Bacterial Contamination caused by Wastewater discharged from of some Hospitals in Basra City, Southeast Iraq

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Abstract

This study seeking for explain a paradox that is while the hospitals being served to many of the people in the terms of their medical services, it could be, in the same time, considered as a major source to contaminated them with acute diseases. Therefore, the present study is concerned with detecting on the presence and concentration of some enteric bacterial pathogens which have resulted in wastewater discharged from hospitals in Basra City. Laboratory findings in water samples taken from selected three hospital sites indicated in that there are a significant increase in pathogens, so that it may lead to a serious bacterial contamination in the aquatic environment of Basra.

التلوث البكتيري الناجم عن المياه العادمة المنصرفة من بعض مستشفيات مدينة البصرة، العراق

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المستخلص

تهدف هذه الدراسة إلى الكشف عن مفارقة غريبة، وهي أنه في الوقت الذي تقدم فيه المستشفيات خدمتها الطبية للناس، فإنها يمكن أن تكون في الحين ذاته مصدراً رئيساً لتلوثهم وإصابتهم بأمراض خطيرة. لذا، عنيت الدراسة الحالية بفحص أعداد البكتريا المرضية المعوية التي تسببها المخلفات السائلة المطروحة من بعض مستشفيات مدينة البصرة. وبعد أخذ عينات من ثلاثة مواقع رئيسة، كشف التحليل المختبري وجود أعداد هائلة من هذه البكتريا، وأنها قد تكون مصدر تلويث جرثومي خطير للبيئة المائية في مدينة البصرة.

Key Words Enteric bacterial pathogens, Contamination, Wastewater, Aquatic environment, Total coliforms, Fecal coliforms, *E.coli*., *Salmonella & Shigella* spp.

Introduction

Some enteric bacterial pathogens, although naturally occurring, are known to cause diseases in humans, especially those with compromised immunity. While these bacterial pathogens are one of the most dangerous contaminants on human health, then the effluents from hospital wastewaters are one of the most serious pollutants discharging to the environment. The earlier being to consider as microbial organisms that once invade, somehow, to the human body, then it leads to many of health disorders or even to death. The former, whereas hospitals as provided as the healing to those whom needs them, it could be, in the same time, considered as a major source to the contamination by medical solid waste and wastewater deposited into the environment. The hazards of these medical wastes lies in that it usually is in contact with many of the diseases, bloods, and human parts, which may deposited in the surrounding, causing outbreak of infections. The present study, however, aims to investigate on this form of contamination through applied to wastewater discharging from some hospitals in Basra City.

This study is concerned with examination and analysis of wastewater effluents from some main hospitals in the point of mixing with streams connected it. Since these streams running, eventually, to Shatt Al-Arab River, then risks to human health would be of importance for inhabitants nearby to this river. The present study seeks to realize the magnitude of

contribution of the medical wastewater effluents in pollute the aquatic environment of Basra City, and how can they affect the public health.

Five species of enteric bacterial pathogens living in wastewater discharged from hospital effluents in the study area were determined here as indicators on pathogenic bacteria contamination in water. These are *Total coliforms*, *Fecal coliforms*, *Escherichia coli*, *Salmonella* and *Shigella s*pp. The definition of each species is as follows:

1- **Total coliforms**: Coliforms are a group of gram-negative, rod-shaped bacteria that are nonpathogenic and nonspore forming. The most common coliform genera are *Escherichia*, *Enterobacter*, *Citrobacter*, *Serratia*, and *Klebsiella*, with *E. coli* being the most abundant in the gut of humans and other warm-blooded animals. Coliform bacteria are identifiable by their ability to ferment lactose to produce acid and gas within 48 h, when incubated at 35^{O}C. Because they are found in the intestines of humans, domestic animals, and wild animals, coliforms are shed in feces along with pathogenic organisms present in the gut of infected animals, and can be detected in water with relative ease; total coliforms have been used by the US Public Health Service since 1914 as the standard for sanitary quality of water (Chigbu and Sobolev, 2007).

2- **Fecal coliforms (FC):** are a subgroup of total coliforms consisting mainly of *E. coli*, *Enterobacter*, and some *Klebsiella*. They inhabit the intestines of warm-blooded animals. Because they can grow and ferment lactose at a relatively high temperature (~ 45.0^{O}C), a characteristic that has earned them the name ‘‘thermotolerant coliforms,’’ they can be differentiated from the other members of total coliform. A high number of

fecal coliforms in water suggests fecal contamination, which might have resulted in the introduction of pathogenic microorganisms in the water that present potential health risks to individuals using the water. Fecal coliforms are better indicators of the presence of pathogenic bacteria in water than total coliforms, but their numbers alone cannot be used to tell whether fecal contamination is from human or nonhuman sources (Chigbu and Sobolev, 2007).

3- ***Escherichia Coli* (*E. coli*):** is found in the intestines of humans and other warm-blooded animals where it performs important physiological functions. They are not normally found living in other environments, but have been reported to multiply in surface waters, especially in temperatures ranged between 44.5-46^{O}C (Chigbu and Sobolev, 2007). Several acute diseases caused by *Escherichia coli* such as *Hemorrhagic colitis*, *Nursery diarrhea*, *Traveler's diarrhea*, and *Urinary tract infection* (Koren, 2005). If an incidence by such diseases, then may lead to death, especially among children and oldsters. In fact, *E. coli* was first proposed as an indicator species and as an indicator bacterium for fecal contamination in 1892 (Chigbu and Sobolev, 2007).

4- ***Salmonella & Shigella* spp**: *Salmonella* is a complex genus of Gram-negative, facultatively aerobic, usually motile, rod-shaped, pathogenic bacteria for humans and animals (Koren, 2005). *Salmonella* is a very large group of bacteria comprising more than 2,000 known serotypes. All these serotypes are pathogenic to humans and can cause a range of symptoms from mild gastroenteritis to severe illness or even death. *Salmonella* are capable of infecting a large variety of both cold- and warm-blooded animals. Typhoid fever, caused by *S. typhi*, is an enteric fever that occurs

only in humans and primates. S*almonellosis* is primarily dueto foodborne transmission, because the bacteria infect beef and poultry and are capable of growing in foods. The pathogen produces a toxin that causes fever, nausea, and diarrhea, and may be fatal if not properly treated It is transmitted by ingestion of the microorganisms in food contaminated by feces of infected animals or people; also may be transmitted through water that is contaminated. It is communicable throughout the time of the infection, which can be several days to several weeks, and the carrier state may last for more than a year; general susceptibility (Gerba and Pepper, 2005; Koren, 2005; Koren & Bisesi, 2003).

Shigella spp., that is closely relative to *Salmonelia*, is a genus of Gram-negative pathogenic bacteria that causes gastroenteritis and bacterial dysentery. *Shigella is* caused by a disease known as *Shigellosis* - an acute bacterial disease involving the large and small intestine with diarrhea, fever, nausea, sometimes toxemia, vomiting, and cramps; may cause convulsions in young children. Also, blood, mucous, and pus are present in the feces due to an enterotoxin produced by the bacteria. Incubation time is 1 to 7 days, usually 1 to 3 days. It is caused by four species of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. It is found worldwide, with most cases occurring in individuals under 10 years of age, usually in overcrowded or poor environmental conditions, institutions for children, daycare centers, jails, mental hospitals, and ships. The reservoir of infection is people. It is transmitted directly or indirectly through the oral fecal route from person to person; the carrier state may exist. It is also transmitted through water, food, flies, or a person's hands. It is communicable for at least 4 weeks and in the

carrier state for many months; general susceptibility (Koren, 2005; Koren & Bisesi, 2003). *Shigella* spp. infect only human beings, causing gastroenteritis and fever. They do not appear to survive long in the environment, but outbreaks from drinking and swimming in untreated water continue to occur in the United States, for example (Gerba and Pepper, 2005).

Materials and Methods

Since the present objective is concerned with bacteriological analysis of hospital effluents and investigate its effect on aquatic environment of Basra city, then wastewater samples from a three hospitals were collected. These hospitals are Al-Tahreer Hospital (popularly known as Al Mouna'a) (sample of No.1), General Hospital of Basra (popularly known as Al Jumhuriy) (sample of No.2), and Educational Hospital of Sader (popularly known as Al Taleemi) (sample of No.3) (see Fig.1). The water samples were exactly taken from mixing points of wastewater discharged from a pipe of each hospital with a stream which discharged into it. Water sample of Al-Tahreer Hospital was taken from a mixing point of wastewater with sewage drain running across Maqal district until eventually discharged into Shatt Al-Arab river, and water sample of General Hospital of Basra was collected from a mixing point of wastewater with sewage drain running until eventually discharged into Al-Khorah canal and then into Shatt Al-Arab river, while water sample of Educational Hospital of Sader was directly selected from a mixing point of wastewater with Shatt Al-Arab river.

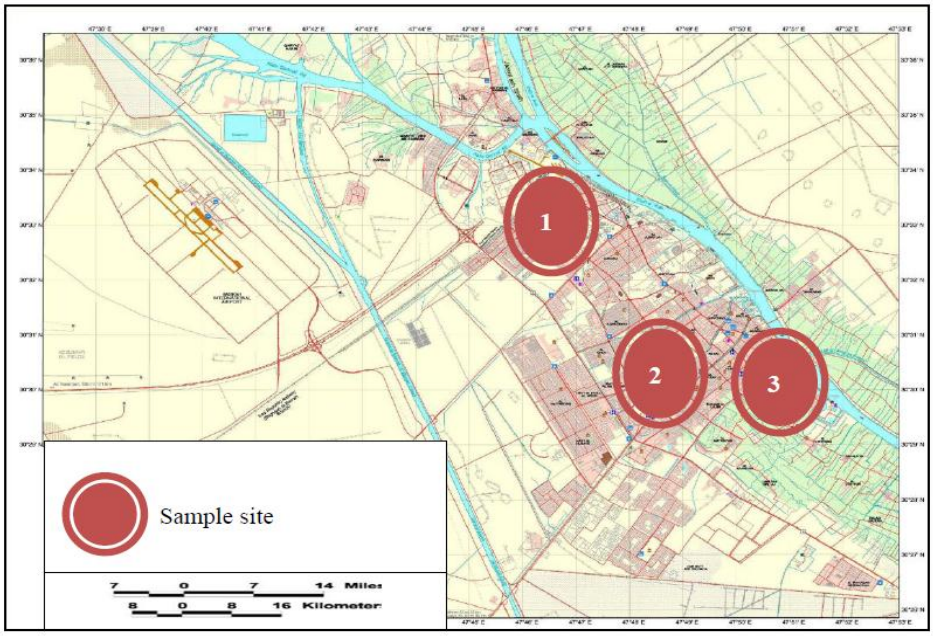


Figure 1: Map of the Study Area (Basra City) and Sampling Sites.

Studied water samples were collected during 2010. As described in Chigbu and Sobolev (2007), scientific procedures used in sampling method have been applied. Firstly, a sterilized 500 ml glass bottle designed to collect water sample were prepared, then proclaimed and rinsed with distilled with water. Samples were unfiltered and unpreserved, except if chlorinated in which case 0.01% v/v sodium thiosulfate were added. Samples have been kept in an icebox at 4^{o}C until it is ready for analysis usually within 24 h of collection, avoiding to occur a biodegradation.

In the laboratory, selected water samples were bacteriologically analyzed, and colonies of *total coliforms*, *fecal colforms*, *Salmonella and Shigella* spp were determined and calculated depending on membrane filter method. In the case of this method, the water sample is filtered through a

0.45 µm filter thereby retaining the organisms on the filter surface. The filter is then simply placed on the surface of a culture medium and incubated under the appropriate conditions and for a specified length of time. Also, water samples should be diluted in 10 ml buffered water dilution blanks before filtration, if necessary. This is preceded by shaking the sample bottle vigorously at least 30 times, and taking 1 ml with a sterile pipette to make sure that 20–80 colonies grow on the membrane. Further serial dilution can be done by taking 1 ml of the diluted sample and adding into another 10 ml buffered water blank using a sterile pipette, and so on.

Bacterial density can be calculated using the following formula:

CFU/ml = (Average number of bacteria counted) × (Reciprocal of the dilution used)

where CFU refers to colony forming units.

In addition to bacteriological examination, other parameters of water sample quality were analyzed. For example, electrical conductivity (EC), salinity, and pH levels were measured by multimeter of WTW 82362 Weiiheim type, Germany. While turbidity was determined by microprocessor turbidity meter of Hi 93703 Hannan type, Italy.

Results and Discussion

The results of laboratory analysis for selected water samples have shown in Tab.1 and 2. As noted from Tab.1 in which the values of some parameters for water sample quality and its spatial variations in the three sampling sites are appearing, its although elevated water salinity in all sampling sites, but the sample No.3 (Educational Hospital of Sader) records a significant

increase with compared to samples No.1 and 2 (Al-Tahreer Hospital and General Hospital of Basra) (see Fig.2a). This may be due to the effects of extreme saline water of Shatt Al-Arab river on that sampling site, as well as effect of severe salinity caused by wastewater discharged from itself hospital sewage and other effluents. Studies (Gerba and Pepper,2005) suggest that some pathogens are protected against heat inactivation by the presence of certain cations.

Table 1: Concentrations of some parameters of selected water sample quality.

No.	Sampling Site	EC (mS/cm)	Sal. (‰ ppt)	pH	Turb. (NTU)	Water Temp. (^{0}C)
1	Al-Tahreer Hospital	14.4	4.8	7.61	193	29.8
2	General Hospital of Basra	5.56	3.0	7.25	64	25.7
3	Educational Hospital of Sader	19.42	11.5	8.27	50	27.1

Source: Fieldwork and Lab work.

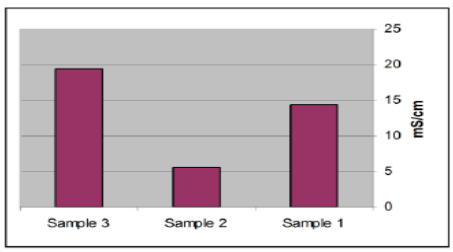
pH values tended to neutral at sampling sites No.1 and 2 (7.61 and 7.25) respectively, while it slightly tended in a sampling site No.3 to basicity (8.27) (see Fig.2b), because of elevated water salinity at that site so as to pH value tends to basicity with an increase in salinity concentration (Alters, 2000). Studies (Gerba and Pepper,2005) mention that enteric bacteria are less stable at pH >9 and < 6.

In respect of turbidity, which expressed an amount of suspended solids in water, sampling site No.1 records a high concentration amounted to 193 NTU, while a concentration in both the sampling sites No.2 and 3 is about 64 and 50 NTU respectively (see Fig.2c). This higher concentration at the sampling site of No.1 is may referred, in turn, to it indirectly

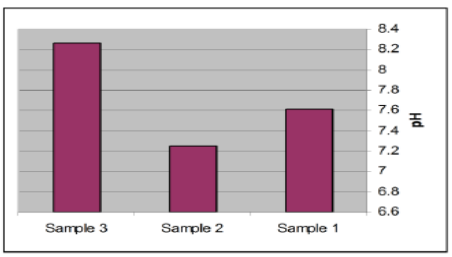
contaminated by solids and sewage rather than the other two sampling sites. Meanwhile, lower concentration of turbidity in the sampling site No.3 is due to a huge body of water for Shatt Al-Arab river that act as dilute suspended solids. However, above three values were, totally, highly exceeded the WHO recommended level of 5 NTU. Studies (Gerba and Pepper,2005) suggest that association with solids prolongs survival of enteric bacteria and virus.

Water temperature of study samples has shown that it was perfect for the growth and survival of enteric bacteria.

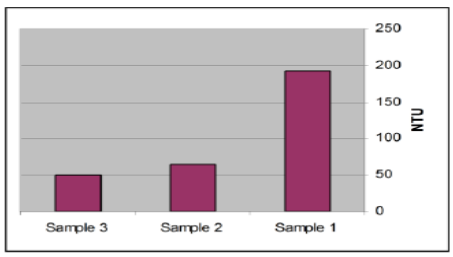
In brief, values of primary parameters indicated that studied water sample quality was severely contaminated, its meaning that stream waters in which wastewater effluents of selected hospitals are discharged into it, maybe as a suitable medium for bacterial contamination.



(a) EC



(b) pH



(c) Turb.

Figure 2: Levels of some parameters for studied water samples represented by EC, pH, Turbidity.
(Based on Tab.1)

As shown in Tab.2 and Fig.3, levels of bacterial contamination resulting in wastewaters discharged from studied hospital effluents appear to so elevated. It is clear that total coliforms and fecal coliforms to be found in enormous numbers at all of the three samples. *E. coli* concentrations amounted to 300, 240 and 40 CFU/100 ml in the samples of No.1, 2 and 3 respectively. However, *Salmonella & Shigella* spp, which some previous studies (Al-Taee and S. M. Shamshoom,2001; Al-Taee,2001; Al-Taee,2002; Al-Taee,2004; Al-Yasseen,2004) were found that it's abundant in the southern Iraq waters, the present study is found that it "Too Numerous To Count" at both the samples of No.2 and 3, while registers about 2600 CFU/100 ml of the sample of No.1.

Table 2: Concentrations of enteric bacterial pathogens in selected water samples (CFU).

NO.	Sampling Site	Total Coliforms	Fecal Coliforms	*E.coli*	*Salmonella & Shigella* spp
1	Al-Tahreer Hospital	TNTC*	TNTC*	300	TNTC*
2	General Hospital of Basra	TNTC*	TNTC*	240	2600
3	Educational Hospital of Sader	TNTC*	TNTC*	40	TNTC*

(*) TNTC refers to "Too Numerous To Count".
Source: Lab work.

Figure 3: Findings of bacterial pathogens of selected water samples, as appeared grown in petri dishes after their nestle for 24h.

(A) Total coliforms,
(B) Fecal coliforms,
(C) E.coli,
(D) *Salmonella & Shigella* spp

Source: Lab work.

In fact, from an environmental viewpoint, these concentrations could not be completely understood unless take their health hazards into account. According to EPA Microbiological Criteria, as shown in Tab.3, that any colony of total coliforms, fecal coliforms and *E. coli* should be not found in the case of drinking water. All selected water samples, however, are absolutely unsuitable for drinking water and represent a confirmed hazard to public health. Furthermore, selected water samples are unsuitable for domestic water uses because of numbers of fecal coliforms exceeds the acceptable exposure level. A comparison the findings with EPA criteria, as well, detected that selected water including Shatt Al-Arab river, are unsuitable for swimming due to it involve on numbers of total coliforms, fecal coliforms and *Salmonella* so that exceeds the recommended exposure

level. Epidemiological studies have demonstrated a relationship between swimming-associated gastroenteritis and the densities of fecal coliforms and other microbes (Gerba and Pepper, 2005).

Table 3: Microbiological criteria for various water uses, according to EPA (modified from Gerba & Pepper,2005; Chigbu & Sobolev, 2007).

Microbiological Parameter	Drinking Water	Domestic Water Supply for Treatment	Bottled Water	Recreational Water
Total coliforms	0 CFU/100 mL	----	----	500 CFU/100 mL
Fecal coliforms	0 CFU/100 mL	----	----	200 CFU/100 mL
E. coli	0 CFU/100 mL	<2000 CFU/100 mL	0 CFU/250 mL	126 CFU/100 mL
Salmonella & *Shigella*	----	----	----	0 CFU/1L

It is worth to mention that there are many routes by which enteric bacterial pathogens transport through aquatic environments. The ability of these pathogens on transport mainly depends on the extent of their resistance to environmental factors governed in their abundance, survival, and transport in the waters. How long a pathogen survives in a particular environment rely on a number of complex factors, such as water salinity, suspended solids, and temperature involved in it , which above mentioned. Tab.4 shows survival times of a variety of enteric pathogens in water, wastewater, soil and on crops. *Shigella* spp, for example, it can survive in environment for almost a month, while *Salmonella* and *E. coli* survival for three months, meaning that their health risks remain in action for a considerable time.

Table 4: Survival times of enteric pathogens in water, wastewater, soil and on crops (Gerba and Pepper,2005).

Microbiological Parameter	Excreted load[a]	Survival (months)[b]											
		1	2	3	4	5	6	7	8	9	10	11	12
1. *Campylobacter* spp.	10^7												
2. *Giardia lamblia*	10^5												
3. *Shigella* spp.	10^7												
4. *Vibrio cholerae*	10^7												
5. *Salmonella* spp.	10^8												
6. *Escherichia coli* (pathogens)	10^8												
7. Enteroviruses	10^7												
8. Hepatitis A virus	10^6												
9. *Ancylostoma duodenale*	10^2												
10. *Taenia saginata*	10^4												
11. *Ascaris lumbricoides*	10^4												

a Typical average number of organisms/g feces
b Estimated average life of infective stage at 20-30°C.

Conclusions

This study concluded with the following findings:

1- It is likely that wastewater discharged from hospitals are contributing to contaminate streams in the study area by a numerous quantity of enteric bacterial pathogens.

2- Deteriorated water quality are contributing, in turn, to promote enteric bacterial pathogen growth and to prolong their survival for a longer period of time.

3- Unsuitability of water quality for many of uses is due to microbiologically contaminated by hospital effluents, it meaning that hazard of infection is potential for Basra inhabitants, particularly those who on a direct contact with above mentioned contaminated streams.

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