QMJ VOL. 4 No.6 Dec. 2008

Occurrence of *Salmonella* serotypes in Euphrates River Water at A-Nassyria city-Iraq.

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الخلاصة

أجريت الدراسة للفترة من نيسان إلى أيلول من عام 2007 لغرض الكشف عن وجود أنماط مصلية لجراثيم السالمونيلا في مياه نهر الفرات وعلاقتها مع اثنين من مؤشرات التلوث الجرثومي (عصيات القولون البرازية والمكورات المعوية). اختيرت ثلاثة محطات للدراسة على النهر عند مدينة الناصرية وواحدة على مصب المجاري. وجد تأثيرا كبيرا لمصبات المجاري على الصفات البكتريولوجية لمياه النهر (من خلال وجود أعداد كبيرة لمؤشرات التلوث) وخصوصا في المحطة 2 والتي تقع في منطقة اتصال مجاري الفضلات بالنهر مما أدى إلى زيادة أعداد المسببات المرضية في النهر. عزلت جراثيم السالمونلا من جميع عينات المحطات 2و3و4 ومن 70% من عينات المحطة 1 وكانت *S. anatum* (22.72%) و *S. typhimurium* (20.9%) أكثر الأنماط المصلية ترددا.

Summary

The study was undertaken from April to September 2007 to detect occurrence of *Salmonella* serotypes in Euphrates river water and its correlation with two indicator organisms (FC & FE) of water contamination. Three stations for study were selected on the river at Al-Nassyriya and one on sewage effluent. High influence for sewage effluents on bacteriological properties of river water (through counting large number of indicators) were found specially in station 2 which lies at the area of sewage effluent outlet with the river and lead to increase of pathogens counts. *Salmonella* were found in all samples of stations 2,3,4 and in 70% of station 1.The most frequently serotypes were *S. anatum* (22.72%) and *S. typhimurium* (20.9%).

Introduction

Contamination of surface waters with disease causing organisms is of great concern to environmental managers and human health. The presence of salmonellas in natural waters constitutes a public health hazard. Detection and identification of these bacteria in waters are important in prevention of salmonellosis outbreaks [1].

Salmonella spp. are ubiquitous enteric bacteria. These gram negative rods are the etiologic agents of food-borne salmonellosis and also the agents that cause typhoid and paratyphoid fevers. *Salmonella* is a prime example of a water- and shellfish-transmitted pathogen [2]. *Salmonella* is a large genus of bacteria including more than 2,300 serotypes environmental isolates represented less than 4.4% of these isolates [3]. Both human and animal excreta are sources of *Salmonella*, and many potential routes are used for the transmission of these excreted enteric pathogens. Survival capacity of Salmonella *spp*. in waters may depend on species and pollution sources. Although most studies have focused on the determination of *Salmonella* strain concentrations in some polluted areas, it was recently shown that the annual bacterial loads of this pathogen in rivers and coastal areas can be very important [4,5].

The sources of fecal indicators and pathogenic bacteria include waste waters from sewage treatment plants; other types of sewage inputs such as combined sewer outfalls and drainage from septic tanks; runoff from agricultural fields or feedlots; effluents from food processing plants (especially meats and beverages); and stromwater runoff (which carries animal and bird droppings). The likelihood that fecal indicator bacteria added to the environment by these means will survive to be counted at a given water quality monitoring site is a function of the distance of the site from such sources, and also a function of the effect of all the environmental factors that influence bacterial survival[6].

In the area where natural water quality has been degraded, field investigation should attempt to identify source of pollution through sanitary survey and appropriate laboratory analysis[7]. Fecal coliform (FC) and fecal enterococci (FE) bacteria have been widely used as indicators of water contamination by humans and other warm-blooded animals [8] and have been included in water quality standards in different parts of the world [9]. Studies have shown that fecal indicator bacteria survive from a few hours up to several days in water, but may survive for days or months in sediments, where they may be protected from sunlight and predators. The survival time of fecal indicator bacteria in water is a function of many environmental influences and there is no number that applies to all water bodies, or even to all times of the year for a single body of water. We assume that pathogens die at the same rate as fecal indicator bacteria. Therefore, if we find relatively high numbers of fecal indicator bacteria in the environment, we assume that there is an increased likelihood of pathogens being present as well. *E.coli* and enterococci showed the strongest relationship with gastrointestinal illness [5, 10].The study was conducted to detect occurrence of *Salmonella* serotypes in Euphrates river water and their correlation with pollution level in the river.

Study area

Euphrates river flows through Al-Nassyria and divide the city into two sides; Al-Jazerah and Al-Shamiah .The river receives discharges from four untreated sewage effluents plants , and from some factories in addition to slaughter effluent.. Four study stations were selected, three of these on the river at the city (north , middle and southern part). Station 1 lies on the river before it insert the city , station 2 was at the junction area of sewage effluents with the river and station 3 south of station 2 with about 500 m. station 4 on sewage effluent .

Materials and Methods

Water samples: Three samples from each station were taken monthly from April to September 2007.The samples were taken according to specification standard methods [9], and stored in a cool box and were transferred to the laboratory within 1hr. for analysis.

Enumeration of indicator organisms : M.P.N method was employed for enumeration of fecal coliform(FC) and fecal enterococci (FE), according to standard methods [9].

Salmonellae analysis were carried out using membrain filter technique described by Alonso,*et al*.[1] . Identification of Salmonella species was done using APIE system. Serological identification was established by slide agglutination with specific sera .O and H antisera (Difco Laboratories, and Detroit, MI, USA) in order to determine the antigenic formula. Polyvalent *Salmonella* O and H antisera were used to obtain a preliminary diagnosis, and the definitive antigenic formula was then determined using monovalent antisera .

Results

The average of indicator organisms and salmonellae counts found at the four stations from April to September 2007 are shown in table 1.The results revealed presence of high numbers of fecal coliform and fecal enterococci in all samples. The greatest number of indicator organisms were found in station 4 (on sewage effluent)with significant differences. Sharp increases of indicators and salmonellae were recorded significantly in samples of station 2 comparing with station 1 and 3 . Ratio of FC/FE ranged between 3.4 to 5.2 for all stations.

Table 1: Average of indicator organisms and salmonellae counts per 100 ml of river water.

Stations	FC	FE	FC/FE ratio	Salmonellae
Station 1	1200	350	3.4	3
Station 2	7400	1800	4.1	12
Station 3	2100	450	4.6	5
Station 4	5.800.000	1.100.000	5.2	1200

Salmonella serotypes were isolated from all samples of stations 2,3,4 and from 70% of station 1 .A total of 110 colonies of *Salmonella* were isolated and identified.The most frequently serotypes were *S. anatum* (22.72%) and *S. typhimurium* (20.9%) (table, 2).

Table 2:Percentages of *Salmonella* serotypes isolated from river water

Salmonella serotype	No. of Strains	Percentage%
S. anatum	25	22.7
S. typhimurium	23	20.9
S. bredeney	16	14.5
S. infantis	10	9.1
S. enteriditis	9	8.2
S. muenchen	8	7.3
S. paratyphi B	6	5.5
S. paratyphi A	5	4.6
S. typhi	4	3.6
S. senftenbery	4	3.6
Total	110	100

Discussion

Waters of Iraqi rivers including Euphrates river receiving large quantities of untreated wastewater discharged from human and industrial sources. In addition to rainfall which introduce enteric pathogens from distant sources into river water.

Fecal coliform and fecal enterococci have been widely used as indicators of water contamination by humans and other warm-blooded animals and have been included in water quality standards in different parts of the world [9,10].

The results revealed presence of high numbers of indicator organisms in Euphrates river water beginning from station 1 which lies on the river before it's insertion the city, and this pointing for high level of contamination due to large amounts of sewage discharged in the river from wastewater effluents and factories through it's flowing in each of Syria and Iraq.

FC/FE ratio ranged between 3.4-5.2 which indicate that human feces is the source of water contamination . when this ratio equal or less than 0.4 mean animal feces is the source of contamination [11].

The greatest numbers of indicator organisms were found in station 4 which represent sewage effluent of Al-Jazerah side of the city including sewage of hospitals. High influence for sewage effluents on bacteriological properties of river water was found in station 2 which lies at the area of sewage effluent outlet with the river(Indian plant) and lead to increase of pathogens counts(Table 2). The influence continued to station 3 south of station 2(500m.) .The standard was set at a geometric mean concentration of 126 colonies of *E.coli* and 33 of enterococci per 100 milliliters (mL) of water, which was estimated to be correlated with a gastrointestinal illness rate [12].

Salmonella serotypes were isolated from all samples of station 2,3,4 and from 70% of station 1 alone study period. Many studies were detected that occurrence of *Salmonella* correlates with proximity to the water contaminated by sewage discharged [13,14]. *Salmonella* is frequently isolated from water sources ,which serve as bacterial reservoirs and may aid transmission between hosts [15]. Like *E. coli*, *Salmonella* is constantly released into the environment from infected humans, farm animals, pets, and wildlife [16].

Detection of *Salmonella* at a distance of approximately 500 meters from the point of discharge , showed the risk of survival of these organisms in river water where many people using it for different purposes. Comparing to other bacteria, *Salmonella* has high survival rates in aquatic environments ,it outlives both *Staphylococcus aureus* and the waterborne *Vibrio cholerae* in groundwater and in heavily eutrophied river water[17]. Despite efforts to contain and sanitize human waste, *Salmonella* can survive for 10 to 15 days in a septic system [18].

Salmonella serotypes which isolated in the present study(table 2) had clinical importance in human salmonellosis in Iraq ,therefore, their presence in the environmental waters may be of epidemiological significance.*S. anatum* and *S. typhimurium* are the predominant isolated serotypes. *Salmonella* are ubiquitous intestinal bacteria, that cause typhoid and paratyphoid fever in humans and animals. Many kinds of vertebrates including farm animals are potential hosts of these bacteria. *Salmonella* infections that are not only foodborne but also waterborne are a cause for concern in terms of public health, especially in developing countries where wastewater is directly discharged into lakes, ponds, and rivers. Contaminated surface water is a potential source of waterborne infections [19].

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