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Detection of Bacterial Pollution from School Drinking Water Tanks in the Habbaniyah District West of Iraq

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Abstract

This study was conducted to evaluate the bacterial contamination in drinking water tanks from 20 randomly distributed primary schools in Habbaniyah district. The most probable number and the total plate count methods were used to determine the numbers of coliform, fecal coliform, streptococci and fecal streptococci. Chemical and physical properties of the water were measured, including residual chlorine, total dissolved solids, temperature and pH. Biochemical methods were used to detect various bacterial species contaminating the water samples and the results were confirmed using the VITEK 2 compact system. High levels of bacterial contamination were indicated. The highest value of coliform numbers reached 12 cells/100 ml. The total plate count recorded the highest values reaching 300 cells/ml. The concentration of residual chlorine was very low or non-detectable in most of samples below the required concentration and the concentrations of total dissolved solids exceeded the permissible limit (855 mg/L). The genetic relationship between environmental isolates of *Escherichia coli* bacteria from tank water and clinical isolates from children's health centers were also studied. The results for *Escherichia coli* demonstrated a similarity ratio between environmental and clinical isolates of 64% by using dendrogram and Enterobacterial Repetitive Intergenic Consensus PCR. Nevertheless, this study recommends the need to monitor drinking water quality that supplies these reservoirs, as well as to prevent and treat problems that result in drinking water contamination and the spread of diseases.

Keywords: *E. coli*, Primary schools, Fingerprinting, ERIC-PCR.

الكشف عن التلوث البكتيري لخزانات مياه الشرب المدرسية في منطقة الحبانية بالعراق

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الخلاصة

أجريت هذه الدراسة لتقييم التلوث البكتيري لخزانات مياه الشرب في 20 مدرسة ابتدائية موزعة عشوائياً في منطقة الحبانية. تم استخدام طريقة العد الكلي لتحديد أعداد بكتريا القولون والمكورات العقدية البرازية. تم قياس الخواص الكيميائية والفيزيائية للماء، بما في ذلك الكلور المتبقي ، والمواد الصلبة الذائبة الكلية، ودرجة الحرارة، ودرجة الحموضة. تم استخدام الأختبارات البيوكيميائية للكشف عن الأنواع البكتيرية المختلفة المسببة لتلوث عينات المياه، تم تأكيد النتائج باستخدام نظام VITEK 2. لوحظ وجود نسبة عالية من التلوث البكتيري. بلغت

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أعلى قيمة لأعداد بكتريا القولون 12 خلية / 100 مل بينما بلغت اعلى قيمة للعدد الكلي للبكتريا 300 خلية / مل. كان تركيز الكلور المتبقي منخفضًا جدًا أو غير موجود في معظم العينات وكان أقل من التركيز المطلوب، بينما تجاوز التركيز الكلي للمواد الصلبة الذائبة الحد المسموح به (855 مجم / لتر). تمت أيضًا دراسة العلاقة الجينية بين العزلات البيئية لبكتيريا *Escherichia coli* في مياه الخزان والعزلات السريرية من المراكز الصحية للأطفال. أظهرت نتائج الإشريكية القولونية نسبة تشابه بين العزلات البيئية والسريرية بنسبة 64٪ .

1. Introduction

Bacteria are among the most important groups of microorganisms that spread in the aquatic environment [1]. In addition to containing bacteria that are autochthonous aquatic bacteria, water contains another group (Where the Autochthonous are microorganisms that inhabit a specific space and live in it, while the allochthonous are the organisms that invade this space and live in it under abnormal conditions), allochthonous bacteria which often invade the aquatic environment from various sources such as rain and the remains of diseased animal and plant tissues that may find their way to water [1, 2]. Prior studies have indicated that most water bacteria are Gram-negative, non-spore forming and bacilli [3,4]. Most water bacteria are heterotrophs that play an important role in degrading the organic compounds in the water and thus actively contribute to the cycling of elements in the aquatic environments [5].

Bacteria live in various freshwater environments such as lakes, rivers, ponds and drinking water reservoirs [6]. Water is the main source of infections with many diseases because it acts as both a vector medium and a carrier of many microorganisms that negatively affect humans [2, 7]. Contaminated water can transmit diseases such diarrhea, cholera, dysentery, typhoid and poliomyelitis. Additionally, most water sources are subject to the ingress of pollutants such as civil and industrial waste and sewage [8]. Recent World Health Organization (WHO) reports have indicated that greater than 2 billion people use drinking water contaminated with feces and nearly half a billion people in the world suffer from the problem of using polluted water annually, and an estimated ten million people, the vast majority of them being children, die annually due to the infections with dangerous diseases such as typhoid, cholera, viral hepatitis, amoebic and bacillary dysentery, caused by the use of unhealthy water [5]. Moreover, WHO has calculated that more than 80% of diseases in the world are caused due to the use of polluted water and that 1 in 3 people globally do not have access to safe drinking water [9]. An estimated 170 million urban dwellers and 770 million rural dwellers in developing countries suffer from a lack of access to safe drinking water [10]. Agricultural water sources are also contaminated, with one study finding the total number of bacteria exceeded 100,000 cells/100 ml [2]. Studies have indicated the presence of many pathogenic bacteria in freshwater such as *Salmonella*, *Shigella*, fecal coliform, fecal streptococcus, and *vibrio*. Another study indicated that *Vibrio cholera* and *V. eltor* were able to live in unsterilized water containing salt at a temperature of 37°C for a period 289 and 423 days respectively [11]. The presence of *E. coli* at any level in a water source requires investigation of likely sources of contamination [12]. Furthermore, resistant pathogens present in the water pose a serious threat to the population as these pathogens seep into the river water through direct expulsion from the sewage treatment plants which in under-developed areas are often not effective at treating such pathogens [13]. In Iraq, the drinking water network suffers from sewage leakage through dilapidated pipes or through river water purification plants during the process of pumping it to consumers as microorganisms leak into the aquatic environment. This generates urgent problems in infection control that require immediate mitigations to minimize or eliminate potential consequences [14]. The presence of safe, potable water of high specifications is essential in maintaining a healthy and safe population, and there is

greater need to maintain surveillance systems to monitor water quality [15]. Given the importance of bacterial contamination of drinking water, and the need to know the levels of contamination to determine appropriate treatment methods and prevent the occurrence of this type of biological pollution [16, 17], we conducted this present study to evaluate the contamination of multiple sources of drinking water in the Habbaniyah district of Iraq. And given the higher burden of water-borne disease on children, this study focused on the drinking water tanks associated with schools. The most probable number (MPN) of microbes method, which is considered an appropriate than test to determine the quality of the water used and its suitability for drinking, was employed [3], in addition to characterizing the content of organic matter, salts, nitrates and nitrites in water.

2. Material and Methods

2.1. Study Area

The study included 20 primary government schools in the Khalidiya City in the Anbar Governorate of Iraq, accounting for about 9,950 students (Supplemental Table 1). Sites were randomly selected within the city limits so that these schools were representative of the whole city. These schools were being provided with potable water through four water purification projects located on the Euphrates River.

Supplemental Table 1: Study site details

School Code	Primary School Name	GPS	Students Number
1	Ebn Albitar for girls	33.38345,43.52932	324
2	Ebn Albitar for boys	33.38163,43.52821	373
3	Ashour	33.39030,43.52169	285
4	Barada	33.38375,43.52945	491
5	Al Taakhi	33.39111,43.52123	454
6	Al ganaen al mualaka	33.39026,43.52160	1005
7	Haneen for girls	33.39033,43.52125	550
8	Haneen for boys	33.38355,43.52830	398
9	Khalydiat al somoud	33.39034,43.52178	357
10	Al Rayaheen	33.38333,43.52929	650
11	Al Shaheed Khamees	33.39419,43.56103	354
12	Al Safa	33.39047,43.52197	495
13	Utba bin gazwan for girls	33.38371,43.52902	410
14	Utba bin gazwan for boys	33.39035,43.52140	563
15	Ukadh	33.38411,43.52910	656
16	Omar El mukhtar	33.39058,43.52113	580
17	Al Gassanya	33.38338,43.52937	525
18	Al Majd	33.38439,43.52459	476
19	Al Mahmoud	33.39020,43.52148	545
20	Al Najeeb	33.38244,43.52967	436

Samples were collected from drinking water tanks from each primary school (for schools with more than one tank, samples were collected from each tank), and triplicate samples were obtained for each tank at each timepoint during the period from April to May 2021. The method described in American Public Health Association [3] was adopted for sample collection, whereby water samples were collected in sterilized glass bottles (250 ml). The samples were distributed into two tubes, where one tube was devoted to bacteriological examinations and the second tube was for chemical and physical examinations. All key information, including time, date, place of collection, and the weather conditions, was recorded on the collection bottles. Samples were collected from eight to eleven AM to avoid variations due to time of day. Samples were kept in ice box after collection and during transport to the laboratory where they were tested within three hours of delivery.

In addition to water samples, stool samples were collected from school children suffering from diarrhea and intestinal colic. Forty samples were collected as part of routine public health surveillance and/or diagnostic activities and data was de-identified prior to analysis by the research team. Cases from students belonging to the relevant primary schools who visited the local health center were included, while those that were not associated with study primary schools or were linked to food poisoning and diarrhea caused by parasites were excluded. In the pathological analysis laboratory of the local health center, stool samples of the infected students were collected in special sterile plastic bags., Care was taken that the samples remained free of urine and water. Samples were examined directly without waiting. After obtaining the stool sample, a bacterial suspension was made using nutrient broth to enhance the growth of bacteria, and then by a loopful, the McConkey broth tubes were inoculated using the MPN as described below. Specimens underwent characterization as detailed in the bacteriological tests section. Clinical specimens positive for *E. coli* disease were further characterized with the genetic tests and ERIC-PCR technique described below.

2.2. Chemical and Physical Tests

Temperature was measured using a graduated mercury thermometer and pH was measured by using a portable Model HI 8428 pH-meter (HANNA Company, USA) directly from the water supply. pH values were adjusted by 4, 7 and 9 buffer solution, and meter parts were sterilized according to the manufacturer's instructions. Total dissolved solids (TDS) were measured by filtration and evaporation method. Briefly the water samples were filtered through a filter paper size of 0.45 µm then the filtrate was poured into a jar with a known weight. The jar was weighed after the water had evaporated. The filter disc was also dried in an oven at 105°C for two hours and weighed using a sensitive balance. The equation described in Abbawi *et al*. [18] was applied to extract the concentration of TDS:

$$TDS\ mg/l = \frac{(a-b)x10^6}{Sample\ volume\ (l)}$$

Where a = weight of the jar and the total dissolved solids in grams, and b = jar weight in grams. The residual chlorine concentration was measured using the iodometric titration method [18], as the residual chlorine concentration in the water is unstable and gradually decreases, the following equation was applied to find the residual chlorine concentration:

$$Residual\ chlorine\ mg/l = \frac{(a \pm b)x\ c\ x\ 35450}{Sample\ volume\ (ml)}$$

Where: a = volume of sodium thiosulfate solution used for sample titration., b = volume of sodium thiosulfate solution used for blank titration. c = standard of sodium thiosulfate solution.

2.3. Bacteriological Tests

Culture media for isolating, growing and diagnosing isolated bacterial species were used according to the instructions of the producing company. The most probable number (MPN) method was used to find the numbers of coliforms, fecal coliforms, streptococci, and fecal streptococci [19]. This method is commonly used to test the quality of water, specifically determining whether a water source is safe in terms of bacteria presence. For example, the presence of very few fecal coliform bacteria would indicate that a water source likely contains no disease‑causing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water could contain disease‑producing organisms making the water unsafe for consumption. In other words, detected microorganisms are not necessarily pathogens, however, they serve as an indicator of possible contamination. However, this method was implemented according to standard methods for examining water and wastewater [3] to detect the coliforms. The same method was applied again, with some changes, to determine the number of streptococci bacteria and fecal streptococci. This time azide dextrose broth medium (Merck, USA) was brought into use without Durham tubes. Additionally, the plate pouring method was used to quantify the total number of bacteria in water samples [3]. The following formula was applied to calculate the total number of bacteria in one milliliter of the sample:

Number of bacteria in 1 ml= Number of Colonies x Dilution inverted

Furthermore, a series of biochemical tests were also conducted, including indole, urea, Simmon citrate (Himedia), Triple Sugar Iron Agar (TSI-Merck) and oxidase tests to investigate and detect other bacterial species present in the studied water samples. A suspension of detected bacterial pure colonies was submitted to biochemical tests following APHA, 1998 procedure. The phenotypic biochemical profiles of the isolates were compared with the WHO criteria for biochemical identification. The results of these tests were confirmed using the VITEK 2 compact system (bioMérieux, France), after differentiation of the gram-positive and -negative colonies, loaded onto the appropriate kit (Bionumber: GN-2411151203-CDT and GP-2420789203-CST) for the VITEK assay.

2.4. Genetic Tests and ERIC-PCR Technique

The Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) is a simplified typing method for an expanding number of organisms which makes this method suitable for localized epidemiology, especially in regions with minimal disease-specific reagents. ERIC-PCR was performed on the complementary test positive results (using EMB agar) for both environmental and clinical case samples. DNA was extracted and purified using the WIZARD PCR DNA extraction kit, according to manufacturer instructions (PROMEGA, USA). PCR was used to amplify parts of the *E. coli hsdR* gene, using ERIC1 and ERIC2 nondegenerate oligonucleotide primers as following: F=5'ATGTAAGCTCCTGGGGATTCAC-3 and R=5'AAGTAAGTGACTGGGGTGAGCG-3, [20,21]. The ERIC-PCR method [22] was employed to compare the genetic profile of isolates of *E. coli* bacteria and to quantify the variances when comparing isolates from different environments using the fingerprinting method [23]. GoTaq Green Master Mix kit was used (PROMEGA Corporation, USA). The PCR solution was prepared from the following materials with a final volume of 25 µL of: 12.5 µL Green Master Mix, 5 µL of extracted DNA template, 1 µL of each primer, 5.5 µL Nuclease-free water. The mixture then was mixed well and placed in a thermocycler with the following program: one cycle for 4 minutes at a temperature of 95°C for the initial denaturation and 35 subsequent cycles comprised of 30 seconds at a temperature of 95°C for DNA template denaturation and 30 seconds at a temperature of 95°C, 50°C for the annealing process, 60 seconds at 60°C for the extension process, and one 5 minute cycle at 72°C for the final elongation of the replicated DNA strand. Subsequently, 10 µl of the multiplier gene

product was transferred for electrophoresis onto 1.5% agarose gel. The fingerprinting technique was performed via whole genome band analysis where the gel electrophoresis resulted image was scanned and maintained as software file that was used to identify the optical density along each band line [24]. The clustering of the isolates was assessed via this method and a dendrogram tree was created to enable the visualization of the degree of genetic relationship and similarity ratio between the isolates using dendrogram.

2.5. Statistical Analysis

The data was analyzed using one-way analysis of variance by Statistical Package for the Social Science (SPSS) program (Version 19.0).

3. Results

Table 1 shows the most probable number for coliform, fecal coliform, streptococci, and fecal streptococci for primary schools during the study period where the range of coliforms was 7 cells/100ml and the average of 6.6 cells/100ml, while the range for streptococci was 3 cells/100ml in an average of 4.75 cells/100ml for all schools under study.

The results revealed high levels of bacterial contamination with coliforms in the drinking water samples of 65% of the schools. The numbers of coliforms reached the highest level in drinking water samples from the Haneen Primary School for Boys (12 cells/100 ml), followed by Al-Safa Primary School for Boys (9 cells/100 ml), and the lowest numbers were recorded in Al-Najeeb, Ukadh, Al Mahmoud, Al Ganaen al Mualaka, Al-Rayaheen and Ebn Al-Bitar Schools for Boys, and Omar El mukhtar (5 cells/100 ml for each). Similarly, fecal coliform bacteria was at the highest level at the Haneen Primary School for Boys and Al-Safa Primary School for Boys (4 cells/100 ml each), while the rest of the schools recorded low values (<2 cells/100ml).

Same pattern held for streptococci bacteria, with the highest numbers recorded in Haneen Primary School for Boys and Al-Safa Primary School for Boys, 7 and 6 cells/100 ml respectively. The remaining schools recorded lower rates (4 cells/100 ml). Haneen and Al-Safa Primary Schools for Boys also had the highest numbers of fecal streptococci which reached 2 cells/100ml, while the rest of the schools recorded less than 2 cells/100ml.

The TPC measurement of bacteria closely correlated with the MPN results in the tested water samples (Figure 1) where the range varied from 33 cells/ml from Al Majd Primary School for Boys to 300 cells/ml from the Haneen Primary School and the average was 81.2 cells/ml. followed by Al-Safa Primary School for Boys (250 cells/ml), while the remaining schools recorded lower rates.

Table 1: The most probable number (MPN) of coliforms and streptococci bacteria in water samples from primary schools expressed in cells/100ml

School Code	Primary School Name	Coliforms	Fecal Coliforms	Streptococci	Fecal Streptococci
1	Ebn Albitar for girls	7	2	5	<2
2	Ebn Albitar for boys	5	<2	4	<2
3	Ashour	7	2	5	<2
4	Barada	8	2	5	<2
5	Al Taakhi	7	2	5	<2
6	Al ganaen al mualaka	5	<2	4	<2
7	Haneen for girls	6	<2	4	<2
8	Haneen for boys	12	4	7	2
9	Khalydiat al somoud	7	2	4	<2
10	Al Rayaheen	5	<2	4	<2
11	Al Shaheed Khamees	7	2	5	<2
12	Al Safa	9	4	6	2
13	Utba bin gazwan for girls	6	<2	4	<2
14	Utba bin gazwan for boys	7	2	4	<2
15	Ukadh	5	<2	5	<2
16	Omar El mukhtar	5	<2	4	<2
17	Al Gassanya	8	2	6	<2
18	Al Majd	6	<2	5	<2
19	Al Mahmoud	5	<2	4	<2
20	Al Najeeb	5	<2	5	<2

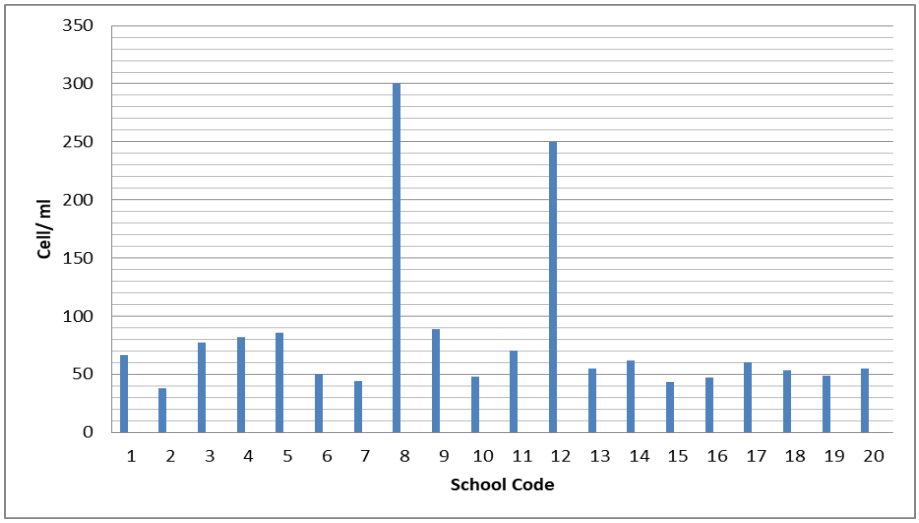

Figure 1: The total plate count (TPC) for the studied primary schools.

In addition to microorganisms, the TDS of water samples (Table 2) was also evaluated. The highest values (p <0.1) of TDS were recorded in Al-Safa Primary School for Boys (855 ± 20 mg/L) and the lowest values were recorded in Al Majd Primary School (508 ± 9.1 mg/L. Residual chlorine was recorded in 10 schools (0.1 mg/L), while none was detected in water samples from most schools. The temperature had minimal variation between the sites with

highest value of 17.3±0.1°C at Al Shaheed Khamees Primary School for Boys while the lowest value was 16±0.22°C at Haneen Primary School for Boys. Water pH varied marginally, with the highest values at Utba bin Ghazwan Primary School for Boys (8.4±0.1) and the lowest value at Ashour Primary Mixed School (7.1±0.1). The temperature and pH were within the applicable limits and annual averages for the study period.

Table 2: Chemical and physical examinations of primary school water samples.

Primary School Name	Residual Free Chlorine (mg/l)	Total Dissolved Solids (mg/l)	pH	Temperature (C°)
Ebn Albitar for girls	0	627±16.3	7.6±0.2	16.9±0.2
Ebn Albitar for boys	0.1	580±7.1	7.5±0.02	17.0±0.1
Ashour	0	669±11.1	7.1±0.1	16.6±0.2
Barada	0	590±11.4	8.3±0.1	17.1±0.3
Al Taakhi	0	612±10.2	7.9±0.1	16.8±0.2
Al ganaen al mualaka	0.1	588±7.5	7.3±0.01	16.9±.15
Haneen for girls	0.1	540±9.9	7.81±0.01	17.0±0.1
Haneen for boys	0	800±12.2	8.2±0.3	16.0±0.2
Khalydiat al somoud	0	705±13.9	8.2±0.1	16.6±0.2
Al Rayaheen	0.1	510±13.1	7.6±0.2	16.9±0.1
Al Shaheed Khamees	0	601±9.6	7.3±0.0	17.3±0.1
Al Safa	0	855±20	7.5±0.1	16.7±0.3
Utba bin gazwan for girls	0.1	600±9.3	7.7±0.2	17.2±0.2
Utba bin gazwan for boys	0	630±17.2	8.4±0.1	17.0±0.1
Ukadh	0.1	522±11.2	8.3±0.02	16.1±0.3
Omar El mukhtar	0.1	713±11.1	8.1±0.04	16.8±0.3
Al Gassanya	0	604±6.1	8.2±0.22	17.1±0.2
Al Majd	0.1	528±9.1	8.0±0.1	16.1±0.1
Al Mahmoud	0.1	533±8.3	8.1±0.2	16.6±0.2
Al Najeeb	0.1	591±12.1	8.3±0.01	16.3±0.2

based on diagnostic tests Several pathogenic bacteria were identified in the water samples (Table 3). *E. coli* detection was particularly pronounced and was found in 52.5% of the drinking water samples.

Table 3: Bacterial species detected in water samples and biochemical tests results for bacteria isolates.

Bacteria	Percent (Number)	Indole	Urea	Simmons citrate	TSI	Oxidase
Escherichia coli	52.5% (21)	+	-	-	+\-	-
Enterobacter aerogenes	10% (4)	-	-	+	-	-
Klebsiella sp.	15% (6)	-	+	+	-	-
Aeromonas sp.	7.5% (3)	+	-	+	-	+
Pseudomonas sp.	15% (6)	-	-	+	-	+

The *E. coli* isolates were analyzed via ERIC-PCR to assess whether an association existed between *E. coli* found in school drinking water and cases of diarrhea and intestinal colic among school children. The results suggested a genetic relationship between the isolates

collected from drinking water tanks and sick school children in local health centers. The isolates shared similar banding patterns. The percentage of genetic similarity was found to be 64% between bacteria isolated from water tanks and those isolated from pathological cases of students (Figure 2).

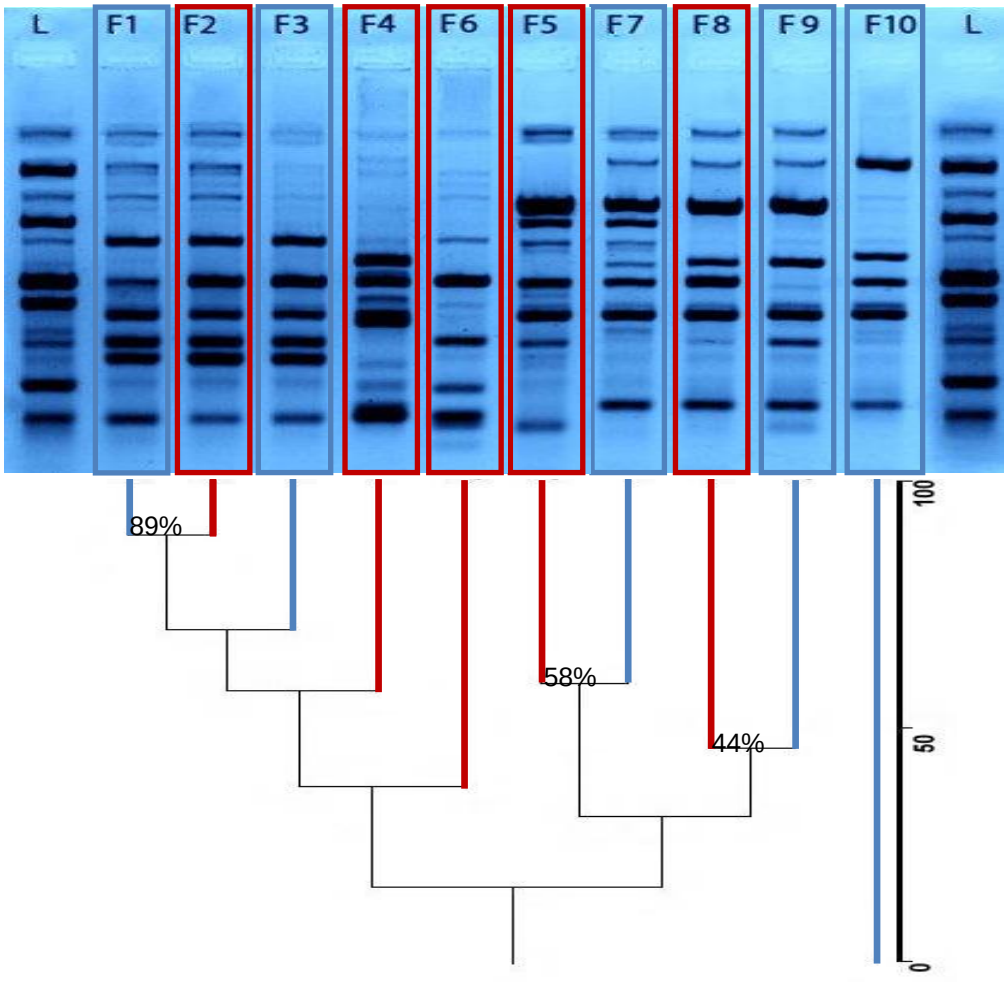

Figure 2: ERIC-PCR assay gel visualization of similarity between isolates of *E. coli* and the genetic relationship between the isolates visualized with a dendrogram; lanes and lines in red (F2, F4, F5, F6, and F8) correspond to pediatric clinical specimens; lanes and lines in blue (F1, F3, F7, F9, and F10) correspond to school water samples. Lanes marked with an "L" are size ladders.

Dendrogram analysis indicated that there were three specific groups among the isolates: F1-2, F5-7, F8-9, and an overall similarity ratio between the isolates was 64% (Figure 2). Isolates F1 and F2 were similar by 89% and isolates F5, F7, F8 and F9 were similar by 58% and 44% respectively. Bacterial isolate 10 was unlike any other isolate in the group.

4. Discussion

The results revealed that there were high levels of biological contamination with coliforms in the drinking water samples of 65% of the schools that exceeded the local permissible limits (0-5 cells/100ml). This indicated that the water was not suitable for drinking, while the presence of bacterial contamination was observed at lower levels in 35% of schools. Where

the values recorded in Haneen and Al-Safa Primary Schools were above the maximum permissible limit for drinking water - 50 bacteria cells/ml were based on Iraqi standards. Almost 70% of schools were over permissible limit where the values of TDS exceeded the permissible limits which should have been less than 500 mg/L. Yet, based on local standards, the residual chlorine in water should be 0.3 mg/L following purification.

The low concentration of residual chlorine (below local standards or entirely absent) in all studied primary schools, was most likely a significant factor that contributed to the high rates of biological pollution. Prior studies in the study region also observed low residual chlorine [7]. Despite the presence of some chlorine in schools, viable microorganisms were found in the studied water samples. This could be explained by the resistance of these organisms to the existing chlorine concentrations [11], also, the storage of drinking water in tanks for several days, especially during the weekends, could participate in chlorine reduction. Additionally, it was noted in the study sites that there were holes in or an absence of covers for some water tanks which increased the evaporation of chlorine, as well as the chance of organic and biological pollution through bird droppings or dead animals. However, even if chlorine is removed from water by leaving it outside for a few days, water remains susceptible to contamination with microorganisms, especially the coliform bacteria.

The greater contamination observed in specific primary schools within Khalidiya City can likely be attributed to several factors, most notably being the deterioration of the pipes equipped for these schools. In addition to the old tubes and the presence of several cracks in the water pipes which could have been due to the military operations that took place in the city in recent times, there was the presence of heavy machinery trucks used by city residents which generally failed to account for the weights allowed on these pipes. Areas around pipe cracks and leakage tend to form holes and gaps around the pipe providing perfect environment for the microorganisms and pathogens to enter the water network as these bacteria naturally existed in the surrounding soil and water environments [25]. Additionally, some of holes and gaps that were observed around the pipes in these areas had formed small pools of water that were frequented by animals such as livestock, dogs, and birds drinking from or had submerged themselves in this water possibly leading to the water pollution with their secretions and microorganisms of these animals [26]. It was also noted that there were many violations of the drinking water networks near schools with the highest contamination attributed to unpermitted and overrun housing. These violations also led to an enhanced possibility of water contamination especially due to the use of plastic and rubber pipes connected with the network to deliver water to these houses. Furthermore, these tubes were also subject to damage as they were generally exposed and not buried properly which could lead to contamination with human and animal feces. Also, there was an observation of water pumps installed directly on the water network which posed an additional risk due to the pressure permeation in the pipes, possibly withdrawing water contaminated with microorganisms from cracks in the network [27]. Beyond the pipes in these unpermitted dwellings, there was an increase in the quantities of liquid waste drainage into the river which created an additional burden on water purification and treatment plants, negatively affecting the efficiency of the sterilization process and periodic filtration operations of river water.

The presence of coliforms supports their ability to resist the lower levels of chlorine found. Many studies have indicated that this species can resist chlorine concentrations up to 0.3 mg/L [28]. The presence of these species in addition to *Enterobacter aerogenes, Klebsiella* sp., *Pseudomonas* sp., and *Aeromonas* sp. serve as an indicator of the biological pollution of these water sources and highlight the need to correct the supply problem to avoid negative health impacts [29]. The high values of these pathogenic species recorded in the water samples reflected the extent of the health hazard for human consumption and provides a

picture of the inadequacy of the water distribution pipes [26, 30]. The genetic fingerprinting clearly indicated a genetic relatedness between bacteria isolated from school drinking water and bacteria isolated from disease cases of students which suggested that contaminated water sources could be a source of infections for students at these schools. The residual chlorine concentrations in all the studied water samples were below the required level. The concentration of dissolved solids was high and there were cracks and others neglected on the water network, all of which likely contributed to the high rates of biological pollution found in school water, including the presence of many potentially pathogenic species. The authors recommend addressing the causes of this pollution, in addition to putting in place systems to allow continuous monitoring.

5. Conclusion

The presence of pathogenic bacteria in drinking water, even in small numbers, gave a vital indication of a failure of biosafety measures in water treatment and purification plants and problems associated with the water network. Detection of microorganisms requires the evaluation of current control measures, evaluation of system for failures and development of additional sterilization systems that operate with high efficiency to ensure the complete sterilization of water. Setting up monitoring stations along the drinking water network to detect the appropriate water quality and measure its bacterial and organic content in a manner that ensures its safe delivery to consumers.

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Ethics Approval: Informed consent was obtained (Administrative order carrying the number 563 in 4-11-2020 by College of Science). The study was approved by the biosafety and biosecurity university committee, as well as oral approval was obtained from the students' parents for the purpose of taking pathological samples from them.

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Supplementary Material

Supplemental material is shown in the supplemental material table. The study included 20 primary schools in the city of Khalidiya in the Anbar Governorate of Iraq. These schools were representative of the whole city and accounted for about 9,950 students. Study sites were approximately 3 km away from each other and were distributed from the east to the west of the city.

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