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Original Research Article

Molecular Diagnosis of Some Gram-Negative Bacterial Genera from the Polluted Tigris River in Samarra

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Abstract: This study aimed to identify bacterial isolates from the Tigris River in Samarra using molecular techniques. The results revealed bacterial species from the genera Escherichia, Enterobacter, Aeromonas, Klebsiella, and Pseudomonas. 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. 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 suggesting the influence of environmental stress and pollution on the genetic structure of these bacteria.

Keywords: 16S rRNA Gene, *Klebsiella Pneumoniae,* Tigris River PCR.

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.

Polymerase chain reaction (PCR) is an important tool 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.

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.

MATERIALS AND METHODS

DNA extraction and PCR

All tools and materials used in the DNA extraction process were autoclaved, genomic DNA extraction According to the company of Geneaid by Genomic DNA Extraction kit, confirmed by agarose gel 1% fig (1).

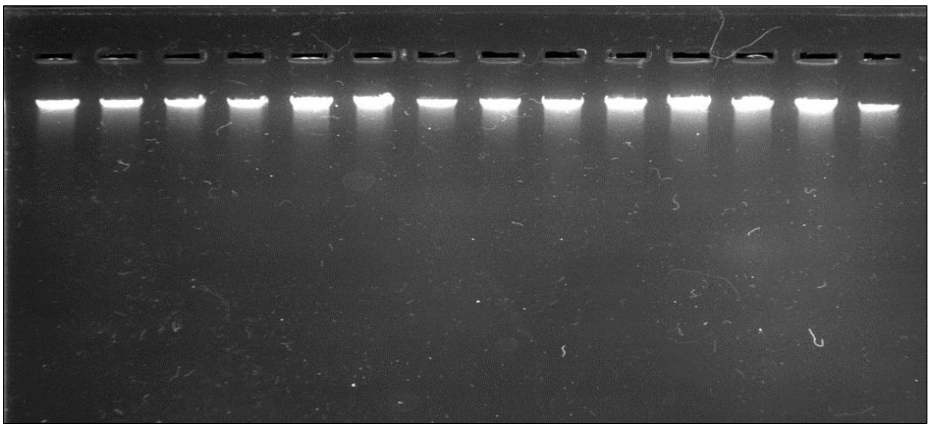

Fig. 1: Genomic DNA

Polymerase Chain Reaction

PCR using specific primers table (1) to amplify (175 bp) 16S rRNA gene with 2% agarose electrophoresis with (Scientific cleaver) to verify the amplification.

Table 1: sequence of specific primers for amplify16 s

Primer	Sequence 5` > 3`	Product	Reference
F		175 bp	
R			

Protocol of PCR fig (2) was first denaturation 95 ˚C for 5 min followed by 35 cycles denaturation 95 ˚C for 30 sec, annealing 63 ˚C for 30 sec, and extension 72 ˚C for 30 sec with final extension 72 ˚C for 7 min and holding 4 C for infinity, by thermal cycler verrati (applied biosystems).

Table 2: Protocol of PCR for amplify16 s

Step	Temp ˚C	Time / h	Cycles
First denaturation	95	5	1
denaturation	95	0·30	35
Annealing	63	0·30	
Extension	72	0·30	
Final extension	72	7	1
Holding	4	∞	

Results of S-rRNA16 gene amplification showed the success of the amplification process using PCR ,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 (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 Fig (2).

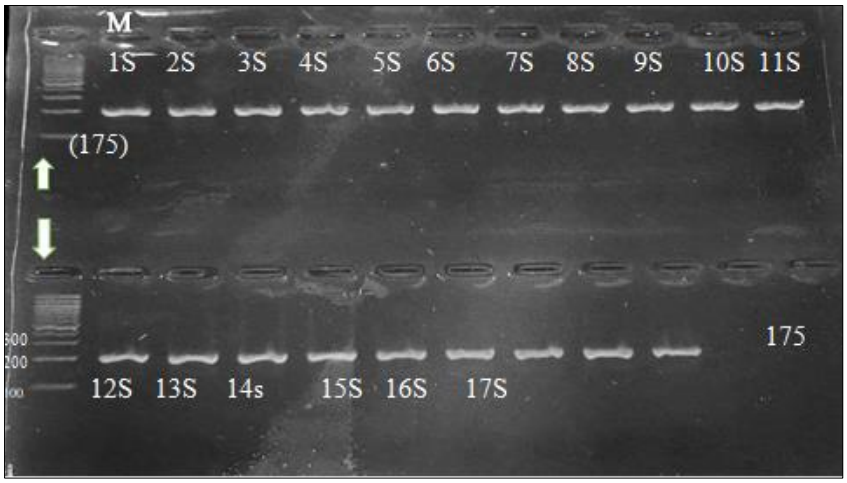

Fig. 2: PCR product electrophoresis with 2% agarose

RESULTS AND DISCUSSION

The results indicate the diagnosis of Enterobacter· *hormaechei* and Enterobacter· *cloacae* bacteria, as the nucleotide sequence of the 16S rRNA gene was 99% identical to the nucleotide sequence of the same gene and the same bacteria recorded in the nucleic acid database in NCBI· It is noted from the figure that there is a complete alignment of the nucleotide bases of the gene under study (sbjct) and the nucleotide bases of the same gene in the BLAST site in the NCBI database, as shown in Figure (3) and Figure (4), which confirms that the isolated bacteria are E· *cloacae* and E· *hormaechei*.

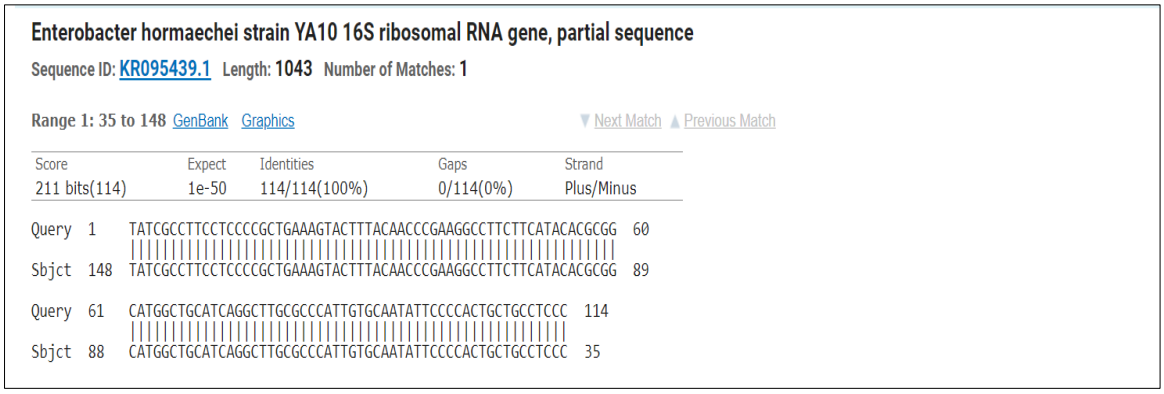

Figure 3: Alignment of nucleotide sequences of the 16S rRNA gene of Enterobacter hormaechei in the NCBI databases

The reason for the spread of species belonging to the genus Enterobacter spp in river waters and throughout the year is attributed to the fact that they are one of the species that grow in moderate water temperatures (mesophilic bacteria) (Gaalova *et al*., 2014).

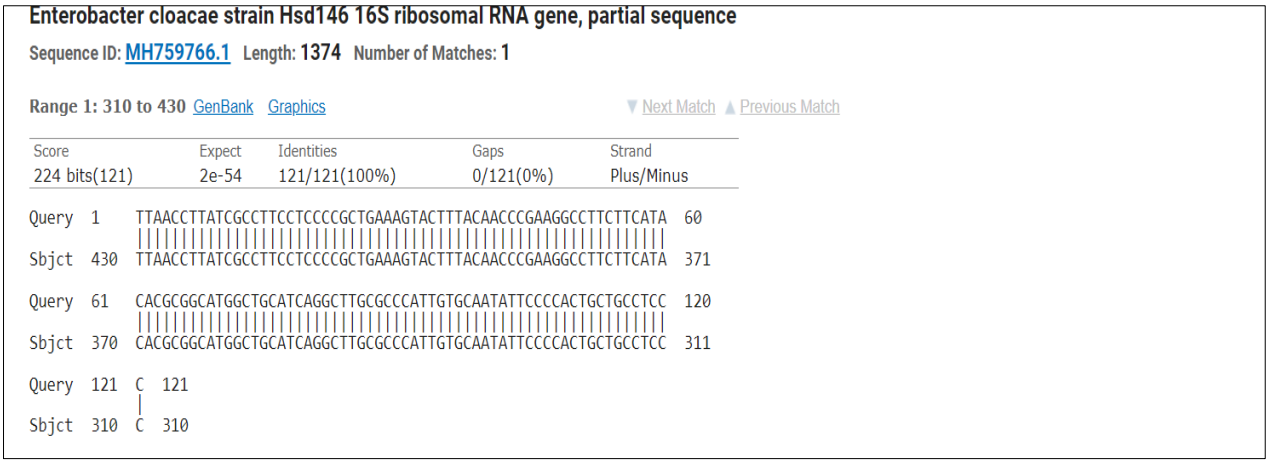

Figure 4: Alignment of nucleotide sequences of the 16S rRNA gene of Enterobacter cloacae with NCBI databases

It can be considered a traditional indicator of microbial pollution of water (UNEP/WHO, 2011) and has the ability to adapt to environmental conditions, as well as antibiotic resistance, in addition to reaching river waters when sewage and industrial wastewater are disposed of indiscriminately, as well as along with increased anthropogenic activities (Akhter *et al*., 2015).

The results shown in Figure (5) indicate the diagnosis of E· coli bacteria based on the molecular diagnosis of the 16S rRNA gene of this bacteria and comparing its nucleotide sequences to the same gene in NCBI with a probability of 99%· The presence of these species is likely due to fecal contamination from untreated wastewater and as a result of population expansion, agricultural activity and pastures (Pall *et al*., 2013).

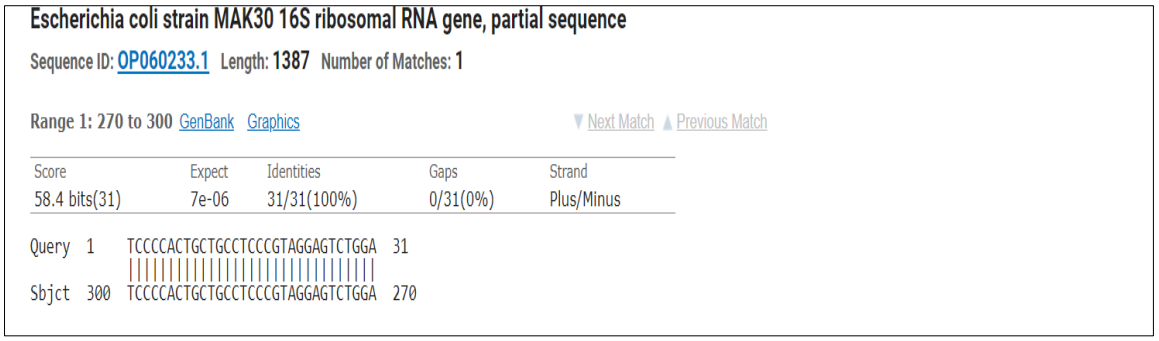

Figure 5: Alignment of nucleotide sequences of the 16S rRNA gene of E·coli bacteria sequences in the NCBI databases

Alignment analysis of the isolated DNA sequence of Klebsiella pneumoniae, using the BLAST tool, produced a high match with the reference sequence of K· pneumoniae strain K8· The match rate reached 98% at genomic positions (4034060–4034188) and 97% at positions (120974–121106), with identity scores of 128/130 and 132/136, respectively, and low E-values (2e-55 and 6e-55), reflecting high statistical significance.

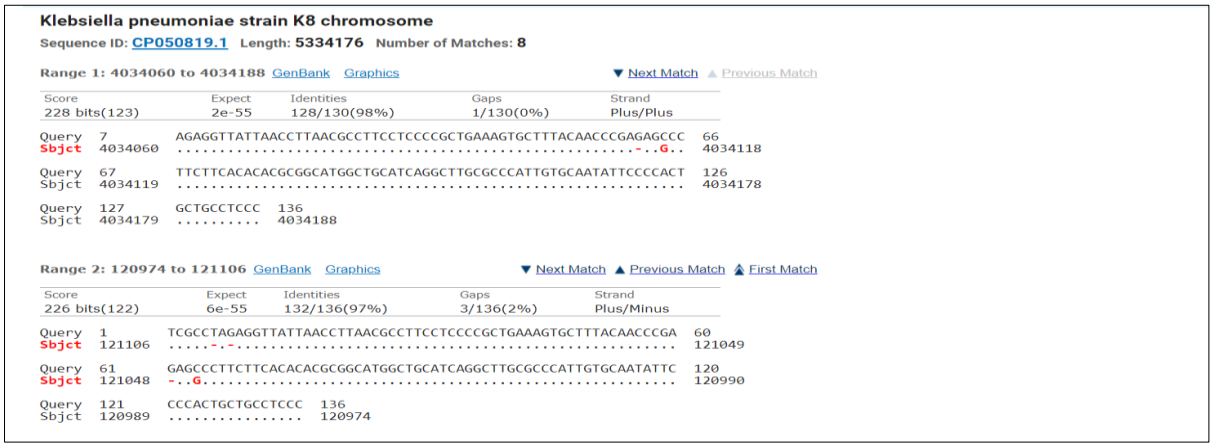

Figure 6: Alignment of nucleotide sequences of the 16S rRNA gene of Klebsiella pneumoniae in the NCBI databases

Despite this high homology, some genetic mutations were observed in the isolate sequence, including single-stranded base substitutions (SNPs), such as G to A translocations, and limited gaps· These mutations are likely the result of environmental stresses resulting from water pollution, such as the presence of chemical compounds, antibiotic residues, or heavy metals, which may lead to the generation of random mutations or stimulate horizontal gene transfer between microorganisms sharing the environment. These results are consistent with the findings of Araújo *et al*., (2024) that environmental isolates of Klebsiella pneumoniae exhibit distinct genotypes that differ from clinical strains, as a result of adaptation to polluted aquatic environments· Larsson and Flach (2022) also demonstrated that different environmental pressures are among the factors driving the emergence of genetic diversity in environmental bacteria, especially those living in environments with high selective pressure.

Molecular Diagnosis of Pseudomonas spp

Alignment results using the GenBank database and the BLASTn tool showed that the molecular sequence of the isolate 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.

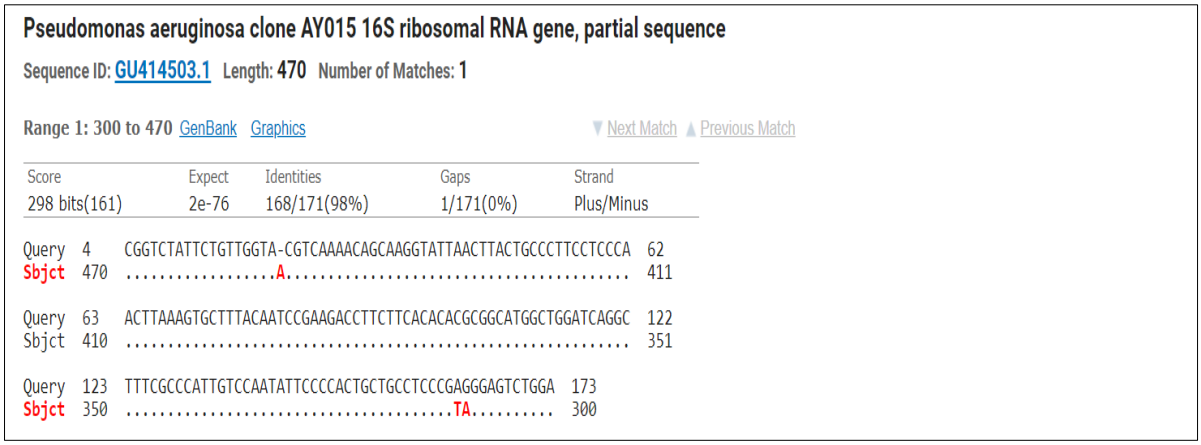

Figure 8: Alignment of nucleotide sequences of the 16S rRNA gene of P· citronellolis (Query) with the primer Univ11750 and sequences of the same gene (sbjct) in the NCBI databases

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