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# Gene Sequencing of Gelatinase among *E. faecalis* Isolates

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## ABSTRACT

**Background:** *Enterococcus* is a genus of Gram-positive, catalase-negative, non-spore-forming, facultatively anaerobic bacteria that may exist alone or in chains. The lactic acid bacteria (LAB) that generate bacteriocins include the enterococci. *gelE* on the *E. faecalis* chromosome codes for gelatinase, a zinc-containing metalloproteinase that is released.
**Objective:** Virulence gene sequencing for gelatinase in *E. faecalis* strains
**Materials and methods:** Patients hospitalized and seen at Baghdad's Al-Karama Hospital and Medical City Hospital throughout a three-month period (May to July 2022) provided the study's 200 participants with urine and vaginal specimens. The samples were cultured for (18-24) hours in various mediums. Then, they were incubated at (37ºC) for (18-24) hours on a number of different selective media. When first trying to identify *E. faecalis*, scientists relied on colony morphology, microscopic examinations, and biochemical assays.
**Results:** All 200 clinical samples cultured positive, however only 44(22%) of the isolates were associated with *E. faecalis*, the automated Vitek 2 system employed GP-ID cards containing 64 biochemical assays to ensure the isolates were really *E. faecalis*. Using this method, it was able to quickly identify 44 different bacterial isolates, with a confidence level ranging from excellent (probability percentage of (94 to 99.7%).The virulence factor *gelatinase* gene in *E. faecalis* was studied in 47 isolates of the bacteria obtained from various environments. Results from our analysis indicated that, only 14 of the 44 isolates (31.8%) tested positive for this gene, with a molecular length of 213bp. The sequencing of *gelE* gene shows for *E. faecalis* having one transversion A/C, and the effect Missense. From the Gene Bank, found part of *gelE* gene having 99% compatibility with the subject of *gelE* gene in NCBI under sequence ID: CP0881981. Another part of sequencing for *gelE* gene to *E. faecalis*, the results shows compatibility of 100% in Gene Bank of *gelE* under sequence ID: CP088198.1, so no recorded change noticed from the gene in this isolate. Furthermore, neighbour phylogenetic distances in this tree indicated a wide biological diversity of *E. faecalis* sequences.
**Conclusions:** The pathogenicity of *E. faecalis* increased by presence of *gelE* gene.

**Keywords**: PCR, *Enterococcus faecalis*, *gelE* gene, UTIs.

## INTRODUCTION

*Enterococcus* is a genus of Gram-positive, catalase-negative, non-spore-forming, facultatively anaerobic bacteria that may exist alone or in chains. The lactic acid bacteria (LAB) that generate bacteriocins include the enterococci. Not only do these organisms not produce endospores, but their little flagellum may also provide them with some degree of motility. They cluster together in a white, creamy color [1].

While the majority of enterococci are anaerobes, there are a few species that need oxygen to survive. Pectin and cellulose are not digested by enterococci, and nitrate reduction is not a typical metabolic process for these bacteria. They are a common, possibly dangerous species that has evolved a resistance to or phenotypic tolerance for various types of disinfectants and physical agents [2].

*gelE* on the *E. faecalis* chromosome codes for gelatinase, a zinc-containing metalloproteinase that is released. Gelatinase is manufactured as a 509-amino-acid pre-properly peptide, and then its pre-sequence or signal sequence and pro-sequence are cleaved off at the amino-terminal end. Macrophages were discovered to respond to collagen peptides by releasing a cascade of inflammatory molecules, including hydrogen peroxide, superoxide anion, elastase, and gelatinase [3].

A virulence factor called serine protease (*sprE*) is hypothesized to contribute to systemic illness in mammals. *sprE*, a gene that encodes a serine protease, is located directly downstream of *gelE* and is co-transcribed with it. This gene produces a 26-kDa serine protease that is secreted and has similarity with *S. aureus* V8 protease [4].

This quorum-sensing mechanism is controlled by an auto inducing peptide termed gelatinase biosynthesis-activating pheromone, and it positively regulates the expression of pathogenicity-related extracellular proteases in *E. faecalis* (GBAP). GBAP is a lactone-linked 11-amino-acid cyclic peptide [5].

Sequencing the areas downstream of *gelE*, the gene coding for the gelatinase, allowed the serine protease to be identified. Protein kinase E (*gelE*) and serine protease E (*sprE*) were shown to be cotranscribed in a Northern blot [6]. The fsr (*E. faecalis* regulator) locus was discovered after sequencing the areas upstream of the *gelE* gene, and it has three open reading frames. Gelatinase and serine protease expression is controlled by the quorum-sensing Fsr system [7]. The gelatinase gene, *gelE*, is part of an operon with the serine protease gene, *sprE*. Direct and indirect harm to host tissues is caused by the action of these proteases, which also nourish bacteria with peptide nutrition [8].

### *Aim of study*

Virulence gene sequencing for gelatinase in *E. faecalis* strains.

## MATERIALS AND METHODS

This study were included 200 patients, the specimens were collected from urine and vagina and from patients who were admitted and visit in Al-Karama Hospital and Medical City Hospital in the capital, Baghdad, during a period of three months from May to end of July, 2022.

### *Ethical Approval*

The necessary ethical approval from ethical committee of the hospitals and patients and their followers must obtained. Moreover, all subjects involved in this work are verbal informed and the agreement required for doing the experiments and publication of this work are obtained from each one prior the collection samples No. BMS/ 0226/016.

### *Clinical specimens*

The proper specimens collected for bacteriological analysis are described below. Those specimens were collected in proper ways to avoid any possible contamination.

### *Urine samples*

The specimen were generally collected from patients suffering from UTIs. Mid-stream urine samples were collected in sterilized screw-cap containers, then the urine samples were inoculated on selective media (Chromo agar) and incubated aerobically at 37°C for 24h [9].

### *Vaginal swabs*

The samples were generally collected from women (pregnant and non-pregnant) suffering from vaginitis. The swabs were inserted into the posterior fornix, upper part of the vagina and rotated there before withdrawing them. A vaginal speculum was also used to provide a clear sight of the cervix and the swabs were rubbed in and around the introits of the cervix and withdrawn without contamination of the vaginal wall. Swab for culture should be placed in tubes containing normal saline to maintain the swab moist until taken to laboratory. The swab was inoculated on selective media (Chromo agar) and incubated aerobically at 37°C for 24 hs. [10].

***Identification of Enterococcus faecalis with Vitek2 System***

Vitek 2 medical microbiology used as an automatic identification (ID) instrument device.

***DNA Extraction***

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (Korea).

***Detection of gelE of E. faecalis virulence gene***

DNA (extract from bacterial cells) was used as a template in specific PCRs for the detection of *gelE* virulence gene. DNA was purified from bacterial cells by using the Geneaid DNA extraction Kit. The primers used for the amplification of a fragment gene were listed in Table (1).

**TABLE 1:** the primers, sequences, and PCR conditions

| Gene name | Primer sequence (5´- 3´) | Size of Bp | Conditions | References |
|---|---|---|---|---|
| ***gelE*** | F: TATGACAATGCTTTTTGGGAT<br>R: AGATGCACCCGAAATAATATA | 213 | 95ºC, 5 min.<br>95ºC, 1 min.<br>56ºC, 1 min.<br>72ºC, 1 min.<br>72ºC, 10 min. | [11] |

***Determinate of DNA sequencing for gelE virulence gene.***

One specific PCR fragment partially covering the coding regions of the gelE proteins were selected in this study. The amplified fragments were directly exposed to direct sequencing experiments to assess the pattern of genetic polymorphism in the collected bacterial samples. Then, specific comprehensive trees were built to assess the accurate discrimination of the observed variants and their phylogenetic distribution.

***Nucleic acids sequencing of PCR amplicons***

The resolved PCR amplicons were commercially sequenced from both (forward and reverse) directions, following to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified.

***Interpretation of sequencing data***

The sequencing results of the PCR products of the targeted samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome. The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the bacterial sequences was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com).

***Translation of nucleic acid variations into anima acid residues***

The amino acid sequences of the targeted protein were retrieved online from the protein data bank (http://www.ncbi.nlm.nih.gov). The observed nucleic acid variants in the coding portions of the analyzed genetic loci were translated into a reading frame corresponding to the referring amino acid residues in the encoded protein using the Expasy online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was conducted between the referring amino acid sequences and their observed mutated counterpart using the "align" script of the BioEdit server.

## RESULTS

Patients hospitalized and seen at Baghdad's Al-Karama Hospital and Medical City Hospital throughout a three-month period (May to July 2022) provided the study's 200 participants with urine and vaginal specimens. At (37ºC), the samples were cultured for (18-24) hours in various mediums. Then, they were incubated at (37ºC) for (18-24) hours on a number of different selective media (Chromo Agar Medium). When first trying to identify *E. faecalis*, scientists relied on colony morphology, microscopic examinations, and biochemical assays. All 200 clinical samples cultured positive, however only 44(22%) of the isolates were associated with *E.*

*faecalis*. As can be seen in Figure (1), the automated Vitek 2 system employed GP-ID cards containing 64 biochemical assays to ensure the isolates were really *E. faecalis* as shown in Figure (1). Using this method, it was able to quickly identify 44 different bacterial isolates, with a confidence level ranging from excellent (probability percentage of (94 to 99.7%). The results were shown in Table (2).

**TABLE 2:** Isolation and identification of *E. faecalis* by specific media, biochemical test and automated Vitek 2 system

| No. of samples | specific media | | biochemical test | | automated Vitek 2 system | |
|---|---|---|---|---|---|---|
| | positive results | Negative results | positive results | Negative results | positive results | Negative results |
| 200 | 44(22%) | - | 44(22%) | - | 44(22%) | - |

Patient Name: medical, noor
Location:
Lab ID: 183202111

Patient ID: 183202111
Physician:
Isolate Number: 1

Organism Quantity:
**Selected Organism : Enterococcus faecalis**

**Source:**

**Collected:**

| **Comments:** | |
|---|---|
| | |
| | |

| **Identification Information** | **Analysis Time:** | 2.85 hours | **Status:** | Final |
|---|---|---|---|---|
| **Selected Organism** | 99% Probability | **Enterococcus faecalis** | | |
| | **Bionumber:** | 156012765773471 | | |
| **ID Analysis Messages** | | | | |

**Biochemical Details**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | + | 4 | PIPLC | - | 5 | dXYL | - | 8 | ADH1 | + | 9 | BGAL | - | 11 | AGLU | + |
| 13 | APPA | - | 14 | CDEX | + | 15 | AspA | + | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | - |
| 20 | LeuA | + | 23 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | PyrA | + | 27 | BGUR | - |
| 28 | AlaA | + | 29 | TyrA | + | 30 | dSOR | + | 31 | URE | - | 32 | POLYB | + | 37 | dGAL | + |
| 38 | dRIB | + | 39 | lLATk | - | 42 | LAC | + | 44 | NAG | + | 45 | dMAL | + | 46 | BACI | + |
| 47 | NOVO | + | 50 | NC6.5 | + | 52 | dMAN | + | 53 | dMNE | + | 54 | MBdG | + | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | - | 59 | SAL | + | 60 | SAC | + | 62 | dTRE | + | 63 | ADH2s | + |
| 64 | OPTO | + | | | | | | | | | | | | | | | |

**FIGURE 1:** Identification of *Enterococcus faecalis* by using automated Vitek 2 system

The virulence factor gelatinase gene in *E. faecalis* was studied in 47 isolates of the bacteria obtained from various environments. Results from our analysis indicated that, only 14 of the 44 isolates (31.8%) tested positive for this gene, with a molecular length of 213 bp as shown in Figure (2).

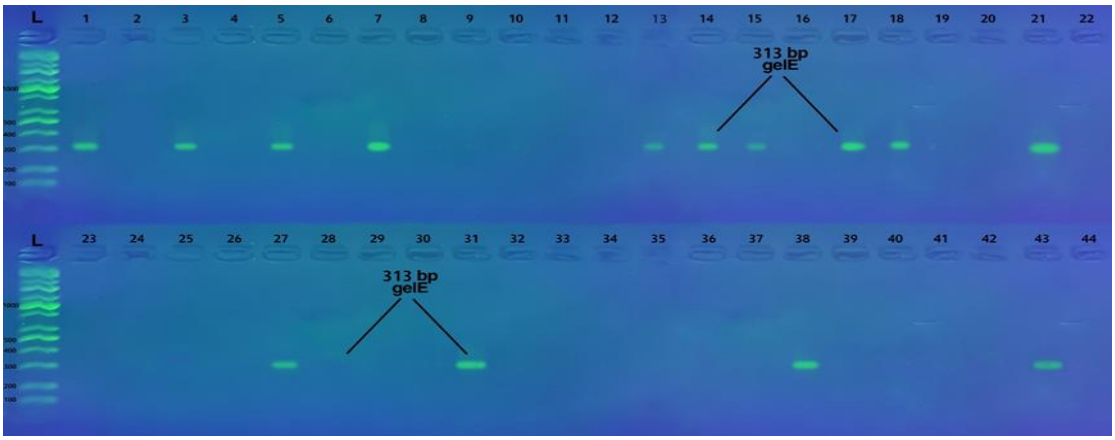


**FIGURE 2.** Agarose gel electrophoresis (1.5%) of PCR amplified of *gelE* gene at (313bp) of *E. faecalis* for (55) min at 5 volt/cm2. 1x TBE buffer for 1:30 hours. L: DNA ladder (100).

***DNA sequencing for of gelE virulence gene***

After confirming the amplification of *gelE* gene by conventional PCR, 20µl from PCR reaction with 50µl of forward primer for this gene was send to Macrogen Company to determine the DNA sequencing in this gene. Homology search was conducted using Basic Local Alignment search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov), and BioEdit program. The results were compared with data obtained from gene bank published ExPASY program which is available at the NCBI online. The *gelE* gene was amplified by PCR method, and send for sequencing service to Macrogen Company Korea. Thr sequencing result of *gelE* gene shows for *E. faecalis* having one transversion A/C in location (1592444 nucleotide) from the Gene Bank found part of *gelE* gene having 99% compatibility with the subject of *gelE* gene in NCBI under sequence (ID: CP0881981) as showed in Table (3), Figures (3A-D in appendix). Another part of sequencing for *gelE* gene to *E. faecalis*, the results shows compatibility of 100% in Gene Bank of *gelE* under sequence ID: CP088198.1, so no recorded change noticed from the gene in this isolate.

**TABLE 3:** Determinate of DNA sequencing for some important virulence genes

| Source : Enterococcus faecalis | | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Type of substitution** | **Location** | **Nucleotide** | **Sequence ID with compare** | **gene** | **Identities** |
| **1.** | **Transvertion** | 1592444 | A\C | **ID: CP088198.1** | ***gelE*** | **99%** |
| **2.** | **Transvertion** | 1592458 | A\C | **ID: CP088198.1** | ***gelE*** | **99%** |
| | **Transvertion** | 1592444 | A\C | | | |
| **3.** | **Transvertion** | 1592444 | A\C | **ID: CP088198.1** | ***gelE*** | **99%** |
| **4.** | ---------- | ------ | ----- | **ID: CP088198.1** | ***gelE*** | **100%** |

***Enterococcus faecalis* strain S11-6 chromosome, complete genome Sequence ID: CP088198.1Length: 2717320Number of Matches: 1**

Range 1: 1592274 to 1592460GenBankGraphicsNext MatchPrevious Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 334 bits(369) | 1e-89 | 186/187(99%) | 0/187(0%) | Plus/Minus |

Query 1
CAATGCGTTATGGTGACACAAGTACACCAACAGGAAAAACGTATGCTTCCTCTTTAGATG 60
**Sbjct** 1592460 .....**A**..... 1592401
Query 61
TAGTTGGTCATGAAATGACACATGGTGTGACGGAACATACTGCCGGTTTAGAATATTTAG 120
Sbjct 1592400 ..... 1592341
Query 121
GACAATCAGGTGCCTTGAATGAATCTTATTCTGATTTGATGGGTTATATTATTTCGGGTG 180
Sbjct 1592340 ..... 1592281
Query 181 CATCTAA 187
Sbjct 1592280 ..... 1592274

**FIGURE (3A):** Alignment analysis of *gelE* gene (four isolates 1, 2, 3, 4) of *E. faecalis* with Gene Bank at NCBI. Query represents of sample; subject represents a database of National Center Biotechnology Information (NCBI).

***Enterococcus faecalis* strain S11-6 chromosome, complete genome Sequence ID: CP088198.1Length: 2717320Number of Matches: 1**

Range 1: 1592274 to 1592460GenBankGraphicsNext MatchPrevious Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 329 bits(364) | 2e-88 | 185/187(99%) | 0/187(0%) | Plus/Minus |

Query 1
CACTGCGTTATGGTGACACAAGTACACCAACAGGAAAAACGTATGCTTCCTCTTTAGATG
60
**Sbjct** 1592460 ..**A**.....**A**..... 1592401
Query 61
TAGTTGGTCATGAAATGACACATGGTGTGACGGAACATACTGCCGGTTTAGAATATTTAG
120
Sbjct 1592400 ..... 1592341
Query 121
GACAATCAGGTGCCTTGAATGAATCTTATTCTGATTTGATGGGTTATATTATTTCGGGTG
180
Sbjct 1592340 ..... 1592281
Query 181 CATCTAA 187
Sbjct 1592280 ..... 1592274

**FIGURE (3B):** Alignment analysis of *gelE* gene (four isolates 1, 2, 3, 4) of *E. faecalis* with Gene Bank at NCBI. Query represents of sample; subject represents a database of National Center Biotechnology Information (NCBI).

***Enterococcus faecalis* strain S11-6 chromosome, complete genome**
**Sequence ID: CP088198.1Length: 2717320Number of Matches: 1**
Range 1: 1592274 to 1592460GenBankGraphicsNext MatchPrevious Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 334 bits(369) | 1e-89 | 186/187(99%) | 0/187(0%) | Plus/Minus |

Query 1
CAATGCGTTATGGTGACACAAGTACACCAACAGGAAAAACGTATGCTTCCTCTTTAGATG
60
**Sbjct** 1592460 .....**A**..... 1592401
Query 61
TAGTTGGTCATGAAATGACACATGGTGTGACGGAACATACTGCCGGTTTAGAATATTTAG
120
Sbjct 1592400 ..... 1592341
Query 121
GACAATCAGGTGCCTTGAATGAATCTTATTCTGATTTGATGGGTTATATTATTTCGGGTG
180
Sbjct 1592340 ..... 1592281
Query 181 CATCTAA 187
Sbjct 1592280 ..... 1592274

**FIGURE (3C):** Alignment analysis of *gelE* gene (four isolates 1, 2, 3, 4) of *E. faecalis* with Gene Bank at NCBI. Query represents of sample; subject represents a database of National Center Biotechnology Information (NCBI).

***Enterococcus faecalis* strain S11-6 chromosome, complete genome**
**Sequence ID: CP088198.1Length: 2717320Number of Matches: 1**
Range 1: 1592274 to 1592460GenBankGraphicsNext MatchPrevious Match

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 338 bits(374) | 3e-91 | 187/187(100%) | 0/187(0%) | Plus/Minus |

Query 1
CAATGCGTTATGGTGAAACAAGTACACCAACAGGAAAAACGTATGCTTCCTCTTTAGATG
60

Sbjct 1592460 ..... 1592401
Query 61
TAGTTGGTCATGAAATGACACATGGTGTGACGGAACATACTGCCGGTTTAGAATATTTAG
120
Sbjct 1592400 ..... 1592341
Query 121
GACAATCAGGTGCCTTGAATGAATCTTATTCTGATTTGATGGGTTATATTATTTCGGGTG
180
Sbjct 1592340 ..... 1592281
Query 181 CATCTAA 187
Sbjct 1592280 ..... 1592274

**FIGURE (3D):** Alignment analysis of *gelE* gene (four isolates 1, 2, 3, 4) of *E. faecalis* with Gene Bank at NCBI. Query represents of sample; subject represents a database of National Center Biotechnology Information (NCBI).

***Phylogeny of gelE gene***

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid variation observed in the amplified 213bp of *gelE* gene amplicons. This phylogenetic tree was contained four samples alongside other relative nucleic acid sequences of *E. faecalis* sequences. These different genera were incorporated to assess the pattern of genera relatedness and to investigate the extent of the possible effect of the observed nucleic acid variation on the actual positioning of the investigated samples in the out-genera regions. Within this tree, investigated samples were incorporate alongside other relative sequences to constitute only one major clade of incorporated sequences within the cladogram. No other related sequences were found to be highly correlated to the incorporated sequences of *E. faecalis*.

This data indicated the highly specific ability of *gelE* gene-based amplicons to detect these bacterial particles without including any noticeable homology with other sequences of other species whether being in the same genus or other out group sequences.

The incorporated sequences within this clade showed the presence of various strains of *E. faecalis* sequences with variable origins within the major clade, the samples were distributed in to closely associated positions closely- related phylogenetic distances. It was observed that, four samples were samples were positioned in the immediate vicinity to several Asian and European strains of E. faecalis, including Chinese (GenBank acc. No. CP028835.1), Japanese (GenBank acc. No. AP018543.1), Viet Nam (GenBank acc. No. MH720045.1), Thailand (GenBank acc. No. CP070621.1), South Korea (GenBank acc. No. CP041344.1), two Switzerland (GenBank acc. No. CP088198.1 and CP015410.2), USA (GenBank acc. No. CP046108.1), United Kingdom (GenBank acc. No. LR607378.1), Spain GenBank acc. No. CP029612.1), Germany (GenBank acc. No. CP022712.1), Czech Republic (GenBank acc. No. CP046247.1), France (GenBank acc. No. CP065784.1) and Canada (GenBank acc. No. CP050491.1). these results were shown in Table (4), Figure (4).

**TABLE 4:** The NCBI-BLAST Homology Sequence identity (%) between local *gelE* gene *E. faecalis* isolates and NCBI-BLAST submitted *gelE* gene *E. faecalis* isolates in other countries

| No. | Accession | Country | Source | Compatibility |
|---|---|---|---|---|
| **1.** | **ID: CP088198.1** | Switzerland | *E. faecalis* (*gelE*) gene | **99%** |
| **2.** | **ID: CP046108.1** | USA | *E. faecalis* (*gelE*) gene | **99%** |
| **3.** | **ID: LR607378.1** | United Kingdom | *E. faecalis* (*gelE*) gene | **99%** |
| **4.** | **ID: CP041344.1** | South Korea | *E. faecalis* (*gelE*) gene | **99%** |
| **5.** | **ID: CP028835.1** | China | *E. faecalis* (*gelE*) gene | **99%** |
| **6.** | **ID: MH720045.1** | Viet Nam | *E. faecalis* (*gelE*) gene | **99%** |
| **7.** | **ID: CP029612.1** | Spain | *E. faecalis* (*gelE*) gene | **99%** |
| **8.** | **ID: AP018543.1** | Japan | *E. faecalis* (*gelE*) gene | **99%** |
| **9.** | **ID: CP022712.1** | Germany | *E. faecalis* (*gelE*) gene | **99%** |
| **10.** | **ID: CP015410.2** | Switzerland | *E. faecalis* (*gelE*) gene | **99%** |
| **11.** | **ID: CP046247.1** | Czech Republic | *E. faecalis* (*gelE*) gene | **99%** |
| **12.** | **ID: CP065784.1** | France | *E. faecalis* (*gelE*) gene | **99%** |

| | | | | |
|---|---|---|---|---|
| **13.** | **ID: CP070621.1** | Thailand | *E. faecalis* (*gelE*) gene | **99%** |
| **14.** | **ID: CP050491.1** | Canada | *E. faecalis* (*gelE*) gene | **99%** |

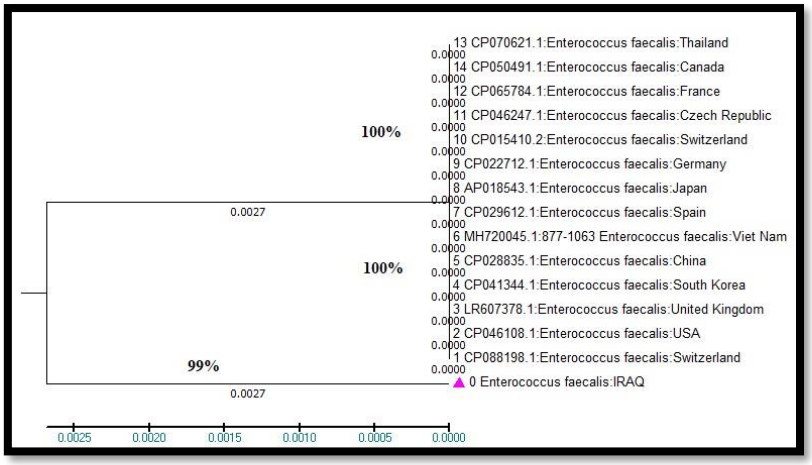


**FIGURE 4:** Phylogenic tree of *gelE* gene

## DISCUSSION

Hashem *et al*., [12], whose findings were corroborated by these findings, showed that 45 of the 58 isolates of *Enterococcus faecalis* from clinical specimens were able to grow on selected *Enterococcus* medium, with the greatest number of isolates detected in urine samples. *E. faecalis* was shown to be a pathogenic bacterium by Stępień-Pyśniak *et al*., [13] after being isolated from several sites of infection, most often UTIs. In line with the findings of Jahansepas *et al*., [14], who discovered that, the majority of *E. faecalis* isolates (22.6%) were detected in urine samples. Results are also consistent with those reported by Wójkowska-Mach *et al*., [15], who discovered that, the prevalence of *E. faecalis* was around 21.1% in urine and 30% from vaginal swabs.

Enterococci have gained more attention in recent years due to their rising resistance to several antimicrobial drugs and their capacity to produce severe infections [16]. Most cases of illness caused by enterococci are urinary tract infections, and this is true both in and out of hospitals [17].

External contamination, maybe by organisms that had multiplied in the gastrointestinal system and become closely linked with the patient during hospitalization, was the cause of urinary tract infection in patients with indwelling devices or infection of sutured surgical wounds [18]. 50 *Enterococcus faecalis* isolates were identified to belong to the species by Toc *et al.*, [19], with 32% of them being derived from feces, 32% from urine, 12% from wounds, and 10% from the vaginal canal.

Ahmed and Hafidh, [20] observed that, 75% of *E. faecalis* isolates came from urine. Studies found that the rate at which *E. faecalis* was isolated from patients varied depending on a number of variables, including the size of the samples used, the regions in which they were conducted, the isolation and identification methods employed, the effect of environmental conditions on patients' health, and the social and cultural background of the patients themselves. These bacterial isolates seemed competent to infiltrate a bladder cell line, as evaluated by an antibiotic protection experiment, and their tight contact with epithelial cells shed from patients was indicative of intracellular colonization [21]. To isolate *E. faecalis*, Weiner *et al*., [22] tested stool, urine, and vaginal samples.

*E. faecalis* strains have produced a variety of biologically active extracellular products similar to the virulence factors of bacteria and these virulence factors associated with health effects in humans [23]. In the clinical *E. faecalis* isolates, Farman *et al*., [24] found that, 14 virulence factor gene patterns were discovered, with *ace-asa1-cylA-frsA-esp-gelE* being the most common.

This study's findings corroborated those of Esmail *et al*., [25], who isolated 15(60%) of 25 *E. faecalis* strains from human urine and discovered that, 15 were connected to this gene. Kaviar *et al*., [26] observed that, 100% of *E. faecalis* isolates had the *gelE* gene, hence these findings contradict their findings. According to the research by Iseppi *et al*., [27], the *gelE* gene

was the most often discovered of the components it examined, being present in 52.8% of all isolates. Urinary tract infections (UTIs) and the development of pus reveal a correlation between the presence of the *gelE* gene in *E. faecalis* isolated from urine and wounds. The *E. faecalis* virulence factor gelatinase is an extracellular metalloprotease that hydrolyzes gelatin, collagen, and casein. This enzyme's capacity to hydrolyze collagens and certain bioactive peptides implies it plays a role in *E. faecalis*-related inflammatory processes, both at the outset and throughout their development. It is a hydrophobic metalloprotease having the capability for cleaving insulin, casein, hemoglobin, collagen, gelatin and fibrin [28].

There have been attempts to link its proteolytic qualities to an increase in the prevalence of enterococci in a variety of settings, including endocarditis and bacteremia [29], urinary infections, and oral infections [30]. Despite having the *gelE* gene, the microbe could not produce gelatinase because of a 23.9 kb deletion in the locus fsr [31]. In addition, 5 of the 7 gelatinase-producing strains identified in illness cases were moderate to strong biofilm makers, suggesting a probable link between gelatinase production and biofilm-forming abilities, propagation of the infectious process, and bacterial persistence in UTIs. Although the role of gelatinase in enhancing biofilm formation is still unknown.

Ferchichi *et al*., [32] described possible models: gelatinase might participate in production of an extracellular signaling peptide by proteolytically processing an inactive secreted peptide precursor to a mature component, or it might proteolytically activate another surface protein involved in some aspects of regulation or the process of biofilm development, such as a protein that participates in secretion of extracellular polymeric matrix material.

The current observation of this tree has confirmed sequencing reactions because it explained the actual neighbour –joining- based positioning in such observed variation. Interestingly, the multinational origins of our investigated samples could not be ignored. This was due to their positioning in the vicinity to different Asian, Europeans and Americans strains belonged to the same organism. Based on the currently analyzed *gelE* nucleic acid sequences analysis is showed that, there were some variation the identity was (99%) when compared with the stranded strains, all this mutation were reduced function because they don't change the genetic code, or stop the protein translation but gave variation.

By applying the UPGMA technique, we were able to deduce the evolutionary history [33]. The best tree is displayed, with a total branch length of 0.00536291. The phylogenetic tree was inferred using evolutionary distances, and its branches are displayed to scale using those distances' units. The evolutionary distances are expressed as the average number of base substitutions per site and were calculated using the Maximum Composite Likelihood approach [34].

Fifteen different nucleotide sequences were used in the study. First, second, third, and noncoding positions of the codon table were included. Blank and empty spots have been filled in completely. The final dataset included 187 distinct roles. MEGA6 was used for the evolutionary analysis [35].

## CONCLUSION

The pathogenicity of *E. faecalis* increased by presence of *gelE* gene. Furthermore, neighbour phylogenetic distances in this tree indicated a wide biological diversity of *E. faecalis* sequences.

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