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Research Article

An Approach Using Non-Conventional Indicators for Detecting Microbial Water Pollution

The present study aims to investigate the suitability of the genus *Aeromonas* as a non-conventional microbial indicator of water quality in different Egyptian water resources; River Nile, drainage wastewater, and chlorinated drinking water. *Aeromonas* was detected in 71.2% of examined samples, being maximum in drainage water and minimum in River Nile and drinking water. Several positive significant relationships between *Aeromonas* and the corresponding heterotrophic plate count, total coliforms, fecal coliforms, and fecal streptococci were recorded, particularly in drainage water and River Nile. 81.4% of presumptive *Aeromonas* species were identified as *A. hydrophila*. The effect of seasonal variation showed maximum recovery in summer (61.5%) and spring (28.9%) compared to autumn (5.8%) and winter (3.8%). The time needed to reduce the population by 90% (T_{90}) was 55 h and the calculated decay coefficient was 0.018 h^{-1}. The residual chlorine efficient to inactivate *A. hydrophila* in drinking water supplies should be maintained at levels not less than 0.7 mg L^{-1}. Susceptibility of 72 isolates to 20 different antibiotics revealed recognizable multiple antibiotic resistance phenomenon toward eight (40%) of the tested antibiotics. Sensitivity was mostly directed to norfloxacin, ofloxacin, ceftriaxone, and cefotaxime. The study concluded that, supplementing the traditional indicators index of water quality with *Aeromonas* levels could be a simple, reliable, and inexpensive valid tool for better microbiological characterization of water.

Keywords: *Aeromonas*; Antibiotics; Chlorination; Microbial contamination; Pathogen

Received: June 24, 2013; *revised:* August 27, 2013; *accepted:* September 18, 2013

DOI: 10.1002/clen.201300503

1 Introduction

One of the most important factors of water pollution is the microbial contamination, especially with pathogenic microorganisms. Enteric pathogens are typically responsible for water borne sickness [1]. Diseases contacted through contaminated drinking water kill about five million children annually and make one-sixth of the world population sick [2].

The microbiological examination of water has a special significance in pollution studies, as it measures directly the deleterious effects of pollution on human health [3]. Direct monitoring of pathogens in water is an attractive option, as it would provide invaluable information regarding public health risks. However, there are hundreds of different types of pathogens that can be found in water due to fecal pollution. Therefore, it is not economically, technically, and practically feasible to routinely monitor all possible pathogens [4].

Alternatively, traditional indicators of microbial water pollution have long been worldwide proposed as a useful tool to detect water safety as well as surrogates for the presence of pathogenic microorganisms. These traditional indicators include heterotrophic plate, total coliforms, fecal coliforms, and fecal streptococci [5, 6].

Although the concept of traditional indicators was adopted by the U.S. Public Health Services as early as 1914 [7], yet there has been a change of focus recently regarding reliance on these indicators only to determine the microbiological quality of water. Several epidemiological studies concerned with public health effects due to microbial pollutants in water showed that coliform bacteria do not adequately reflect the occurrence of pathogens [8]. Numerous limitations associated with their application include: short survival time in water body [9], non-fecal source [10], ability to multiply after releasing in water column [11], great weakness to the disinfection process [12], and low levels of correlation with the presence of pathogens [13].

In response to the growing understanding and acceptance of these limitations, the World Health Organization discussed in details the inadequacies of traditional indicators, and debates the merits of their use in combination with alternative non-conventional indicators in order to ensure increasing both the detection sensitivity and specificity of fecal pollution and associated pathogens [14].

During the last decade scientific interest has turned to members of the genus *Aeromonas* as human and animal pathogens. Recent researches showed that they are almost equally important as coliforms in water quality monitoring programs. Even more, in

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Abbreviations: **CFU**, colony forming unit; **MAR**, multiple antibiotic resistance

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some countries *Aeromonas* are now considered better indicator of contamination than general coliforms [15–18].

Aeromonas spp. are common aquatic microorganisms, their presence have been reported frequently in a wide range of water sources such as rivers, lakes, wells, sewage, sea water, chlorinated drinking water, and in some countries in bottled mineral water [19]. The importance of their detection has increased recently due to their emergent human pathogens properties. *Aeromonas* spp. can cause gastroenteritis (traveler's diarrhea), bacterimia, septicemia, wound infections as well as respiratory tract infections [20, 21]. Individuals at the greatest risk of infection are children, the elderly, and the immune compromised people. The common routes of infection suggested are the ingestion of contaminated water or contact of the organism with a skin break [22]. Another important aspect to take into account in relation to these bacteria is that some of them have been associated with sea food contaminations and exclusively with severe fish diseases, thus causing loss of an important economic resource [23, 24]. In view of previous findings, the World Health Organization and the US Environmental Protection Agency proposed *Aeromonas* as one of the contaminants of concern in water-born diseases [25, 26].

The present study aims to generate a base line data concerning the prevalence of *Aeromonas* species as potential human pathogens in different Egyptian water sources, as well as to evaluate its importance as a non-conventional indicator for microbiological water pollution. Some factors affecting and controlling occurrence and survival of this pathogen in water were also investigated.

2 Materials and methods

The present investigation was started with samples collection in which, a total of 146 water samples were collected over a twelve-month period from December 2011 through November 2012. The collected samples represented three different types of water resources including, River Nile water ($n = 60$), drainage wastewater ($n = 36$), and chlorinated drinking water ($n = 50$). River Nile water was collected from five different sites, starting from Helwan to upstream of Delta Barrage in El-Kanater, at km 902.0, 922.0, 928.0, 938.0, and 947.0 from Aswan High Dam. Drainage wastewater was chosen to represent agricultural, industrial, and sewage mixed wastes from three different drains, namely El-Tahreer, El-Tibeen, and El-Rahawy drains, respectively. Samples of drinking water were collected from the distribution network represented by tap water from five different districts throughout great Cairo governorate, namely Helwan, El-Maadi, El-Roda, Shoubra, and El-Kanater.

2.1 Sampling procedure

Water sampling was carried out according to standard methods for examination of water and wastewater [5]. Samples were collected in polyethylene containers for physico-chemical analysis, and in clean sterilized glass containers for bacteriological analysis. Bottles containing drinking water samples were supplemented with sterile sodium thiosulfate (0.1 mL of 3% $Na_2S_2O_3$ solution in 120 mL bottle) to inactivate up to 5 mg L^{-1} residual chlorine. The purpose of inactivating residual chlorine is to prevent continuation of its bactericidal action during sample transit to lab. The examination then will indicate more accurately the true microbial content of the water at time of sampling [5].

2.2 Bacteriological analysis

Water samples from different sources were assayed for traditional bacterial indicators and *Aeromonas* according to standard methods recommended by American Public Health Association (APHA) [5]. The heterotrophic plate count at 37°C was determined by spread plate method No. 9215C on plate count agar medium. For enumeration of total coliforms, fecal coliforms, fecal streptococci, and *Aeromonas* spp., the membrane filter technique was applied. Water samples of appropriate volumes were filtered through sterile, surface girded Sartorius membrane of pore size 0.45 μm and diameter 47 mm, according to standard methods No. 9222B, 9222D, 9230C, and 9260L on M-Endo agar LES, M-FC agar, M-*Enterococcus* agar, and ampicillin dextrin agar media, respectively. Dilutions are considered through the selected sample volume for filtration [5]. All media used were obtained in a dehydrated form, Difco (USA). Results were recorded as colony forming unit (CFU/100 mL) using the following equation:

$$\frac{\text{Colonies}}{100}(\text{mL}) = \frac{\text{counted colonies}}{\text{mL of sample filtered}} \times 100 \quad (1)$$

2.2.1 Genus confirmation and species differentiation

Typical yellow colonies (dextrin fermentation) developed from overnight incubation at 35°C on ampicillin-dextrin agar medium were recorded as presumptive *Aeromonas* species. Random colonies were chosen from each sample and streaked on nutrient agar (oxoid) to ensure purity. Gram-negative, oxidase-positive, and glucose fermenting isolates were further identified by biochemical characteristics using the analytical profile index 20 E strip system obtained from BioMereux (France) [27].

2.2.2 Factors affecting occurrence and survival of *Aeromonas* in water

2.2.2.1 Physico-chemical characteristics

Physical and chemical analysis of different water samples were carried out according to standard methods for examination of water and wastewater [5]. Field parameters including temperature, pH, electric conductivity, and dissolved oxygen were measured in situ using multi-probe system, model Hydralab-Surveyor and rechecked in laboratory. In lab, total dissolved solids were determined by the gravimetric method, turbidity by the HACH-RATIO/XR-turbidimeter, and the biochemical oxygen demand by using ORION BOD fast respirometry system model 890.

2.2.2.2 Seasonal variation

River Nile water was chosen to study the effect of seasonal variation on occurrence and survival of *Aeromonas* species in water. Samples were collected monthly to cover the four seasons in one year survey. Monthly mean counts of *Aeromonas* as well as the mean surface water temperature were recorded.

2.2.2.3 Survival time (T_{90}) and decay coefficient (K)

The survival time (T_{90}) is the time needed to reduce the initial bacterial population by 90%. Usually, population dynamics for bacteria are modeled by first-order kinetics:

$$\frac{dN(t)}{dt} = -KN(t)\, K > 0 \quad (2)$$

i.e., an exponential decay of N with time t. This model is both simple and efficient. Its single parameter, decay coefficient K, is often replaced by $k = k_m/2.3$, which corresponds to the use of decimal logarithms for bacterial count:

$$\log N(t) - \log N(t_0) = -K(t - t_0) \quad (3)$$

K, usually expressed in h^{-1}, and thus it is the inverse of the period of time T_{90} (h) necessary for reducing the bacterial population by 90% [28–30].

Determination of T_{90} values for *A. hydrophila* was studied in unifactorial experiments carried out in laboratory. The experiments were conducted in 1 L sterile flasks filled with 500 mL of raw River Nile water. Flasks were placed in front of a window receiving plenty of solar radiation to simulate the natural conditions. The initial count (CFU/100 mL) of *A. hydrophila* was recorded as well as water pH and temperature. All steps were done in triplicates ($n = 3$) at different time intervals (15, 24, 36, 48, 60, and 72 h). Percentages of reduction in bacterial count (mean ± SE) were recorded for all replicates and the survival time (T_{90}) was estimated from the plotted survival curve (% of reduction in count versus t (h)). The decay coefficient (K) was calculated as $1/T_{90}\ h^{-1}$.

2.2.2.4 Residual chlorine

To investigate residual chlorine efficiency to inactivate *A. hydrophila*, drinking water samples collected from the distribution network represented by tap water were assayed for residual chlorine concentrations *in situ*, by colorimetric method using portable data logging spectrophotometer (HACH DR/2010). Different obtained concentrations ($mg\ L^{-1}$) were compared to the corresponding recorded counts (CFU/100 mL) of *A. hydrophila*.

2.2.2.5 Antibiotic susceptibility

Twenty antimicrobial antibiotic discs obtained from Oxoid (UK), and belong to eleven different groups were chosen for investigating their potency against 72 *A. hydrophila* isolates from drinking water samples according to the method as described in [31, 32]. These antibiotic classes are most commonly used in human and veterinary medicine in Egypt (Tab. 1). The results obtained were interpreted according to protocols standardized for the assay of antibiotic compounds as guided by National Committee for Clinical Laboratory Standards. The results were categorized as: R (resistant), I (intermediate sensitive), and S (sensitive).

2.3 Statistical analysis

Data interpretations involving many variables were carried out through: correlation coefficient matrixes between all pairs of bacteriological parameters, the Pearson's correlation coefficient (r) was used to correlate levels of *A. hydrophila* with seasonal variations, mean values, standard errors, and log transformed values were calculated using MINITAB statistical software program. The variables considered in the statistical analysis are traditional indicators count (e.g., heterotrophic plate count, total coliforms, fecal coliforms, and fecal streptococci) and *Aeromonas*, water type (e.g., River Nile, drainage water, and drinking water), physico-chemical characteristics of water samples and seasonal variations.

3 Results and discussion

3.1 Levels of *Aeromonas* species in different water sources

The significance and suitability of *Aeromonas* spp. versus traditional indicators in assessing microbiological quality of different water sources were evaluated. The monthly recorded bacterial counts in CFU/100 mL were expressed as mean $\log_{10}$(CFU/100 mL) for better data illustration.

3.1.1 River Nile

Results, illustrated by Fig. 1, showed that *Aeromonas* spp. were recovered for all examined samples, and the detected counts were accompanied by positive evidence for traditional indicators. Meanwhile, in about 80 and 100% of studied locations, *Aeromonas*

Table 1. Antibiotics for sensitivity test

Group	Scientific name	Trade name	Symbol	Disc potency (μg)
Penicillins	Amoxycillin/clavulanic acid	Augmentin	AG	30
	Ampicillin	Ampicillin	AM	10
	Carbenicillin	Pyopen	PY	100
	Methicillin	Methicillin	MET	5
	Piperacillin	Pipril	PRL	75
Cephalosporins	Cephalothin	Keflin	KF	30
	Cefotaxime	Claforan	CTX	30
	Ceftriaxone	Rocephen	CRO	30
Glycopeptides	Vancomycin	Vancocin	VA	30
Aminoglycosides	Amikacin	Amikin	Ak	30
	Tobramycin	Nebcin	TOB	10
	Kanamycin	Kanatrex	K	30
Tetracyclines	Tetracycline	Tetracycline	TE	30
Macrolides	Erythromycin	Erythromycin	E	10
Lincosamides	Clindamycin	Lincocin	DA	30
Quinolones	Norfloxacin	Noroxin	NOR	10
	Ofloxacin	Tarivid	OFX	10
Sulfa drugs	Trimethoprim/sulfamethoxazole	Septrin	SXT	25
Nitrofurans	Nitrofurantoin	Colifuran	F	300
Chloramphenicol	Chloramphenicol	Chloramphenicol	C	30

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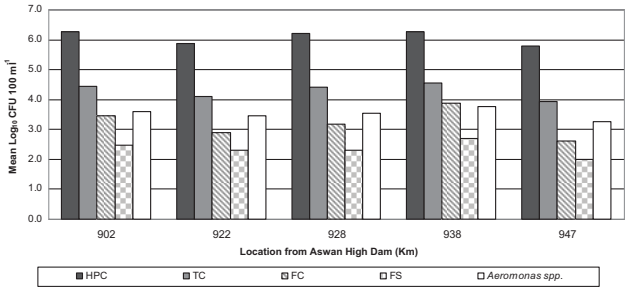

Figure 1. Levels of *Aeromonas* spp. in River Nile compared to traditional indicators.

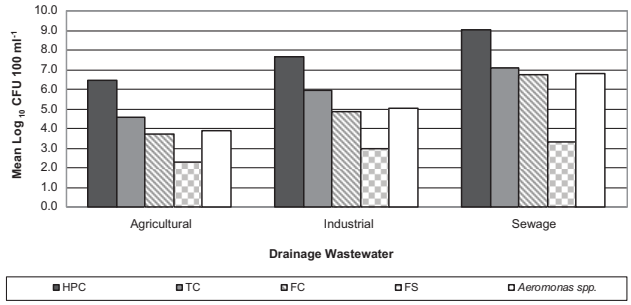

Figure 2. Levels of *Aeromonas* spp. in drainage wastewater compared to traditional indicators.

Table 2. Classification of the trophic state based on the densities of *Aeromonas* spp. [36]

Trophic state	Densities of *Aeromonas* spp. (CFU/100 mL)
Oligotrophic	<1500
Oligo-mesotrophic	1510–6500
Mesotrophic	6510–32 500
Meso-eutrophic	32 600–57 500
Eutrophic	57 600–340 000
Hypereutrophic	>340 000

spp. were detected in higher counts than fecal coliforms and fecal streptococci, respectively. This was somewhat not surprising, since most fecal coliforms and fecal streptococci decay as soon as they leave their hosts, in contrast to *Aeromonas* spp. that can persist well in aquatic systems, thus reflecting the actual quality of the water body. In this respect, a study was conducted on Mfoundi River watershed at Yaounde in Cameroon, in which the recorded values of *Aeromonas* were about twofold higher than fecal coliforms [16]. Our results were also in harmony with those previously reported by Kivanc et al. [19] Dumonter et al. [33], and Di Bari et al. [34].

Counts of *Aeromonas* detected in studied area of River Nile ranged between 18×10^2 and 60×10^2 CFU/100 mL. Normally, in water not affected by heavy sewage pollution, *Aeromonas* numbers ranged between 1000 and 10 000 CFU/100 mL [35]. Based on the density of *Aeromonas* [36], an evaluation of the degree of eutrophication has been proposed for classifying tropical aquatic systems (Tab. 2). Accordingly, River Nile in our area of study could be assigned to the oligo-mesotrophic level.

3.1.2 Drainage wastewater

Figure 2 compares the levels of *Aeromonas* spp. detected in different wastewater sources to the levels of traditional indicators. *Aeromonas* were detected in all types of wastewater, being variable according to the extent of pollution. The counts were maximum in sewage (62×10^5 CFU/100 mL), followed by industrial (11×10^4 CFU/100 mL) and agricultural wastewater (8×10^3 CFU/100 mL). *Aeromonas* levels were superior to those recorded for both fecal coliforms and fecal streptococci.

Similarly, earlier investigators reported high numbers of *Aeromonas* associated with sewage pollution. The counts varied from 10^3 up to $>10^6$ CFU/100 mL [16, 37, 38]. *Aeromonas* tends to flourish in aquatic habitats of highly trophic nature, presumably due to the presence of high organic matter of various origins as well as the stable environmental conditions of light and temperature [39]. Consequently, these bacteria could serve well as pollution indicators in waters receiving human and industrial sewage [40, 41].

3.1.3 Drinking water

The percentages of positive and negative samples for traditional bacterial indicators as well as *Aeromonas* spp. are shown in Fig. 3. Heterotrophic bacteria were positive (100%) in all tested samples, and their densities ranged between 1 and 82 CFU/100 mL. Meanwhile, a total of 23 (46%) samples were positive for total coliforms, with cell number ranging between 1 and 7 CFU/100 mL. It is worth mentioning that, fecal coliforms and fecal streptococci, indicators of fecal pollution, were not detected in any of the tested samples.

Clearly, Fig. 3 and Tab. 3 demonstrate, respectively, the percentage of positive samples and numbers of recovered *Aeromonas* spp. from tap water. *Aeromonas* were detected in eight (16%) out of 50 examined samples, and their densities ranged between 2 to 32 CFU/100 mL. In this respect, the European community has established a drinking water standard for *Aeromonas* of no more than 20 CFU/100 mL in water leaving the treatment plant and 200 CFU/100 mL in distribution systems [42]. Similarly, *Aeromonas* have been detected in chlorinated drinking water from distribution systems in Italy, Mexico City, Brazil, and the United States [17, 18, 37, 43]. This shows their capacity to survive and multiply within biofilms on the surfaces of pipes [44], as well as their relative resistance to chlorination [45–47].

The occurrence of *Aeromonas* in drinking water throughout this study was coupled by observing complete disappearance for fecal coliforms and fecal streptococci in all tested samples, although eight

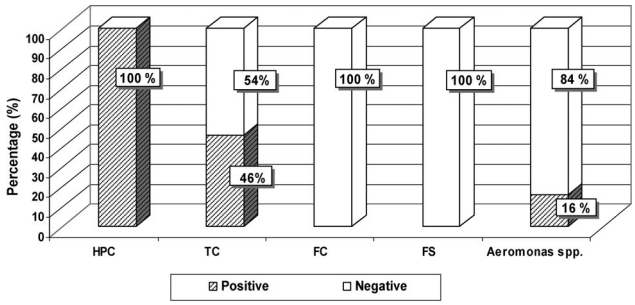

Figure 3. Percentages of positive and negative drinking water samples for *Aeromonas* spp. and traditional indicators.

Table 3. Bacteriological analysis of drinking water samples showing positive results for *Aeromonas* spp.

Sample no.	HPC (CFU/mL)	TC	FC	FS	*Aeromonas* spp.
		(CFU/100 mL)			
1	35	2	ND	ND	10
2	27	ND	ND	ND	5
3	75	5	ND	ND	25
4	63	4	ND	ND	15
5	10	ND	ND	ND	3
6	82	7	ND	ND	32
7	10	ND	ND	ND	2
8	18	1	ND	ND	4

HPC; heterotrophic plate count; TC, total coliforms; FC, fecal coliforms; FS, fecal streptococci; ND, not detected.

of them (16%) were positive for *Aeromonas*. The same observation was recorded for three samples (6%) showing negative results for total coliforms (Tab. 3).

These observations matched those recorded by Ahmed et al. [4] and Massa et al. [15] and show that traditional indicators alone are inadequate markers for microbiological water quality, *Aeromonas* could possibly be present when they are absent. Based on this idea, the United States Environmental Protection Agency included *Aeromonas* in the contaminant candidate list as a potential health risk through drinking water that need to be evaluated for possible regulations [44, 48].

The correlation coefficient matrix (Tab. 4) established to compare the degree of association between *Aeromonas* spp. and the indicators bacteria indicated significant positive correlation between the numbers of *Aeromonas* recorded in River Nile and the corresponding heterotrophic plate count, total coliforms, fecal coliforms, and fecal streptococci. The same relationship was observed in samples from different types of drainage water.

Nearly similar trends were observed in the spatial distribution of *Aeromonas* and traditional indicators within a wide range of water sources, particularly moderate and highly polluted ones. The same observations were also reported by Djuikom et al. [16] and Araujo et al. [49]. Contrastively, the correlation between *Aeromonas* and traditional indicators in drinking water were mostly lacking in data obtained during this study. Absence of correlation supports the earlier results demonstrated in Tab. 3, which recorded occurrence of *Aeromonas* and complete disappearance for fecal coliforms and fecal streptococci. Our results agree with those reported by Ahmed et al. [4] and Fernandez et al. [50].

3.2 Identification of *Aeromonas* species

Ninety-two presumptive *Aeromonas* isolates (81.4%) out of 113 were confirmed and identified as *A. hydrophila* (Fig. 4). These results show that *A. hydrophila* is the most predominant species among isolated Aeromonads. Earlier investigations supported our results and

Table 4. Correlation coefficient matrix between *Aeromonas* spp. and traditional indicators

		HPC	TC	FC	FS	*Aeromonas* spp.
River Nile	HPC	1.00				
	TC	0.98	1.00			
	FC	0.73	0.84	1.00		
	FS	0.77	0.87	0.98	1.00	
	Aeromonas spp.	0.86*	0.94*	0.96*	0.98*	1.00
Drainage wastewater	HPC	1.00				
	TC	0.9998	1.00			
	FC	0.9997	0.9989	1.00		
	FS	0.9111	0.9197	0.9008	1.00	
	Aeromonas spp.	0.9998*	0.9991*	0.99999*	0.9025*	1.00

HPC, heterotrophic plate count; TC, total coliforms; FC, fecal coliforms; FS, fecal streptococci.
*Significant positive correlation.

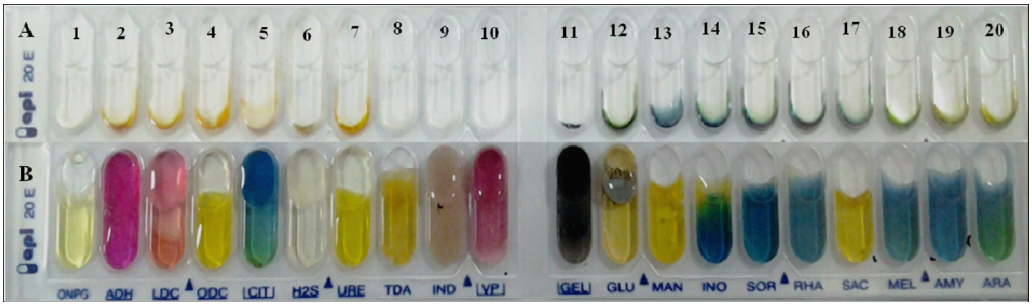

Figure 4. Analytical profile index 20 E strips of *A. hydrophila* and reaction tested. (A) before inoculation; (B) after inoculation; 1: ONPG, β-galactosidase; 2: ADH, arginine dihydrolase; 3: LDC, lysine decarboxylase; 4: ODC, ornithine decarboxylase; 5: CIT, citrate utilization; 6: H_2S, production of H_2S; 7: URE, urea hydrolysis; 8: TDA, deaminase; 9: IND, indole production; 10: VP, acetoin production; 11: GEL, gelatinase; 12: GLU, glucose fermentation; 13: MAN, mannose fermentation; 14: INO, inositol fermentation; 15: SOR, sorbitol fermentation; 16: RHA, rhamnose fermentation; 17: SAC, sucrose fermentation; 18: MEL, melibiose fermentation; 19: AMY, amygdalin fermentation; 20: ARA, arabinose fermentation.

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demonstrated the association between *A. hydrophila* and several human infections [34, 51, 52]. This organism can produce entertoxins, hemolysins, and cytotoxins that can potentially act as virulence factors [53].

3.3 Factors affecting occurrence and survival of *A. hydrophila* in water

3.3.1 Physico-chemical characteristics

Results demonstrated in Tab. 5 showed that *A. hydrophila* is ubiquitous in the environment. This organism has been detected in all studied water sources showing different physico-chemical characteristics, and its concentrations varied with the environment being investigated. It could survive a pH range (6.3–8.2), electric conductivity (395–1500 $\mu S\,cm^{-1}$), total dissolved solids (253–900 $mg\,L^{-1}$), turbidity (1.9–78.6 NTU), dissolved oxygen (0.3–7.6 $mg\,L^{-1}$), and biochemical oxygen demand (2–130 $mg\,L^{-1}$). This highlights its biological tolerance to a wide scale of physico-chemical factors and underlines its capacity to adopt environments with different trophic levels [41]. Its survival in chlorinated drinking water correlates with its capacity to cope with nutrient limitation conditions by entering into a starvation survival state [54].

3.3.2 Seasonal variation

Sunlight is among the most potent abiotic factors in the inactivation or killing of bacteria in aquatic environment. The growth of *A. hydrophila* is significantly influenced by the water thermal scale, which changes according to seasonal variations [55]. Clearly, Fig. 5 illustrates the percentages of recovered *A. hydrophila* from River Nile during four different seasons. Maximum recovery was recorded during hot and warm seasons (summer: 61.5%, spring: 28.9%), while minimum recovery was recorded during winter (3.8%) and autumn (5.8%). This might be due to temperature increase during summer and spring, which are ideal for prolonged survival and multiplication of bacteria in water [56].

The mean surface water temperature (19.6, 27.5, 31.3, and 23.6°C) for winter, autumn, summer, and spring, respectively, were found to be strongly correlated ($r = 0.826$) with the monthly mean counts recorded for *A. hydrophila*. The above results justify temperature change and seasonal variations as master key factors for survival of Aeromonads and point out the increasing probabilities for infections and risk during summer season, when these microorganisms are multiplying more rapidly [35].

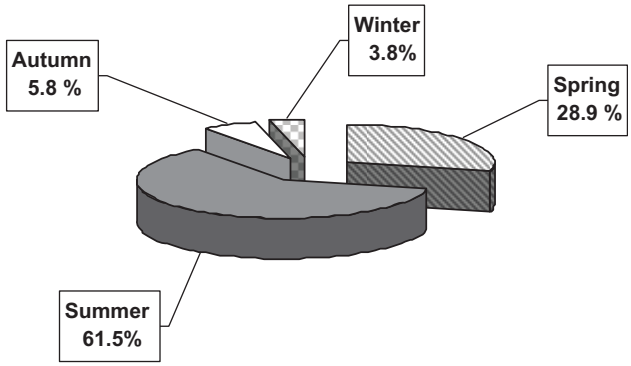

Figure 5. Effect of seasonal variation on *A. hydrophila* occurrence in River Nile.

3.3.3 Survival time (T_{90}) and decay coefficient (K)

T_{90} constitutes an important parameter for water resources conservation. It helps forecast the abatement of bacteria in certain aquatic system, and guide decision makers in water treatment issues [30]. The study reported here contributes to knowledge of the survival patterns of *A. hydrophila* within temperate fresh water climates. A unifactorial laboratory experiment was carried out using raw River Nile water. The recorded initial count for *A. hydrophila* was 36×10^2 CFU/100 mL, at pH 8.2 and room temperature 25°C.

As shown in Tab. 6, the pattern of *Aeromonas* survival with time indicated gradual decrease in count, which attained its maximum level (99.6%) within 72 h. The survival time (T_{90}) estimated from the plotted survival curve (% of reduction in count vs. t (h)) was about 55 h (Fig. 6) indicating that this period is necessary for reducing *Aeromonas* population by 90%. Similar study was conducted by Mezrioui and Baleux [28] and almost reported the same time needed ($T_{90} = 53$ h). The decay coefficient (K) calculated was 0.018 h^{-1}.

3.3.4 Residual chlorine

Chlorination is a treatment method employed primarily for microbial disinfection. The presence of *A. hydrophila* in drinking water supplies, including in those chlorinated, is a matter of public health concern due to their capacity to produce toxins, regrow in distribution systems, colonize biofilms, and resist chlorine application [37, 57, 58].

Results illustrated in Fig. 7 showed that the residual chlorine maintained in the distribution network under study ranged between 0.1 and 1.3 $mg\,L^{-1}$. This vast range of chlorine concentrations was

Table 5. Physico-chemical analysis of some representative water samples positive for *A. hydrophila*

Parameters	Unit	River Nile	Agricultural wastewater	Industrial wastewater	Sewage wastewater	Drinking water
pH	–	8.2	7.7	6.3	7.2	8
EC	$\mu S\,cm^{-1}$	450	670	1500	1300	395
TDS	$mg\,L^{-1}$	260	418	900	660	253
Turbidity	NTU	6.5	8.9	78.6	60.8	1.9
DO	$mg\,L^{-1}$	7.6	4.8	4.2	0.3	7.5
BOD	$mg\,L^{-1}$	6.0	8.0	9.0	130.0	2.0
A. hydrophila	CFU/100 mL	36×10^2	8×10^3	11×10^4	62×10^5	32

EC, electrical conductivity; TDS, total dissolved solids; DO, dissolved oxygen; BOD, biochemical oxygen demand.

Table 6. Effect of time on the survival of *A. hydrophila* in River Nile

Time (h)	15	24	36	48	60	72
% Reduction (mean ± SE)	7.5 ± 0.29	16.7 ± 0.16	38.2 ± 0.12	77.3 ± 0.12	98.1 ± 0.12	99.6 ± 0.16

comparable to positive and negative evidences of *A. hydrophila* whose count ranged between 0 and 32 CFU/100 mL. Fortunately, chlorination applied was sufficient to control Aeromonads provided that the free chlorine residual remains $>0.7\,mg\,L^{-1}$ at distal ends of the system. Free chlorine residuals <0.9 always showed positive contribution for the Aeromonads within the total bacterial budget. In this respect, the World Health Organization recommended chlorine residual for centrally treated water of 0.2–$0.5\,mg\,L^{-1}$ [59].

Similar studies reported complete inactivation with total chlorine between 0.71 and $0.90\,mg\,L^{-1}$ [60]. Others showed even higher demands up to $2.5\,mg\,L^{-1}$ [45]. The overall situation is mostly governed by several factors including, organic matter content, temperature, residence time of water in distribution network, and level of residual chlorine.

3.3.5 Antibiotic susceptibility

The spread of drug resistance among *Aeromonas* species is a matter of major concern since recent surveys have implicated some of these organisms as primary human pathogens [25, 26]. The problem is rendered more complicated in case of resistance to more than one type of antibiotics, a case known as multiple antibiotic resistance (MAR).

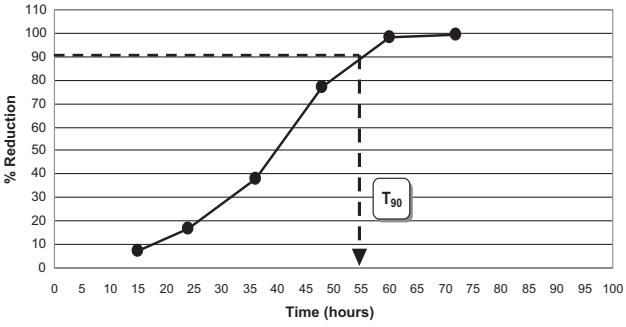

Figure 6. Survival curve (T_{90}) for *A. hydrophila* in River Nile.

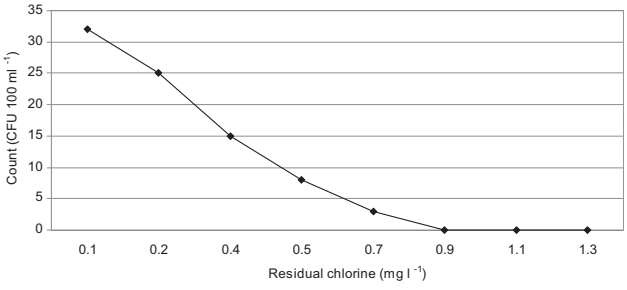

Figure 7. Effect of residual chlorine on *A. hydrophila* in drinking water supplies.

In the present study, a total number of 72 *A. hydrophila* isolates from drinking water samples were tested for their sensitivity to 20 antibiotic agents. As shown in Fig. 8, 100% of the isolates were sensitive to nine (45%) out of 20 tested antibiotics. These antibiotics were: cefotaxime, ceftriaxone, amikacin, tobramycin, kanamycin, tetracycline, norfloxacin, ofloxacin, and chloramphenicol. Similarly, some investigations reported the susceptibility of *Aeromonas* to tetracycline, aminoglycosides, third generation of cephalosporins and the quinolones [61].

On the other hand, recognizable resistance (100%) was demonstrated toward eight (40%) of the tested antibiotics. These antibiotics included amoxycillin/clavulanic acid, ampicillin, carbenicillin, methicillin, cephalothin, vancomycin, erythromycin, and clindamycin. Moderate resistance patterns (36.1, 27.8, and 16.7%) were observed for only three (15%) antibiotics. These antibiotics were, respectively, nitrofurantoin, trimethoprim/sulfamethoxazole, and piperacillin. MAR phenomenon was clearly observed throughout the results. Supporting data from recent studies concluded the importance of taking proper measures to control MAR Aeromonads, particularly in water sources used by humans [62–65]. Antibiotic resistance leads to increase in the risk of inappropriate therapy as well as increase of bacterial transmission between humans due to long periods of infection [66, 67].

Collectively, as given by the National Health and Medical Research Council [68] and the World Health Organization [20], an appropriate indicator should be: applicable for all water types, always present in fecally contaminated water and correlated well with the degree of pollution, more resistant to environmental stress or treatment, persist for greater length of time than pathogens, and detectable by simple, reliable, and inexpensive methods compared to those used for other pathogens.

Based on the results obtained in this study, this provides our monitoring for *Aeromonas* population to meet the above requirements.

4 Concluding remarks

Supplementing the traditional indicators index of water quality with *Aeromonas* levels could be a simple, reliable, and inexpensive valid tool for better microbiological characterization of water in order to control and prevent health risks. Chlorine residual may inactivate

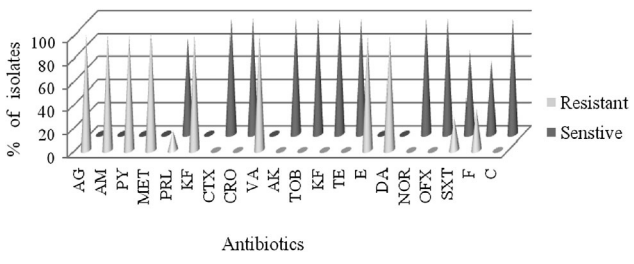

Figure 8. Percentages of resistant and sensitive *A. hydrophila* isolates to some tested antibiotics.

the basic fecal indicators, but do not eliminate other important bacteria in water, consequently chlorine concentration must be constantly maintained at levels not less than $0.7\,mg\,L^{-1}$. It is recommended to prevent unregulated use of antibiotics in different aspects and control pollution levels to restrict the dissemination of multiple antibiotic resistant bacteria in water environment.

Acknowledgments

This research has been supported by the Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), Cairo, Egypt. The author is most grateful to Dr. Amr R. Abdel Tawab and Dr. Karam Farrag for their valuable and sincere cooperation.

The authors have declared no conflict of interest.

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