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Genetic relationship of clinical bacterial isolates with their environmental spreading using ERIC-PCR

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Abstract---The study examined the relationship between environmental bacterial and the pathological isolates of inward patients. It identified the biological safety level procedures followed. This work was conducted during the period from December 2021 till May 2022 in Ramadi city (Anbar Governorate). Samples were divided into 30 environmental samples collected from the hospital environment, sewage and water, and 100 clinical samples collected from inpatients. These patients were in The Ramadi Teaching Hospital and in The Women's and Children Hospital. Bacterial isolates were diagnosed using biochemical tests and confirming using VITEK2 technique. Some chemical and physical variables were detected. This work studied the Genetic fingerprinting of selected bacterial species by the Enterobacterial repetitive intergenic consensus of Polymerase Chain Reaction ERIC-PCR and conducted the dendrogram program was conducted for showing the relationships between environmental and pathological isolates of *E. coli*. As for the genetic fingerprint examinations environmental *E. coli*, the statistical analysis of the dendrogram program using ERIC-PCR technique had indicated that there was a similarity ratio 74.58% with the clinical *E. coli*. The study found that the hospital environment and the sewage water flowing from it are polluted to a large extent by pathogenic bacteria referring the weakness of biological safety procedures followed in the studied hospitals, which eventually might affect the quality of the environment.

Keywords---*E. coli*, ERIC-PCR, biosafety.

Introduction

Pathogenic microorganisms appear in various environments posing big health problems besides the recently spread of the Coronavirus and the viruses that cause hemorrhagic fever. Healthcare waste are the wastes of healthcare facilities, laboratories, and research centers related to medical treatment. Of these wastes, 75–90% are known as general healthcare waste or non-hazardous waste, yet 10–25% are hazardous (Behnam et al., 2020). Managing such wastes is a serious issue as it is intrinsic toxic and infectious; so, unsafe mismanaging or disposal of wastes is risky to all front-line workers. Improperly managing healthcare waste pollute and contaminate soil, air, and surface and groundwater (Halling-Sørensen et al., 1998). Yet measures against drug-resistant bacteria at clinical sites like hospitals (Aleksun & Levy, 2007) are taken. Many scholars studied these sewage and river water bacteria; (Huijbers et al., 2015); (Rizzo et al., 2013); (Rizzo et al., 2013). The problems are linked Antimicrobial-Resistant Bacteria (AMRB) and are common in modern societies (Mills & Lee, 2019).

It is obvious that sewage from hospitals contains pathogenic microorganisms in particular due to its inclusion of genes coding for resisting in the sewage systems enabling its adoption to various settings (Kumari, 2020). The direct greywater releases contain ARB and antibiotics and help efficiently to the antimicrobial resisting transmissions in the microbial eventually to humans. The discharge with not treatment is because of the lack of legislation to regulate the final disposal of these wastes (Noman et al., 2022).

In the Enterobacterial repetitive intergenic consensus of Polymerase Chain Reaction technique (ERIC-PCR) according to amplifying random distributions of inter-genome parts, different organisms could be differentiated. So, the bacteria are different in the frequency of sequences. So, this technique shows various patterns of specific primers in electrophoresis. Here, the designed primers 4 attach to repeated sequences expanding the distances in those attached primers to repeated sequences (Ardakani & Ranjbar, 2016). Repetitive DNA sequences are used in prokaryotic genomes to study diversity and determine relatedness similarities and differences between strains of the same species, and thus fingerprinting of genomic DNA enables the identification of rapid analysis of a large number of bacterial isolates and determination of the degree of kinship between them using primers complementary to sequences repeated amplification of fragments of various sizes with DNA (Uelze et al., 2020). The current work aims to investigate the microbial content of pathogenic microorganisms present in the wastewater of Ramadi General Hospital and Maternity Hospital, which are thrown directly into the river and explore the relationship or similarity between selected clinical isolates with the environmental isolates using Dendrogram program for fingerprinting and genetic tree design.

Materials and Methods

Sample collection

Clinical specimens were obtained from cerebrospinal fluid, wound, and urine of patients attending Ramadi General Hospital as well as the General Women's and

Children's Hospital in Ramadi following procedures presented by (Sleigh & Timbury, 1986). To collect the wastewater samples, we used two sets of bottles, the first was used to collect samples for bacteriological examinations, the second was used for the purpose of conducting physical and chemical tests which was carried out by holding the bottle from its base and immersing it in water to a depth of approximately 20 cm in the direction of water stream. To measure the bottles of the concentration of dissolved oxygen and the vital requirement. We used oxygen, opaque bottles with a narrow nozzle, and the samples were collected by filling an opaque glass bottle to the nozzle, while continuing to allow water to flow out of it until all air bubbles were removed inside the water. After that, the bottles were kept inside a box containing pieces of ice, and the laboratory samples were transferred rapidly so that physical and chemical tests were conducted in 24 hours, while bacteriological tests were performed within three hours from the sampling time (APHA & Association, 1995). The sampling of the soil (approx 10g) is conducted in clean, dry and sterile polythene bags by sterilized spatula (Burh, 2011) that reduce the contamination chances as much as possible, and transferred to the laboratory for further analysis. The soil microorganisms were isolated by serial dilution method on nutrient agar medium. Other specimens were collected from surrounding environment, including operating theaters, patient lobbies, corridors, and laundry rooms, were isolated and identified.

Isolation and identification

Sample specimens were cultured on MacConkey agar and blood agar and incubated at $37 \pm 1^\circ\text{C}$ for 24 h. The typically different colonies were subcultured and diagnosed according to cultural, morphological, microscopic examinations, biochemical, and physiological characterizations (Abed SM., Yasin LQ., 2021). Identification at the species level was confirmed via VITEK2 compact system

Fingerprinting of isolates using ERIC-PCR technique

Initially, genomic DNA extraction was performed using wizard kit purchased from Promega, Korea. Purity and concentration of DNA was checked using Drop-Nano device at wavelengths 260/280 nm. *E. coli* was only selected for the purpose of fingerprinting. Based on the gene sequence found in NCBI, ERIC primers; ERIC 1: 5'-TGTAAGCTCCTGGGGATTAC-3', ERIC-2: 5'-AAGTAAGTGACTGGGGTGAGCG-3' imported from Macrogen Korea. were used at 10 picomole/microliter in a total volume of reaction of 25 μL using GoTaq Green Master Mix (Promega, USA). The reaction mixture included 12.5 μL of Master Mix, 5 μL DNA, 1 μL of each forward and reverse primers and 5.5 μL of Nuclease-free water. PCR program used in this work was 94°C for initial denaturation, 30 seconds at 95°C for the DNA for the denaturation of DNA template, 40 second at 55°C for annealing 60 second at 72°C for elongation 35 cycles. We electrophoresed PCR produces on 1.5% agarose gel and visualized by gel documentation devices (Abed et al., 2020).

Statistical Analysis

The statistical program Genstat V.15 (Factorial Analysis) was used to analyze the results obtained, as the arithmetic mean and standard deviation $\text{SD} \pm$ for all measurements were used and F. Test was used to find out the differences among

results obtained and the correlation coefficient was calculated within the study groups, as well as the L.S.D test was used to find out the least significant difference between the means of the coefficients at probability level $P < 0.05$.

Results and Discussion

We collected 100 Bacterial isolates from patients in Al-Ramadi Teaching Hospital and Women and Children Teaching Hospital from December 2021 till May 2022, yet the number of bacterial isolates from the environments surrounding the hospital was 30 bacterial isolates which the table (8) distributed.

Table 8
The number of samples examined and isolated bacteria percentage of from environmental and pathological models

Type of sample	Clinical samples				Environmental samples				The total
	Urine	CSF	Wounds	UTI	Waste water	Soil	River water	Environmental smears	
Total number of samples	70	10	10	10	6	4	5	15	130
Total number of positive growth	60	7	6	6	5	3	3	10	100
Number of samples that did not give growth	8	3	5	5	1	1	2	5	30
Gram negative isolates	59 (98%)	5 (71%)	5 (83.3%)	5 (83%)	4 (80%)	2 (67%)	2 (67%)	8 (80%)	90
Gram positive isolates	1 (2%)	2 (29%)	1 (16.6%)	1 (16.6%)	1 (20%)	1 (33%)	1 (33%)	2 (20%)	10
Number of E.coli isolates	5	2	2	3	4	2	3	3	24

MacConkey agar is used to culture the samples on blood agar and medium for 24 hours at 37°C Bacterial colonies were diagnosed based on phenotypic characteristics growing on the culture media. Then, we conducted a microscopic examination of the following staining them with gram stain. The results of diagnosing samples revealed the predominance of Gram-negative bacilli over the gram-positive, as the Gram-negative isolates reached 90, while the gram positive isolates was 10. It was found that *Escherichia coli* is the most isolate with a percentage of 24% for both clinical and environmental samples, and the findings indicated that *Enterococcus faecalis* was the least frequent of the isolates from patients with 1.96%. As for (*Yersinia enterocolitica*, *Pantoea spp*, *Enterobacter cloacae*, *Pseudomonas luteole*, *Streptococcus ubevis*, *Proteus mirabilis*, *Acinetobacter haerndytica*, *Pseudomonas alacaligenes*) It was the least frequent of the isolates taken from sewage water samples.

ERIC-PCR Fingerprinting technique

A total of 24 selected strains of *E. coli* were subjected to ERIC-PCR analysis, where we isolated 12 of them from patient samples and 12 were isolated from wastewater. This technique is known as repeating nucleotide sequences in genes of intestinal bacterial groups to know the various genetic variations of bacterial isolates for showing the true sources of the isolates and the degree of kinship by using the polymerase chain reaction kits and the specialized primer to diagnosing the affinity genes in which we performed the reaction. After transferring the PCR product onto an agarose gel at a concentration of 1.5%, many bands showed the several genes in the bacterial isolates with clear contrast between environmental and pathological isolates and between isolates from the same source. The data were analyzed by the statistical program Past the affinity and the level of similarity between bacterial isolates and their division into groups, as picture (5) and figure (3-8) show. The data that appears in figure (3-8) was analyzed using the computerized statistical program, and it was obtained 8 specific groups G1, G2, G3, G4, G5, G6, G7, and G8 isolated bacteria *E. coli* the similarity level was 63%, as shown in Figure (3.8). the panding pattern gave 70 packages, the number of clinical sample packages was 32, and the number of environmental sample packages was 38, ranging (200-100000 bp) and converted to digital numbers (0-1) and then subjected dendrogram analysis.

Picture 5

Electrophoresis of the DNA polymerase chain reaction produce by the ERIC-PCR system, Environmental isolation (1-12) and Clinical isolation (13-24)

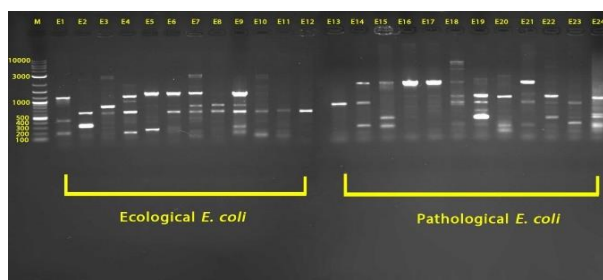
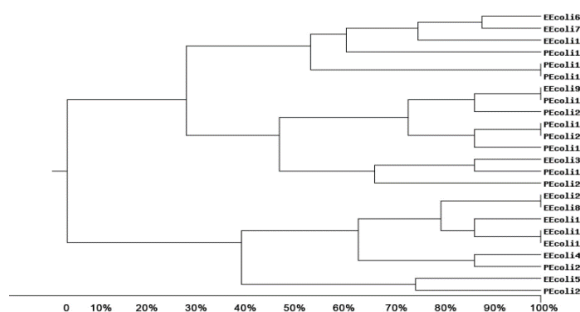


Figure 3.8.

The dendrogram showing the relatedness of the *E. coli* isolates was determined by the DNA fingerprints analysis performed by ERIC-PCR



Cluster examinations are exploratory data analysis tools to solve classification problems. The tool aims to classify the data into groups or clusters. The cluster reveal high internal homogeneities and high external heterogeneities (Abdi, 2003). By remarking the figure (3.8) bacterial isolates are classified into 8 genotypes with a similarity ratio (74.58) % according to the degree of their affinity and similarity with each other despite the different sources of isolate, Where the first cluster G1 included environmental isolates isolated from different environmental subcluster E6,E7 with a similar percentage 90% , Also, the environmental subcluster E1 was similar to the two isolates E6,E7 in a percentage 75% , and the pathological subcluster P15 was similar to the two isolates E6,E7 in a percentage 62% .

Yet the second cluster G2 contained pathological isolates P16,P17 resembling in percentage 100% , while the third cluster G3 included environmental cluster E9 and pathological cluster P19, which were similar in percentage 100% , as well as pathological subcluster P22 that were similar to them in a percentage 86% . The fourth cluster G4 included pathological clusters P14,P21 that were similar in percentage 100% , as well as pathological subcluster P18 that were similar to them in a percentage 86 % . The fifth cluster G5 included environmental cluster E3 and pathological cluster P13 isolates, which were similar in percentage 86% . As for the pathological subcluster P24 , which was similar to the two clusters E3, P13 with a percentage 76% .

The sixth cluster G6 included the environmental clusters E2,E8 which were similar with a percentage 100% , as well as the environmental clusters E11,E12 with a similar percentage 100% , As for the environmental subcluster E10 , it is similar to the two clusters E11,E12 with a percentage 86% . Regarding the seventh cluster G7 , which included environmental clusters E4 and pathological clusters P23 , which were similar in a percentage 86% . Finally, the eighth G8 cluster included environmental cluster E5 and pathological clusters P20 , which were similar in proportion 75% .

It is necessary to refer to the sources of isolation for these bacterial isolates, because the environmental and pathological isolates of the third group resembled the pathological bacterial isolate by 6-8, yet there are bacterial isolates that are not similar to them and are not divided into specific groups. In the end result, a genetic affinity between the pathological and environmental bacterial isolates at 82% and the pathogenesis did not rely on a genetic pattern a certain.

The similarity between environmental and pathological isolate percentage revealed that there is a genetic affinity between them, and this proves the transmission of the source of infection to sewage, showing no real processing of the hospital wastes in the form ensuring free of germs wastes and residues. Therefore, its presence in the wastewater of hospitals and their similarity in high proportions with pathological isolates confirms that safety and biosecurity conditions are not applied where it is necessary providing sewage stations with sterilization systems represented by chlorine, as a significant factor to sterilize the killing of pathogenic microorganisms, the survival of which increases their virulence, and the transfer of virulence factors between species bacteria, including plasmids, are more ground-based and resistant to antibiotics.

The previous findings show no stages of waste treatments, as no sedimentation units were observed filters and sterilization systems in sewage stations besides their wear and lack of regular maintenance, which affects the physical, chemical, and biological indicators of water, where the findings are outside the permissible ranges to Iraqi and international standards, the open ponds are vulnerable to pollution, animal excrement, and dust that increases the turbidity of the water because it is an excellent factor in the growth and reproduction of microorganisms. This could be a potential source for the transmission of pathogenic bacteria to humans.

References

- Abdi, H. (2003). Multivariate analysis. *Encyclopedia for Research Methods for the Social Sciences*. Thousand Oaks: Sage, 699–702.
- Abed SM., Yasin LQ., M. WS. (2021). No Title. *Tropical Journal of Natural Product Research*, 6. <https://doi.org/http://www.doi.org/10.26538/tjnpr/v1i4.5>
- Abed, S., Assie, A., Abu-Elteen, K., Dheeb, B., & Abu-Qatouseh, L. (2020). Molecular Characterization of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Isolates Obtained from Human-Skin Samples in Iraq. *Biomedical and Pharmacology Journal*, 13(2), 737–746.
- Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 128(6), 1037–1050.
- APHA, A., & Association, W. P. H. (1995). *Water Environment Federation, Standard Methods for the Examination of Water and Waste Water*. American Public Health Association, Washington DC, USA.
- Ardakani, M. A., & Ranjbar, R. (2016). Molecular typing of uropathogenic *E. coli* strains by the ERIC-PCR method. *Electronic Physician*, 8(4), 2291.
- Aryani, L. N. A., & Lesmana, C. B. J. (2019). Neuropsychiatric factor and polymorphism gene in internet addiction. *International Journal of Health & Medical Sciences*, 2(1), 39-44. <https://doi.org/10.31295/ijhms.v2n1.90>
- Behnam, B., Oishi, S. N., Uddin, S. M. N., Rafa, N., Nasiruddin, S. M., Mollah, A. K. M. M., & Hongzhi, M. (2020). Inadequacies in hospital waste and sewerage management in Chattogram, Bangladesh: exploring environmental and occupational health hazards. *Sustainability*, 12(21), 9077.
- Burh, P. M. (2011). *Microbial analysis of soil and water samples from Koel river in Rourkela, Odisha*.
- Halling-Sørensen, B., Nielsen, S. N., Lanzky, P. F., Ingerslev, F., Lützhøft, H. C. H., & Jørgensen, S. E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment-A review. *Chemosphere*, 36(2), 357–393.
- Huijbers, P. M. C., Blaak, H., de Jong, M. C. M., Graat, E. A. M., Vandenbroucke-Grauls, C. M. J. E., & de Roda Husman, A. M. (2015). Role of the environment in the transmission of antimicrobial resistance to humans: a review. *Environmental Science & Technology*, 49(20), 11993–12004.
- Kumari. (2020). Hospital wastewater treatment scenario around the globe. In *Current Developments in Biotechnology and Bioengineering* (pp. 549–570). Elsevier.
- Mills, M. C., & Lee, J. (2019). The threat of carbapenem-resistant bacteria in the environment: Evidence of widespread contamination of reservoirs at a global scale. *Environmental Pollution*, 255, 113143.

- Noman, E. A., Mohamed, R. M. S. R., Al-Gheethi, A. A., Al-Shaibani, M. M., Al-Wrafi, F. A., Al-Maqtari, Q. A., & Vo, D.-V. N. (2022). Antibiotics and antibiotic-resistant bacteria in greywater: Challenges of the current treatment situation and predictions of future scenario. *Environmental Research*, 212, 113380.
- Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total Environment*, 447, 345–360.
- Sleigh, J. D., & Timbury, M. C. (1986). Notes on Medical Bacteriology. CHURCHILL LIVINGSTONE, NEW YORK, NY(USA). 1986.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, 6(1), i-v. <https://doi.org/10.53730/ijhs.v6n1.5949>
- Uelze, L., Borowiak, M., Deneke, C., Szabó, I., Fischer, J., Tausch, S. H., & Malorny, B. (2020). Reply to Li et al., “GC content-associated sequencing bias caused by library preparation method may infrequently affect Salmonella serotype prediction using SeqSero2.” *Applied and Environmental Microbiology*, 86(18), e01260-20.