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Article

Isolation and Identification of Enterococcus faecalis and Enterococcus lactis from Human Clinical Specimens

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Abstract: *E. faecalis* and *E. lactis* are opportunistic Gram-positive pathogens associated with soft tissue infections and are characterized by multidrug resistance and virulence potential. The aim of this study was to evaluate antimicrobial resistance patterns and to detect selected virulence genes in *E. faecalis* and *E. lactis* isolated from soft tissue infections. Two hundred and ten clinical specimens (biopsies, aspirates, and swabs) were collected and cultured on MacConkey, blood, chocolate, and brain heart infusion agars under aerobic and anaerobic conditions. Identification was performed by conventional methods, the VITEK2 system, 16S rRNA gene sequencing, and PCR detection of *agg*, *esp*, and *ace* genes. A total of 98 bacterial isolates were collected. VITEK2 identified two isolates as *E. faecalis* and two isolates as *E. lactis*, but 16S rRNA sequencing confirmed one strain per species. Genes (*agg* and *esp*) were present; *ace* was absent. All isolates that were confirmed were resistant to the antibiotics tested. Soft tissue infections harbor multidrug-resistant *E. faecalis* and *E. lactis* possessing key virulence genes, underscoring their clinical importance and the need for continued surveillance and infection prevention.

Keywords: *Enterococcus faecalis*, *Enterococcus lactis*, Soft Tissue Infections, Antimicrobial Resistance, Virulence Genes, 16S rRNA

Introduction

Enterococci are Gram-positive bacteria identified in 1899 as natural inhabitants of the human intestinal microbiota. These organisms had been taxonomically classified for many decades in the genus *Streptococcus*, specifically as serogroup D streptococci. *Streptococcus faecalis* is described in 1906 following its isolation from a clinical case of endocarditis. *Streptococcus lactis* is described in 1919. Major taxonomic differences among serogroup D streptococci have been identified with the advent of biochemical characterization techniques and molecular approaches, including DNA–rRNA homology studies and 16S rRNA gene sequencing. Based on these findings, they were reclassified into a separate genus *Enterococcus*, in 1984. The genus currently includes more than 50 recognized species, including *Enterococcus faecalis* and *Enterococcus lactis*, which are regarded as clinically important due to their

frequent association with human infections and their increasing importance in medical microbiology [1], [2].

E. faecalis and *E. lactis* are clinically important opportunistic pathogens, particularly in hospitals. *E. faecalis* is the principal cause of enterococcal infections, accounting for approximately 80% of human cases, and is most frequently implicated in urinary tract infections, wound infections, bacteremia, and endocarditis [3], [4], [5].

In contrast, *E. lactis* is increasingly recognized for its capacity to adapt to healthcare environments, driven by resistance to antibiotics and environmental pressures. Genomic studies have identified two distinct clades, including hospital-adapted lineages closely linked to nosocomial outbreaks and opportunistic infections [2].

Due to the presence of virulence factors, antimicrobial resistance genes, and biofilm-forming ability, both species possess high pathogenic potential, which contributes to their persistence and clinical impact, and therefore pose a growing public health concern [6], [7], [8].

agg is one of the genes encoding an important virulence factor, the aggregation substance (AS), a plasmid-encoded adhesin regulated by pheromone signaling. AS is conducive to intimate contact between donor and recipient bacterial cells so that plasmids can be transferred successfully. AS is on the surface of the donor cell, but there must be a complementary binding substance on the recipient cell, encoded chromosomally, for the interaction to be successful [3].

The *esp* gene encodes the enterococcal surface protein (Esp), a protein that plays a role in bacterial adhesion to host and abiotic surfaces. The protein is involved in the formation of a biofilm that increases the persistence of bacteria in biopolymeric matrices [9].

ace is a virulence determinant of enterococci on their surface; the bacteria have a protein that helps them to adhere to several host components, including type IV collagen in the human body, type I bovine and rat collagen, murine laminin, and human dentin. In vivo, increased Ace expression has been demonstrated in the presence of serum and constituents of the extracellular matrix [10].

Materials and Methods

Ethics Statement

Ethical permission was granted to undertake this study by the Ministry of Health and Environment in Ninawa Health Department with approval number (272) on 1/10/2025.

Sample Collection

Two hundred and ten samples from surgical biopsies, aspirates, and swabs from different clinical sources were received. Samples were collected from Al-Salam Teaching Hospital, Al-Jumhouri General Hospital, and Ibn Sina Hospital of Mosul City during the period from 1/10/2025 to 1/2/2026. Samples of the three categories of the specimens were divided into equal parts to make 70 biopsies, 70 aspirates, and 70 swabs. All the samples were cultured on MacConkey, blood agar, chocolate agar, and brain heart infusion agar. Biopsy samples and anaerobic aspirates were kept in an anaerobic jar in the incubator.

Diagnostic Tests

The samples were incubated and then subjected to a series of morphological tests (Gram stain, catalase, oxidase, carbohydrate fermentation, morphology on different media). Identification of bacterial isolates was done by the VITEK2 automatic identification system on 24 hr old pure isolates. Colonies were reactive in sterile 0.45% sodium chloride solution, and turbidity was determined using a Densichek using the McFarland standard. Suspension was loaded into VITEK 2 cards with biochemical tests, and sample data were typed into the computer attached to it. The test included automated incubation, reading and interpretation to give a profile of the bacteria being tested.

Antibiotic Sensitivity Test (AST)

Antimicrobial susceptibility testing of bacterial isolates obtained from soft tissue infections was performed using the VITEK 2 Compact system (Bio analyse, Turkey). Manual susceptibility testing was additionally carried out to confirm the results and to include antibiotics commonly used in clinical practice.

Molecular Identification

Polymerase chain reaction (PCR) with the entire 16S rRNA gene was performed using the following reaction mixes (Go-Taq G2 Green Master Mix, Promega, USA): 16S rRNA 27F primer (AGAGTGATCMTGGCTCAG), 1522R primer (AAGGAGGTGATCCARCCGCA). The 16S rRNA gene study was determined by sending the products of the PCR reaction of the predetermined genes with the primers of the resulting package at Psomagene sequencing company (USA). The sequences of the genes were blasted against the sequences of the genes deposited in the National Centre for Biotechnology Information (NCBI). Then, the presence of the *agg*, *esp*, and *ace* genes was investigated in *Enterococcus faecalis* and *Enterococcus lactis* isolates.

Table 1. Thermal Programs Used to Investigate Genes Using PCR

Genes	Initial denaturation	Denaturation	Annealing	Extension	N Cycles	Final extension (Min/°C)	Reference
Agg	94 °C / 5 min	94 °C 40 sec	50–68 °C 60 sec	72 °C 60 sec	30	72 °C / 7 min	[11]
Esp							
Ace							

Results

Distribution of Bacterial Isolates

Based on the phenotypic diagnosis and using the VITEK2 system, we obtained 98 bacterial growth samples from 210 clinical samples taken from patients suffering from soft tissue infections (abscesses, boils, surgical wounds, burns, diabetic foot ulcers), divided into swabs, aspirates, and biopsies as in Table 2.

Table 2. Distribution of bacterial isolates according to sample type.

Bacterial Isolates	Swabs	Aspirates	Biopsies
Staphylococcus aureus	13	4	3
Coagulase Negative Staphylococci	7	3	1
Streptococcus pyogenes	5	3	2
Streptococcus agalactiae	3	1	0
Enterococcus	3	0	1
Clostridium spp.	0	2	5
Proteus spp.	5	2	3
Klebsiella spp.	5	2	1
Pseudomonas spp.	4	2	2
Escherichia coli	3	1	0
Aeromonas spp.	2	0	0
Bacteroides spp.	0	4	5

Fusobacterium spp.	0	2	0
Total	50	25	23

Cultural Examination

The results of cultural examination of bacterial colonies showed bacterial colonies of *Enterococcus* spp. on blood agar presented small to medium, circular, smooth, greyish white colonies, showing mainly non-hemolytic (γ-hemolysis) activity with occasional weak α-hemolysis; on brain heart infusion agar, the isolates showed good growth with smooth, circular, slightly moist colonies that appeared creamy white to pale grey (Figure 1).

Figure 1. Appearance of A: Enterococcus faecalis, B: Enterococcus lactis on different agar.

Microscopic Examination

The *E. faecalis* and *E. lactis* are Gram-positive cocci bacteria, as shown in Figure 2.

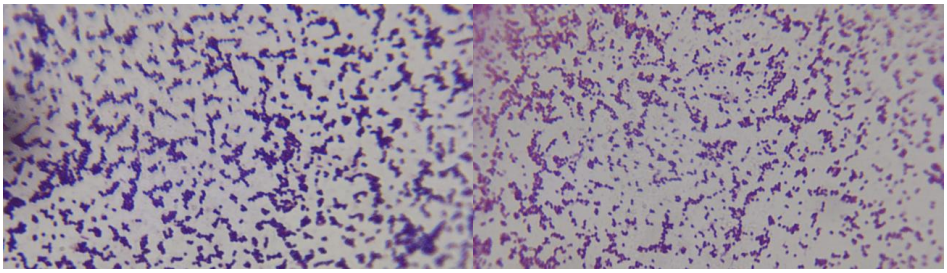

Figure 2. Microscopic examination for A: Enterococcus faecalis, B: Enterococcus lactis.

Antimicrobial Susceptibility

The antimicrobial susceptibility profile of *Enterococcus faecalis* and *Enterococcus lactis* isolates demonstrated resistance to ampicillin, clarithromycin, vancomycin, linezolid, tobramycin, erythromycin, levofloxacin, clindamycin, doxycycline and imipenem (Figure 3).

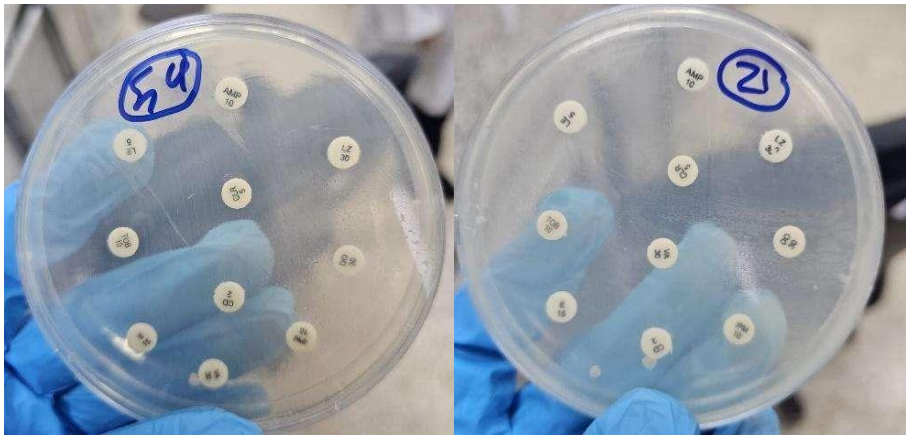

Figure 3. Disk diffusion showing the antibiotic resistance pattern of E. faecalis (54) and E. lactis (21).

Detection of agg, esp and ace Genes

Molecular analysis by PCR showed that the isolates of *E. faecalis* and *E. lactis* were positive for the virulence genes *agg* and *esp* and negative for the *ace* gene. The results of the present study showed the presence of *agg* and *esp* genes in isolates of both species (Figure 4).

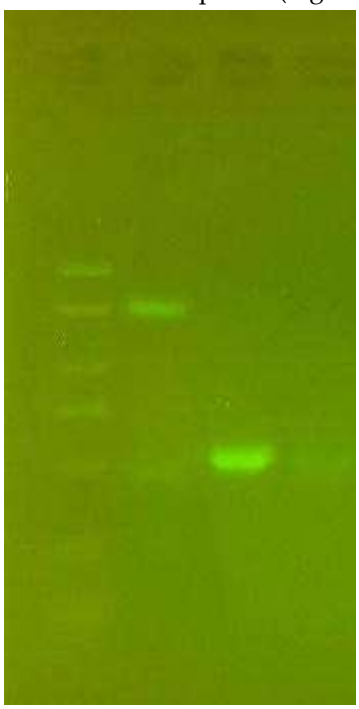

Figure 4. Gel electrophoresis of Enterococcus faecalis and Enterococcus lactis. L: ladder 5000 bp on 1% agarose. 1: agg (1553), 2: esp (510), 3: ace.

Discussion

E. faecalis and *E. lactis* were resistant to all the antibiotics used in the present study. Enterococci are especially proficient at acquiring antibiotic resistance through mobile genetic elements (MGEs) such as plasmids and transposons [12]. Hence, *E. faecalis* and *E. lactis* are often multi-drug resistant, including to clinically relevant antibiotics such as ampicillin and vancomycin [13]. Consequently, the World

Health Organization (WHO) has designated VRE as a high-priority pathogen [14]. Vancomycin resistance is mainly the result of the *vanA* gene, which is part of the *vanA* operon and is often located on transposons, like Tn1546 [15].

Regarding the virulence determinants, the results of the present study showed the presence of *agg* and *esp* genes in isolates of both species. However, variability across studies has been well documented. For example, some authors reported a higher frequency of *esp* in *E. lactis* isolates than in *E. faecalis* ones, indicating species- and strain-dependent differences in the distribution of virulence genes [16].

In addition, Ace, described as a common adhesin in previous studies, particularly in invasive isolates, was not detected in any of the isolates analyzed in the present study. Such absence has also been reported elsewhere and may be due to geographic variation, differences in clinical origin, or the genetic diversity of circulating strains. Overall, these findings highlight the heterogeneous distribution of virulence determinants among *Enterococcus* spp. and their possible variability according to the local epidemiological condition. The presence of the *agg* (aggregation substance) gene suggests enhanced ability of the isolates to adhere to host cells and to induce bacterial aggregation, which promotes colonization and contributes to the transfer of virulence determinants. This gene has also been strongly linked to biofilm formation, thus enhancing the persistence of enterococci in clinical environments and their resistance to host immune responses [3].

Likewise, the presence of the *esp* (enterococcal surface protein) gene indicates the ability of these isolates to adhere to epithelial surfaces and medical devices. The *esp* gene is important for biofilm formation and is often associated with an increased pathogenicity and chronicity of enterococcal infections, especially those acquired in hospitals [9].

However, the absence of the *ace* (adhesion to collagen of Enterococcus) gene indicates that collagen-binding is not a primary adherence mechanism in the isolates studied. This suggests that other adhesion-related virulence factors such as *agg* and *esp* could compensate for the loss of *ace*, indicating the diversity of pathogenic strategies used by *Enterococcus* species. In general, these results suggest that *E. faecalis* and *E. lactis* possess important virulence-related genes that might be important for their pathogenic potential, even without some adhesion factors.

Conclusion

This study shows the presence of multidrug-resistant *E. faecalis* and *E. lactis* in soft tissue infections and emphasizes their clinical significance as emerging opportunistic pathogens. The combination of the presence of important virulence genes (*agg* and *esp*) and the absence of the *ace* gene indicates a unique virulence profile that may be involved in colonization and persistence in infected tissues. Furthermore, the complete resistance of the confirmed isolates to all antibiotics tested underlines the serious therapeutic problems raised by these organisms. The findings underscore the importance of ongoing epidemiological surveillance, effective infection control policies, and judicious use of antimicrobial agents to prevent the spread of resistant enterococcal isolates in clinical settings.

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