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Occurrence of *Yersinia enterocolitica* in Different Egyptian Water Resources: the Relation with Bacterial Indicators, Survival and Resistance to Antibiotics and Disinfectants

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Abstract: Deterioration in water quality below the established standards is mainly due to human activities and untreated or inadequately treated wastes discharge in the environment. Untreated or inadequately treated sewage discharges to receiving water have often been shown to contain a variety of pathogens with density and variety being related to the size of population served, seasonal patterns for certain diseases and the extent of community infections. *Y. enterocolitica* causing outbreaks of gastroenteritis similar to those produced by *Salmonella* and *Shigella*. Eighty five isolates of *Yersinia* spp. were isolated from different water samples collected from Nile River, canals and groundwater. All the isolates were biochemically identified as Yersinia enterocolitic. These isolates were furtherly serologically identified to subgroups: 0:9, 0:6, 0:3 as well as untyped strains. The same water samples were examined for heterotrophic plate counts, total coliform, fecal coliform, as well as fecal streptococci as indicators for fecal pollution.Survival experiments for *Y. enterocolitica* were conducted to study the effect of different environmental conditions on its behavior. The study includes the effect of changing the natural habitat to tap water, sea water, tap water contaminated with sewage, temperature, pH, organic matter, starvation, heterotrophic bacteria and sun light, as well as different disinfectants. Finally, the sensitivity of different serogroups against 26 different antibiotics was determined.

Key words: Antibiotic resistant · Bacterial indicators · Disinfectants · Survival · *Yersinia enterocoloitica*

INTRODUCTION

Y. enterocolitica, the causative agent of yersiniosis, is receiving increased recognition as a significant food-borne pathogen [1], causative agent for gastroenteritis in children [2] and diarrhea in humans [3]. In addition, it has been associated with water-borne diseases [4] and was isolated from human as well as non-human sources including water and drinking water [5]. It was suggested that *Y. enterocolitica* can reach the water supplies through fecal material being transported via runoff water to nearby streams or by seepage into the groundwater [6].

Ideally,the finding of such indicator bacteria should denote the possible presence of all relevant pathogens. In practice, the full criteria for the ideal indicator can not be met by any one organism, especially *E.coli* as the essential indicator of pollution [7]. Schiemann [8] in his study on surface and well water found that many samples that showed positive results for *Y. enterocolitica* were negative for coliform. The survival of disease causative agent under different environmental conditions represents one of the factors which determine the spread of diseases between the consumers of contaminated food or water. Disinfection process using different disinfectant (chlorine, chloramines and ultraviolet) at the effective dose represent the last barrier against the spread of water-borne diseases. A variety of sublethal stressors cause a reversible form of physiological injury in enteric bacteria [9]. In a study performed by Bissett [10] six out of 24 examined strains belong to serotype 0:8 of *Y. enterocolitica* were sensitive to Ampicillin, Carbenicillin and Cephalothin while two strains of serotype 0:11 were resistant to Cephalothin whereas the remaining serotypes (1, 3, 5, 6, 10, 13, 16) were resistant to Ampicillin.

The aims of the present study are to attract the attention to *Y. enterocolitica* as causative agent for health impact by studying its presence in the Egyptian aquatic environment and its survival under different environmental conditions. As bacterial indicators of pollution were used as an indication for the possibility of pathogens presence, the correlation between the bacterial indicators and the presence of *Y. enterocolitica* in aquatic

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environment should be studied. To control, evaluation of different disinfectants as well as the sensitivity to antibiotics should be investigated.

MATERIALS AND METHODS

Samples Collection: Water samples collection was carried out in accordance to the *Standard Methods for the Examination of Water and Wastewater* [11]. Seasonal variations in temperature through the year were considered during sample collection. The tested samples include:

Nile River Samples: Subsurface (30 cm) samples were collected in duplicates of 2 liter sterile brown glass bottles from the two banks and middle of the River at two locations namely El-Maadi and Rod El-Farag (at Greater Cairo). The samples from the two banks of one location were mixed to form a composite sample.

Canal Water Samples: Composite water samples were collected from the two banks of Ismailia Canal (Near Al-Ameriah Water Works at Greater Cairo) as well as from Al-Zomor Canal (El-Giza Governorate) at a location near El-Giza Square. Other samples were collected from the middle of the water course of both canals.

Groundwater Samples: Groundwater samples were collected from hand pumps (27-30 meter deep) located at a small village near Banha (El-Qualiubia Governorate) and from Al-Haram region (El-Giza Governorate).

Bacteriological Examinations: Most Probable Number (MPN) values for total coliforms, fecal coliform and fecal streptococci and *Y. enterocolitica* counts by membrane filter technique (MF) were determined according the *Standard Methods* [11].

Biochemical Tests and Serological Typing: Oxidase, catalase, nitrate reduction, sugars fermentation and citrate utilization were carried out as confirmatory biochemical tests for *Yersinia* colonies to be identified as *Y. enterocolitica*. Somatic serotyping was performed on 50 of confirmed *Y. enterocolitica* isolates using slide agglutination test with anti-*Y. enerocolitica* 0:3, O:6, O:9 *serum* (Institut fur Immunprapatate und Nahmedien, Berlin).

Survival Experiments: Cell suspension was prepared from *Y. enterocolitica* that isolated from different water sources and suspended in saline to be used as inoculum's for the different vehicles used for survival studies. The inoculum's used was adjusted to give density ranged between 10^3-10^4 cells /ml in the water vehicle used (autoclaved Nile River, Ismailia Canal, Al-Zomor Canal and groundwater).

Cell counts was determined directly after inoculation and after exposure intervals by cultivation an inoculum on three replicates Tryptic Soy Agar plates which incubated at 30°C for 48 hr and the mean colony counts was calculated. The vehicles used were: autoclaved Nile River water, canal water and groundwater, unsterile dechlorinated tap water, Red Sea water and tap water contaminated with sewage. Effect of changes in environmental conditions (pH, the presence of organic matter and the presence of other organisms represented by different levels of heterotrophic plate count (HPC) bacteria on the survival of *Y. enterocolitica* was considered.

Sensitivity of *Y. enterocolitica* to Disinfectants and Antibiotics: Water samples contaminated with specific level of *Y. enterocolitica* were exposed to direct sun light between 9 a.m and 1.30 p.m. Each half an hour, water sample was withdrawn and the survived cells were determined as cfu/ml. The means of light intensity during a week was determined as Lux unit.

Exposure of *Y. enterocolitica* held in water samples (sterile and non-sterile) from different sources to direct ultra violet light (UV) from UV lamp (5 W/m^2) using 3 different UV doses (3.2, 6.7 and 12 mJ/sec./cm^2) and its effect on cells viability was studied *Y. enterocolitica* strains were held in the original water habitat (the water source from which it was isolated) and exposed to chlorine or mono-chloramine at different doses (1, 2, 3, 4 and 5 ppm). After definite contact time (5 to 60 min. and extended to 6 and 24 hr in case of chloramine) a water sample withdrawn and neutralized with sodium thiosulphate and viable cells were determined. Representatives for the three serotypes of *Y. enterocolitica* (0:3, 0:6 and 0:9) were surveyed against 26 antibiotics using sensitivity disks and measuring zone of inhibition formed.

RESULTS

***Occurrence of Y. enterocolitica* and its Relation with Bacteria Indicators:** Regarding the bacterial indicators, fecal streptococci MPN values were generally lower than that for fecal coliform except in case of Al-Zomor Canal.

Table 1: Bacterial Indicators (MPN/100 ml) and *Yersinia enterocolitica* (cfu/250 ml) densities in Various Water

	Parameters			
Locations	Total coliform	Fecal coliform	Fecal stereptococci	*Yersinia enterocolitica*
Nile El-Maadi Bank	$2.8x10^3$	$3.3x10^2$	$2.4x10^2$	7(s),4(c)
Nile El-Maadi Center	$3.4x10^2$	$1.7x10^2$	$2.0x10^2$	10(s),3(c)
Nile Rod El-farag Bank	$1.6x10^4$	$1.8x10^3$	$1.4x10^2$	10(s),3(c)
Nile Rod El-farag Center	$1.6x10^3$	$3.3x10^2$	$1.4x10^2$	9(s),4(c)
Ismailia Canal Bank	$5.3x10^3$	$6.2x10^2$	$3.2x10^2$	9(s),4(c)
Ismailia Canal	$4.7x10^2$	$4.5x10^2$	$1.7x10^2$	8(s),5(c)
Al-Zomor Canal Bank	$1.6x10^4$	$4.0x10^2$	$2.8x10^3$	17(s),10(c)
Al-Zomor Canal Center	$1.6x10^4$	$4.0x10^2$	$2.2x10^3$	13(s),7(c)
Ground water Banha	$3.9x10^2$	$9.2x10^1$	$1.4x10^1$	Less than 1
Ground water El-haram	$2.4x10^1$	$2.4x10^1$	$1.4x10^1$	Less than 1

(s) suspected colonies (c) confirmed colonies

Nile River seemed somewhat less polluted at El-Maadi than at Rod El-Farag location (Table 1). The highest density of confirmed *Y. enterocolitica* (10 cfu/250 ml) was attained from the two banks composite samples collected from Al-Zomor Canal at 15°C as ambient temperature. On the other hand, the lowest density (3 cfu/250 ml) was attained from the Nile River composite samples collected from Rod El-Farag location at 15°C. Generally, the Nile River water samples showed the lowest bacterial parameters values while Al-Zomor Canal samples were the highest. Ismailia Canal was in between the Nile River and Al-Zomor Canal. The banks of the water courses showed higher bacterial parameters values than the course middle (Table 1). There are some evidence could say that groundwater at both locations was polluted from fecal sources and not suitable for drinking. Generally, bacterial indicators were somewhat low compared with the same of the examined surface water samples. No high observable variations in the density of bacterial indicators could be due to the sampling location. Finally, *Y. enterocolitica* was undetectable at both locations (Table 1).

Regarding to the statistical analysis correlation coefficient, in Nile River water samples there are highly significant values (0.76 and 0.66 at 0.01% level) between *Y. enterocolitica* and both total and fecal coliforms, respectively. *Y. enterocolitica* in Ismailia Canal water samples showed highly significant correlation with both total and fecal coliforms (0.92 at 0.01 % level). In case of Al-Zomor Canal, *Y. enterocolitica* was significantly correlated with fecal coliform (0.77 at 0.05% level). 14 isolates out of 40 (35%) and 26 isolates out of 73 (35.61%), from River Nile and canals respectively were confirmed biochemically as *Y. enterocolitica*. The 40 confirmed *Y. enterocolitica* isolates were subjected to serological typing. Only 29 strains out of the 40 (72.5%) were serotyped. Serotype 0:3 was dominated (6 from the examined 14 from the Nile River and11 out of the 26 from the canals) followed by serotype 0:9 (4 from Nile River and 4 from canal water samples) and finally serotype 0:6 (1 strains from Nile River and 3 strains from canal water samples).

Survival of *Y. enterocolitica* under Different Environmental Conditions: In autoclaved tap water, viability of the examined *Y. enterocolitica* strains was negatively affected as a constant decline in numbers. The reduction in numbers was never exceeded than 12% when the isolates were held in its original environment (control). In autoclaved Red Sea water, *Y. enterocolitica* was not detectable after 24 hr, while little decline in numbers was observed for the control.When autoclaved tap water was fortified with 3% sterile sewage, slight decline in *Y. enterocolitica* numbers was observed during the first 60 min of exposure followed by 72.8-89.8% decline at the end of 48 hr, depending on the serotype. In presence of glucose at 1% concentration the reduction was ranged between 3-12% after 4 days while by raising the concentration to 5% less reduction was observed. Peptone at 1% concentration cannot support any growth but an increase in survival was observed by increasing the concentration to 3%. The optimum and the lowest viability were achieved at 25 and 45° C, respectively. Viability was drastically affected at pH 5.0 and less affected at pH 9.0, while it was not affected at pH 7.0. The survival of *Y. enterocolitica* was supported by the presence of microorganisms and the decline in numbers was 30, 0 and 13% in the presence of 3.3×10^2, 7.6×10^3 and 1.03×10^5 cfu/ml, respectively.

Sensitivity to Disinfectants and Antibiotics: Exposure of *Y. enterocolitica* to sun light caused somewhat slight but steady decline in numbers reaching 21-43% after 3.5 hr of exposure depending on the serotype and the type of vehicle.

Ultraviolet at different doses of 3.2, 6.7 and 12 mJ/sec/cm^2 was effective against *Y. enterocolitica* but affected by the presence of other organisms. Most strains belonging to *Y. enterocolitica* types cannot persist exposure to chlorine (1-5 ppm) or mono-chloramine (1-4 ppm). The more effective antibiotics against *Y. enterocolitica* were Nitrofurantion, Novobiocine, Rifampicine and Chloramphenicol.

DISCUSSION

In the present study there are some evidence demonstrated that the higher count of bacteriological parameter (inducators) in the examined water samples, the higher possibility of confermed *Y. enterocolitica* existence (Table 1). This is clear especially in case of Al-Zomor Canal which receive a huge amount of domestic as well as animal wastes. Ground water from hand pumps has a protection barrier against pollution depending on depth, soil structure and presence of pollution sources. *Y. enterocolitica* was not detectable in groundwater samples. According to the methodology followed in this study, only 35% of *Yersinia* spp. isolates has been identified as *Y. enterocolitica.*

Y. enterocolitica was significantly correlated with total coliform as well as fecal coliforms. Fecal streptococci is considered as supplementary indicator of fecal pollution. Serotypeing and biotyping of isolates are important since some *Y. enterocolitica* found in the environment may be non-pathogenic for human beings [12]. The results documented the prevalence of serotype 0:3 followed by 0:9 and finally 0:6. These three serotypes represent the most common human types [13]. It was mentioned that enrichment medium may define the predominant serotype because its specicity [14]. The phenomenon of viable but nonculturable cells and injury may explain the failure in *Y. enterocolitica* enumeration after somewhat short period of its transfer from the original habitat to a new aquatic environment (Nile River or canal water to salty water). Bacteria initially presented with adequate nutrients to support growth and thereafter exposed to environment with low nutrient concentrations appear to adapt to the environmental challenge by utilizing one or more mechanism for survival [15].

Chlorine as traditional water disinfectant was effective against *Y. enterocolitica.* The present results confirmed the previous results that demonstrated the slow inactivation for enteric bacteria by chloramines than free chlorine [16]. Our results on the effectiveness of UV against *Y. enterocolitica* are in agreement with the finding of Lund and Ormerod [17] that 0 : 3 serotype was nearly completely inactivated with UV as dose of 6 mJ/sec/cm^2. The use of antibiotics against the cause of infectious diseases should based on a strict bioassay determination. The most effective antibiotics are Nitrofurantoin, Novobiocine, Rifampicine and Chloramphenicol. The results are somewhat in agreement with the result of Toma and Laffeur [18].

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