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Association between *Aeromonas* spp. and Classical Bacterial Indicators of Pollution in Different Aquatic Environments

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THE DENSITY of *Aeromonas hydrophila* and classical bacterial indicators of pollution (total bacterial counts at 22 and 37°C, total coliforms, faecal coliforms, and faecal streptococci) were determined at different aquatic environments for one year during February, 2000 to March, 2001. The results showed that, the density of *A. hydrophila* was greater than those of faecal bacterial indicators (total and faecal coliforms, and faecal streptococci) in samples of chlorinated effluent, agricultural drainage, River Nile water, sea water, and ground water. Meanwhile, in treated effluent and polluted sea water samples, the density of *A. hydrophila* was lower than that of total coliforms but higher than or equal to those of faecal coliforms and faecal streptococci. In domestic wastewater and ground water samples *A. hydrophila* and faecal streptococci had similar trends of fluctuation in cell densities throughout the period of study. Linear correlation coefficients analyses for the investigated parameters were used to clarify the relationship between *Aeromonas hydrophila* and classical bacterial indicators of pollution. The results showed that in case of agricultural drainage significant correlations ($r=0.84$, $b=1.48$, $P \leq 0.05$) were obtained between *A. hydrophila* and all the classical bacterial indicators of pollution. On the other hand, in case of chlorinated effluent and River Nile water there was no significant correlations. However, relations between *Aeromonas* and members of the classical indicators of pollution in the other aquatic environments were different.

Keywords: *Aeromonas hydrophila*, Classical bacterial indicators of pollution, Correlation, River Nile, Aquatic environments, Slope, Water, Wastewater.

Aeromonas hydrophila is a ubiquitous bacterium frequently found in a wide range of aquatic environments. In general, it is not considered a normal inhabitant of the human gastrointestinal tract. In recent years, the importance of *Aeromonas* as a human pathogen associated with a broad spectrum of diseases, including gastroenteritis. bacteriaemia and wound infections, has been noted

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(Merino, *et al.*, 1995; Wadstrom and Ljungh, 1991). Several authors have suggested that water may be an important source of human infection (Burke, *et al.*, 1984; Havelaar, *et al.*, 1990). When present in sufficiently large numbers, motile *Aeromonas* (*A. hydrophila, A. sobria* and *A. caviae*) might infect susceptible hosts, including young children and immunocompromised persons (Van der Kooij, 1988). Although in the epidemiology of infection caused by *Aeromonas* spp very little is known about the origin of the contamination, the role of aquatic ecosystems as sources of these bacteria is in any case recognized (Cugno and Pasquale, 1994). In fact, strains of *A. hydrophila* producing virulence factors have been isolated from municipal water (Handefield, *et al.* 1996; Krovacek, *et al.* 1992; Kuhn, *et al.*, 1997) and a study by Burke *et al.* (1984) reports that there is a correlation between cases of diarrhea and the presence of *A. hydrophila* in drinking water. In water free from faecal pollution there was no correlation but in polluted waters there was significant relationship between the numbers of aeromonads, faecal coliforms and the concentration of organic matter (Araujo, *et al.*, 1989a). Also, the relationship between water pollution and bacterial flora in river water was studied by Wada (1993), who showed that, the coliform group and *Aeromonas* were strongly related to the organic pollution. *Aeromonas spp.* showed a significant positive correlation with classical bacterial indicators of pollution and total suspended solids in the Buffalo River during the summer (Pettibone, 1998).

The wide distribution of *A. hydrophila* in different aquatic environments such as surface water and ground water (Kersters, *et al.*, 1995), estuarine and seawater (Kaper, *et al.*, 1981), sewage and wastewater (Boussaid, *et al.*, 1991) underlines the capacity of this species to adapt to environments that differ in terms of nutrients or presence of other aquatic microorganisms. Moreover, this bacterium has been recovered in chlorinated and unchlorinated drinking water (Knochel and Jeppesen, 1990; Van der Kooij, 1988), and some authors consider that the current technologies for production of drinking water could provide environmental conditions favoring the growth of this organism (Van der Kooji and Hijnen, 1988). The frequent isolation of *A. hydrophila* in drinking water supplies and mineral water may suggest that re-evaluation of current water-quality standards is required (Knochel and Jeppesen, 1990; El-Taweel and Shaban, 2001a; Messi, *et al.*, 2002).

The author therefore initiated a study of the concentration and distribution of *A. hydrophila* in a variety of waters (domestic wastewater, treated effluent, River Nile water, sea water, agricultural drainage, ground water, and tap water). Also, the population dynamics of this organism were then compared with those of classical bacterial indicators of pollution.

Material and Methods

Sampling sites and procedures

Samples were collected monthly for one year (Feb., 2000 - Mar., 2001) from the River Nile, ground (well) water, drinking (tap) water, raw and treated

municipal wastewater (Zenin wastewater treatment plant, allocated in El-Giza governorate), agricultural drainage water (Hados and Ramsis drains allocated in El-Sharkeya governorate), and sea water (at two sites from Suez Canal one of them polluted by domestic wastewater). The samples (0.5-2 L) were collected in sterile glass bottles and kept in ice box. Bacteriological examination was carried out within 2-6 hr after collection.

Bacteriological examination

The following bacteriological parameters were determined: total bacterial counts at 22 and 37°C on Plate Count Agar (TBC), total coliforms on Lauryl Tryptose Broth and Brilliant Green Broth (TC), faecal coliforms on EC medium (FC), faecal streptococci Azide Dextrose Broth and Pfizer Selective Enterococcus Agar (FS), and *Aeromonas hydrophila* onto mA Agar (Ah). These parameters were determined according to APHA (1998), except *A. hydrophila* was determined according to Rippey and Cabelli (1979).

Total bacterial counts were counted by using pour plate method as colony forming unites (CFU/ml), while the most probable number (MPN) five tubes and three set technique was used to determine total and faecal coliforms as well as faecal streptococci in 100 ml sample, *A. hydrophila* was determined using surface streaking technique for polluted water and wastewater samples, and membrane filtration technique for the other samples. To estimate the proportion of *A. hydrophila* in different water samples, colonies were picked from the *A. hydrophila* agar medium. This colony sample was taken from around 50 colonies in a single petri dish. Each colony was sub-cultured onto nutrient agar for 24 hr at 37°C before identification. Isolates with the following reactions confirmed the genus *Aeromonas* (Monfort and Baleux,1990): motility (+), Gram stain (-), cytochrome oxidase (+), D-glucose fermentation (+) (37°C, 24 hr), arginine dihydrolase (+) (30°C, 24 to 48 hr), ornithine decarboxylase (-) (30°C, 24 to 48 hr), and o-nitrophenyl-B-D-galactopyranoside test (+) (30°C, 2hr). The *Aeromonas* species was determined by using the following screening tests (30°C, 24 to 48 hr) (Popoff, 1984): esculin hydrolysis, L-arabinose utilization, fermentation of salicin, acetoin from glucose (Voges-Proskouer), gas from glucose, and $H_2S$from cysteine.

Statistical analysis

In order to study the relationship between A. hydrophila and indicator microorganism densities linear correlation analysis were used. The quantitative analysis for *A. hydrophila* and classical bacterial indicators of pollution were carried out with 6 to 12 samples replicates at each sampling site during the period of study. All the data were transformed in decimal logarithms and processed by the Microsoft Excel (Office 2000) under Microsoft Windows 98, computer application.

Results and Discussion

The isolated strains from different aquatic environments were biochemically identified (Table 1). *Aeromonas hydrophila* was found represent 75-83% from

total isolates of *Aeromonas* spp. In this study the majority of *Aeromonas* spp. was *A. hydrophila*, thus it is the dominant strain in *Aeromonas* spp.

TABLE 1. Biochemical identifications of *Aeromonas* spp. Isolated from different aquatic environments.

Reactions	*A .hydrophila*	*A. caviae*	*A. sobria*
Esculine hydrolysis	+	+	-
L-Arabinose utilization	+	+	-
Fermentation of Salicin	+	+	-
Acetoin from glucose (VP)	+	-	±
Gas from glucose	+	-	+
H2S from cysteine	+	-	+

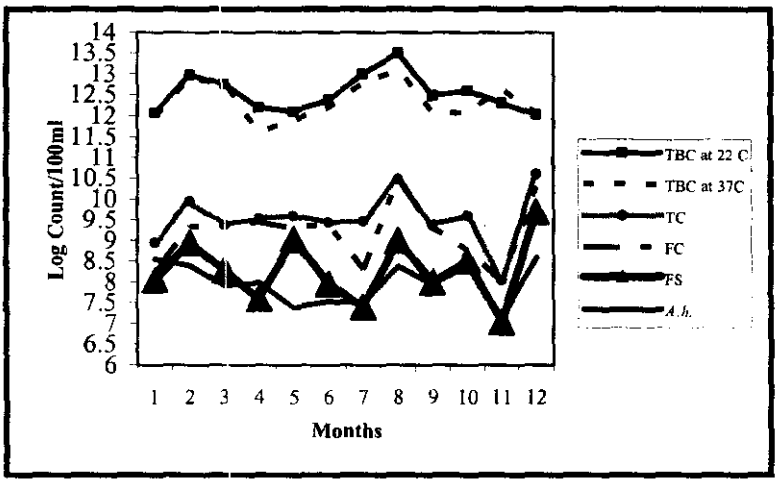
The study divided in two sections, the first was distribution of *A. hydrophila* and classical bacterial indicators in different aquatic environments, and the second was relationship between *A. hydrophila* and classical bacterial indicators in different aquatic environments.

Distribution of A. hydrophila and classical bacterial indicators in different aquatic environments

The log counts of the classical bacterial indicators of pollution and *A. hydrophila* in the investigated waters are summarized in Fig.1-4. *A. hydrophila* comprised 0.03 to 3.5 %, 0.01 to 0.1 %, 0.02 to 3.8 %, 0.05 to 2.0 %, 0.01 to 2.1 %, 4.6 to 13.3 %, 0.004 to 3.3 % and 0.5 to 20.5 % of total bacterial counts in samples from domestic wastewater, treated effluent, chlorinated effluent, agricultural drainage, River Nile water, sea water, sea water polluted by sewage, and ground water, respectively. Davis and Sizemore (1981) reported that, presumptive *A. hydrophila* comprised less than 0.5 % of total plateable heterotrophic counts in the samples of high- salinity and 3.4 % of the total population in the samples of low-salinity. Van der Kooij (1988) found that, aeromonads constituted a minor fraction of the heterotrophic bacterial population in drinking water. While, Gavriel *et al.* (1998) demonstrated that aeromonads are known to constitute a considerable fraction of the heterotrophic population of raw water. They added that, heterotrophic plate counts at either 22 or 37°C did not demonstrate any notable association with *Aeromonas*.

The density of *A. hydrophila* was fluctuated between 10^4 and 10^5, 10^4 and 10^6, 10^2 and 10^5,10^2 and10^4 and10^3 and10^6 cfu /100ml in samples of chlorinated effluent, agricultural drainage, River Nile water, sea water, and ground water, respectively and it was greater than total and faecal coliforms, and faecal streptococci (Fig. 2 (A,B), Fig. 3 (A,B), and Fig. 4 (B).

(A) Domestic Wastewater.



(B) Treated Effluent.

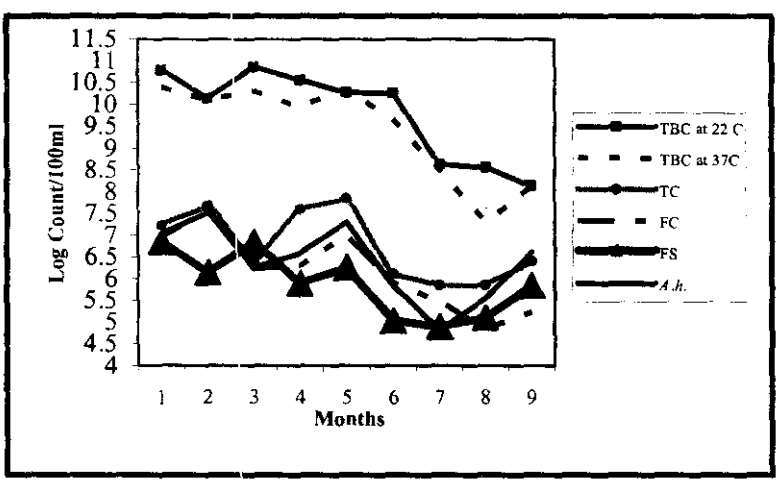
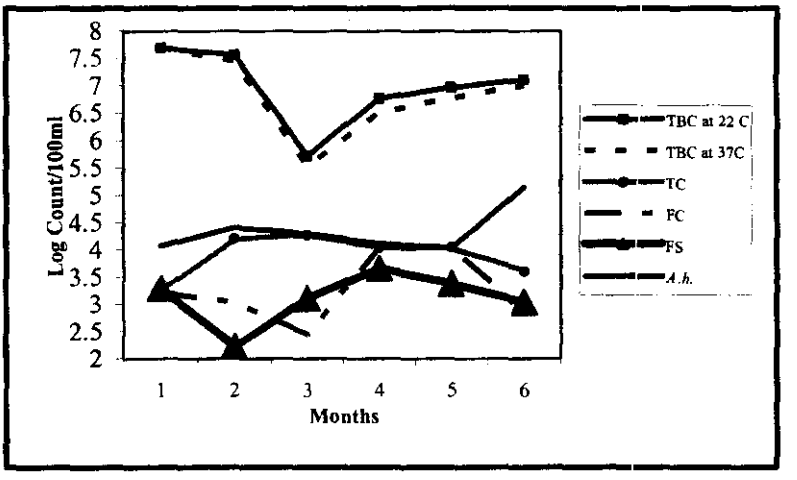


Fig.1. Log Counts of Classical Bacterial Indicators and *A. hydrophila* per 100ml in Domestic Wastewater (A) and Treated Effluent (B).

(A) Treated Effluent With Chlorine.



(B) Agricultural Drainage.

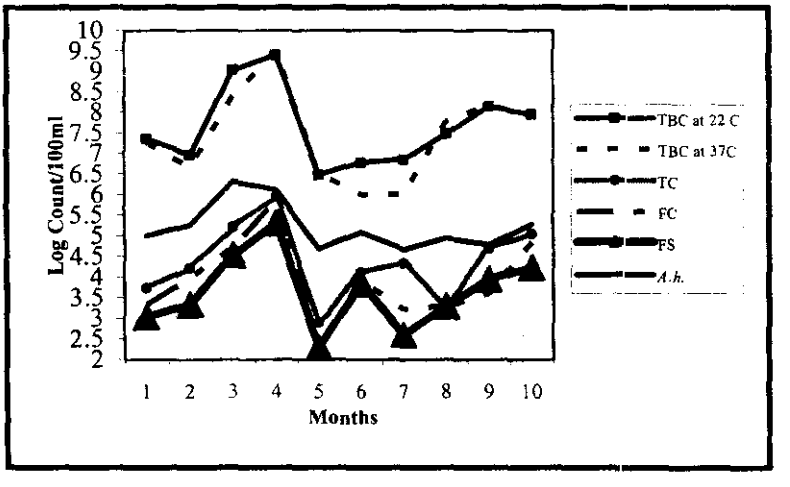
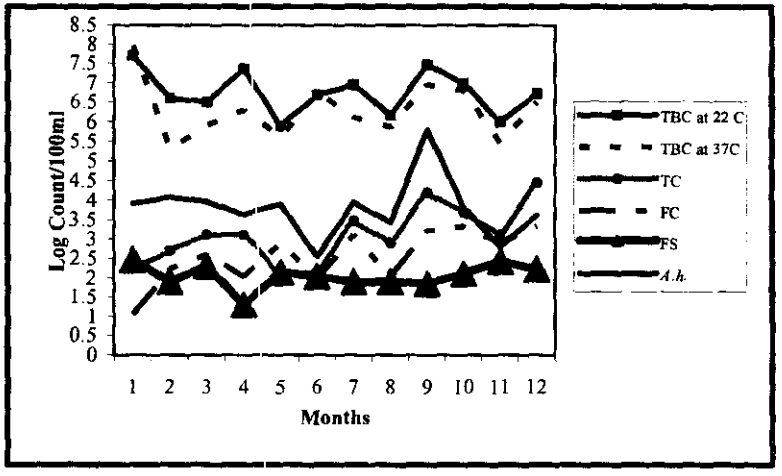


Fig. 2. Log Counts of Classical Bacterial Indicators and *A. hydrophila* per 100ml in Treated Effluent with Chlorine (A) and Agricultural drainage (B).

(A) River Nile.



(B) Sea Water.

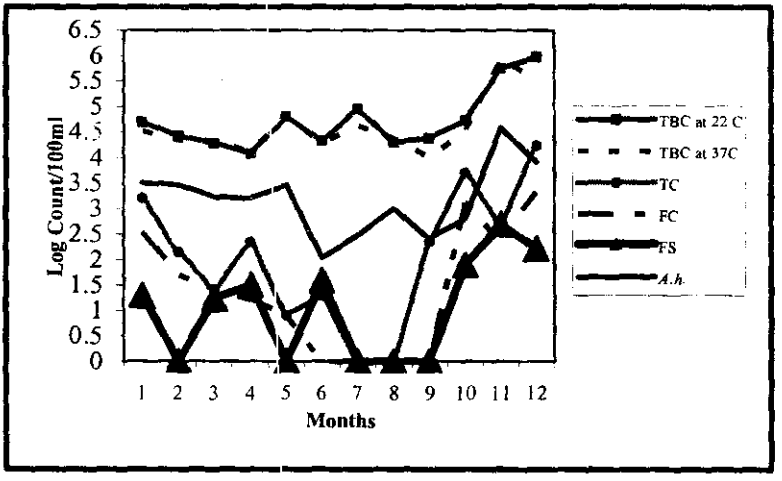
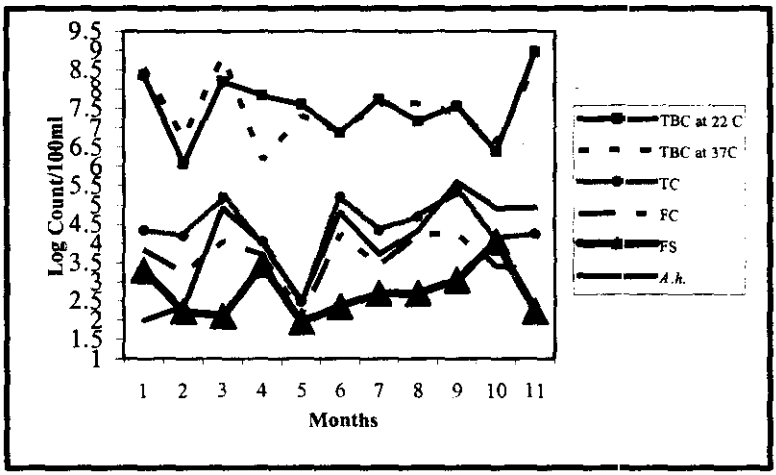


Fig. 3. Log Counts of Classical Bacterial Indicators and *A. hydrophila* per 100ml in River Nile water (A) and Sea Water (B).

(A) Sea Water Polluted with Domestic wastewater.



(B) Groud Water.

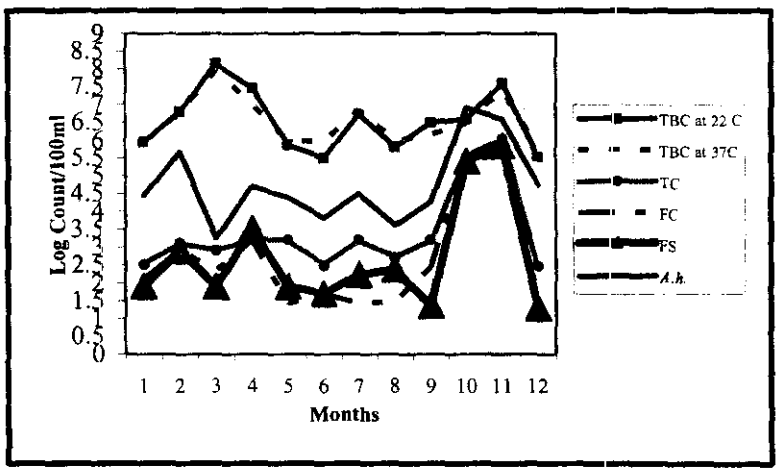


Fig. 4 . L og C ounts o f C lassical B acterial I ndicators and *A. h ydrophila* per 100ml in Sea Water Polluted with Domestic Wastewater (A) and Ground Water (B).

The high counts of *A. hydrophila* in the above mentioned aquatic environments might be referred to:-

1- Because A. hydrophila was more resistant to chlorine than faecal bacterial indicators.
2- The agriculture drainage water contains nitrate and phosphorous which increase the eutrophication in the water system.
3- The river water contains different nutrient materials which increase the trophic situation.
4- The sea water receives a large amount of pollutants coming from rivers, wastewater discharge, and salinity increase the temperature which support the growth of A. hydrophila.
5- The ground water polluted by subsurface seepage of sewage from septic tanks and sewers lines or from land application of wastewater.
6- This might be also to increase the organic matter to the suitable levels, antagonisms with other microorganisms and effect of toxicants in aquatic environments.

Bacteria of the genus *Aeromonas* are ubiquitous in the different aquatic environments and have been found in a wide variety of conditions including oligotrophic upland waters, eutrophic low land rivers, sewage effluents and estuarine and marine waters (Poffe and Op de Beeck,1991; Ashbolt *et al.* 1995). Menon (1985) attributed the higher counts of *A. hydrophila* in the Cornwallis River to the cumulative effect of pollution from untreated and inadequately treated sewage from several communities ,separate storm sewers ,as well as agricultural and food processing wastes . Araujo *et al* (1989 b) reported that, the frequency of isolation of *Aeromonas spp* were consider lower in the samples taken from the plant using chlorination. They added that, this emphasizes the efficacy of chlorine in controlling the numbers of these organisms in water.

A. hydrophila were isolated from domestic wastewater samples at a constant density (10^7to10^8 cfu/100ml) and this density was lower than that of faecal bacterial indicators (Fig.1, A). This because the main composition of wastewater were faecal materials and A. hydrophila was not common in faecal matter. In treated effluent and sea water samples polluted by sewage, the density of *A. hydrophila* was lower than those of total coliforms but greater than or equal to those of faecal coliforms and faecal streptococci (Fig.1, B and Fig. 4, B). Araujo *et al.* (1989b) reported that the nearly constant isolation of *Aeromonas spp.* in the different samples was probably due to the fact that these bacteria tend to flourish in aquatic habitats of highly trophic nature, the presence of highly organic matter of various origins.

In the case of sea water faecal bacterial indicators were detected in 70% of samples, while *A. hydrophila* was detected in 100% of samples. Hazen and Esch (1983) found that the concentration of *A. hydrophila* in fresh and marine water was greater than that of coliforms. The higher numbers have been found

associated with sewage pollution (Shubert, 1967), higher nutrients contents (Rippey and Cabelli, 1985), and higher temperature (Seidler *et al.*, 1980).

In domestic wastewater and ground water samples *A hydrophila* and faecal streptococci had similar trends regards fluctuation in cell densities throughout the period of study. While in case of treated effluent and River Nile water samples similar trend of fluctuations was between *A. hydrophila* and, total and faecal coliforms. Several investigators (Kaper *et al.*, 1981; Seidler *et al.* 1980) observed that, the number of aeromonads often approached, or exceeded *Escherichia coli* in surface waters and sewage. Alonso *et al.* (1991) found analogous results in Valencia coastal waters and the number of aeromonads exceeded the faecal coliforms all the year round. Brion *et al.* (2000) reported that *Aeromonas spp* are wide spread in nutrient rich waters and sewage that the increase in concentration might indicate faecal contamination in surface waters.

The present results indicated that *A. hydrophila* was detected at high densities in waters polluted by sewage. This might be due to the high nutrient levels of sewage and this is in agreement with many studies (e.g. Alonso *et al.*, 1991; and Pianetti *et al.*, 1998). *Aeromonas* hydrophila was found at high densities in raw and treated sewage although it was not commonly isolated from human feces (Moyer, 1987). Presumably, the organism multiplies in sewage due to the high nutrient levels found therein. This is consistent with the response of the organism reported in nutrient rich fresh waters (Rippey and Cabelli, 1985). Thus, *Aeromonas* hydrophila has been shown to be strongly associated with the trophic states of fresh waters (Rippey and Cabelli,1980).

The examination of water samples from the final effluent from El-Giza Water Treatment Plant showed that *A. hydrophila* was present in low numbers (1-8 cfu/100ml) in 60% of the samples. Similar results were reported by other authors (Havelaar *et al.*, 1987; Araujo *et al.* 1989b; Kuhn *et al.* 1997; El-Taweel and Shaban, 2001 b), which indicate that chlorine at the concentrations used for routine disinfection is not 100 % effective in killing *A. hydrophila.*

Relationship between A. hydrophila and classical bacterial indicators in different aquatic environments

In order to study the relationship between *A. hydrophila* and the concentrations of classical bacterial indicators of pollution, linear correlation coefficients analyses were used (Table 2).

In domestic wastewater samples, *A. hydrophila* counts yielded significant correlations with total coliforms and faecal streptococci ($r = 0.60$, $P \leq 0.05$), where the last had the highest value of regression line slope. In treated effluent samples. *A. hydrophila* counts showed a significant correlation with total and faecal coliforms, and faecal streptococci where $r = 0.89$, 0.80 and 0.74 and $P \leq 0.05$, respectively. Coliform group in general showed the best slop values compared to faecal streptococci. On the other hand, in case of ground water

samples opposite results were obtained, where faecal streptococci showed the best slope values (Table 2).

In agricultural drainage samples, significant correlations appeared between *A. hydrophila* and the classical bacterial indicators of pollution, with the highest correlation coefficient and slope ($r = 0.84$, $b = 1.48$, $P \leq 0.05$) for faecal coliforms. Similar results were found in the Buffalo River during the summer as pointed out by Pettibone (1998). From thirteen fresh water aquacultural farms in Denmark, Knochel (1990) reported that the correlation was week between total viable counts and the *Aeromonas* counts.

TABLE 2 - Linear correlation between *A. hydrophila* and classical bacterial indicators in Different Aquatic Systems.

Sample Type	n	Total bacterial count at 22°C		Total Bacterial Count at 37° C		Total coliforms		Faecal coliforms		Faecal streptococci		% A. h.
		R	b	r	b	R	B	r	b	r	b	
Domestic waste-water	12	0.12	0.11	-0.04	-0.04	0.60*	0.81	0.42	0.65	0.60*	0.94	100
Treated effluent	9	0.51	0.61	0.63	0.83	0.89*	0.83	0.80*	0.83	0.74*	0.62	100
Chlorinated Effluent	6	0.04	0.07	0.06	0.11	-0.16	-0.15	-0.53	-0.82	-0.37	-0.44	100
Agricultural Drainage	10	0.82*	1.41	0.68*	1.36	0.74*	1.18	0.84*	1.48	0.82*	1.31	100
River Nile	12	0.48	0.34	0.22	0.21	0.45	0.43	0.29	0.26	-0.22	-0.59	100
Sea water	12	0.63*	0.54	0.77*	0.63	0.42	0.82	0.66*	1.19	0.45	0.65	70
Sea water + sewage	11	0.08	0.06	0.02	0.01	0.62*	0.40	0.53	0.28	0.15	0.08	100
Ground water	12	0.20	0.15	0.14	0.09	0.84*	0.87	0.85*	1.11	0.84*	1.12	100

*Statistically Significant ($p \leq 0.05$)

n : Number of samples, r: Correlation coefficient, b: Slope

% A.h.: % of positive detection of *Aeromonas hydrophila*

In agricultural drainage samples, significant correlations appeared between *A. hydrophila* and the classical bacterial indicators of pollution, with the highest correlation coefficient and slope (r=0.84, b=1.48, P≤ 0.05) for faecal coliforms. Similar results were found in the Buffalo River during the summer as pointed out by Pettibone (1998). From thirteen fresh water aquacultural farms in Denmark, Knochel (1990) reported that the correlation was week between total viable counts and the *Aeromonas* counts.

In sea water samples, there was a significant correlation between *A. hydrophila* counts showed and total bacterial counts at 22, and 37°c and faecal coliforms (r= 0.63, 0.77 and 0.66, respectively and P≤ 0.05). While total bacterial count at 37°c showed the highest correlation coefficient, faecal coliforms the best slope value. Alonso *et al.* (1991) reported that, significant correlations were obtained between coliform group and motile *Aeromonas*, whereas when faecal pollution increased the correlation was not significant. They added that, the number of *A. hydrophila* isolates increased in less polluted sites at Puzol beaches in Valencia - Spain.

In case of chlorinated effluent and River Nile water samples, there was no significant correlations between *A. hydrophila* and the classical bacterial indicators of pollution, while significant correlation was observed in polluted sea water samples between *A. hydrophila* and total coliforms (r=0.62, P≤ 0.05) . No correlation was found between the indexes of faecal contamination and *Aeromonas* spp. for water samples at Metauro and Foglia Rivers in Italy (Piantti *et al.*,1998). In addition, Rippey and Cabelli (1979) found that the concentrations of *A. hydrophila* were not correlated with the total number of heterotrophic bacteria in fresh waters. Seidler *et al.* (1980) suggested that, the numbers of *Aeromonas* cells showed a significant correlations with total and faecal coliform counts (r=0.95 to 0.96 and 0.90, respectively and P≤ 0.05) for both sediment and water samples at Anacostia River in Washington. And they concluded that *Aeromonas* are useful indicators of pollution.

From the point of view of sanitation, the incidence of *Aeromonas* strains may be more important than that of *Escherichia* coli. Where, *E. coli* being present in very high numbers in the gut of warm-blooded animals and widely used as an indicator of faecal contamination to estimate the risk of exposure to other types of pathogenic organisms present in animal or human wastes. However, the effectiveness of *E. coli* as a bioindicator for water quality has recently been questioned (Lund, 1994). Compelling epidemiological evidence has shown that total coliform, faecal coliforms or *E. coli* counts do not correlate well with pathogenic incidences caused by other types of waterborne pathogens (Lund, 1994). Wu (1999) stated that, conventional water testing methods are only outdated but also cannot afford adequate protection to public health. Several reports have demonstrated that wound infections caused by *Aeromonas* spp. are linked with recreational and other activities in aquatic environments (Seidler *et al.*, 1980; Thonnon *et al.*, 1987; Kong *et al.*, 2002), and these bacteria could therefore pose a problem for public health (Seidler *et al.*, 1980; Handfield *et al.*,

1996). In addition, Chaurent *et al.* (2001) concluded that, although *Aeromonas hydrophila* was never detected in the treatment plant effluent or distributed bulk water, showing disinfectant efficiency on suspended bacteria; isolates of *A. hydrophila* were identified in 7.7% of the biofilm samples, indicating a potential for re-growth and contamination of drinking-water distribution systems. This could help to permit reevaluation of water quality standards in order to control the presence of *Aeromonas spp.* in drinking water supplies.

Conclusions

1- Results showed that *A. hydrophila* was detected at high densities in waters polluted by sewage.
2- Significant correlations were obtained between *A. hydrophila* and classical bacterial indicators in case of agricultural drainage.
3- *A. hydrophila* more resistant for chlorine and environmental stress than those of faecal indicators.
4- *A. hydrophila* can be considered as indicator for pollution supplemented with classical bacterial indicators.
5- The *A. hydrophila* in water must be reduced as much as possible, where it can cause enteric and non-enteric diseases for humans.

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التلازم بين تواجد الايروموناس هيدروفيلا و كشافات التلوث التقليدية فى أنواع مختلفة من المياه.

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فى هذه الدراسة تم تقدير كثافة ميكروب الايروموناس هيدروفيلا وكشافات التلوث التقليدية (العد الكلي للبكتيريا علي درجتي حرارة٢٢، ٣٧° م ،مجموعة القولون الكلية، مجموعة القولون البرازية، والمجموعة السبحية البرازية) فى أنواع مختلفة من المياه خلال الفتـــرة من فبراير ٢٠٠٠ الى مارس ٢٠٠١. أوضحت النتائج ان كثافة الايروموناس هيدروفيلا أعلى من كثافة كل من مجموعة القولون الكلية، مجموعـــة القولـــون البرازيـــة وكـــذلك المجموعة الســبحية البرازيـــة فـــى عينـــات المخلـــف النهـــائي المكلـــوره (الســـيب المكلور)،الصرف الزراعي،مياه نهر النيل،مياه البحر،بالإضافة الى المياه الجوفية. بينمـــا فى مياه السيب ومياه البحر الملوثة بمياه المجارى كانت كثافة الايروموناس هيدروفيلا أقل من كثافة مجموعة القولون الكلية وتساوى أو أكبر من مجموعة القولون البرازية وكذلك المجموعة السبحية البرازية.أما فى حلة عينات مياه الصرف الصحي والمياه الجوفية كانت أعداد الايروموناس هيدروفيلا تسلك نفس سلوك المجموعة السبحية البرازية فـــى حالـــة الزيادة أو النقصان خلال فترة الدراسة. وفى محاولـــة لإيجـــاد علاقـــة بـــين ميكـــروب الايروموناس هيدروفيلا وكشافات التلوث التقليدية تم إجراء تحليل إحصـــائي باســـتخدام معامل الارتباط (r) والميل(b)عن طريق تحويل الأعداد الحقيقية للميكروبات تحـــت الدراسة الى أعداد لوغاريتمية حتى يسهل دراسة العلاقة بينها كل هذه التحاليل الإحصائية تمت باستخدام الكومبيوتر على الأكسل (Excel).أوضحت نتائج التحليل الإحصـــائي أن العلاقة كانت معنوية بين عناصر المقارنة فى حالة مياه الصرف الزراعي أما فى حالـــة مياه النيل والسيب المكلور فلم توجد علاقة معنوية بينهما. أما باقي أنواع المياه التي تحت الدراسة أظهرت النتائج علاقات معنوية وغير معنوية مختلفة بين ميكروب الايروموناس هيدروفيلا وكشافات التلوث التقليدية. وعلى ذلك نتيجة لهذه الدراسة يوصى بإعادة تقيـــيم المواصفات القياسية والمعايير التي تتحكم فى صلاحية المياه للشرب وكذلك المعايير التي تتحكم فى إعادة استخدام مياه الصرف الصحي المعالجة .