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Molecular Detection of Gram-Negative Bacterial Species in Polluted Water Samples from the Tigris River within Samarra City

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Abstract

This study aimed to identify bacterial isolates from the Tigris River in Samarra using molecular techniques targeting the 16S rRNA gene. The results revealed bacterial species from the genera *Salmonella*, *Pseudomonas aeruginosa*,. Identification was confirmed via 16S rRNA sequence analysis, showing high similarity (97–100%) with reference strains in the NCBI database. Point and insertion mutations were particularly noted in Klebsiella pneumoniae and Pseudomonas aeruginosa, suggesting environmental stress impact. The study highlights the value of molecular tools in environmental monitoring and emphasizes the need for continuous water quality assessment due to the potential presence of multidrug-resistant bacteria.Bacterial identity was confirmed through 16S rRNA gene sequence analysis, where most isolates showed a high similarity (97–100%) to reference strains in the NCBI database. Several point mutations and insertion mutations were detected in some isolates, particularly in *Salmonella* and *Pseudomonas aeruginosa*, suggesting the influence of environmental stress and pollution on the genetic structure of these bacteria.

Key words:,16S rRNA gene sequence analysis, *Salmonella*, *Pseudomonas aeruginosa*, Tigris River

الكشف الجزيئي عن أنواع البكتيريا سالبة الجرام في عينات المياه الملوثة من نهر دجلة في مدينة سامراء

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الملخص

هدفت هذه الدراسة إلى تحديد عزلات بكتيرية من نهر دجلة في سامراء باستخدام تقنيات جزيئية تستهدف جين S rRNA16. كشفت النتائج عن أنواع بكتيرية من أجناس السالمونيلا والزائفة الزنجارية. تم تأكيد التحديد من خلال تحليل تسلسل S rRNA16، حيث أظهر تشابهًا كبيرًا (97-100%) مع السلالات المرجعية في قاعدة بيانات المركز الوطني لمعلومات التكنولوجيا الحيوية. لوحظت طفرات نقطية وإدخالية بشكل خاص في بكتيريا الكلبسيلة الرئوية والزائفة الزنجارية، مما يشير إلى تأثير الإجهاد البيئي. تُبرز الدراسة أهمية الأدوات الجزيئية في الرصد البيئي، وتُشدد على ضرورة التقييم المستمر لجودة المياه نظرًا لاحتمالية وجود بكتيريا مقاومة للأدوية المتعددة. تم تأكيد هوية البكتيريا من خلال تحليل تسلسل جينات الرنا الريبوسومي S16، حيث أظهرت معظم العزلات تشابهًا كبيرًا (97-100%) مع السلالات

المرجعية في قاعدة بيانات المركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI). تم الكشف عن العديد من الطفرات النقطية والإدخالية في بعض العزلات، وخاصةً في السالمونيلا والزائفة الزنجارية، مما يشير إلى تأثير الإجهاد البيئي والتلوث على التركيب الجيني لهذه البكتيريا.

الكلمات المفتاحية: تحليل تسلسل جينات الرنا الريبوسومي S16، السالمونيلا، الزائفة الزنجارية، نهر دجلة

Introduction

Intestinal bacterial pathogens have been recognized for over a century, and at the beginning of the 20th century, it was demonstrated that modern drinking water treatments, including filtration and disinfection, are highly effective in controlling intestinal bacterial diseases such as typhoid fever and Today, outbreaks of waterborne bacterial diseases are uncommon in developed countries and

Polymerase chain reaction (PCR) is an important biotechnology in molecular biology. It is widely used to detect mutations and classify microbes based on genetic sequence variations. It is also used to amplify millions of copies of a specific DNA fragment in a short time. PCR is widely recognized for its sensitivity and specificity in microbial identification (Na et al., 2021; Ingalagi et al., 2022; Budreikaitė and BaSevičienė, 2024), BoSe and Moore (2023) reported that 16S-rRNA gene sequences in bacteria are used in taxonomic and evolutionary studies, because they contain variable regions that can contribute to the distinction between different genera and even species.

The objectives of molecular diagnostics of bacteria in the Tigris River water in Samarra are diverse and include several aspects, including identifying bacterial diversity and the different types of bacteria present in the water, which helps in understanding the biological distribution of bacteria in the region. Diagnosing and detecting pathogenic bacteria, such as bacteria that cause intestinal or infectious diseases, and using molecular diagnostic techniques such as polymerase chain reaction (PCR) to analyze bacterial DNA and more accurately identify their species.

Materials and methods

Molecular diagnosis of bacteria

DNA extraction

All tools and materials used in the DNA extraction process were autoclaved, and the work area was sterilized before starting the procedure.Molecular diagnosis steps for bacterial isolates were carried out by extracting genomic DNA using specialized primers to amplify the 16S rRNA gene by polymerase chain reaction (PCR) technology, followed by determining the nitrogenous base sequences of the target genes, and comparing them with the

reference sequences in the National Center for Biotechnology Information (NCBI) database.

According to the instructions of Geneaid, the manufacturer of the extraction kit,

Electrophoresis of PCR product

%2agarose gel electrophoresis was performed to verify the success of the amplification

Process.

8µl of each PCR product was loaded into the wells, along with a DNA Ladder Marker (100–1500 bp) at one end.

The device was operated at 100 V for 75–80 min, and DNA bands were observed using UV light.ity.

Results and discussion

Molecular diagnosis of bacterial isolates

DNA extraction

Seven isolates were selected for accurate molecular diagnosis after phenotypic and microscopic examination and diagnosis of the bacterial isolates.Genomic DNA was extracted from these isolates to analyze their 16S rRNA gene sequences. The results showed the success of DNA extraction from pure cultures of the isolates using the extraction kit provided by Macrogen (South Korea(

The presence of DNA in the selected isolates was confirmed by electrophoresis on agarose gel at a concentration of 2%, using an electric potential of 70 volts for one hour. The presence of DNA was confirmed by the appearance of distinct bands on the agarose gel in all isolates, as shown in Figure (4-1(.

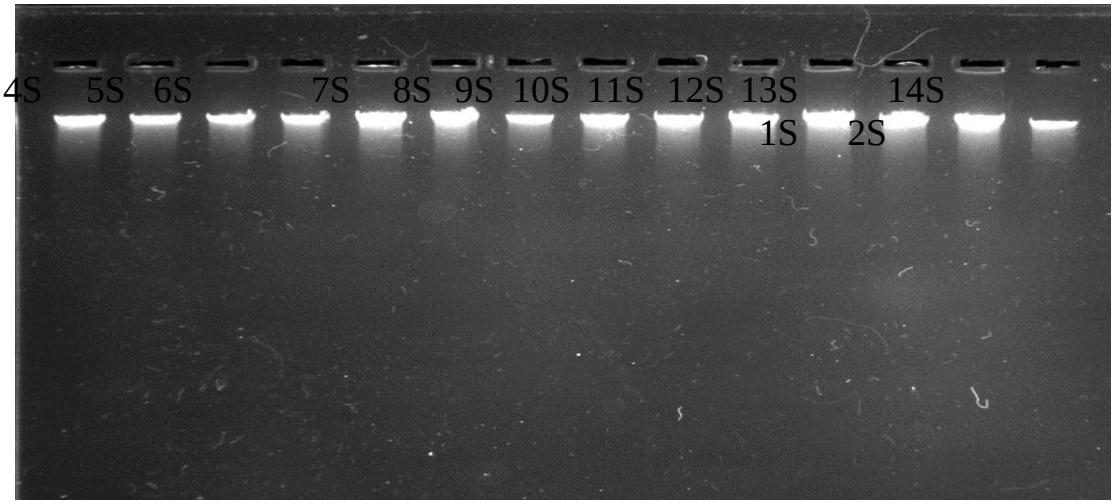
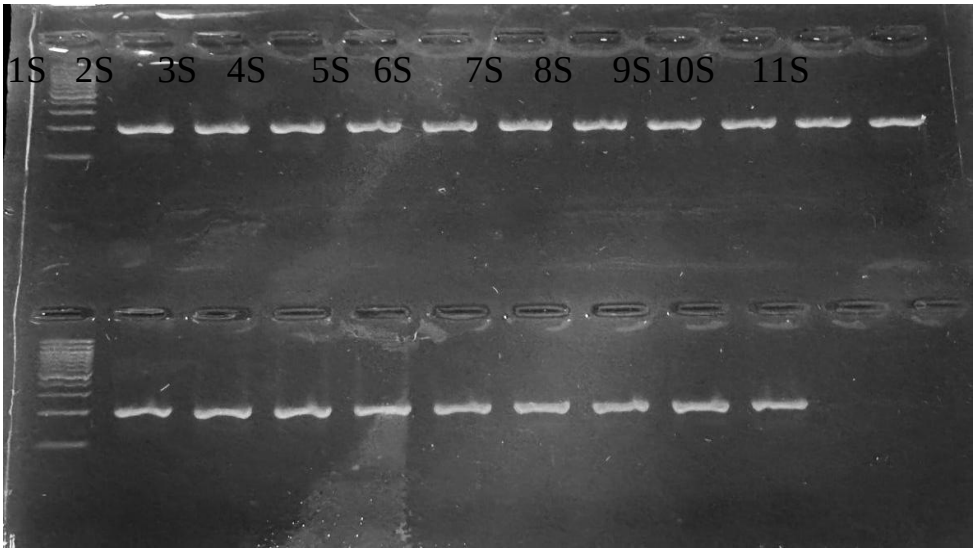


Figure (4-1): Electrophoresis of genomic DNA after the extraction process and the presence of DNA in the selected isolates through electrophoresis on agarose gel at a concentration of 2%, using an electrical voltage of 70 volts for one hour.

Electrophoresis of PCR products

The results of the S-rRNA16 gene amplification of DNA samples extracted from bacterial isolates showed the success of the amplification process using PCR technology,This was done by relying on specific primers, and the reaction products were analyzed using electrophoresis, where all samples showed clear bands at a molecular size of about 175 base pairs (bp), compared to the molecular size ladder (DNA ladder), which confirms the success of the primer binding and the occurrence of the replication process, as shown in Figure (4-2).



DNA LADDAR (175) R

300 175
200
12S 13S 14s 15S 16S 17 s
100

Figure 4-2 Electrophoresis of PCR amplification products of 16S-rNA gene from 18 bacterial isolates on 2% agarose gel at 70 V for 1.5 hours where the molecular size of the bands is 175 base pairs.

Molecular diagnosis of some types of *Salmonella enterica*

The results of 16S rRNA sequence analysis using the BLAST tool showed a high match with *Salmonella enterica* subsp. enterica serovar Kentucky strain KCID6, with a 99% identity (113/114 identical bases) and no alignment gaps. This statistical significance value was very high (E-value = 6e-49), indicating high diagnostic reliability. This genetic match confirms that the studied sample belongs to the *Salmonella enterica* Kentucky strain, which is consistent with previous studies that have demonstrated the presence of this strain in contaminated environmental aquatic sources (Fabre et al., 2018).

The presence of a single point mutation (SNP) in the studied sequence compared to the reference sequence may be attributed to several environmental factors, most notably environmental stresses in polluted water such as the presence of heavy metals, chemical pollutants, and antibiotics, which may stimulate mutation rates by affecting the accuracy of DNA replication enzymes (Martínez, 2009)

Oxidative stress resulting from pollution, which leads to the formation of reactive oxygen species (ROS) that cause damage to nitrogenous bases (Imlay, 2019)

Horizontal gene transfer is common among bacteria in aquatic environments, which may contribute to genetic changes or the emergence of new variants.

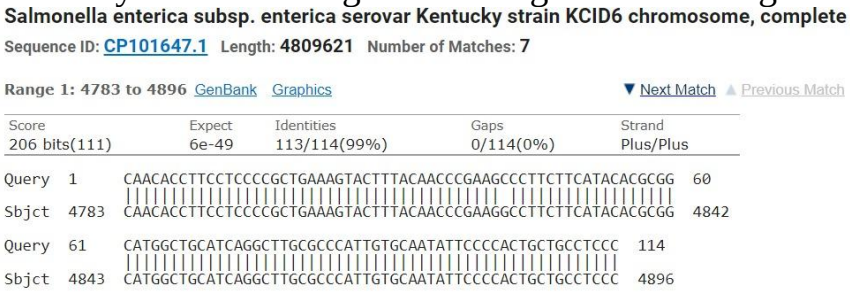


Figure (3) Alignment of nucleotide sequences of the 16S rRNA gene of *Salmonella enterica* (Query) with the primer Univ11750 and sequences of the same gene (sbjct) in the NCBI databases.

Molecular diagnosis of Pseudomonas spp.

Alignment results using the GenBank database and the BLASTn tool showed that the molecular sequence of the isolate (470 base pairs in length) is 98% (168/171) identical to the 16S rRNA gene sequence of the reference isolate Pseudomonas *aeruginosa* clone AY015 (GU414503.1). The E-value was

approximately 2e-76, which is a very low value indicating a strong statistically significant match. The high Identities value indicates a clear genetic closeness between the studied sequence and the reference sequence.

These results support the classification of the isolate as *P. aeruginosa*, as recent molecular studies have shown that ≥98.7% 16S rRNA sequence identity is an acceptable criterion for taxonomic identification at the bacterial species level (Kim et al., Commichaux et al., 2024). Despite the strong identity, three several minor genetic mutations were observed: one substitution and two insertion bases.

Recent research suggests that such mutations may be due to several causes, most notably environmental stress. Bacterial isolates that arise in polluted aquatic environments may be exposed to evolutionary pressures due to the presence of chemicals, antibiotics, or heavy metals, leading to the emergence of simple genetic mutations (Sharma et al., 2022).Mutations may result from uncorrected errors during DNA replication, a common process in bacteria, particularly in genes that do not directly code for proteins .Genetic variation is present even within a single species; small amounts of genetic variation (1–2%) can exist without affecting genetic classification or biological function (Martínez-Porchas et al., 2020).Recent ecological studies indicate that P. aeruginosa isolates from different environmental sources exhibit minor mutation patterns in conserved genes such as 16S rRNA in response to environmental adaptation (Qiao et al., 2025).

The results of this study are consistent with what has been documented in numerous local and international studies.

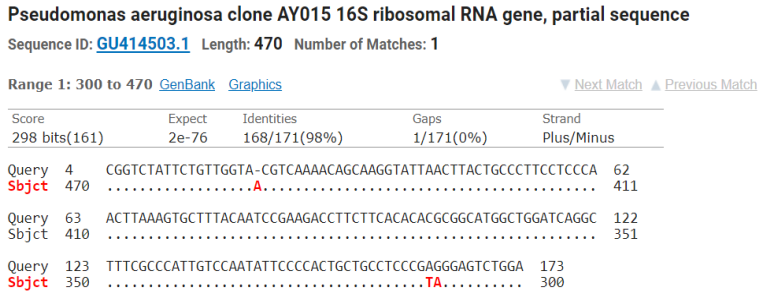


Figure (4) Alignment of nucleotide sequences of the 16S rRNA gene of P. citronellolis (Query) with the primer Univ11750 and sequences of the same gene (sbjct) in the NCBI databases.

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