



Morphological and Molecular Characterization of Multidrug-Resistant Bacteria Isolated from Burn Infections in Samarra City of Iraq

Harith Ahmed Mustafa¹, Amidah Ali Atyah², Yousif Awad khalaf³, Haqi Ismael Jasim⁴

¹Department of Biology Sciences, University of Samarra, Samarra, Iraq; ²Department of Pathological Analysis, College of applied sciences, University of Samarra, Samarra, Iraq; ³Department of Biology Sciences, University of Samarra, Samarra, Iraq; ⁴Department of Biology Sciences, University of Samarra, Samarra, Iraq

Abstract

Background: Burn infections are a major cause of morbidity and mortality due to the high risk of bacterial colonization, antimicrobial resistance, and subsequent invasive infections, making accurate morphological and molecular characterization of multidrug resistant bacterial pathogens essential for guiding effective treatment and infection control strategies. **Objective:** The aim of this research was to isolate and perform morphological and molecular characterization of bacteria contaminating burns, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Citrobacter* species and to compare the molecular characterization of these bacteria before and after they developed antibiotic resistance and to determine if any mutations occurred at the molecular level. **Methodology:** This was a cross-sectional study that was conducted in the Department of Biology Sciences at University of Samarra, Samarra, Iraq. This study was carried out from November 2024 and February 2025. Bacteria were isolated and identified from 40 burn samples. Morphological and biochemical diagnosis revealed the presence of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Tests showed high resistance to common antibiotics such as penicillin and cefotaxime, with relative sensitivity to azithromycin. Molecular diagnosis using 16S rRNA gene sequencing confirmed the identity of the isolates, indicating the presence of other species such as *Pseudomonas aeruginosa* and *Enterobacter cloacae*. **Results:** These results confirm the prevalence of multidrug-resistant bacteria in burn injuries and highlight the importance of accurate molecular diagnosis and susceptibility testing for improving treatment and genetic surveillance of resistance. **Conclusion:** Analysis also revealed ribosomal gene mutations in some isolates following antibiotic exposure, suggesting the influence of selective pressure in the development of resistance. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):319-330]

Keywords: Burn wound infection; Multidrug resistance; 16S rRNA sequencing; Antibiotic susceptibility; resistance; sensitive; Polymerase Chain Reaction; NCBI (National Center for Biotechnology Information)

Correspondence: Harith Ahmed Mustafa, Department of Biology Sciences, University of Samarra, Samarra, Iraq; Email: harith.a.m@uosamarra.edu.iq; ORCID: <https://orcid.org/0000-0003-3516-6644>
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Introduction

Human skin is the layer that separates all the body's internal organs, tissues, and bodily fluids from the external environment. In addition to being an effective barrier in the immune system and playing a role in nerve sensation, it also maintains the body's

water balance. Therefore, any tear in the skin's surface layers due to a burn or wound will disrupt the skin's function, allowing microorganisms to penetrate the internal tissues and bloodstream. This provides them with a suitable, moist, and protein-rich environment that aids their growth¹.

Hospital-acquired infections are among the most difficult health problems faced by physicians who constantly deal with critical cases today.

Despite the use of modern technologies in hospitals during various surgical procedures, and the excellent and continuous care provided by medical staff to patients, hospital-acquired infections have become one of the most prominent and widespread problems globally, causing increasing economic losses. Humanity exacerbates the suffering of patients, prolongs their hospital stays, and increases the costs of their treatment. The development and progression of an infection depend on several factors, including the number, source, and virulence of the bacteria causing the infection, as well as the host's susceptibility, the mode and nature of exposure, and the route of bacterial transmission. These various factors are collectively referred to as the Chain of Infection. Resistant strains of *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella aerogenes* have emerged against many antibiotics. This is attributed to the presence of vector plasmids that contribute to the production of new resistant strains, or to the presence of genes that confer self-resistance, in addition to mechanisms that alter the drug target site.

This phenomenon has become an increasing medical problem due to the difficulty in controlling the diseases it causes. Furthermore, the indiscriminate and excessive use of antibiotics in treating or preventing bacterial and viral infections has led to a global increase in antibiotic resistance rates². In recent years, oxidative stress and microbial infections have emerged as key factors in wound repair and complications arising from these infections, especially when caused by multi-drug resistant bacteria. Comparative studies of inflammatory and oxidative stress markers in infectious diseases have revealed marked changes in these markers in bacterial conditions^{3,4}, and new products like polyherbal mouthwash have been reported to have antibacterial and antioxidant properties, and to decrease oxidative damage and inflammation in infected wounds that could be used in wound healing treatment⁵. The research aimed to isolate and perform morphological and molecular diagnosis of bacteria contaminating burns, and to compare the molecular diagnosis of bacteria before

and after antibiotic resistance, and whether mutations occurred at the molecular level.

Methodology

Study Design & Settings: This was a cross-sectional study that was conducted in the Department of Biology Sciences, University of Samarra, Samarra, Iraq. This study was carried out from November 2024 to February 2025.

Isolation and Identification of Bacteria: In this study, 40 samples were collected from patients with burn infections, of both sexes and various ages, admitted to the Specialized Burns Hospital at Samarra City. Samples were collected from patients in the outpatient clinic, emergency department, operating room, internal medicine department, and wound dressing department between mid-November 2024 and mid-February 2025. The swabs taken included 40 burn swabs from patients aged 18 to 65 years, with 20 swabs from females and 20 from males. The results showed bacterial growth in 40 samples on the media used, representing 100% of the total samples collected. Of the 20 burn swabs that showed positive growth, we selected for molecular examination from a total of 40 samples, distributed as follows: 14 males and 6 females.

Microscopic Diagnosis: The examination was performed by observing the bacteria's ability to stain with Gram stain, based on the instructions⁶.

Cultural Characteristics: Rapid diagnosis of the growing bacteria and their colonies was made by observing their shape, size, color, texture, edge shape, and smell.

Biochemical Test: Biochemical tests were conducted to identify the types of bacteria isolated⁷.

Antibiotic Sensitivity Testing: A sensitivity test was performed on all isolates against 5 commonly used antibiotics (Table 2). The diffusion method described by Kirby-Bauer was used, using Mueller-Hinton agar medium⁸. The resistance of the isolates under study to antibiotics was determined based on measuring the diameter of the inhibition zone using an eye ruler, as stated in a study⁸.

DNA Extraction and Polymerase Chain Reaction (PCR): Sterilization of the materials used and all instruments in the DNA isolation and extraction process was carried out, and confirmation of

Table 1: Types of Bacteria

Types of Bacteria	Oral	Urine	Wound	Total
<i>Pseudomonas aeruginosa</i>	2 (4.0%)	6 (12.0%)	8 (16.0%)	16 (32.0%)



Figure I: Agarose gel electrophoresis, which indicates PCR amplification bands of 16S rRNA gene at 175 bp

genomic DNA extraction was performed, based on Geneaid instructions, through a genomic DNA extraction kit, using a 1.0% agarose gel (Figure I). The amplification of the bacterial diagnostic marker gene *16S rRNA* was done using gene-specific primers to perform polymerase chain reaction (PCR), which produced a 175-base-pair (bp) amplification. The agarose gel electrophoresis of the amplification products was then done to ensure successful amplification by resolving the agarose gel products with a Cleaver Scientific electrophoresis system on a 2.0% agarose gel (Table 1).

Table 1: PCR Cycling Conditions to Amplify the *16S rRNA* Genes

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	63	30 sec	35
Extension	72	30 sec	35
Final Extension	72	7 min	1
Hold	4	∞	-

The thermal cycling conditions were as follows: firstly, an initial denaturation at 950 C was performed with 35 cycles of denaturation at 950 C, 30 seconds; primer annealing, 630 C, 30 seconds; and extension, 720 C, 30 seconds. An extension step was done at 720 C, 7 minutes, and the reaction was maintained at 4°C at the end. All amplifications were done with the help of a Veriti thermal cycler (Applied Biosystems) (Figure II).

The *16S rRNA* gene amplification was positive in all the isolates tested successfully. Electrophoresis of the PCR products showed clearly and distinctly formed bands with a molecular size of about 175 bp, which is in line with the expected size of the amplicon when compared with a molecular weight

ladder of the DNA. These findings validate the selectivity of primer binding and the effectiveness of the amplification reaction (Figure II).

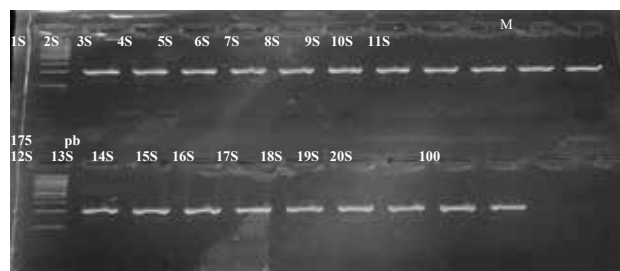


Figure II: Polymerase Chain Reaction (PCR) product electrophoresis with 2.0% agarose gel

Identification: Isolates were identified by observing their morphological characteristics on media, such as colony color, size, and shape, Gram staining, and microscopic arrangement. Biochemical tests were then performed, and the results were recorded for later comparison with established references to characterize each isolate. The results showed bacterial growth in 40 samples on the aforementioned media, representing 100 % of the total collected samples. Twenty burn swabs that showed bacterial growth were selected for molecular examination from a total of 20 samples, distributed as follows: 14 males and 6 females.

Identification of Gram-Positive Bacterial Species: Three isolates of *Staph. aureus* identified. They appeared on blood agar as smooth, medium-sized, yellow to golden circles (Figure II).



Figure III: Showing *Staph. aureus* Bacteria Fermenting Mannitol

After Gram staining, they appeared as grape-like clusters or in pairs. As shown in Figure III, when cultured on mannitol saline agar at 37°C, the isolates fermented mannitol, appearing as a distinct golden yellow color (Figure IV to Figure VII). They were positive for the catalase test and negative for the oxidase test. Six *Staphylococcus epidermidis* isolates were identified as non- γ -hemolyzed on blood agar. They were spherical, shiny, white to gray in color, and arranged in clusters. Their identification results were positive for the catalase test and negative for the oxidase and coagulase tests. They did not ferment mannitol.

Ethical Consideration: Research Ethics Approval No. 20240032, No. 32, dated 22/12 / 2024.

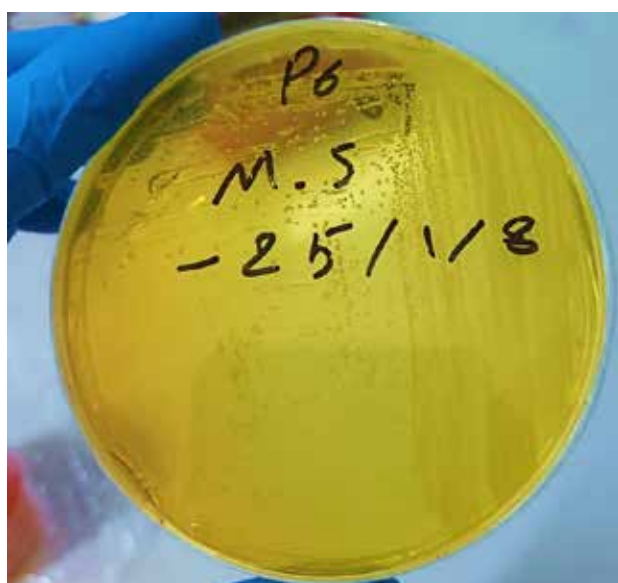


Figure IV: Showing *Staphylococcus epidermidis*

Results

Antibiotic Resistance Testing: One of the most serious and significant medical problems related to antibiotic resistance is the emergence of strains

resistant to more than one antibiotic. This makes it difficult to select the appropriate treatment for these bacteria. A major cause of multidrug resistance is the indiscriminate use of antibiotics without prior sensitivity testing, which increases bacterial adaptation and, consequently, resistance to these antibiotics⁹. The response of isolated bacteria to five antibiotics was studied by measuring the diameter of the inhibition zone.

Resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis* to antibiotics: The results, as shown in Figures VI to II, indicate that *Staph. aureus* exhibited 100.0% resistance to penicillin. This may be attributed to the presence of plasmids carrying specific genes that code for changes in the ribosomal structure, which inhibit its binding to this antibiotic. The bacteria also showed 100.0% resistance to cefotaxime. Regarding azithromycin, the bacteria showed sensitivity,

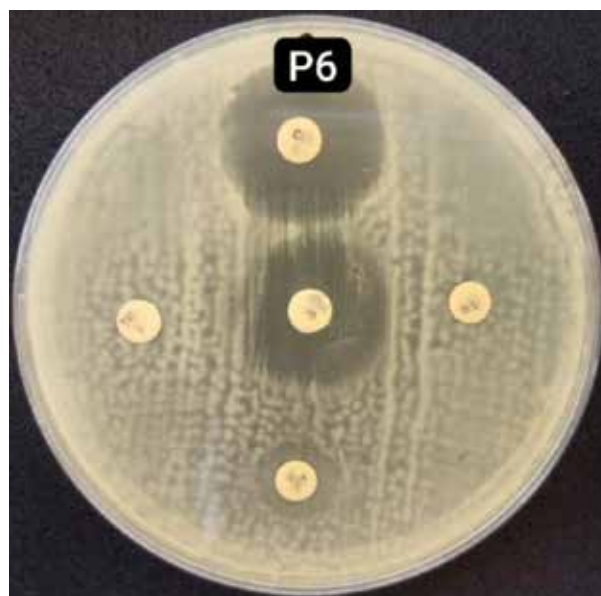


Figure V: Showing *Staphylococcus aureus* on Mueller-Hinton agar

Table 6: Inhibition Zone of *Pseudomonas aeruginosa* by the Well Diffusion Method

Samples	Amoxicillin	Tetracycline	Azithromycin	Cefotaxime	Penicillin
P1	0.0 (R)	12m (S)	30m (S)	0.0 (R)	0.0 (R)
P2	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)
P3	0.0 (R)	0.0 (R)	28m (S)	0.0 (R)	0.0 (R)
P4	0.0 (R)	0.0 (R)	25m (S)	0.0 (R)	0.0 (R)
P5	0.0 (R)	13m (S)	22m (S)	0.0 (R)	0.0 (R)
P6	0.0 (R)	0.0 (R)	30m (S)	0.0 (R)	0.0 (R)
P7	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)
P8	0.0 (R)	0.0 (R)	12m (S)	0.0 (R)	0.0 (R)
P9	8m (S)	0.0 (R)	9m (S)	0.0 (R)	0.0 (R)
P10	0.0 (R)	0.0 (R)	11m (S)	0.0 (R)	0.0 (R)

R =resistance , S = sensitive

measured at a diameter of 30 mm for *Staph aureus* causing burn infections. They were completely resistant to amoxicillin and to tetracycline, measured at a diameter of 9 mm on a ruler. *Staph. epidermidis* bacteria showed complete resistance (100.0%) to cefotaxime, tetracycline, and amoxicillin. Azithromycin showed sensitivity up to a full 25 mm diameter on a ruler, and penicillin showed resistance (Table 2).

Molecular diagnosis of *Staphylococcus* species:

These results show the complete nucleotide sequences of the gene under investigation, and the nucleotide sequence of the same gene in the BLAST program is also added to them. locus is within the National Center for Biotechnology Information (NCBI) database for the *Staph* genus. The isolate of *Staph aureus* showed a 96.0% concordance with only two gaps (1.0%) in the alignment. Several single nucleotide polymorphisms (SNPs) were observed, including substitutions of nitrogenous bases such as C to G and G to A, as well as other minor variations. These mutations are largely

normal and reflect intraspecific variation. Molecular alignment analysis of the 16S rRNA gene of the studied isolate showed a 94.0% concordance with the reference sequence of *Staph. haemolyticus*. There were 132 concordances out of 141 bases, with three gaps (2.0%) and three point mutations. Point mutations were observed, consisting of nitrogenous base substitutions at specific positions, such as G↔T and A↔G, along with insertion/deletion gaps. These mutations are likely due to a combination of environmental and genetic factors that may affect the bacteria. In polluted environments or those containing antibiotics or heavy metals, environmental stress induces genetic changes that aid survival, such as mutations in conserved genes. These mutations occur during DNA replication due to errors in polymerase enzyme function and are normal, even in conserved genes like 16S rRNA. Conjugation, transformation, or transduction processes can introduce new mutations or foreign sequences into the bacterial genome. Minor, non-significant mutations may accumulate in

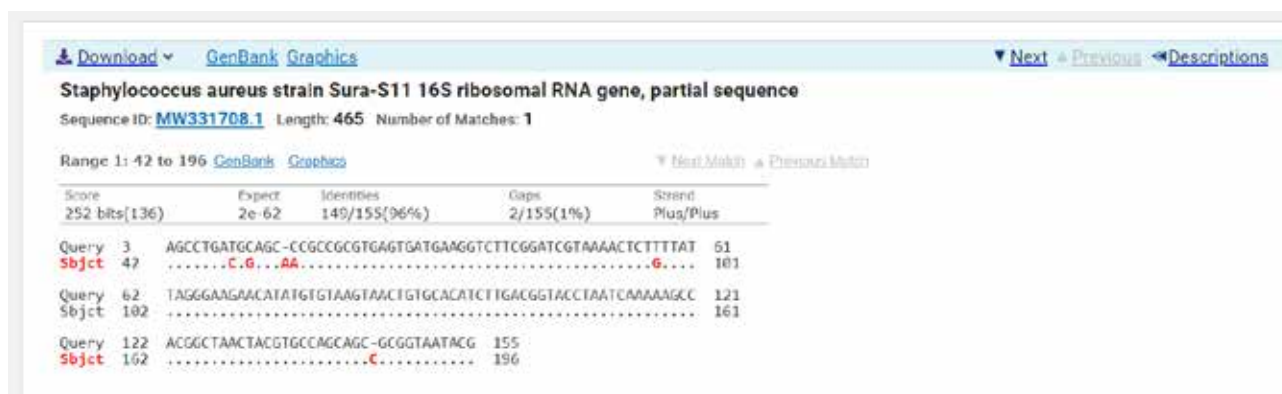


Figure VI: Nucleotide sequence comparison between the 16S rRNA gene of *Staphylococcus aureus* isolates (Query) and universal primer Univ11750 against reference sequences of that gene available in the NCBI GenBank database (Subject).

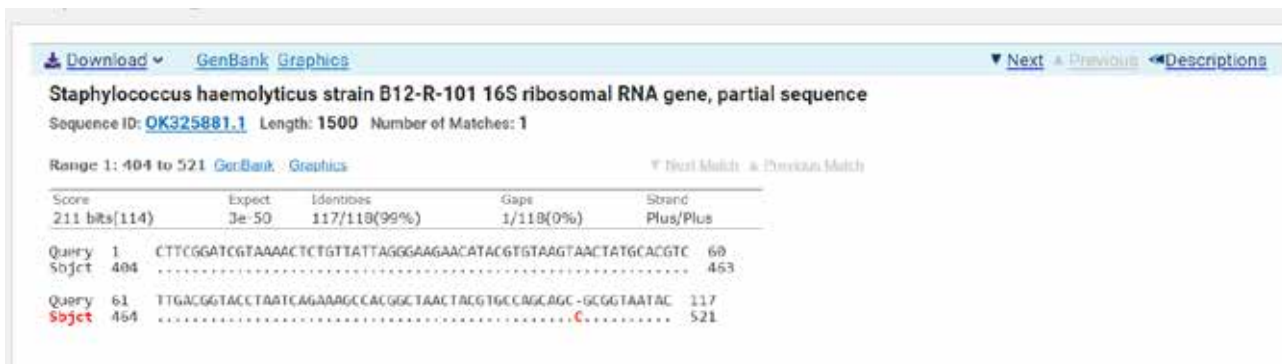


Figure VII: Nucleotide sequence comparison of 16S rRNA gene of *Staphylococcus haemolyticus* isolates (Query) with using universal primer Univ11750, compared with the reference sequences of the same gene obtained in the NCBI GenBank database (Subject).

non-vital regions of the gene over time, leading to slight variation between strains without altering the gene's biological function. A recent study¹⁰ confirmed that the presence of 16S rRNA mutations with a 93 to 97.0% match warrants classifying the isolate as very close to the known species, with the need for further sequencing to definitively confirm the identity (Figure VII).

***Pseudomonas* species Molecular Identification:**

The analysis of the sequences alignment showed an exact match between the nucleotides sequences of the target gene obtained in the clinical isolates and the appropriate reference sequences available in the NCBI GenBank database. The 100.0% sequence identity was observed with all the isolates to 16S rRNA gene of *Pseudomonas aeruginosa*, and no replacements, additions, or gaps of the nucleotides were identified. These results are not only unambiguous molecular identification of the genus *Pseudomonas aeruginosa* (Figure XI).

These findings play into the other findings made

by¹¹, who confirmed that 16S rRNA gene sequencing is a very effective and valid technique of identifying bacterial species, especially when isolates are obtained in an environmental and clinical setting. Moreover, the method has been commonly used in the diagnosis and differentiation of more closely related bacterial taxa which would otherwise be hard to differentiate by means of more traditional biochemical and phenotypic phenotypes.

Molecular Identification of Selected *Enterobacteriaceae* Species:

The presence of *Enterobacter cloacae* was established through molecular diagnostic analysis with a sequence identity to the 16S rRNA gene of the recovered isolate and the corresponding reference sequence in NCBI nucleotide database being 98.0%. Analysis done using BLAST alignment revealed that the query sequence had a high concordance with the subject sequence but the three substitution point mutations were observed in the 3rd line of the alignment, with a final result of 152 same bases out

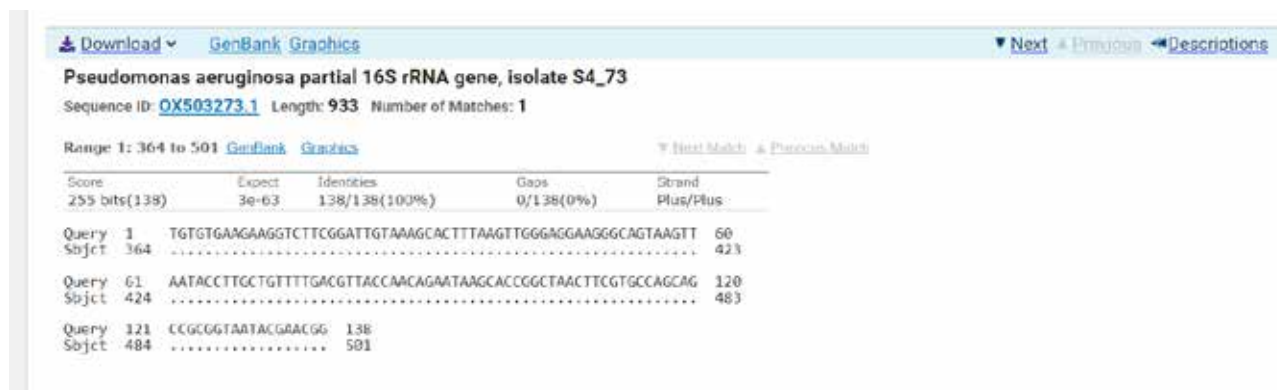


Figure VIII: Alignment of the 16S rRNA gene of *Pseudomonas aeruginosa* isolates (Query) and a universal primer Univ11750 and aligned against reference sequences of the same gene found in the NCBI GenBank database (Subject).

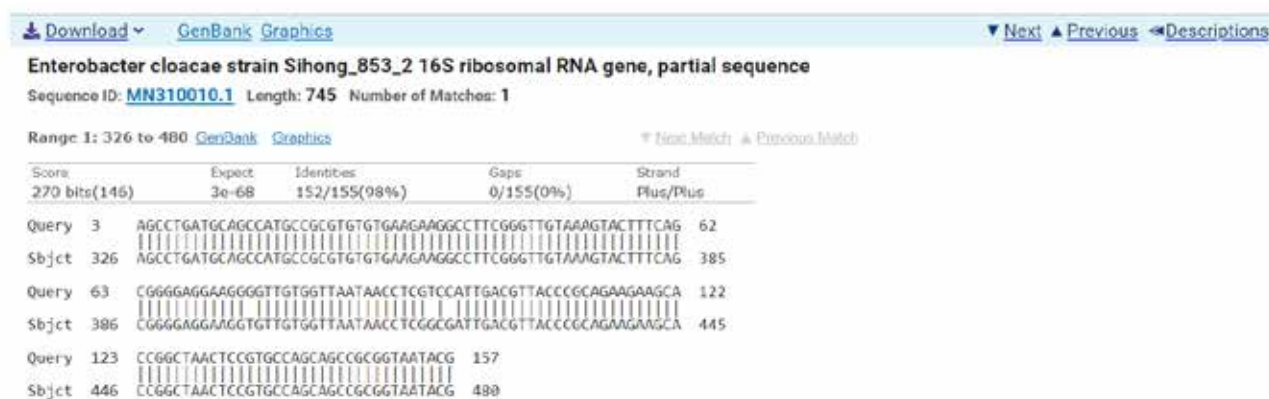


Figure IX: Showing the alignment of the nucleotide sequences of the 16S rRNA gene of *E. cloacae* (Query) with the primer Univ11750 and the sequences of the same gene (sbjct) in the NCBI databases.

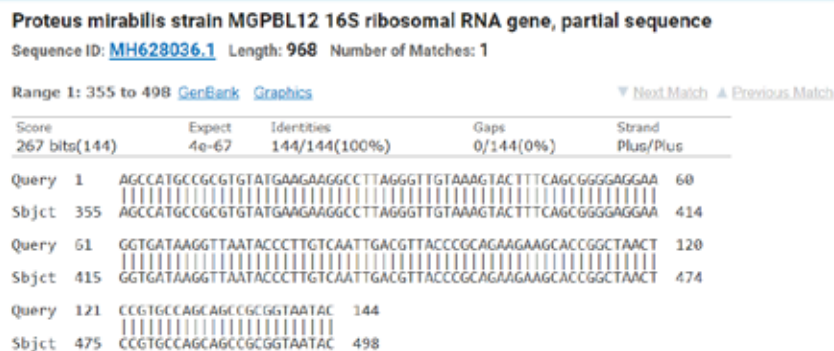


Figure X: The figure shows the alignment of the nucleotide base sequences of the 16S rRNA gene of the bacterium *Proteus mirabilis* (Query) with the primer Univ11750 and the sequences belonging to the same gene (sbjct) in the NCBI databases.

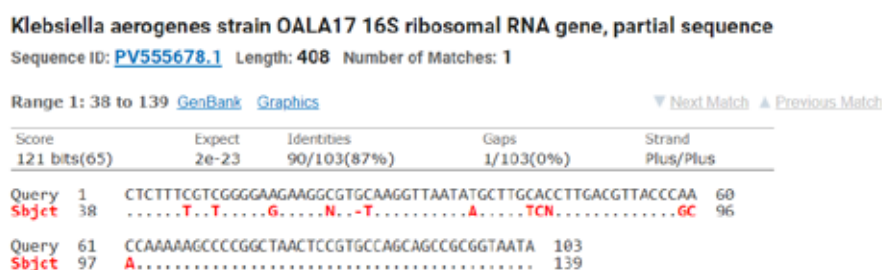


Figure XI: Alignment of the nucleotide sequence of the 16S rRNA gene of the isolates of *Klebsiella aerogenes* (Query) with the universal primer Univ11750 in comparison with the reference sequences of the same gene of *Klebsiella aerogenes* (Subject) stored in the NCBI GenBank database.

of 155 bases. No gaps of insertions or deletions were observed. These results confirm the identity of the isolated organism as *E. cloacae*, whereas the type of point mutations indicates the existence of small intraspecific genetic variation¹².

Molecular diagnosis also allowed discovering the *Proteus mirabilis*, which has its sequence of the 16S rRNA gene that is 100 per cent similar to the corresponding reference sequence that is also stored in the NCBI nucleotide database. The result of BLAST alignment indicated 100 percent concordance between query and subject sequences and no nucleotide substitute, insertion, and/or gaps. These findings clearly prove the identification of the isolated organism as *Proteus mirabilis*¹³.

The sequence analysis of 16S rRNA gene of one of the bacterial isolates found at the site of a skin burn wound showed that the genetic similarity between a sample and a reference sequence of *Klebsiella aerogenes* registered in NCBI database was 87.0% with 90 identical bases out of 103 total positions. Nine point mutations and one insertion/deletion gap have been detected which suggests that there is a certain level of partial genetic divergence and it could be attributed to the specificity of phenotype of

this isolate. Several substitution mutations were observed in various locations in the sequence including the base transitions and transversions like T-G, T-N, and A-G at positions 38, 61 and 97 of the reference sequence respectively. Such mutational patterns could be explained by the selective pressure of the environment on the site of the burn wound which is a highly stressful microenvironment characterized by high temperatures, necrotic tissue accumulation, presence of reactive oxidizing agents, and a strong host immune reaction¹⁴.

Molecular Diagnosis of *Citrobacter* spp.: The identification of *Citrobacter* spp. by molecular diagnostic analysis was verified by the sequence of 16S rRNA gene of the recovered isolate, which has shown the 97.0% sequence identity with the respective reference sequences of the same gene and organism in the NCBI nucleotide database. The alignment of the BLAST sequence showed that there is a high concordance between the nucleotide sequence of the analyzed gene (Query) and the reference subject sequence (Subject), which, in turn, proves that the isolated organism is of genus *Citrobacter* sp. Five mutations were detected in the alignment region that consisted of four nucleotide

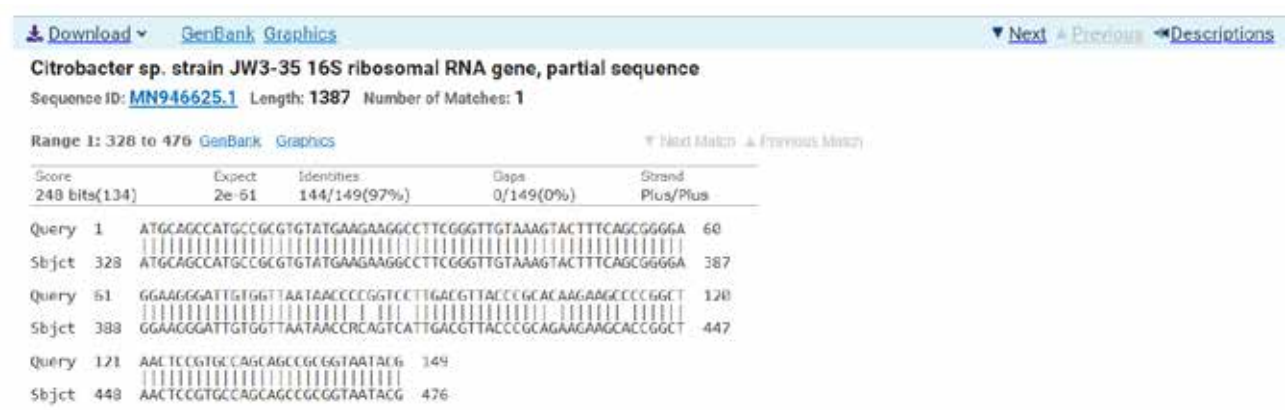


Figure XII: Nucleotide sequences comparison of the 16S rRNA gene of *Citrobacter* spp. isolated (Query) with the universal primer Univ11750 and reference sequences of the 16S rRNA gene of the same gene obtained in the NCBI GenBank database (Subject).

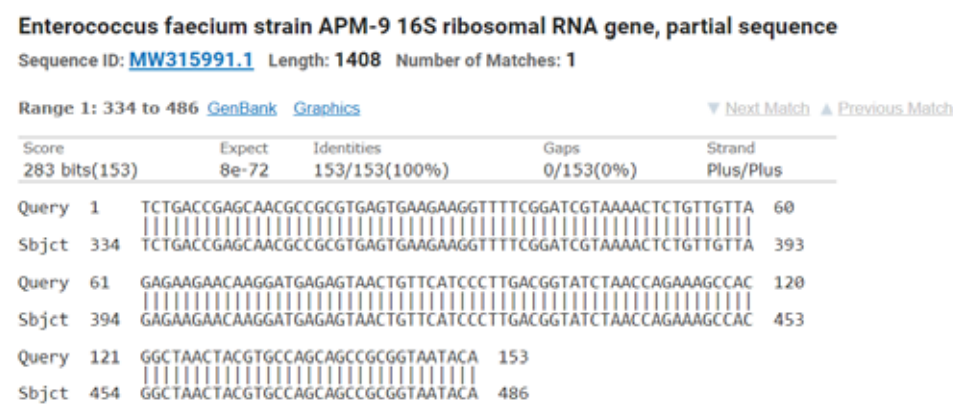


Figure XIII: The figure showed alignment of the nucleotide base sequences of the 16S rRNA gene in *Enterococcus faecium* (Query) bacteria mediated by the use of the primer Univ11750 and the subordinate sequences of the same gene (sbjct) in the NCBI subordinate databases

transition substitutions and a deletion event that had happened at the first position of the alignment¹⁵.
Enterococcus faecium Molecular identification:
Molecular diagnostic analysis was used to identify the organism as *Enterococcus faecium* as the 16S

rRNA gene sequence of the recovered isolate showed full sequence homology with the corresponding reference sequence of the same gene and the same organism that is contained in the NCBI nucleotide database. Analysis of BLAST sequence

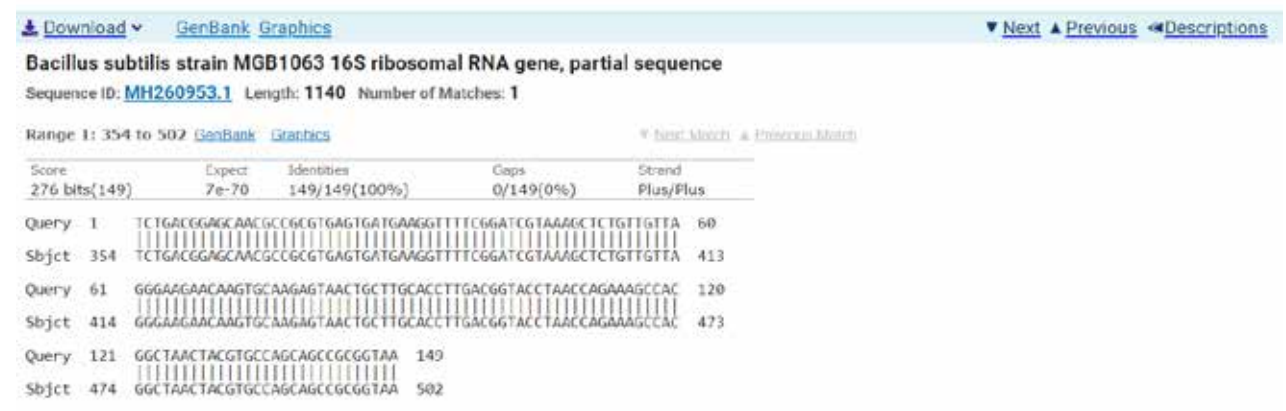


Figure XIV: The alignment of the sequences of the 16S rRNA gene of *Bacillus subtilis* isolates (Query) with the universal primer Univ11750, and compared against the reference sequences of the same gene obtained in NCBI GenBank database (Subject).

Citrobacter sp. strain BGS9 16S ribosomal RNA gene, partial sequenceSequence ID: [MN209834.1](#) Length: 1388 Number of Matches: 1Range 1: 309 to 481 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
300 bits(162)	5e-77	170/173(98%)	3/173(1%)	Plus/Plus
Query 6	TATTGC-C-ATGGGCGC-AGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGG	62		
Sbjct 309	A.A.....A.....	368		
Query 63	GTTGTAAGTACTTTCAGCGGGGAGGAAGGGATTGTGGTTAATAACCGCAGTCATTGACG	122		
Sbjct 369		428		
Query 123	TTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGTAATACG	175		
Sbjct 429		481		

Figure XV: The figure contains an alignment of the nucleotide base sequences of the *16S rRNA* gene in *Citrobacter* (Query) bacteria with the primer Univ11750 and the sequences belonging to the same gene (sbjct) in the NCBI bases

Enterobacter cloacae strain Sihong_853_2 16S ribosomal RNA gene, partial sequenceSequence ID: [MN310010.1](#) Length: 745 Number of Matches: 1Range 1: 309 to 479 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
279 bits(151)	1e-70	165/171(96%)	3/171(1%)	Plus/Plus
Query 7	ATTGC-C-ATGGGCGC-AGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCT			
Sbjct 309	A.A.....A.....			
Query 64	TTGTAAGTACTTTCAGCGGGGAGGAAGGGGTTGTGGTTAATAACCTCGGTCATT			
Sbjct 369	T.....CG....			
Query 124	TACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGTAATAC	175		
Sbjct 429		479		

Figure XVI: The figure contains an alignment of the nucleotide base sequences of the *16S rRNA* gene in *Enterobacter cloacae* (Query) with the primer Univ11750 and the subordinate sequences of the same gene (sbjct) in the NCBI databases

alignment showed that there were no deletions, insertions, or gaps between the nucleotide sequence of gene being studied (Query) and that of the reference subject sequence (Subject). These observations confirm beyond any doubts that the organism is *Enterococcus faecium*, which is isolated¹⁶.

Pantoea agglomerans are identified by molecular means: Sequencing of 16S rRNA gene of one bacterial isolate obtained in wound samples indicated that it had 95 percent sequence identity with the reference sequences of the genus *Pantoea*, specifically *Pantoea agglomerans*. BLAST analysis alignment revealed the presence of single nucleotide polymorphisms (SNPs) and a single gap in the area of alignment, which is one of the signs of intraspecific genetic diversity. This diversity is often found in human-derived *Pantoea agglomerans* isolates, and can be attributed to adaptive changes in response to tissue microenvironmental, infection-related selection pressure, antibiotic stress

via wound treatment protocol, and in response to environmental and nosocomial contaminants¹⁷. There are growing indications that these mutations can confer increased survival ability in clinical isolates in the inflammatory microenvironment, compared to group counterparts in the environment¹⁸.

Article Discussion

Mutational discussion of the 16S rRNA Gene After exposure to antibiotics.

Enterobacter cloacae

The *16S rRNA* gene sequencing of *Enterobacter cloacae* isolates showed that the nucleotide point mutations appeared after the exposure of the bacteria to antibiotics, and no mutations were observed before the treatment. This time series is very indicative that antibiotic exposure was an act of selection / mutagenesis. The mutational patterns are usually clustered in hypervariable regions of the 16S rRNA

gene that regulate the ribosomal secondary structure and the conformation of binding sites to antibiotics¹⁹. Transition mutations which were mostly found were adenine to guanine (A-G) and cytosine to thymine (C-T) mutations. These are the most common mutation mutations observed on the ribosomal genes, which occur due to the process of transcriptional errors during the rRNA generation under the condition of oxidative stress caused by exposure to antibiotics²⁰. It is worth noting that these mutations are often functional, at sites 1408 and 1491 of the *rrs* gene, directly involved with the binding of aminoglycosides to the decoding center of the 30S ribosomal subunit²¹.

The A1408G replacement, specifically, leads to a change in conformations at the aminoglycoside binding site, significantly decreasing the binding affinity of gentamicin and amikacin to the ribosome and therefore, increasing the high antimicrobial resistance²². These mutations that occur after exposure to antibiotics can be explained by two major mechanisms. The former is the selective pressure, in which exposure to antibiotics kills the vulnerable bacterial cells, and leaves surviving, growing, and clonal expansion of cells which already possess pre-existing resistance conferring mutations. The second is the antibiotic-induced mutagenesis, whereby some antimicrobial agents stimulate bacterial stress response pathways, mainly the SOS response which raises the rate of error in DNA repair process and enhances the chance of a de novo mutational event²³.

Mutations in the *16S rRNA* gene signify one of the most clinically relevant resistance mechanisms to the aminoglycoside antibiotics, such as gentamicin, amikacin, and kanamycin, which have their bactericidal effect on high-affinity binding to particular locations in the *16S rRNA* to alter bacterial translational fidelity. Similar mutations have been identified in clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* recovered off wound, urinary, and blood that additional causes resistance-associated mutations in this gene^{24,25}.

Interestingly, new evidence suggests that the resistance provided by *16S rRNA* mutations can be enhanced by other mechanisms in particular, the ribosomal RNA methylation provided by *16S rRNA* methyltransferases e.g., ArmA and NpmA. These enzymes are catalysts at the identical sites of methylation in the rRNA targeting sterically inhibiting the binding of antibiotics, generating high-level resistance phenotypic levels²⁶ Based on this, post-exposure mutations can be detected which would indicate a synergistic relationship between

direct gene mutation and ribosome chemical modification.

These results are also of significant clinical implications since mutations in the *16S rRNA* gene can predict stable and heritable genetic resistance that cannot be reversed after the removal of the selective pressure. Moreover, their occurrence in isolates related to wounds increases the risk of horizontal gene transfer through the dissemination of plasmids, which is a significant epidemiological hazard in the hospital environment²⁷.

Enterobacter Cloacae

The *16S rRNA* gene sequence of *Citrobacter* sp. strain BGS9 and this base substitution NCBI reference sequence (MN209834.1) on comparison showed few point substitutions (A→G and C→A base substitutions) and minor gaps (1.0% of the alignment) in the conserved region (Range: 309481). Such transitions are in line with the existence of transition and transversion mutations in which the latter is a well-described mutational category, which develops in the course of the selection pressure of antibiotic exposure²⁸.

The A to G replacement is considered as a transition mutation which in most cases causes a very little change in the *16S rRNA* secondary structure in normal circumstances. But in situations where they are found within functional sensitive regions - especially the secondary loop structures that border the decoding center of the 30S ribosomal subunit - the presence of such substitutions can greatly impair the binding affinity of the aminoglycoside antibiotics, including gentamicin and amikacin²⁹. Conversely, C 2 A transversion mutation is linked with a stronger alteration of inter-base hydrogen bonding and, possibly, a loss of the spatial stability of the rRNA, which leads to structural changes in the antibiotic binding site³⁰.

Such mutations are important molecular cues of the presence of selective pressure due to antibiotics. It is important to note that no mutations were observed in pre-treatment samples but their blatant occurrence after antibiotic exposure is a strong indication that the strain went through clonal selection that favored those cells with pre-existing resistance-conferring mutations. Past research has already determined that point mutations in the *16S rRNA* gene are an initial and crucial channel in the development of bacterial resistance when subjected to frequent antibiotic attack³¹. The *16S rRNA* gene as a whole is sensitive to any change in the sequence as it is a gene that encodes functional domains that are directly related to the construction of the antibiotic binding site in the

ribosome. Substantial amounts of evidence in the literature are supportive of the fact that aminoglycoside resistance in various bacterial species, such as *Citrobacter* spp., *Pseudomonas aeruginosa*, and *Escherichia coli*, is mediated by mutations in homologous areas of the *rrs* gene. In addition, some mutant strains can develop a higher resistance capacity due to the simultaneous expression of *16S rRNA* methyltransferases (RMTases) including ArmA and NpmA that chemically modify the same rRNA targets and consequently enhances resistance^{32,33}.

Altogether, these results support the idea that the *16S rRNA* sequence changes observed in *Citrobacter* sp. isolates after exposure to antibiotics are adaptive molecular responses aimed at reducing the drug-target binding efficacy to result in the formation of stable and transmissible drug resistance. The fact that mutations were only present in 1.0% of the sequence whereas 98.0% were not was an indication that mutational events were not random but the mutations were only restricted to some very important loci - a pattern that is indicative enough to cause functionally important changes without disrupting the overall structural integrity of the ribosomal gene³⁴.

Conclusion

The results demonstrate the significant impact of multidrug-resistant bacteria on burn wound infections. Molecular identification using *16S rRNA* sequencing provided precise characterization of bacterial isolates and revealed genetic variations associated with antibiotic exposure. Mutations observed following antibiotic exposure suggest that antibiotic pressure may drive the development and persistence of resistance. These findings highlight the importance of routine antibiotic susceptibility testing and molecular surveillance in guiding treatment and preventing the spread of resistant bacteria within burn units.

Acknowledgements

None

Conflict of interest:

The authors declare that they have no conflict of interest.

Financial Disclosure

None.

Authors' contributions

None.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can

provide it upon justifiable request.

Ethics Approval and Consent to Participate

The study received ethical approval from the Research Ethics Committee of the College of Education for Pure Sciences, Samarra University, Salahaddin, Iraq (Reference No. 20240032, No. 32; December 22, 2024). The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

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How to cite this article: Mustafa HA, Atyiah AA, Khalaf YA, Jasim HI; Morphological and Molecular characterization of multidrug-resistant bacteria isolated from burn infections in Baghdad Medical City/Iraq; Bangladesh J Infect Dis 2026;13(1): 319-330

ORCID:

Harith Ahmed Mustafa: <https://orcid.org/0000-0003-3516-6644>

Amidah Ali Atyiah: <https://orcid.org/0000-0002-9374-0408>

Yousif Awad Khalaf: <https://orcid.org/0000-0002-3501-822>

Haqi Ismael Jasim: <https://orcid.org/0009-0003-0360-255>

Article Info

Received on: 1 June 2025

Accepted on: 12 October 2025

Published on: 1 June 2026

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