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Enterococcus faecalis Virulence Spectrum in UTI: Phenotypic and Genotypic Insight from Al-Najaf City

Samer A. MH. Al-Hilali[1], Zainab Jaber Hadi[2] and Ahmed A. Hussein[3]

[1,2] *Faculty of Science, University of Kufa, Iraq.*

[3]*Faculty of Medicine, University of Kufa, Iraq.*

Email: sameram.hussein@uokufa.edu.iq, zainabj.hadi@uokufa.edu.iq, ahmed.altwali@uokufa.edu.iq

ABSTRACT

Background: *Enterococci are a predominant cause of Gram-positive urinary tract infections (UTIs) globally. Key virulence mechanisms, such as particular factors and biofilm formation, hinder antimicrobial efficacy during infection.* ***Objective:*** *We investigated the incidence of UTI and characterized virulence determinants in clinical Enterococcus faecalis isolates.* ***Methods:*** *Twenty E. faecalis isolates recovered from UTI patients in Al-Najaf city were phenotypically and genotypically characterized.* ***Results:*** *Isolates exhibited diverse virulence profiles. All produce biofilm. Phenotypic assays showed gelatinase in 30%, protease in 55%, and hemolysin in 85% of isolates. The virulence genes esp and ace were present in 60% and 50% of isolates, respectively.* ***Conclusion:*** *The study demonstrates that E. faecalis isolates from Al-Najaf City possess a variety of virulence factors, the frequent detection of these factors, notably the esp and ace genes, supports their significant role in the pathogenesis of UTIs caused by these bacteria.*

Keywords: Urinary tract infection, Enterococcus faecalis, Pathogenesis, Virulence factors, esp gene, ace gene, Biofilm.

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INTRODUCTION

Urinary tract infections (UTI) are highly prevalent bacterial infections, second only to respiratory infections, with women experiencing higher rates than men.[1] Gram-negative bacteria are the predominant cause, but recognized Gram-positive pathogens include *Enterococcus* species (especially *E. faecalis*), *S. saprophyticus*, and group B *Streptococcus*. Globally, *E. faecalis* represent a major cause of these Gram-positive UTIs. [2,3] The pathogenic potential of enterococci is enhanced by their remarkable adaptability and intrinsic resistance to multiple classes of antimicrobials (e.g. cephalosporins) and tolerance to harsh environmental conditions, including, exposure to chlorine, alcohol-based disinfectants, extreme temperatures, pH variations, oxygen tension, humidity fluctuations, and nutrient scarcity.[4]

The severity of infections is significantly influenced by virulence factors. Adhesins such as collagen-binding protein (Ace) and enterococcal surface protein (Esp) contribute to pathogenesis by mediating adhesion and colonization, avoiding immune detection, and promoting extracellular enzyme production.5 Capsule antigens also represent a critical virulence factor, particularly in UTIs, by facilitating adhesion, promoting biofilm development, and conferring resistance to neutrophil phagocytosis.[6] Biofilm formation is a key virulence factor underpinning the pathophysiology of enterococcal infections.

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Biofilms not only facilitate colonization and persistence but also significantly impede the penetration and efficacy of antimicrobial agents, alongside other virulence determinats.[7]

We aimed to quantify UTI prevalence attributable to *Enterococcus faecalis* in hospitalized patients correlate with distinct virulence gene profiles in recovered isolates.

METHODOLOGY

Bacterial isolates

Urine samples were collected from patients clinically diagnosed with Urinary tract infections at some of main hospitals in Al-Najaf city (Al-Sader Medical City, Al-Forat General Hospital, Al-Zahra Maternity and Children and Al-Hakeem General Hospital). Sample collection was conducted over three-months period starting from November 2023. Clean catch midstream urine samples were obtained in sterile container and transported to laboratory for processing.

This study followed all the ethical guidelines set out by the Institutional Review Boards and National Research Committee, in accordance with the principles of Declaration of Helsinki.

Upon receipt, each urine sample was thoroughly mixed. A portion was subjected to microscopic examination to assess pyuria.[8] the other portion was cultured quantitively using calibrating loop. Streaking eas performed onto blood agar and MacConkey agar plats, which than incubated aerobically at 37°C for 18-24 hours.

Bacterial growth was assessed, and isolates were initially identified based on colony morphology, Gram staining. Gram-positive cocci presumptively identified as Enterococci based on negative catalase activity, ability to hydrolyze esculin. Presumptive Enterococci were sub cultural on Hicrome UTI agar (HiMedia Laboratories, India) for further differentiation based on chromogenic characteristics. Final identification and confirmation was completed via VITIC-2 compact system (bioMerieux, France) with ID-GP cards.

Production of Gelatinase

E. faecalis isolates were inoculated by stabbing into tubes filled with nutritional gelatin (12% gelatin) and incubated at 37ºC for up to 72 hours to assess gelatinase production. following incubation, tubes were refrigerated at 4ºC for 30 minutes. Gelatinase-producing isolates liquefied the medium despite refrigeration.in contrast, the gelatin medium remained solid in gelatinase-negative tests after refrigeration.[9]

Production of Protease

Samples were inoculated by streaking onto skim milk agar plates, followed by incubation at 37ºC for 48 hours. Protease production was indicated by formation of clear zone surrounding the bacterial growth.[10]

Production of Hemolysin

Haemolysin production was phenotypically assessed on blood agar incubated at 37ºC for 24 hours. A zone of β-haemolysis around colonies demonstrated haemolysin activity.[11]

Biofilm formation

We used the Tissue Culture Plate Method (TCPM) as biofilm detection assay, following established protocol.[12]

Virulence genes detection

The existence of virulence genes *esp* and *ace* was investigated in E. faecalis isolates. (**Table 1**) details the primer used. PCR reaction were performed according to established protocols.[13,14] Amplification products were electrophoresed at 70 volts fore 90 minutes on ethidium bromide-stained 2% agarose gels and visualized under UV illumination.

Table (1): primers used in this study.

Primers	Sequence (5'-3')	Product size(bp)	References
ace	F:GGAATGACCGAGAACGATGGC R:GCTTGATGTTGGCCTGCTTCCG	616	13
esp	F:TTGCTAATGCTAGTCCACGCC R:GCGTCAACACTTGCATTGCGAA	933	14

RESULTS

Patient and bacterial isolates

Out of the 412 processed samples, 110 showed positive urine cultures. Among these, 78 (71.0%) were from females patients and 32(29%) from males. The identified etiological agents of UTI were follow: *E. faecalis* (20 isolates, (18.8%) and other pathogens (90 isolates, (81.2%). For farther details see (**Table 2**).

Table (2): Distribution of etiological agents in patients with UTI.

Bacterial isolate	No. of isolates		Total
	Female	Male	
E. faecalis	14 (70%)	6(30%)	20 (18.8%)
other species pathogens	64 (68.9%)	28 (31.1%)	90 (81.2%)
Total	78(71.0%)	32(29%)	110 (100%)

Virulence factors (phenotypic detection)

All *E. faecalis* were shown to be capable of forming biofilms, however two (1%) showed strong biofilm formation. There are nine (45%) with mild biofilm formation and nine isolates (45%) with weal biofilm formation. Furthermore, 55% of E. faecalis isolates tested positive for protease synthesis, whereas 30% showed gelatinase activity. 85% of E. faecalis isolates produced haemolysin, according to phenotypic characterization. The result are presented in (**Figure 1**).

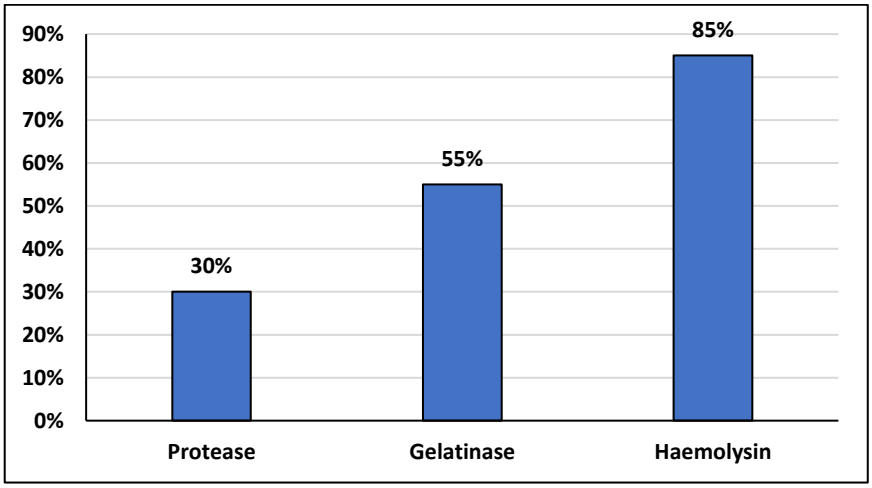

Figure (1): Percentage of virulence factors among *E. faecalis* isolates.

PCR-based genes detection

The following gene-specific primers were used to identify the genes encoding virulence factors in E. faecalis isolates. The majoraty of *E. faecalis* isolates (12 out of 20; 60%) harbored the *esp* gene which codes for the Enterococcal surface protein (**Figure 4)**, according PCR amplification results (**Figure 2**).

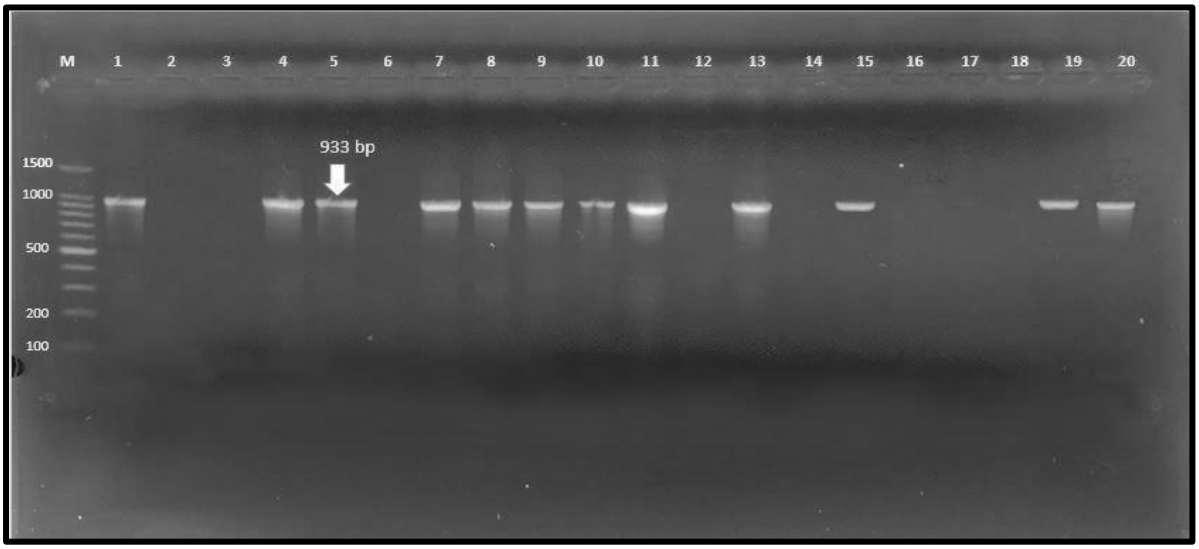

Figure (2): Electrophoresis diagram of PCR amplified products for extracted DNA of 20 isolates of *E.*

Faecalis using specific esp gene primer show positive products at 933 bp. Line M: molecular size DNA marker and products migrated at 75 volt for 80 minutes and stained with ethidium bromide.

Detection of *ace* gene in *E. faecalis*

The collagen-binding protein gene (*ace*) was detected in half of the isolates (10 out of 20, 50%)(**Figure 4),** through PCR amplification (**Figure 3**).

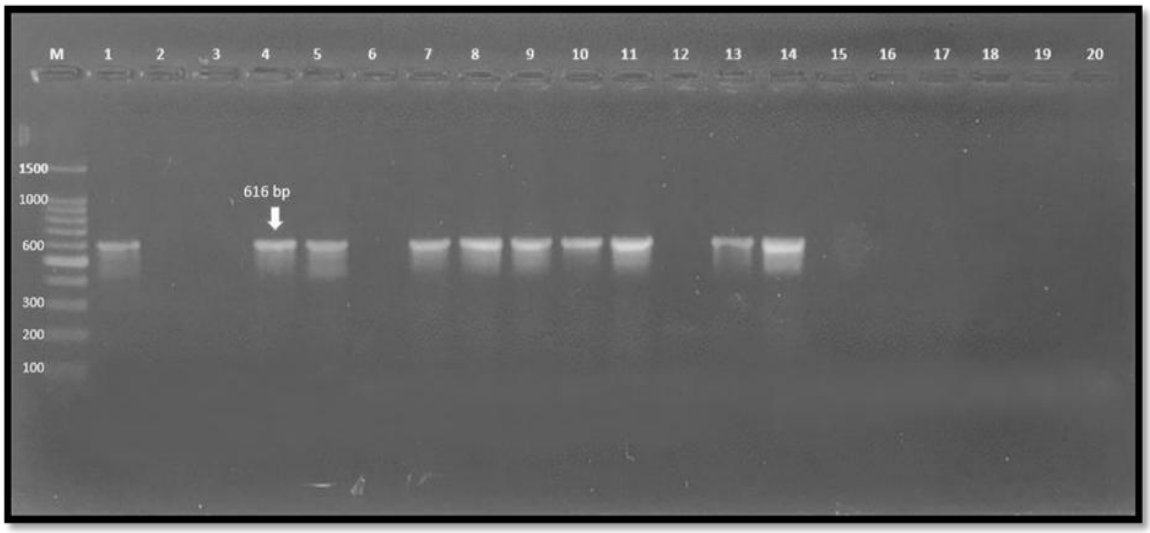

Figure (3): Electrophoresis diagram of PCR amplified products for extracted DNA of 20 isolates of *E.*

Faecalis using specific ace gene primer show positive products at 616 bp. Line M: molecular size DNA marker and products migrated at 75 volt for 80 minutes and stained with ethidium bromide.

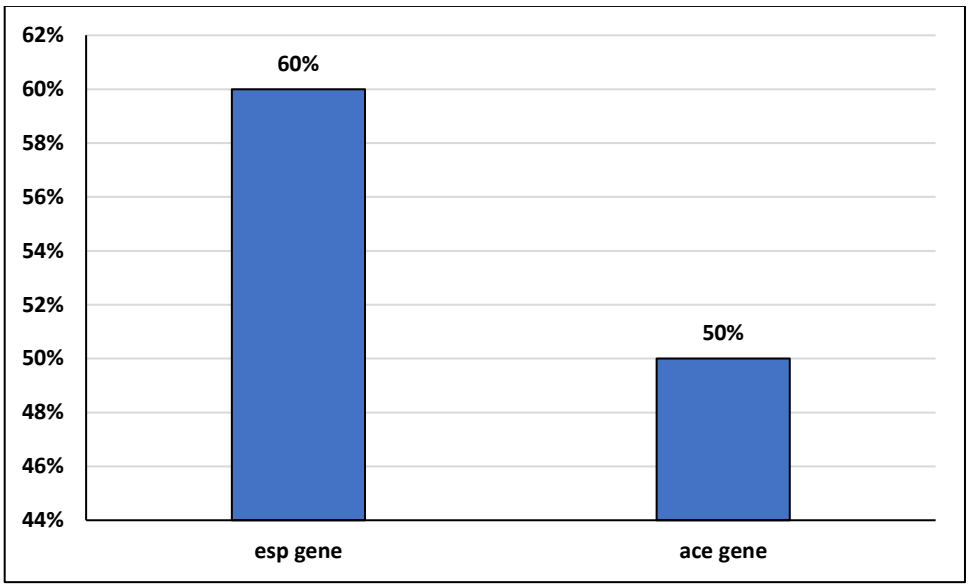

Figure (4): Distribution of virulence factors genes among *E. faecalis* isolates.

DISCUSSION

Local epidemiological data confirm the established understanding that Gram-negative bacteria are the predominant causative agents of UTIs.[15] However, *E. faecalis* consistently emerges as the most frequently isolates species from human clinical specimens in our sitting.16 This observation is corroborated by both international and local studies, which identify E. faecalis as primary pathogen responsible for human infections, with particular significance in UTIs.[15,17]

Our findings regarding *E. faecalis* align with its well-documented role as a nosocomial pathogen.[18,19] Critically, its capacity for biofilm formation, confirmed in this study, inherently limited the efficacy of antimicrobial therapies, thus posing a significant therapeutic challenge. Furthermore, the demonstrated production of gelatinase by our isolates is consistent with multiple previous reports. [7,20,21]Similarly, the obvious protease activity corroborates the finding of Worsztynowicz *et al.*[22] The expression of β-hemolytic activity, mediated by haemolysin toxins, is the key virulence mechanism. These toxins lyse erythrocytes and impair host immune defence,[23] and variation in hemolytic pattern we detected align with the known diversity among *E. faecalis* isolates reported elsewhere.[24,25]

Analysis of virulence genes revealed variable reported prevalence for *esp* gene in *E. faecalis*, with studies citing frequencies of 53.5%, 81.9%, and 68.7% respectivly.[9,26,27] The epidemiological data concerning the *asa*1 gene in clinical enterococcal isolates present a conflicting picture. Several studies indicate a significantly higher prevalence of *ace* in clinical isolates compared to strains colonizing healthy individuals.[28,29] conversely, other research found no statistically significant differences in asa1gene occurrence between invasive and commensal strains.[30,31]

CONCLUSION

Enterococcus faecalis is well-established, clinically significant pathogen in UTIs, particularly challenging to treat due to its biofilm-forming capability. Its pathogenicity is driven by a combination of potent virulence factors such as (gelatinase, protease, hemolysins), although the prevalence and clinical association of key virulence genes (*esp, ace*) remain areas of variability and ongoing investigation. This underscores the complexity of E. faecalis infections and the need for continued research into its pathogenesis and epidemiology.

Conflict of Interest

There is no financial or personal relationships with other people or organizations that could inappropriately influence the authors' actions.

Financial Disclosures

There is no specific financial interests, relationships and affiliations relevant to the subject of the manuscript.

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