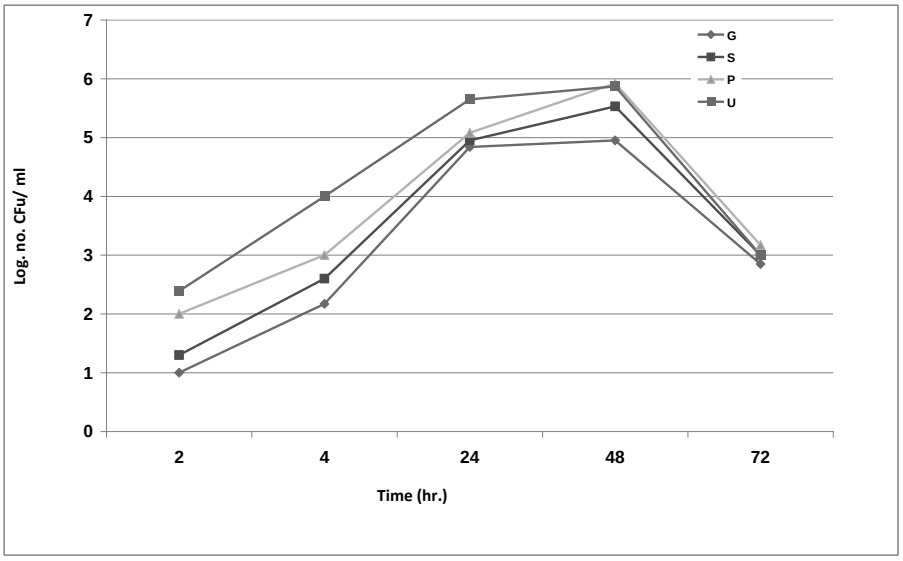

Figure 4- Relationship between time and log.no. of *A.hydrophlia*.(*DW* isolate) on four solid surface at 37°C and pH 7. stainless steel (S), galvanized iron(G) adhered cells, polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

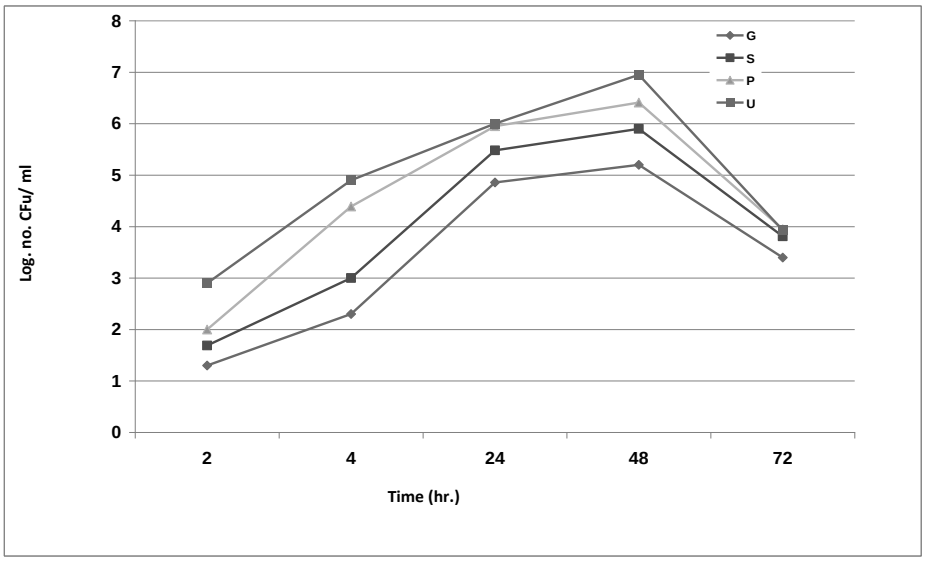

Figure 5- Relationship between time and log.no. of *A.hydrophlia* on four solid surface at 37°C and pH 7. stainless steel (S), galvanized iron(G) (*RW* isolate) adhered cells, polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

In general, on smooth surfaces, biofilms form at a slower initial rate than on rough ones, but biofilm formation after a period of days is inevitable [18].Other study [19] studied the adhesive properties of *A.hydrophila* isolated from unchlorinated communal water distribution system in Poland, and he concluded that biofilm formation by species of *Aeromonas* in water-distribution systems can play a key role in the contamination of drinking water and transmission of different pathogens.

Investigation biofilm formation on stainless steel, polyvinyl chloride and glass surfaces using recycled water systems (potable water systems) gave biofilm formation on all materials at 1-2 log10 CFU/cm^2 [20].

Our results came in accordance with the finding of other study which reported higher biofilm formation on PVC surfaces than on galvanized iron piping.[21].

The present work is in agreement with astudy that found that significantly higher number of aeromonads on uPVC than on SS coupons) also observed a higher number of *Aeromonas* cells on plastic substrata (polybutylene) compared with SS.[22].

Bomo *etal*(20) reported that *Aeromonas* were detected on 100% of oupons investigated and the biofilm biomass was significantly high in the model recycled water distribution system(devices supplied with drinking water)and higher number of aeromonads were culturable from uPVC compared with SS coupons.

A study was conducted in India to bring out the pattern of biofilm formation by heterotrophic bacteria on, polyvinyl chloride (PVC) sheet fitted wooden rack that was immersed in seawater, bacterial enumeration was made by spread plate method on nutrient agar medium. The second dominating bacteria in the biofilm were found to be *Aeromonas spp.* It is interesting to note that the *Aeromonas spp.*was found to be co-aggregating with *Pseudomonas spp.* i.e. the period of occurrence and disappearance of these genera is essentially the same as exhibited by *Pseudomonas spp*.[23].

A questionnaire relating to the type of pipe materials used in Iraq for the distribution of potable water was carried out, information collected from engineers in water treatment plants in Baghdad .Data collected from this questionnaire revealed that types of pipe materials are used in the following percentage: ductile iron (50 %), galvanized iron GI (30%), unplasticised polyvinyl chloride UPVC(10%), and other (asbestos cement, PVC)(10%).

Similar questionnair conducted by Momba and Makala [15] in South Africa found that percentage of polyvinyl chloride-PVC (25%), asbestos cement-AC (21%), asbestos (19%), unplasticised polyvinyl chloride-UPVC (16%), steels (8%), cement-C (4%), bitumen coated (3%), high density polyethylene-MDPE (2%), copper (1%), galvanised mild steel
(1%), mortar lined steel (1%), cast iron (1%).

3.2.3.Effect of temperature on *A.hydrophlia* adherence to four solid surface

The effect of different temperature on the number of adherent cells on solid surface was studied for (*DW, RW*) isolates by using different temperature 4, 37 and 40ºC .

The solid surfaces exposured to bacterial suspension 1x10^9CFU⁄ml at each temperature, figures (6, 7, 8, 9).

Stainless steel (SS), galvanized iron(GI) support less fixed bacteria than plastic -based materials (PVC, and uPVC).This observation supported by Momba. and Makala[15].

The results showed the ability of *A.hydrophlia* (*DW, RW*) to adherence on four solid surfaces at low temperature 5°C greater than 37°C., these results may be due to the stress on bacterial growth which lead to form more slime layer and biofilm to protect bacteria cells against this physical stress .Such phenomenon was explained in detail previously by the tendency of bacteria to regulate genes expression in respone to environmental signals, such as temperuture, pH, oxygen, carbone dioxide concentration, and nutrients aviability[24] **.**

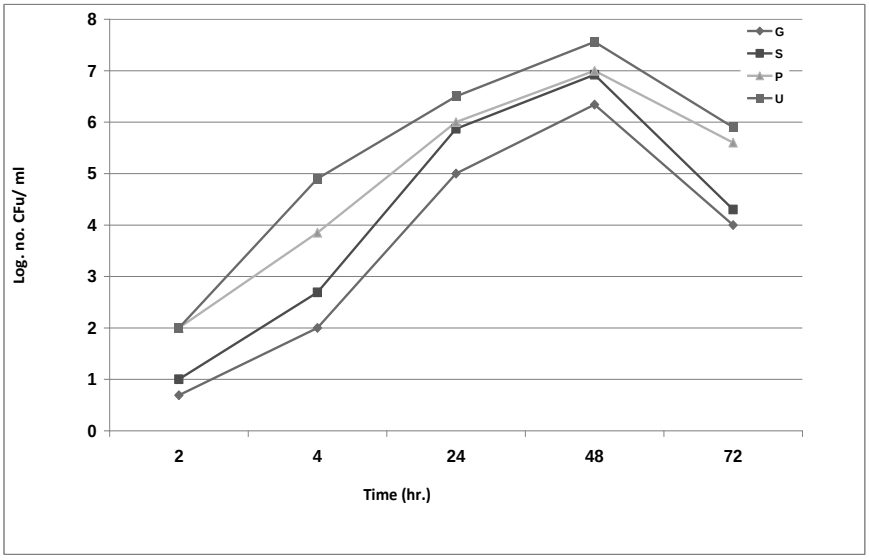

Figure 6- Relationship between time and log.no. of *A.hydrophlia*.(*DW* isolate) adhered cells on four solid surface at 5 °C. stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

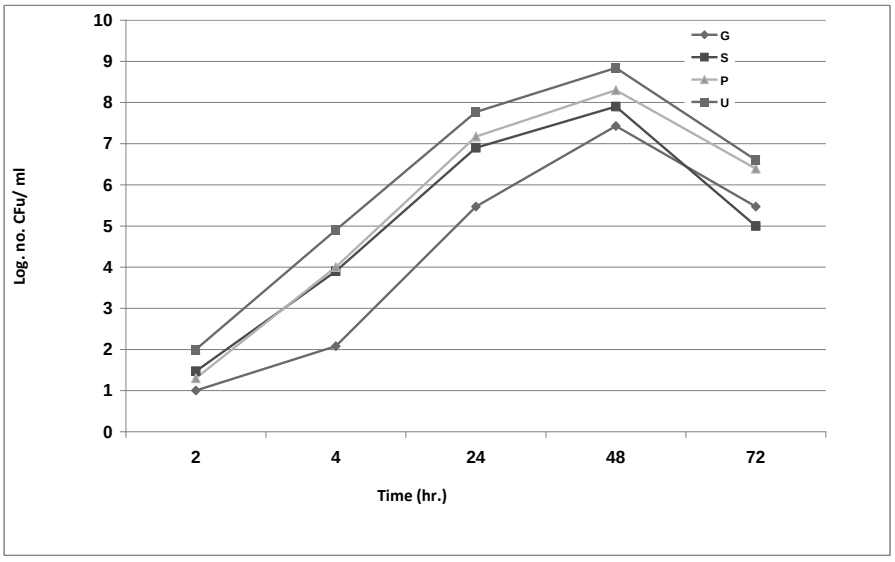

Figure 7- Relationship between time and log.no. of *A.hydrophli(RW) isolate* adhered cells on four solid surface at 5 °C. stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

Its important to note that *RW* isolate appeared larger ability to produce biofilm on all surfaces at all temperatures than *DW* isolate. *Aeromonas* cells could easily attach to distribution system material such as stainless steel, copper, polybutylene after exposures as short as 1-4 hours at temperatures 4 °C. [22].

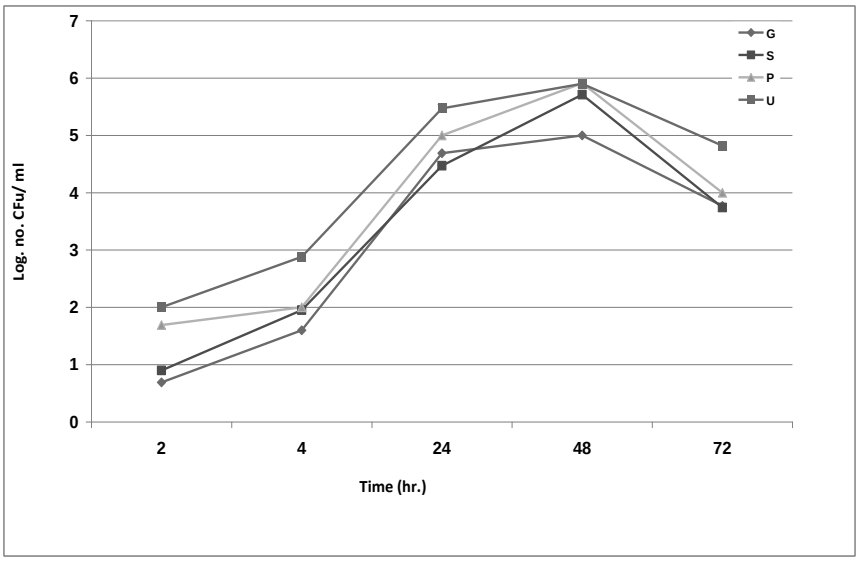

Figure 8- Relationship between time and log.no. of *A.hydrophlia* A.(*DW* isolate) on four solid surface at 40°C adhered cells stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

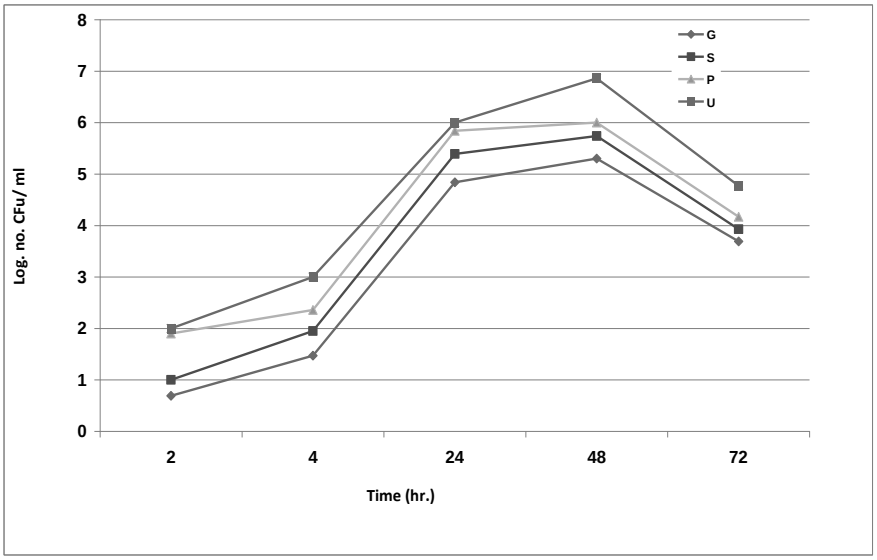

Figure 9- Relationship between time and log.no. of *A.hydrophlia* (RW) isolate on four solid surface at 40°C adhered cells stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U)

3.2.4 Determination of minimal inhibitory cocentration(MIC) of disinfectants

The MICs of two disinfectants (Chloroxyleno(Dettol**)** and hypochlorite (Bleach**)**were determined for the raw water *(RW*)and drinking water (*DW)* isolates of *A. hydrophila*, the results showed that two type of isolates have same MIC. (table 2). The MIC values varied according to the type of disinfectants in the range (1550-7500) µg/ml. In this experiment, Dettol was found as the best antiseptic against both isolates of *A. hydrophila* recording a minimum value of MIC (1550) µg/ml, follows by Bleach with MIC(7500) µg/ml **.**

Table 2- MIC of chemical disinfectants for *A. hydrophila* isolates

Commercial name of Chemical Disinfectants	Scientific name of chemical disinfectants	MIC (µg/ml)
Dettol	**oxylenolChlor**	**1550**
Bleach	**Sodium hypochlorite**	**7500**

The results indicated to high resistance of *A. hydrophila* isolates to the common disinfectants**.**The explanation to these resistance is that microorganisms can be adapted to a variety of physical and chemical conditions. Beside this, increased biocide MICs due to acquired mechanisms have also been reported . Moreover, residual concentrations of antiseptics or disinfectants in clinical situations and that discharged into the river water may cause subtle changes in the bacterial outer structure, thereby stimulating cell-to-cell contact, as is occur in antibiotics .[25].

Mazzola *etal* [26] studied and compared the behavior of selected microorganisms(*S. aureus S. Marcescens, E. coli, E.cloacaeA.calcoaceticus, B. subtilis, B. stearothermophilus*), they were submitted to minimal inhibitory concentration (MIC), the MIC were 1250 to 6250 mg/L of iodine 150 to 4491 mg/L of chlorine, this MIC values appeared to be lower than of recent study that considered as a best indicator to high resistance of local isolates against the disinfectants**.**

3.2.5 Effect of use chemical disinfectants on *A. hydrophila* adherence on solid surfaces

The method of is used in the study which showed effect of two infectants at (MIC) [10, 11] and analysis of these results are shown on figures (10, 11, 12, 13)

At MIC concentration the sodium hypochlorite remove all adherent *A. hydrophila(DW*) isolate from solid surfaces after 24 hour from exposure to adherent bacteria on solid surfaces, except adherent *A. hydrophila(RW*) isolates on UPVC were removed after 48 hr.

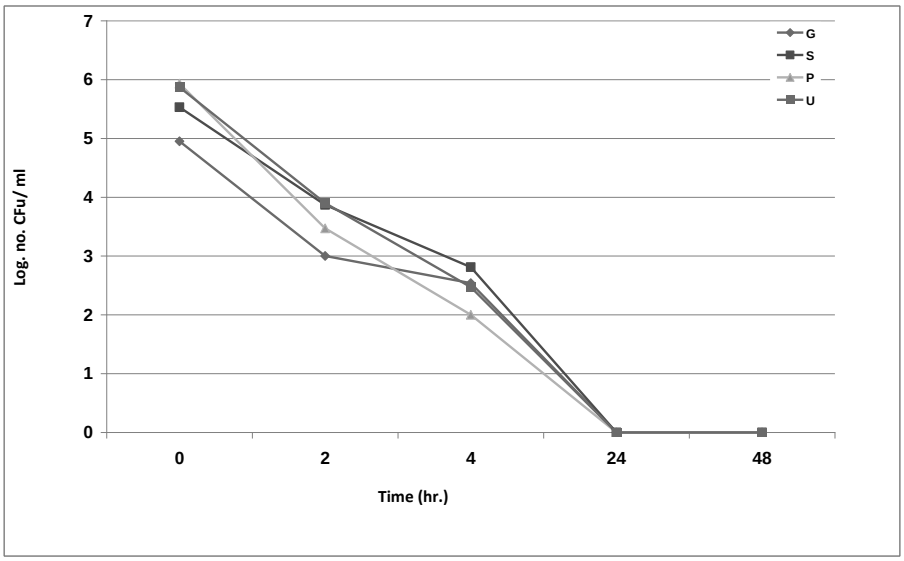

Figure 10- Relationship between log. No and time of of adherent *A.hydrophila(DW*) isolate cells/cm² in presence of (bleach) at MIC concentration at 37°C. initial number for(GI) $9x10^4$-, (SS) $3.4x10^5$cfu⁄cm², PVC $8.5x10^5$cfu⁄cm² and UPVC $7.5x10^5$cfu⁄cm².
stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U).

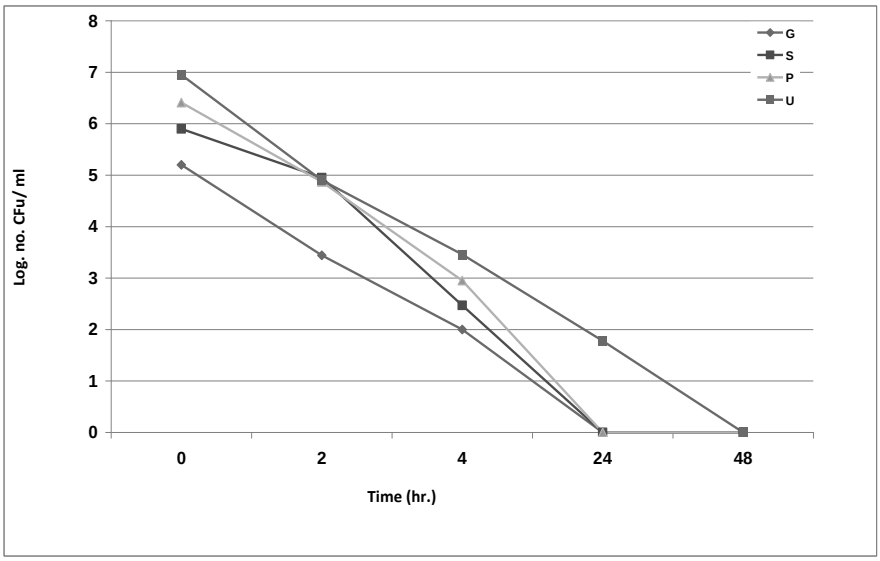

Figure 11- Relationship between log. No of of adherent *A.hydrophila(RW*) isolate cells/cm² and time in presence of (bleach) at MIC concentration at 37°C. initial number for(GI) 1.6 x10^5, (SS) 8x10^5cfu⁄cm² PVC 2.6x10^6cfu⁄cm² UPVC 9x10^6cfu⁄cm².
stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U).

Bleach, a main constituent of sodium hypochlorite, its effects by oxidizing the cell of microorganisms and attacking essential cell components including lipid, protein, and DNA .
The hypochlorite bleach is effective in cleaning and provides the possibility of a level of safety for the personnel handling of these devices.[27**]**

The colonization of pipe materials (PVC, uPVC, MDPE, cement and asbestos cement) by heterotrophic bacteria occur within 20 min under chlorination treatment.[8]

The addition of monochloramine in the chlorinated water system resulted in the removal of adherent bacteria attached to the pipe materials except for PVC was observed on the surface of test pipes between 48 and 168 h.

The ineffectiveness of chlorine in inhibiting bacterial biofilm formation is due to the neutralisation of chlorine by biofilms. [29]

Momba *etal* [30], demonstrated that the presence of an effective disinfectants(chlorine or monochloramine) residual in water system remains one of the most important factors in controlling the effect of pipe materials on biofilm formation.

Experimental studies have shown that biofilms attach to the inner surface of the distribution system even in the presence of free residual chlorine concentration higher than 2.5 mg /-1, [29-31].

The results of the effect of dettol at MIC concentration were stated that *A. hydrophila* cells*(DW*and *RW)*) were removed from both GI and SS surfaces after 24 hour whereas removed from PVC and UPVC surfaces after 48 hour from exposure to adherent bacteria solid surfaces.

Chloroxylenol is used in antiseptic or disinfectant formulations, (called commercially dettol) . Because of its phenolic nature, it would be expected to have an effect on microbial membranes. [32]but the microorganisms in biofelm may exhibit a greater degree of resistance to the chemical or natural cleaning and disinfecting agents compared to bacteria in suspension [33].

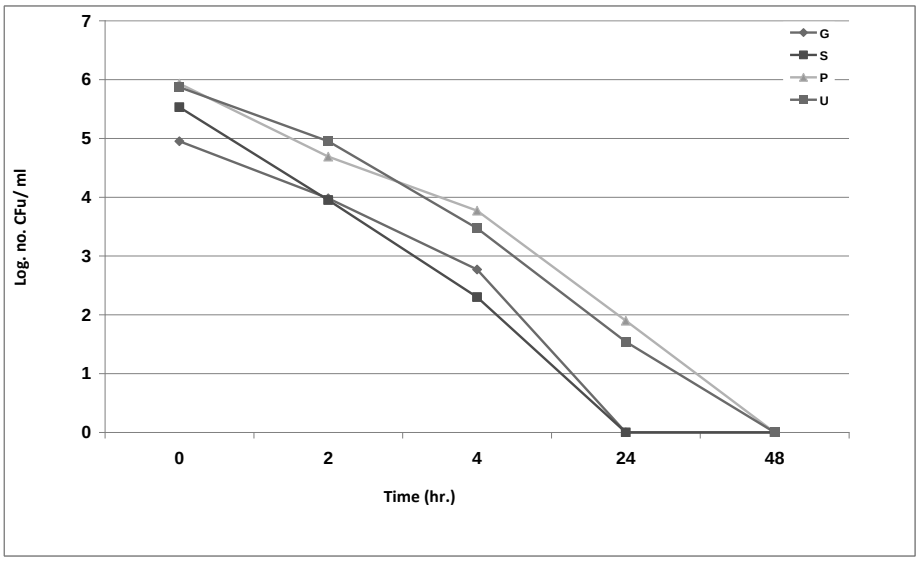

Figure 12- Relationship between log. No of of adherent *A. hydrophila(DW*) isolate cells/cm² and time in presence of (dettole)at MIC concentration at 37°C. initial number for(GI) $9x10^4$-, (SS) $3.4x10^5$cfu⁄cm², PVC $8.5x10^5$cfu⁄cm² and UPVC $7.5x10^5$cfu⁄cm².
stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U).

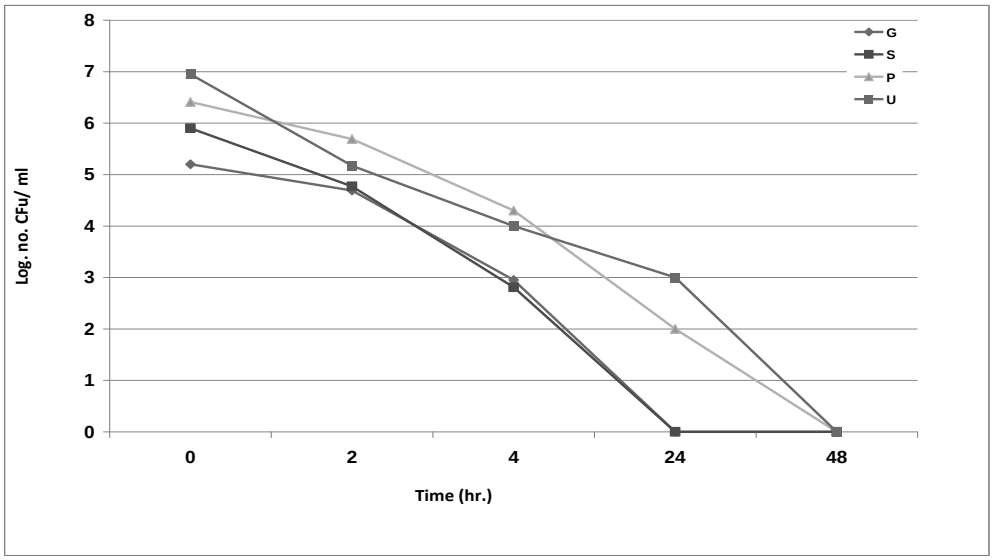

Figure 13- Relationship between log. No of of adherent *A.hydrophila(RW*) isolate cells/cm² and time in presence of dettole at MIC concentration at 37°C. initial number for(GI) 1.6 $x10^5$, (SS) $8x10^5$cfu⁄cm² PVC $2.6x10^6$cfu⁄cm² UPVC $9x10^6$cfu⁄cm².
stainless steel (S), galvanized iron(G), polyvinyl chloride (P) and unplasticised polyvinyl chloride(U).

From the obtained results, it is observed that the decrease in bacterial adherence on four solid surface occur in presence of two disinfectants. The more effective disinfectants was the sodium hypochlorite, then dettol. The effects of these disinfectants on bacterial adherence are due to active effectiveness on bacterial surface ability of these disinfectants to decrease of surface tension between the bacteria and adherence surface [34].

Also Ayliffe, [35] observed that the effect of hypochlorite was effctive directly and its effectivness reachs 99% in repeated use**.** Hypochlorite is excellent for biofouling control as it weakens the extracellular polysaccharide (EPS) structure, leading to sloughing and removal sections of the biofilm. The use of chlorine as a means to remove the EPS has been discussed by Samrakandi and his colleagues [36]. Further, Kumar and Anand[37] list chlorine as one of the chemicals that depolymerizes the EPS.

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