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Biosafety and Bio-security level in some Ramadi City Hospitals and Evaluation of The Pathogenic Environmental Impact of Its Sewage Water Effluents

Omar Lateef Jumaa*, Sufyan Mohammed Shartooh
Biology Dept. College of Science, University of Anbar - Iraq
*Email of corresponding author: omer84omer1984@gmail.com
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Abstract:

Pathogenic microorganisms present in different environments pose a major health risk to human societies, as the wastewater flowing from hospitals is an ideal environment for pathogenic microorganisms that are resistant to different conditions, and thus create a new generation of such microorganisms difficult to deal with and transmit their harmful effects to all society individuals. The study aims to determine the level of bacterial contamination of wastewater flowing from some hospitals in Ramadi City - Iraq, and to find the relationship of similarity between environmental bacterial isolates and pathological isolates for recumbent patients, and thus determine the level of biosafety procedures followed, as well as study the effect of some physical and chemical factors on the present of these bacteria. Physical and chemical examinations included some variables such as Biochemical Oxygen Demand (BOD), pH, Temperature, Electrical Conductivity (EC), Total Dissolved Solids (TDS), and Nitrate NO_3). Additionally, the isolating and diagnosing of 100 bacterial genes using biochemical tests and confirming them using the VITEC 2 compact system device. Also, the genetic fingerprint of some isolated bacterial species was studied using ERIC-PCR technique and Dendrogram program to find the similarity relationship between environmental isolates and pathological isolates of *E. coli* and *Klebsiella pneumonia*, where 60 samples were collected from sewage water, and 60 samples were collected from patients of the Women's and Children's Hospital, and the Ramadi Teaching Hospital for the period from September 2020 to November 2020 in the Ramadi City (Anbar Governorate-Iraq) The results showed the contamination of wastewater with many pathological bacterial types, and the results of the tests (BOD, pH, Temp, EC, TDS, NO_3) included high values in the study area whose rates were (42.25 ppm, 7.32, 22 ° C, 2520.75 µs / cm, 1755.25 ppm, 0.34 ppm) respectively. As for the genetic fingerprint tests for *E. coli* and *K. pneumonia* bacteria isolated from different environments, the statistical analysis of the Dendrogram program indicated a similarity rate of 63% for *E. coli* and 48% for *K. pneumonia* for both samples isolated from wastewater and from inpatients using ERIC-PCR technique. In conclusion, the results obtained from this study revealed that the wastewater is highly contaminated with pathogenic bacteria, the majority of which are from infected patients lying in the hospital, which indicates the poor safety and bio-security procedures followed in the

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studied hospitals, which negatively affected the quality of the resulting wastewater from these hospitals.
Key words: Biosafety, *E.coli*, ERIC-PCR, Ramadi hospitals.

Introduction:

There is no doubt that the wastewater flowing from hospitals is an ideal environment for pathogenic microorganisms, especially as it carries the resistant gene in the sewage system that enables it to be adopted for various conditions (Astha *et al*, 2020). However, large amount of antibiotics are used to treat patients lying in the concerned hospitals, and these factors find their way into the wastewater, which is associated with many pathogenic bacterial species that have adopted resistance for such antibiotics, and thus create a new generation of these resistant bacteria in the water that is difficult to deal with and transmit its harmful effects to all environments of the community it accesses (Asfaw, 2018).

The residues of medicines and resistant pathogens pose a serious threat to aquatic environments, as wastewater from hospitals is considered one of the main sources of this pollution, which overwhelms its dangerous impact on the quality of river water, which constitutes a great burden on drinking water treatment plants that may not be equipped to deal with such pollutants carry certain characteristics, and thus it may transmit to all society individuals. Yet, pathogens such as *Escherichia coli* and *Klebsiella* bacteria and others can pose a risk to human health if they are present in drinking water, which leads to an increase in the number of individuals exposed to disease, thoroughly an increase in the load on hospitals. Therefore, the presence of safe, drinkable, and high-quality relevant water is essential in order to maintain a safe and healthy environment (Prezant, 2016). Hospitals and other associated institutional buildings routinely suffer from internal sewage leakage directly into the river, despite all efforts to control this flow, especially large releases of wastewater laden with various pathogens and various chemicals that find their way into the aquatic environment without passing through any treatment units that reduce the risk resulted from these pollutants. Hence, generating urgent infection control problems, appropriate advance planning, and rapid response to reduce or eliminate potential consequences (George *et al*, 2010).

The foregoing data shows us the great role of the issue of Biosafety and Biosecurity, which all foundations that release their polluted waters to the external environment must adhere to, as all concerned institutions, whether local or global, emphasized the need to adhere to the terms and instructions of safety and biosecurity, in the way that provide a safe environment for workers and people consuming this water at the same time, through the management of such facilities with redundant engineering for safety and security features, strict administrative supervision, biosecurity procedures, and intensive training in emergency situations, and everything that is designed to reduce the risk of exposure to such pathogens and prevent them from being in Society (Gilpin

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2000; Astha *et al*, 2020).

Nevertheless, it can be said that the issue of biosafety in hospitals includes the protection of employees and visiting individuals from infection with biologically dangerous materials that may be transmitted from patients, their equipment, and the hospital environment, to find their way to the external environment, as biological materials are microorganisms or their products that can cause disease to humans or animals (Muhammad *et al*, 2017).

Materials and Methods:

1. Sample collection:

Sixty samples of wastewater were collected for the Women's and Children's Hospital and the Ramadi Teaching Hospital for the period from September 2020 to November 2020, and for the purpose of collecting samples, two sets of bottles were used, the first group for collecting samples for bacteriological tests, and it included bottles with a tight-fitting 250 ml capacity sterile with an autoclave. Secondly, for the purpose of collecting samples for the physical and chemical tests, another group of bottles with a tight cap was used. The samples were collected by following the method mentioned in (WHO, 1995). As for the bottles for measuring the concentration of dissolved oxygen and the biochemical oxygen demand, opaque bottles with a narrow mouth were used for this examination. After that, the bottles were kept in a cool-box, and the samples were transported to the laboratory quickly, so that the physical and chemical examinations are performed within a 24-hour period, while the bacteriological examinations are performed within three hours from the time of sample collection (APHA, 1995). In addition, 60 samples distributed between 30 stool samples and 30 urine samples were collected from the patients of the Women's and Children's Hospital and Ramadi Teaching Hospital for the period from September 2020 to November 2020. However, only 100 samples gave a positive growth result, special sterile and sealed bottles were used in the collection of the samples. Stool samples were handled according to the method described by the World Health Organization (WHO, 1995). While urine samples followed the method described in (Sleigh and Timbury, 1994).

2.Chemical and physical characteristics:

The temperature was measured locally with a graduated mercury thermometer (10-100°C). The pH value was measured with a pH-meter. The electrical conductivity (EC) was measured using the electrical conductivity device, and it is expressed in units of µs / cm. To measure the dissolved solids (TDS), the method of drying in a thermal oven (150 ° Celsius) and dry weight was applied, then the following equation was applied to extract the value concerned (Islam *et al*, 2016).

$$\text{TDS mg/l} = \frac{(A-B)\times 1000}{ml\ of\ sample}$$

Where:

A= weight of dish + dried residue (mg) / B= weight of dish (mg)

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Nitrate concentration was measured using a Tintometer in mg / l. The Biochemical Oxygen Demand (BOD) was measured by the Winkler method described in (APHA, 1999), and the results were expressed in units (mg / liter).

3.Biochemical tests:

Biochemical tests were performed on the studied bacterial isolates, where the Catalase test and the Oxidase test were performed by following the method described in (Brown, 2005), and the Indole production test, Methyl red test, Citrate utilization test, Voges - Proskauer test, motility test and production of Gelatinase enzyme was done by following the method approved in (Macfaddin, 2000), while the method described in (Collin *et al*, 1989) was adopted to test urea consumption. Also, the results of the biological tests were confirmed by using the Vitek 2 compact system (appendix 1 &2) to identify and diagnose the bacterial isolates.

4.Similarity ratio, fingerprinting, and ERIC-PCR Technique:

DNA was extracted and purified using the Wizard® PCR Preps Extraction Kit, according to the instructions of the US manufacturer Promega.

The primers were imported, and the primers solutions prepared from the Korean company Macrogen, depending on the gene sequence found in NCBI, as follows (Versalovic *et al*, 1991):

ERIC-1:5'-ATGTAAGCTCCTGGGGATTCAC-3'

ERIC-2:5'-AAGTAAGTGACTGGGGTGAGCG-3'

Then the standard primers solutions were prepared according to the instructions of the supplied company, using sterile distilled water to obtain a concentration of 100 picomole / microliter. Then the solution of each primer was prepared at a concentration of 10 picomole / microliter, by taking 10 microliters of the stock primer solution and adding 90 microliters of distilled water to it and mixing by vortex mixer, then stored well with the stock primer solutions at a temperature of approximately (-20°C), considering the mixing of the primer solution after removing it from the ice by using a mixer to homogenize it before use.

5.Polymerase Chain Reaction PCR:

The technique of polymerase chain reaction (PCR) was used to amplify different parts of specific genes, where the genes were placed in a single tube to detect the genes of the study, and the ERIC-PCR method was used to obtain the genetic profile of *E. coli* and *K. pneumpniae* isolates and to know the different genetic variances when comparing isolates isolated from environments using the genetic fingerprinting method (Ardakani and Ranjbar, 2016) for fingerprinting, using the GoTaq Green Master Mix kit supplied by the US company Promega.

Accordingly, a PCR mixture of 12.5 µl of Green master mix was prepared, 5 µl of extracted DNA Template, 1 µl of F-Primer, 1 µl of R-primer, and 5.5 µl of Nuclease-free water for the final volume of 25 Microliter, and after that the contents of the PCR tubes were mixed well using a Vortex mixer and then placed in the PCR device

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according to the specified program shown in Table (1):

Table (1) shows the programming of the PCR device

Step.	Process	
1	One cycle for four minutes at 95 ° C for the initial DNA template denaturation	
2	35 cycles include:	
	A	30 seconds at 95 ° C for a DNA template denaturation
	B	30 seconds at 50 ° C for primer binding to DNA template
	C	60 seconds at a temperature of 72 ° C to denaturation annealing/ extension
3	One 5-minute cycle at 72 ° C for final the final extension of DNA	

Then 10 µl of the gene duplicating product was transferred to electrophoresis onto a prepared 1.5% agarose gel. The Dendrogram & Fingerprint genetic tree was drawn to determine the degree of similarity between the bacterial isolates using PAST software version 2009.

Results and Discussion:

1.Isolation and Diagnosis:

Forty nine bacterial isolates were obtained from the sewage water of Ramadi Teaching Hospital and the Women's and Children's Teaching Hospital for the period from September 2020 to November 2020, while the number of bacterial isolates isolated from the patients was 51 bacterial isolates, distributed as in Table (2).

Table 2 shows the number of tested samples, the number and percentage of bacteria isolated from sewage water and pathological samples

No. of *K. pneumoni* a isolates	**No. of *E.coli* isolates**	**Gram +ve isolates number (%)**	**Gram -ve isolates number (%)**	**Number of samples that did not give growth**	**Positive growth total samples**	**Total samples**	**Isolation source**
12	12	1 (2)	48 (98)	11	49	60	**Sewage**
3	2	4 (18.2)	18 (81.8)	8	22	30	**Stool**
9	10	6 (20.7)	23 (79.3)	1	29	30	**Urine**
24	24	11	89	20	100	120	**Total**

Samples were cultured on Blood agar and MacConkey agar for a period of 24 hours and at a degree of 37 ° C. After the incubation period ended, the bacterial isolates were diagnosed based on the phenotypic characteristics of the colonies growing on the

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culture media, then microscopic examination of the samples was carried out after staining them with the Gram stain, the non-growth may be due to the presence of anaerobic bacteria that need Anaerobic incubation conditions, insufficient incubation time, or inadequate of MacConkey agar or Blood agar used in samples culturing.

The isolating results showed the dominance of gram-negative bacilli over the positive, as the number of negative isolates reached 89, while the number of gram-positive isolates was 11, and that *Klebsiella pneumoniae* and *Escherichia coli* were the most isolated by 24% for each, and the results indicate that, *Enterococcus faecalis* was the least frequent of bacteria isolated from patients with 1.96%. While *Yersinia enterocolitical*, *Pantoea spp*., *Enterobacter cloacae*, *Pseudomonas luteole*, *Streptococcus uberis*, *Proteus mirabilis*, *Pseudomonas alcaligenes*, and *Acinetobacter alseudalate* were the least frequent microorganisms isolated from the sewage samples.

2.Bacteria Identification and Biochemical tests

Isolates were diagnosed based on the results of bacteriological examinations and biochemical tests, as 24 isolates of *K. pneumoniae* and 24 isolates of *E. coli* were initially diagnosed. Table (3) shows the biochemical tests that were used to diagnose *K. pneumoniae* and *E. coli* bacteria. *Klebsiella spp.* isolates with rather large colonies and mucous in consistency because they contain the capsule, they are lactose fermenters and gram-negative bacilli (Collee *et al*, 1996). To differentiate the isolates of *Klebsiella spp.* Biochemical tests were performed that distinguish different types of the same genus.

K. pneumoniae was characterized by a negative result for the indole ring production test, while *K. oxytoca* gave a positive result for this test. But both *Klebsiella pneumoniae* and *K. oxytoca* gave a negative result for the Methyl Red test, depending on the approved diagnostic systems as shown in Table 3 (Hansen *et al*, 2004), the bacterial isolates isolated from different clinical samples were diagnosed and isolated from sewage, as the genus *Klebsiella spp.* is similar to the genus *Enterobacter spp.* with the exception of the motility test and the Ornithine decarboxylase test. *Klebsiella spp.* is negative for the motility test, while *Enterobacter spp.* is positive for this test. *Pseudomonas aeruginosa* is distinguished from the rest of the isolates by being the only one that is positive for the test for oxidase and often secretes a bluish green dye, with a distinct odor that resembles the smell of damaged fruit.

The isolation rate of *E. coli* was 27%. The colonies of *E. coli* bacteria growing on the medium of the blood agar and MacConkey agar are characterized by being flat rounded, medium-sized, smooth-edged, milky-white colonies, non-hemolysis on blood agar, and rosy on MacConkey agar, lactose sugar fermenter, the colonies of some isolates are large and mucous, similar to *Klebsiella*, but biochemical tests confirmed that they belong to *E. coli* bacteria, as some of them possess the capsule, so it is mucus. As for EMB media, colonies appeared in a dark brown color with a green metallic shine, which is a characteristic of it from the rest genus of the intestinal family, and this is due to the two pigments of eosin, methyl blue and lactose sugar, as the pigments are linked together and precipitated to form this phenomenon, as well it

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was negative for oxidase test, urease, lysis of gelatin, Voges–Proskauer , H_2S production, and citrate consumption test, and positive for catalase test, methyl red, and indole ring production, as shown in Table (3). As for *K. Pneumoniae*, it was characterized by large, pink mucous colonies on MacConkey agar due to the fermentation of lactose sugar and its containment of a capsule, immobile where no turbidity appeared around the loop stab area in the semi-solid medium, positive for the catalase test, Voges–Proskauer,urease, citrate consuming and not producing H_2S.

Table (3) Laboratory diagnosis of bacterial isolates isolated from sewage patient samples

DNase	H_2S production	Gas	Motility	Urease	Gelatinase	Nitrate reduction	Oxidase	Catalase	IMVC				Number of isolates	Tests / Bacterial Isolates
									Indol	Methyl red	Vogas proskaur	Simmon citrate		
-	-	+	-	+	-	+	-	+	-	-	+	+	24	*Klebsiella pneumonia*
-	-	+	+	-	-	+	-	+	+	+	-	-	24	*Escherichia coli*
-	-	+	-	+	+	+	-	+	+	-	+	+	2	*K. oxytoca*
-	-	+	+	V	-	+	-	+	-	-	+	+	13	*Enterobacter spp.*
-	-	+	+	-	+	+	+	+	-	+	-	+	2	*Pseudomonas aeruginosa*
-	+	+	+	+	+	+	-	+	+	+	+/-	+/-	1	*Proteus mirabilis*
-	+	+	-	+	+	+	-	+	+	+	-	+	3	*Raoultella ornithinolytica*
+	-	+	-	-	-	-	+	+	-	+	-	-	3	*Aeromonas salmonicida*
-	-	+	+	-	+		-	+	-	+	+	+	1	*Pseudomonas luteole*
-	+	-	+	-	-	+	+	+	-	+	-	+	1	*Pseudomonas alcaligenes*
+	+	-	+	-	+	-	+	+	+	+	-	-	2	*Aeromonas veronii*
+	-	+	+	+	+	+	+	+	-	+	+	+	4	*Aeromonas hydrophila*
+	-	-	+	-	+	-	-	+	-	-	+	+	3	*Serretia odorifera*
-	-	-	-	+	-	+	-	+	+	-	-	-	1	*Yersinia enterocolitical*
-	-	-	-	-	-	-	-	+	-	-	-	+	1	*Acinetobacter haerndytics*
+	-	-	-	-	-	+	-	+	-	-	-	+	7	*Staphylococcus aureus*
-	-	-	-	+	-	+	-	+	-	+	+	-	2	*Staphylococcus haemolyticus*
-	-	-	-	-	-	-	-	-	-	-	+	-	1	*Streptococcus uberis*
-	+	-	+	-	+	+	-	+	-	-	+	+	1	*Pantoea spp*
-	+	+	+	-	+	+	-	+	-	+	-	+	3	*Salmonella enterica*
-	-	-	-	-	-	-	-	-	-	-	+	-	1	*Enterococcus faecalis*
													100	Total sum.

3.Chemical and Physical measurements of under study hospitals sewage water

Some chemical and physical tests were carried out for the wastewater treatment unit

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plant of Ramadi Teaching Hospital and the Women's and Children's Teaching Hospital and it emerged that there is a variation in the values of the physical and chemical parameters of water, and that each stage of the treatment unit has its own physical and chemical characteristics, and the characteristic of the change in the properties of this water is relatively due to the stress conditions that this water is subjected to, which affects its physical, chemical and biological properties.

Table (4) shows the levels of the BOD for the sample collection sites from the water of the treatment unit plant at the Ramadi Teaching Hospital and the Women's and Children's Teaching Hospital, where the BOD values of the Sewage samples for Ramadi Teaching Hospital ranged between 40-45 mg / l, while it ranged between 36-39 mg / l in the samples from the Women's and Children's Teaching Hospital, and these values were very high, indicating high pollution and an increase in the number of microorganisms that consume a large amount of oxygen by oxidation process of organic matter (Wen *et al*, 2017). The results also showed that the pH levels of the sample collection sites from the water of the treatment unit plant in the Ramadi Teaching Hospital and the Women's and Children's Teaching Hospital, as the pH values were 7.32 and 7.26 respectively, and the results also showed that the temperature values of the studied sites of wastewater for Ramadi Teaching Hospital, ranged between 20 - 23 ° C. With an average of 21.75 ° C for all the studied sites, while the temperatures of the Women's and Children's Hospital samples ranged between 21-23 ° C and an average of 22 ° C.

As for the electrical conductivity levels of the sewage treatment plant sites for Ramadi Teaching Hospital, they ranged between 1925-1956 µs / cm, with an average of 1942.75 µs / cm. As for the samples of the Women's and Children's Teaching Hospital, the value of the electrical conductivity ranged between 2506 - 2544 µs / cm, with an average of 2520.75 µs / cm, and that the difference in conductivity values is due to the conditions of the water presented to the treatment unit, and among the reasons that lead to an increase in electrical conductivity is the use of salts, chemicals and detergents in hospitals and their disposal to the sewage network without going through the treatment unit.

Regarding the concentrations of total dissolved solids in the sewage water of the study area, the values ranged from 1303 - 1334 mg / l, with an average value of 1322.25 mg / l for samples from Ramadi Teaching Hospital, while the values ranged from 1750 - 1763 mg / l, with an average value of 1755.25 mg / l for the samples of the Women's and Children's Teaching Hospital, and this due to the discharge of wastes of different materials used in the hospitals into wastewater, which leads to an increase in their high percentage. However, the NO_3 concentration in the wastewater of Ramadi Teaching Hospital ranged from 0.26 to 0.28 mg / l with an average value of 0.26 mg / l, while it ranged between 0.33-0.35 mg/l for the samples of the Women's and Children's Teaching Hospital with an average value of 0.34 mg /l, Table (4).

Through the above, we find that there are differences in the values of the studied physical and chemical properties of this water, and this rise and fall of the values studied in hospital wastewater reflects the different quality of the wastewater offered,

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in addition to the size and quality of human activities, and other reasons resulting from the area in which the study was conducted. Yet, these hospitals are characterized by the dense population in which chemicals, sterilizers, disinfectants, and antibiotics polluting the hospital environment are used, which are all thrown into such waters, and thus raise the levels of pollution in hospital sewage, and there is another feature of this water being its old founding date and the lack of regular maintenance, as it is an old building, and the worn-out of buildings and pipes may play a major role in increasing pollution.

The results of the physical and chemical measurements agree with the results of researchers Salah and Ghaida (2008) of wastewater in Al-Alwiya area hospitals in Baghdad, as the results showed an increase in the values of pH, BOD, TDS and EC. The results of our current study are in agreement with the study of Al-Dulaimi (2016) regarding the increase in total dissolved salts and electrical conductivity of wastewater in Ramadi Teaching Hospital, while the results of our current study showed great agreement with (Cest *et al*, 2015) who studied wastewater in Nigeria and attributed the variation in physical and chemical values in hospital wastewater through human activities as a result of increased consumption of detergents, disinfectants and antibiotics, as well as increased microbial activity as a result of the discharge of untreated medical wastes, also the increasing of living organisms in wastewater leads to an increase in the consumption of waste in different ways; Through bio-sorption or bio-accumulation, or it may lead to produce chemical complexes.

Table (4) Physical and chemical characteristics of wastewater for Al-Ramadi Teaching Hospital and the Women's and Children's Teaching Hospital ± Standard deviation

NO_3	TDS	EC	Temp (C°)	pH	BOD	Sample collection sites
mg/l		µS/cm				
0.28	1330	1956	23	7.4	43	**A-1**
0.26	1303	1925	20	7.2	40	**A-2**
0.26	1322	1943	22	7.4	41	**A-3**
0.27	1334	1947	22	7.3	45	**A-4**
0.26 ±0.021a	**1322.25 ±136.69a**	**1942.75 ±152.46a**	**21.75 ± 0.68a**	**7.32±0.073a**	**42.25 ±9.88 a**	**Average**
0.34	1755	2506	22	7.2	39	**B-1**
0.34	1750	2510	23	7.25	37	**B-2**
0.33	1753	2544	21	7.3	36	**B-3**
0.35	1763	2523	22	7.3	37	**B-4**
0.34 ±0.026 a	**1755.25 ±173.95b**	**2520.75 ±362.37b**	**22 ± 0.85a**	**7.26±0.042a**	**37.25 ±8.53a**	**Average**

Where:

The letter A refers to the samples from Ramadi Teaching Hospital, while the letter B ▪

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represents samples from the Women's and Children's Teaching Hospital.
These values represent the average of three replications.
Similar letters indicate that there are no significant differences between the rates of transaction numbers and the different letters indicate the presence of significant differences at a probability level (P <0.05).

4.Genetic similarity relationship for bacterial isolates:

Fingerprinting was determined to amplify different parts of the relative genes of 24 bacterial isolates belonging to *E. coli*, 12 of which were isolated from patient samples and 12 isolated from wastewater of Ramadi Teaching Hospital and Women's and Children's Teaching Hospital, and the same numbers were of *K. pneumoniae*, where the ERIC-PCR method, known as nucleotide sequencing in the genes of the intestinal group, was used to identify the different genetic variances of the bacterial isolates and to reveal the real sources of isolates and the degree of similarity relationship through the use of polymerase chain reaction kit and a specialized primer to diagnose similar genes. Where the reaction of PCR was carried out, and after electrophoresis on agarose gel with a concentration of 1.5%, many bundles appeared, showing the presence of many genes in the bacterial isolates, and the contrast was clear between environmental isolates and pathological isolates, as well as the case between isolates from the same source.

The data obtained using the statistical program Past was analyzed and the degree of relationship and the level of similarity between the bacterial isolates were determined and divided into groups, as shown in pictures (1) and (2) and Figures (1) and (2).

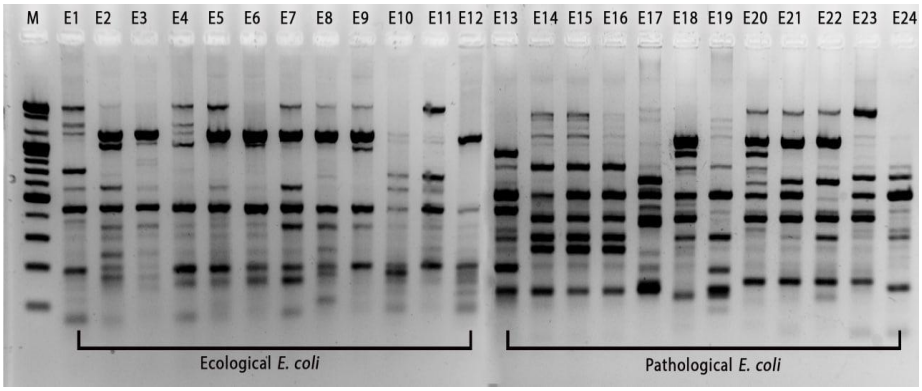

Plate (1) Electrophoresis of PCR product of the DNA using the ERIC-PCR system, Environmental isolate numbers (1-12), and pathogenic isolate numbers (13-24) of *E. coli*

The data shown in plate (1) were analyzed using the computerized statistical program, and 6 specific groups G1, G2, G3, G4, G5 and G6 were obtained from the isolated bacteria *E. coli* and the similarity level was 63%, as shown in Figure (1).

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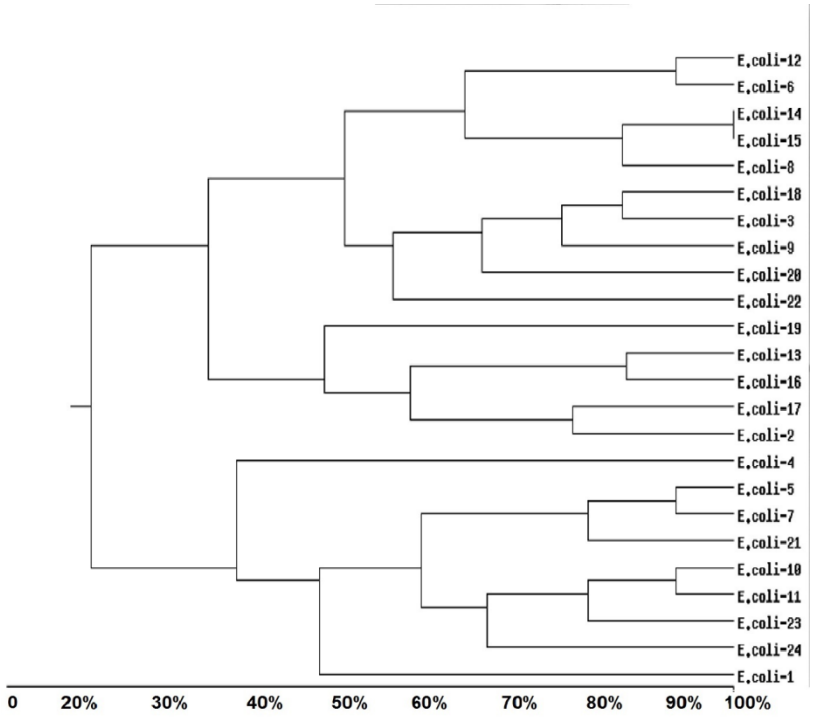

Figure (1) Statistical analysis of 24 *E. coli* isolates, with a similarity ratio of 63%

By observing figure (1), we can divide the bacterial isolates into 6 genotypes with a similarity ratio of 63% depending on the degree of their closeness and similarity with each other despite the different sources of isolation, as the first group G1 included environmental bacterial isolates isolated from wastewater E6 and E12, with a similarity rate of 90%, while the second group, G2, included the pathological isolates E14 and E15, which were similar by 100%, as well as the environmental isolate E8, which was similar with them by 82%, The third group G3 included pathological isolation E18 and environmental isolation E3 and the percentage of similarity between them was 82%, as well as environmental isolation E9, which was similar to the two isolates E18 and E3 by 75%, as well as the pathological isolates E20 and E22, which were similar by 55%. As for the fourth group G4, it includes the pathological isolates E13 and E16 by similarity of 83% and the two pathological isolates E17 and E2, which were similar by 76%.

As for the pathological bacterial isolation E19, it was similar with the two groups by 46%, while the environmental bacterial isolation E4 was not organized with a specific group. As for the fifth group G5, it included environmental bacterial isolates E5 and E7 with a similarity rate of 89% and pathological bacterial isolate E23, which was similar to the two environmental isolates by 66%, As for the sixth group, G6, it included the environmental bacterial isolates E10 and E11, which were similar by 90%, and the pathological bacterial isolate E23, which was similar to the two environmental isolates by 78%, As for the pathological bacterial isolate E24, it was 67% similar to the same environmental isolates, while the percentage of similarity between the pathological bacterial isolate E23 and E24 was 66%, but the pathological bacterial isolate E1 was not organized with a specific group. It is necessary to refer to

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the isolation sources for these bacterial isolates, as it was found that the pathological bacterial isolates in the second group were similar to the environmental bacterial isolation by 82%, while there were pathological bacterial isolates that did not resemble them and were not organized into a specific group. Thus, the result indicates that there is a genetic convergence between pathological and environmental bacterial isolates, at rates of 82%, and that the pathogenesis did not depend on a specific genotype.

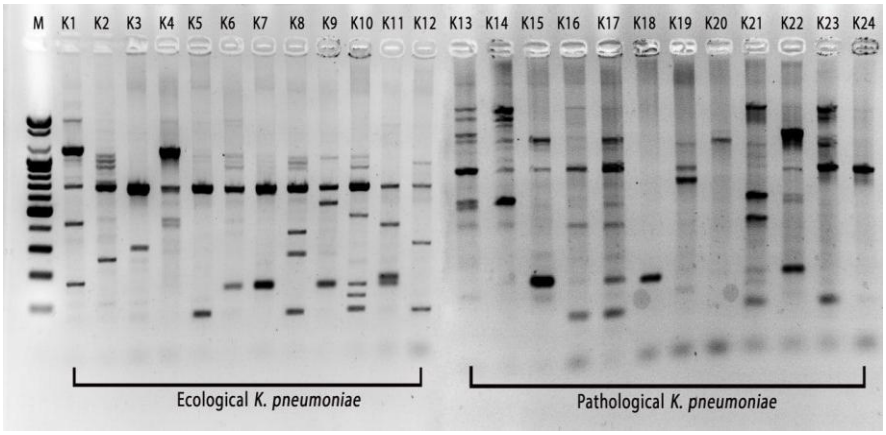

Plate (2) Electrophoresis of DNA polymerase chain reaction product using ERIC-PCR technique, Environmental bacterial isolate numbers (1-12), Pathological bacterial isolate numbers (13-24) of *K. pneumoniae*

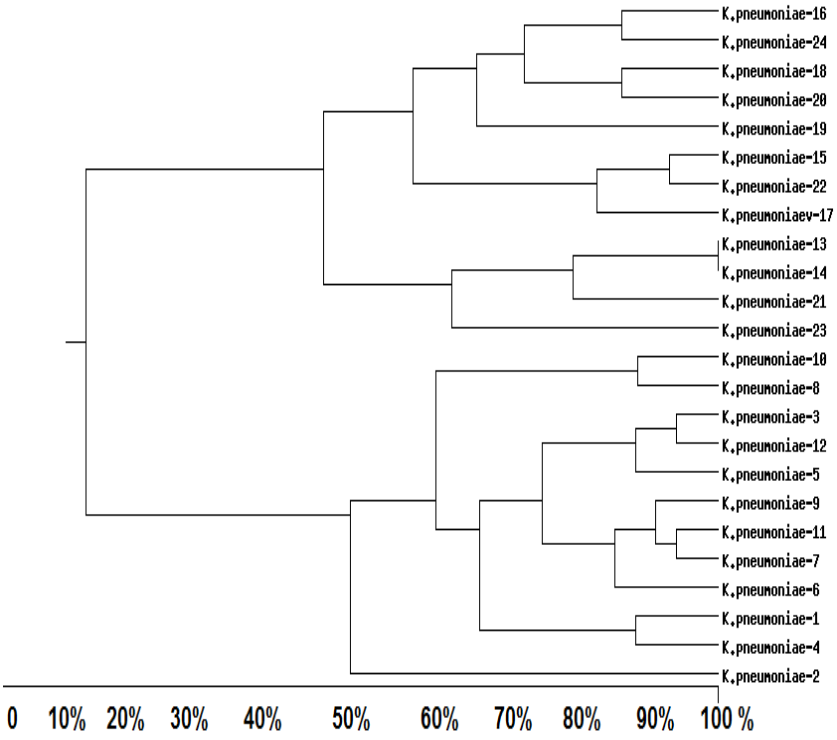

Figure (2) Statistical analysis of 24 *K. pneumoniae* isolates, with a similarity ratio of 48%

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Figure (2) shows that *K. pneumoniae* isolates are divided into seven G1-G7 genotypes with a similarity ratio of 48% depending on the degree of their genetic affinity despite the different sources of isolation. The first group G1 included the same pathological K16 and K24 isolates with a ratio of 86% as well as the case of pathological isolates K18 and K20, with a similarity rate with the pathological isolates K16 and K24, which amounted to 73%, As for the pathological bacterial isolation K19, it was similar to the pathological bacterial isolates K18, K20, K16 and K24 by 65%, while the second group G2 included the pathological bacterial isolates K15 and K22 that are similar by 92% and the pathological bacterial isolation K17 was similar with them by 83%, As for the third group G3, it included the pathological bacterial isolation K13 and K14, which were similar by 100%, while the pathological bacterial isolation K21 was similar with them by 80%, while the pathological isolation K23 was similar by 63%. This difference in percentages can be attributed to the isolation site, as the two isolates K13 and K14 are isolated from the urine, while the isolate K23 is isolated from the feces, which explains the similarity ratio that reached 63% compared to the isolate K21, which was similar with them by 80% as it was isolated from the urine as well. Yet, the fourth group G4 included the environmental bacterial isolation K10 and K8, which were similar by 88%, while the fifth group G5 included the two environmental isolates, K3 and K12, which were similar by 95% and the environmental isolation K5, which was similar with them by 87%.

Nevertheless, the sixth group G6, included the two environmental isolates, K11 and K7, with a similarity rate of 95%, and the environmental isolation of K6, which was similar to them at a rate of 85%. Close similarity ratios indicate isolation of these species from the same source. As for the seventh group G7, it included the two environmental isolates, K1 and K4, and they were similar and closely related genetically by 89%. The percentage of similarity with the groups G4, G5 and G6 was (87, 87, 85) %, respectively, while the environmental bacterial isolation K2 was not organized in a specific group.

The percentage of similarity between environmental and pathological isolates indicates the existence of a genetic convergence between them, and this is an evidence of transmission of the source of infection to wastewater, which shows that there is no real treatment of laboratory and hospital waste in a manner that ensures that these wastes are free of germs, so their presence in hospital wastewater and its similarity of high rates with pathogenic isolates confirming the non-application of safety and biosecurity conditions, as it is necessary to provide sanitation stations with sterilization systems represented by chlorine, which is an important factor in sterilization and killing pathogenic microorganisms, whose survival leads to an increase in their virulence and the transmission of virulence factors between bacterial species, including plasmids which is more pathogenic and antibiotic resistance.

It is noticeable from the previous results that there are no treatment stages for wastes, as sedimentation units, filters and sterilization systems were not observed in

the sewage stations, in addition to their wear and the lack of periodic maintenance of them, which affects the physical, chemical, and biological indicators of water as the results were outside the permissible ranges of Iraqi and international specifications. Additionally, the open ponds are exposed to pollution, animal excreta, and soil that increase the turbidity of the water, as it is a good factor for the growth and reproduction of microorganisms, and this may constitute a potential source for the delivery of pathogenic bacteria to humans, directly or indirectly.

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