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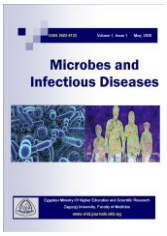

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Original article

Characterization of quorum-sensing and virulence factors associated genes among uropathogenic *Enterococcus faecalis*

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ABSTRACT

Background: *Enterococcus faecalis* (*E. faecalis*) is a significant concern in healthcare due to its ability to cause serious infections and increasing antibiotic resistance, commonly causes urinary tract infections (UTIs) and possesses various virulence factors. The study aimed to assess the antimicrobial sensitivity of *E. faecalis* isolates from UTIs, as well as investigate the presence of *fsr* quorum sensing (QS) and virulence-related genes. Additionally, the correlation between antimicrobial susceptibility and the presence of QS and virulence genes was examined. **Methods** Thirty-seven uropathogenic *E. faecalis* isolates were tested for susceptibility to ten antibiotics using Kirby-Bauer disk diffusion. Then PCR was conducted to detect the presence of *fsr* QS and virulence associated genes. The correlation between QS genes, virulence genes and antimicrobial sensitivity was analyzed. **Results** Resistance rates were high for ampicillin (100%), kanamycin (94.5%), ciprofloxacin (86.5%), levofloxacin (83.8%), erythromycin (83.8%), and tetracycline (78.4%). Approximately half of the isolates were resistant to chloramphenicol (48.6%) and amoxicillin-clavulanate (43.3%). Nitrofurantoin (10.8%) and vancomycin (8.10%) had the lowest resistance rates. PCR analysis detected fsrB and fsrC genes in 81% and 83.7% of isolates, respectively. The gelE and sprE genes were present in 72.9% and 89% of isolates, respectively. The efaA gene was amplified in all isolates, while the esp, ebpA, and asa1 genes were prevalent in 91.8%, 83.7%, and 64.8% of isolates, respectively. The cylLL gene was found in 48.6% of isolates. **Conclusion** These findings emphasize the concerning issue of antimicrobial resistance and the presence of virulence factors in multi-virulent *E. faecalis* isolates from UTIs.

Introduction

Urinary tract infections (UTIs) are common bacterial infections that can be serious and potentially life-threatening if not treated promptly. Urinary tract infections have the potential to progress to severe conditions like bacteremia, which can lead to mortality [1].

Enterococcus faecalis (E. faecalis) is a Gram-positive bacterium commonly found in the gastrointestinal tract but has become a major cause of healthcare-associated infections, particularly in the urinary tract [2]. *Enterococcus faecalis* infections are a significant risk due to its ability to survive harsh conditions, resist antibiotics, adapt genetically and express virulence factors in both community and healthcare settings [3].

The rise of multidrug-resistant *E. faecalis* strains is a significant public health risk due to limited treatment option [4]. *Enterococcus faecalis* has both intrinsic and acquired antimicrobial

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resistance, making it resistant to antibiotics like aminoglycosides, β-lactams, cephalosporins, and lincosamides. This, along with its ability to survive in harsh environments, makes enterococci well-suited for long-term survival in healthcare settings [5].

Enterococcus faecalis strains possess several virulence factors involved in adhesion to host cells, colonization, invasion into host tissues and ability to modulate the host immune response [6].

A major quorum sensing (QS) system in *E. faecalis* is the *E. faecalis* system regulator (*fsr*) regulator locus, which controls the expression of gelatinase (*gelE*) and serine protease (*sprE*), playing a key role in the bacterial pathogenicity by coordinating gene expression and regulating various virulence factors. The *fsr* locus contains four genes: *fsrD, fsrB, fsrC* and *fsrA. FsrD* codes for the propeptide that is processed to produce the gelatinase biosynthesis-activating pheromone (GBAP) with the help of FsrB. FsrC is part of a regulatory system that responds to extracellular GBAP by phosphorylating the intracellular response regulator FsrA. Phosphorylated FsrA then activates the expression of *gelE* and *sprE* within the *fsr* locus [7]. The study aimed to evaluate the susceptibility pattern of *E. faecalis* isolates from UTIs, along with examining the existence of *fsr* QS genes and virulence associated genes.

Methods

Bacterial strains

In this study, a retrospective cohort analysis was conducted to determine the prevalence and characterize the *E. faecalis* isolated from urine cultures. Thirty-seven isolates were obtained from urine cultures from patients at Mansoura University Hospitals using a simple random sampling approach. The collection of specimens followed a protocol approved by the Research Ethics Committee of the Faculty of Pharmacy, Mansoura University with the ethical codes 2024 – 72. Isolates were initially streaked on bile esculin agar (Biolab®), confirming their classification as *Enterococcus* [8]. Further confirmation of the specific species *E. faecalis* was achieved through genotypic analysis using PCR targeting species-specific (*eda1* and *eda2*) using the primer pairs listed in **table 1** [9].

Isolated strains were stored in double-strength tryptic soy broth (TSB) with 50% (v/v) glycerol at -80°C. They were routinely cultivated in double-strength TSB at 37°C with agitation for 24 h [10].

Antimicrobial susceptibility testing for *E. faecalis* isolates

The antimicrobial sensitivity testing was assessed using ten antimicrobial agent disks belonging to different classes by the Kirby-Bauer disk diffusion method on Muller Hinton agar (MHA), following the criteria set by the Clinical and Laboratory Standards Institute [11]. A pure colony of *E. faecalis* was cultured in TSB overnight at 37 °C. The inoculum density was adjusted to an OD600 nm of 0.1 (1.5×10^8 CFU/mL). Lawn culture was performed on MHA plates using sterile cotton swabs dipped in the culture. Antimicrobial discs were added to the plates and incubated overnight at 37 °C. The diameter of the inhibition zones around each disc was measured, and isolates were classified as resistant, intermediate or susceptible based on CLSI 2021 guidelines.

Enterococcus faecalis isolates were classified as multidrug-resistant (MDR) or extensively drug-resistant (XDR) based on specific criteria for acquired resistance. MDR strains were non-susceptible to at least one antimicrobial agent in three or more classes, while XDR strains were non-susceptible to at least one agent in all categories except one or two [12].

Polymerase chain reaction (PCR)

DNA extraction

The rapid DNA extraction method, known as colony PCR, was carried out following the protocol described by **Dashti et al.** Bacterial DNA was prepared by boiling fresh bacterial colonies suspended in RNase- and DNase-free water for 10 min, then centrifuging the suspension at 10000 xg for 10 min to remove cellular debris [13].

Molecular detection of *E. faecalis fsr* QS genes

The presence of *fsr* QS genes (*fsrB* and *fsrC*), gelatinase (*gelE*) and serine protease (*sprE*) were identified using the oligonucleotide primers in **table 1.** The amplification of the genes was accomplished by employing the following reaction: 12.5 µL Dream Taq Green PCR Master Mix-2X (Thermo scientific, US), 2 µL of bacterial DNA, 1 µL of each primer (10µM) and 3.5 µL nuclease free water for a total of 20 µL per reaction. Negative control without a DNA template was also included. The amplification protocol started with an initial denaturation at 95 °C for 2 min, followed by 40

cycles, each consisting of three steps: denaturation at 95 °C for 30 s, annealing at temperatures specified in **table (1)** for each primer pair for 30 s, and extension at 72 °C for 30 s, with a final elongation step at 72 °C for 5 min [14].

Molecular detection of *E. faecalis* virulence genes

PCR was also used to detect and amplify virulence genes in *E. faecalis*. Specific primers were used for the pilus protein gene (*ebpA*), the aggregation substance gene (*asa1*), *E. faecalis* endocarditis antigen (*efaA*), the enterococcal surface protein (*esp*) and the cytolysin (*cylLL*) listed in **table (1)**. The protocol followed standard procedures [14].

Analysis of PCR products

Agarose gel electrophoresis was performed with 1.5% agarose gels to analyze PCR products. Ethidium bromide staining and UV radiation were used for visualization, and the amplicons were compared with the GeneRuler 100 bp plus DNA marker from ThermoFisher ScientificTm, UK.

Correlation analysis

The correlation between QS associated genes, virulence genes, and antimicrobial sensitivity was analyzed using Pearson's correlation analysis. The values were transformed into pseudo-numeric variables represented by 1 and 0 to construct a correlation matrix, which is used to quantify the strength and direction of the correlations. Additionally, to assess the significance of the observed correlations, a t-test was performed.

Statistical analysis and data interpretation

Data was analyzed using GraphPad Prism version 8. Statistical analysis was conducted using a two-tailed t test with significance defined as $p < 0.05$.

Results

Bacterial isolation and identification

Thirty-seven clinical *E. faecalis* isolates were obtained from urine specimens collected from Mansoura University Hospitals. The *E. faecalis* isolates were purified on bile esculin agar, where they formed small black colonies surrounded by a distinct black halo. Gram staining was performed on the pure colonies, revealing their characteristic Gram-positive nature as small cocci in clusters or diplococci when observed under a microscope. To verify the identification of the species, PCR analysis was conducted to detect the presence of *eda1* and *eda2* genes, which are specific to *E. faecalis* species with amplicon sizes of 404 and 389 bp, respectively **(Figure 1)**.

Antimicrobial susceptibility pattern of *E. faecalis* isolates

The antimicrobial susceptibility of all isolates was tested using the Kirby-Bauer disk diffusion method for ten antimicrobial agents of different classes.

The results showed that all isolates were resistant to ampicillin (100%). A high prevalence of resistance was also observed for kanamycin (94.5%), ciprofloxacin (86.5%), levofloxacin (83.8%), erythromycin (83.8%) and tetracycline (78.4%). A moderate resistance percentage was obtained with chloramphenicol (48.6%) and amoxicillin-clavulanate (43.3%). In contrast, a lower level of resistance was observed for both nitrofurantoin (10.8%) and vancomycin (8.10%) **(Figure 2A)**.

The antimicrobial susceptibility test of the current study exhibited that out of 37 *E. faecalis* isolates, 28 (75.7%) were classified as MDR. While only 2 (5.4%) isolates were susceptible to almost all antimicrobial agents. Furthermore, the results showed that 7 (18.9%) isolates were classified as XDR **(Figure 2B)**.

Polymerase chain reaction (PCR)

a. Prevalence of *fsr* QS genes

PCR was conducted to detect the presence of *fsr* QS genes (*fsrB* and *fsrC*), gelatinase and serine protease genes (*gelE-sprE*) among tested *E. faecalis* isolates. PCR analysis demonstrated that the *fsrB* gene was detected in 81% of the isolates, whereas the *fsrC* gene was identified in 83.7% of the isolates . The *gelE-sprE* genes were detected in 72.9% and 89% of the isolates, respectively **(Figure 3)**.

b. Prevalence of virulence factors associated genes

Five virulence genes encoding different virulence phenotypes were screened by PCR **(Figure 4)**, including the pilus protein gene (*ebpA*), aggregation substance gene (*asa1*), endocarditis antigen gene (*efaA*), surface protein gene (*esp*) and cytolysin gene (*cylLL*).

PCR analysis results showed that the *efaA* gene was the most detected gene among the *E. faecalis* tested isolates (100%), followed by the *esp* gene, which was found in 91.8% isolates. The *ebpA* gene was found in 83.7% of the tested isolates, while the *asa1* gene was found in 64.8% of the isolates and 48.6% of isolates harbored the *cylLL* gene.

***Fsr* QS pattern associated with uropathogenic *E. faecalis* isolates**

Ten different patterns (P1- P10) were observed among uropathogenic *E. faecalis* isolates **(Table 2)**. The most frequent profile associated with *E. faecalis* isolates was P10 which exhibited complete *fsr* QS genes (*fsrB* + *fsrC* + *gelE* + *sprE*) and was found in 54% of the isolates. Only one isolate, no. 13, lacked the *fsr* QS associated genes. Moreover, four patterns, P5 (*fsrC* + *sprE*), P6 (*fsrB* + *gelE* + *sprE*), P7 (*fsrC* + *gelE* + *sprE*) and P9 (*fsrB* + *fsrC* + *sprE*) were observed in 8% of the isolates.

Virulence pattern associated with uropathogenic *E. faecalis* isolates

The *E. faecalis* isolated from UTIs possessed two or more virulence traits **(Table 2)**. Among these traits, the adhesion-associated factors *efaA* and *esp* genes were found to be widely present. The presence of all tested virulence genes was the most frequent pattern (29.7%), P9 (*efaA* + *esp* + *ebpA* + *asa1* + *cylLL*). The second most frequent virulence pattern was P7 (*efaA* + *esp* + *ebpA* + *asa1*) in 24.3% of the isolates followed by P3 (*efaA* + *esp* + *ebpA*) among 16.2% of the tested isolates.

Correlation between antimicrobial resistance and virulence genes

Initially, the association between antimicrobial resistances was evaluated. Kanamycin resistance was found to be associated with resistance to two other antimicrobials: tetracycline and levofloxacin ($p < 0.01$). Resistance to chloramphenicol was correlated with levofloxacin resistance ($p < 0.05$).

Next, the correlation between QS and virulence genes was examined. Significant correlations were found between QS genes and virulence associated genes. Notably, there was a significant link between the presence of *fsrB* and *gelE* genes ($p < 0.01$). Additionally, the *sprE* gene was directly correlated with *fsrC* and *gelE* genes ($p < 0.05$). The detection of the *ebpA* gene was significantly associated with the presence of *fsrC*, *gelE* and *sprE* ($p < 0.01$).

Furthermore, the relation between antimicrobial resistance and QS and virulence related genes was also evaluated. The study revealed a direct relationship between resistance to tetracycline and the presence of the *asa1* gene ($p < 0.05$) as well as the *ebpA* gene ($p < 0.01$) in *E. faecalis* strains. Additionally, a significant correlation ($p < 0.01$) was detected between the presence of *fsrB*, *fsrC*, and *ebpA* genes and ciprofloxacin resistance, with *sprE* showing a slightly lower significance level ($p< 0.05$) **(Figure 5**

Table 1. List of oligonucleotides utilized in PCR

Gene	Type	Nucleotide sequence (5` to 3`)	Amplicon size (bp)	Temperature	References
eda1	***Fw***	GGGGACAGTTTTGGATGCTA	404	52	[9]
	Rv	TCCATATAGGCTTGGGCAAC			
eda2	***Fw***	GCCGAAGCTTCATCTTCTTG	389		
	Rv	AGGCGCAGGAACTGTTAGAA			
gelE	***Fw***	AGTGAACGCTACAGATGGAAC	145	60	[15]
	Rv	CGTTCCGTGTAAAGCAATTCC			
sprE	***Fw***	AAGATCGTTACTGGACCCTGAG	239	59	[16]
	Rv	GACCTGGATAAAACCAAGCATC			
fsrB	***Fw***	TCTTCTGTGAGCTTACCGTTT	210	61	[17]
	Rv	GACCGTAGAGTATTACTGAAGCA			
fsrC	***Fw***	TGACGAAACATCGCTAGCTC	194	61	[18]
	Rv	ATGCGAGGATTTGTCACGGT			
Esp	***Fw***	GGAACGCCTTGGTATGCTAAC	100	61	[19]
	Rv	GCCACTTTATCAGCCTGAACC			
asa1	***Fw***	GCACGCTATTACGAACTATGACC	378	57	[20]
	Rv	TAAGAAAGAACATCACCACGAGC			
efaA	***Fw***	TGGGACAGACCCTCACGAATA	100	61	[21]
	Rv	CGCCTGTTTCTAAGTTCAAGCC			
ebpA	***Fw***	CAACAACACCAGGGCTTTTTG	126	61	[22]
	Rv	ACCGGACCAGTCAACGACTAAG			
cylLL	***Fw***	CTGTTGCGGCGACAGCT	53	61	[19]
	Rv	CCACCAACCCAGCCACAA			

***Fw*:** forward ***Rv*:** reverse **bp:** base pair

Table 2. Distribution of *fsr* QS and virulence genes among uropathogenic *E. faecalis* isolates

Pattern No.	No. of isolates	*Fsr* QS pattern	Pattern No.	Virulence pattern	No. of isolates
P1	**1**	**No *fsr* QS genes**	**P1**	***efaA* + *esp***	**1**
P2	**1**	***fsrB***	**P2**	***efaA* + *asa1***	**1**
P3	**1**	***fsrB* + *fsrC***	**P3**	***efaA* + *esp* + *ebpA***	**6**
P4	**1**	***fsrB* + *sprE***	**P4**	***efaA* + *esp* + *asa1***	**1**
P5	**3**	***fsrC* + *sprE***	**P5**	***efaA* + *esp* + *cylLL***	**3**
P6	**3**	***fsrB* + *gelE* + *sprE***	**P6**	***efaA* + *ebpA* + *asa1***	**2**
P7	**3**	***fsrC* + *gelE* + *sprE***	**P7**	***efaA* + *esp* + *ebpA* + *asa1***	**9**
P8	**1**	***fsrB* + *fsrC* + *gelE***	**P8**	***efaA* + *esp* + *ebpA* + *cylLL***	**3**
P9	**3**	***fsrB* + *fsrC* + *sprE***	**P9**	***efaA* + *esp* + *ebpA* + *asa1* + *cylLL***	**11**
P10	**20**	***fsrB* + *fsrC* + *gelE* + *sprE***			

Figure 1. Amplification of species-specific genes in *E. faecalis* clinical isolates (1-37); (A) *eda1* gene (404 bp) (B) *eda2* gene (389 bp). Lane M is a 100 bp DNA marker, C is negative control.

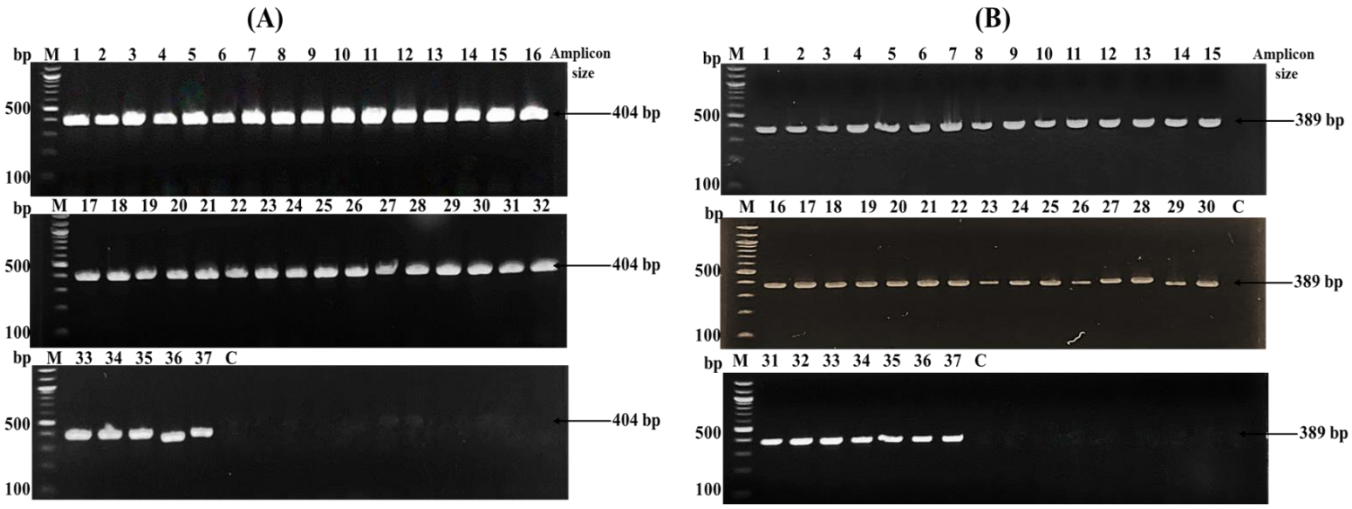


Figure 2. Antimicrobial susceptibility of the tested *E. faecalis* isolates; (A) Sensitivity pattern **(B)** Distribution of multidrug resistance (MDR) and extensive drug resistance (XDR).

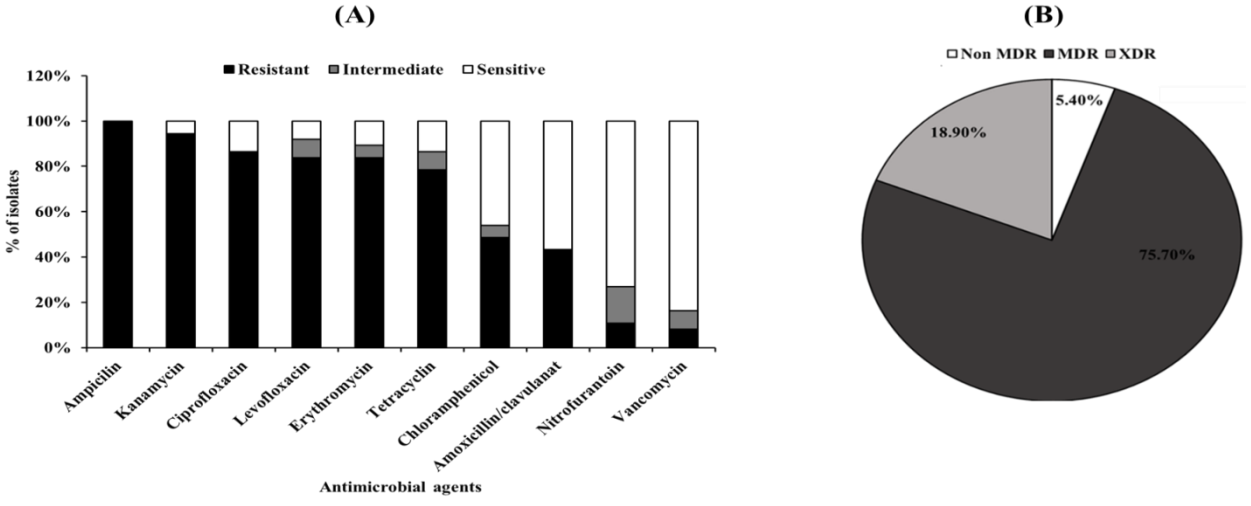


Figure 3. Amplification of *fsr* QS genes in *E. faecalis* isolates; (A) *fsrB* gene (210 bp) **(B)** *fsrC* gene (194 bp) **(C)** *gelE* gene (145 bp) **(D)** *sprE* gene (239 bp). Lane M is 100 bp DNA marker.

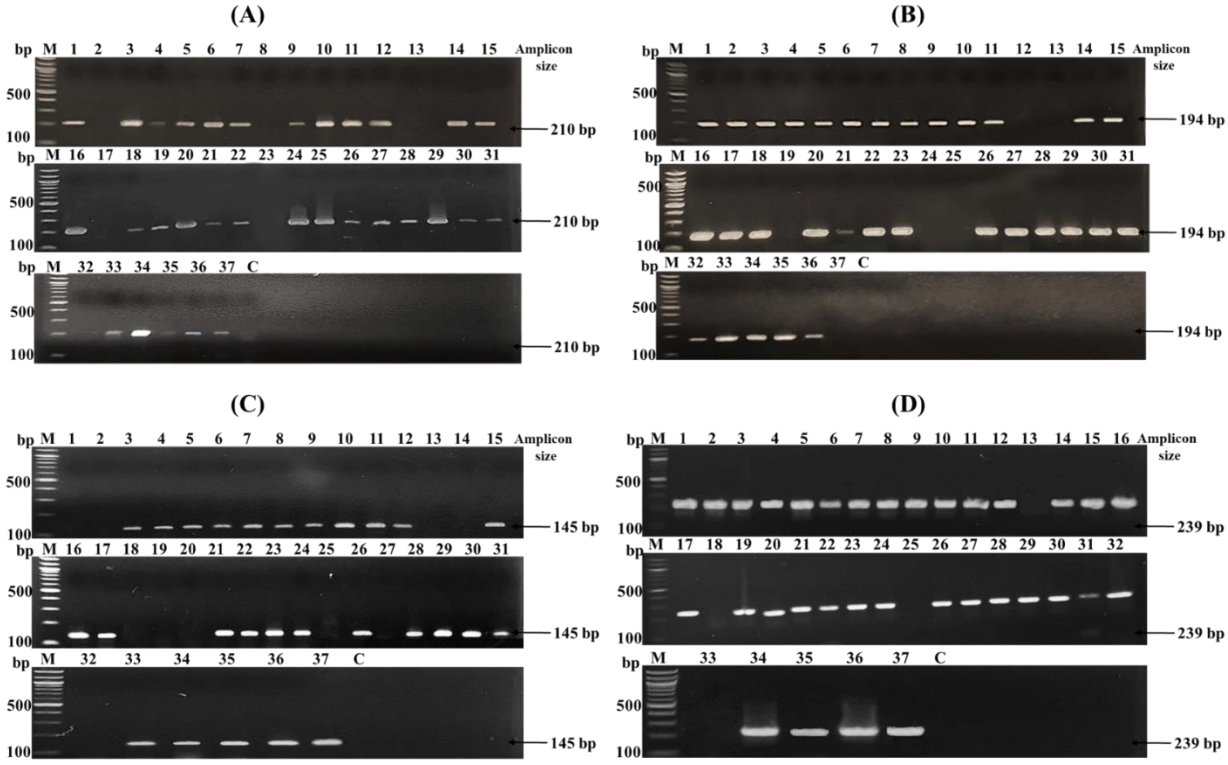


Figure 4. Amplification of virulence genes in ten representative *E. faecalis* isolates by PCR; (A) *efaA* gene (100 bp). **(B)** *esp* gene (100 bp). **(C)** *ebpA* gene (126bp). **(D)** *asa1* gene (378 bp). **(E)** *cylLL* gene (53 bp). Lane M is 100 bp DNA marker

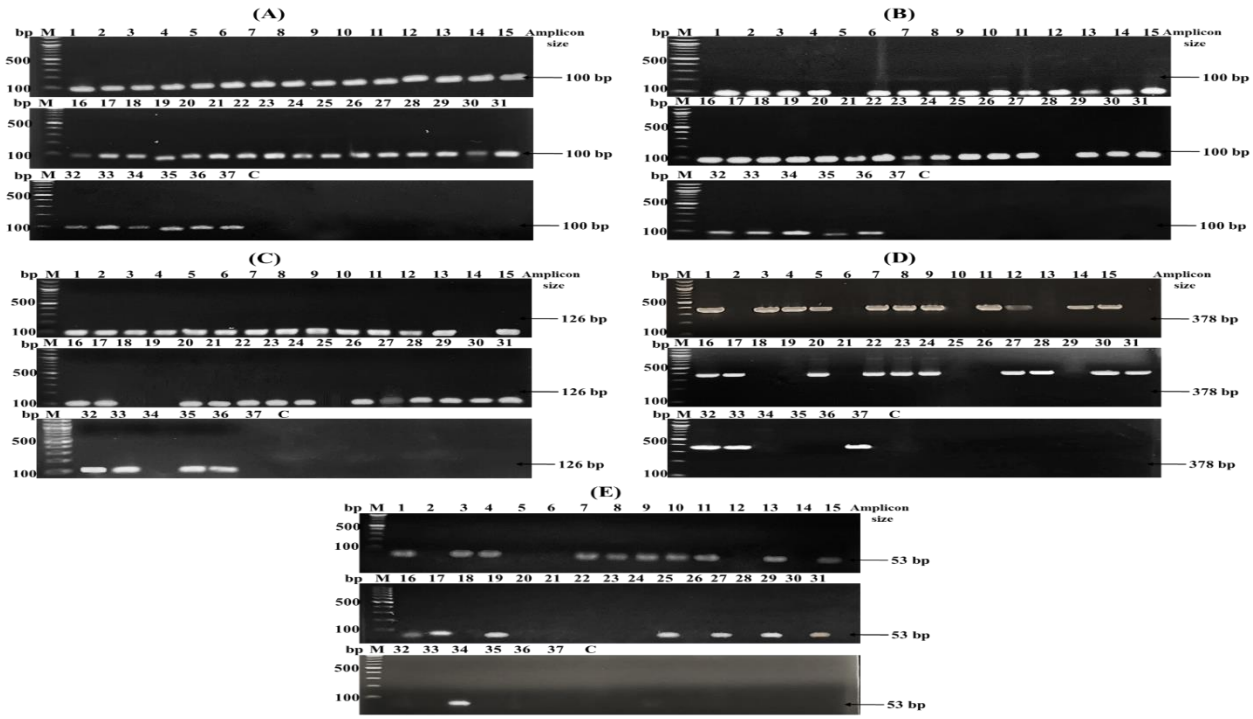
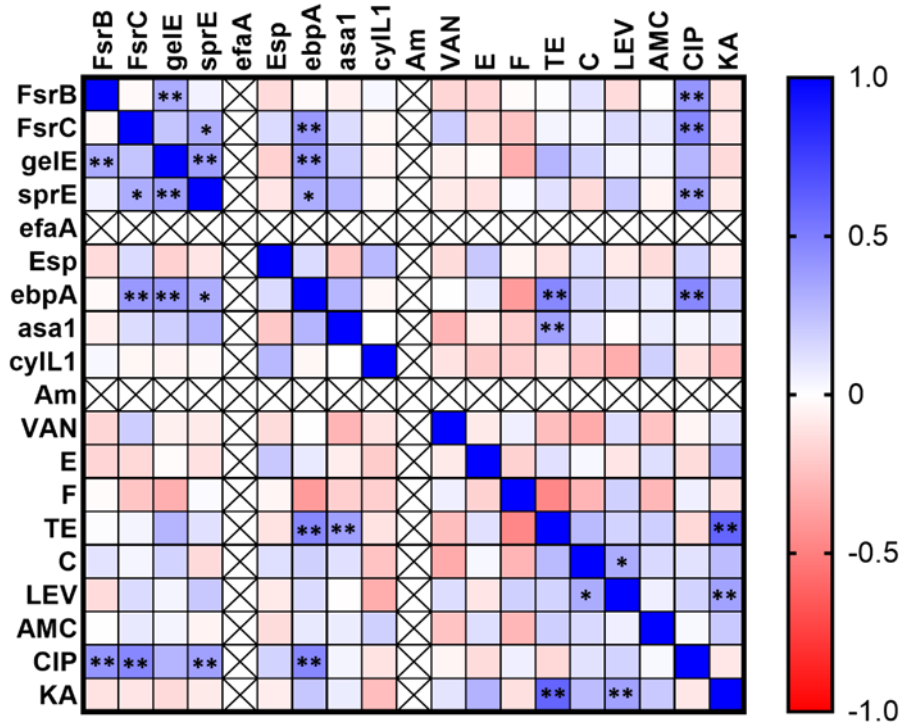


Figure 5. Correlation matrix of antimicrobial resistance and virulence genes in *E. faecalis*. Blue color represents positive correlation, while red color represents negative correlation (**P < 0.01, *P < 0.05), Am: Ampicillin, VAN: Vancomycin, E: Erythromycin, F: Nitrofurantoin, TE: Tetracycline, C: Chloramphenicol, LEV: Levofloxacin, AMC: Amoxicillin-clavulanic acid, CIP: Ciprofloxacin, KA: Kanamycin.



Discussion

Enterococcus faecalis is a bacterium that can be found in the human microbiota and can cause infections in healthcare settings. Its pathogenicity is due to various virulence factors such as surface proteins, adhesion factors, aggregation substances, extracellular proteases and toxins [23].

Enterococcus faecalis is one of major causes of antimicrobial resistant UTIs in healthcare settings due to its virulence factors that help it adhere to tissues and invade, making it more pathogenic and infectious [24].

The *fsr* QS system in *E. faecalis* regulates pathogenic traits like biofilm formation and enzyme production, making it a potential target for combating bacterial infections and reducing antimicrobial resistance. This system controls the proteases GelE and SprE, which are related to pathogenicity, through the secretion of an autoinducing peptide GBAP. Activation of the FsrC-FsrA two-component regulatory system by GBAP regulates the expression of two transcripts: *fsrBDC* and *gelE-sprE* [15].

The study aims to assess antibiotic resistance and virulence genes in *E. faecalis* causing UTI. Thirty-seven isolates were collected from urine samples and tested for sensitivity to ten antibiotics. Results showed high resistance to most anti-enterococcal antibiotics, posing challenges for treatment **(Figure 2A)**. Here in, full resistance (100%) was shown to ampicillin. This was in line with the report from Minia, Egypt with complete resistance to ampicillin [25]. The high rate of resistance to ampicillin might be the sign of prolonged and indiscriminate use of the drug in Egypt.

High rates of resistance were also detected for kanamycin (94.5%), ciprofloxacin (86.5%), levofloxacin (83.8%), erythromycin (83.8%) and tetracycline (78.4%). These resistance percentages were comparable with the study report from Saudi Arabia, where the *E. faecalis* isolates showed high resistance to tetracycline (86.36%), erythromycin (81.81%) and levofloxacin (77.27%) [26]. Amal Talib Al Sa'ady demonstrated that *E. faecalis* isolates from Iraq exhibited lower resistance rate to

kanamycin, tetracycline (64.3%) and ciprofloxacin (14.3%) [27].

This study found that around half of the isolated *E. faecalis* were resistant to chloramphenicol (48.6%) and amoxicillin clavulanate (43.3%). This level of resistance was similar to *E. faecalis* isolates from burn infections in Iraq, which showed 42.9% resistance to chloramphenicol and complete resistance to amoxicillin clavulanate [27].

The *E. faecalis* isolates showed low resistance to nitrofurantoin (10.8%) and vancomycin (8.10%), indicating these drugs could be effective for treating *E. faecalis* infections. Similar findings were reported in a study from Riyadh, where 10% and 4% of *E. faecalis* isolates were resistant to nitrofurantoin and vancomycin, respectively [28].

In the current study, most of the tested *E. faecalis* isolates were classified as MDR, accounting for 75.7% of the isolates. Furthermore, 18.9% of the isolates were categorized as XDR, indicating a higher level of resistance. In contrast, only a small proportion of isolates (5.4%) were found to be sensitive to the tested antimicrobial agents as shown in **figure 2B**. These findings are consistent with a study conducted by **Said and Abdelmegeed**, which reported that 74.6% of clinical *E. faecalis* isolates from Egypt were MDR, and a mere 2.8% were identified as XDR [29].

The presence of genes associated with *fsr* QS and putative virulence factors was studied in the present study by PCR. In QS circuit of *E. faecalis*, the *fsrB* gene encodes a transmembrane protein that processes a propeptide to produce a peptide pheromone. The *fsrC* gene encodes a histidine kinase sensor that responds to the peptide-signaling molecule, phosphorylates its response regulator, and then activates the *gelE-sprE* encoding gelatinase and serine protease enzymes [7].

It was found that *fsrB* and *fsrC* genes were harbored by most of the *E. faecalis* isolates 81% and 83.7%, respectively **(Figure 3A & 3B)**. As well, **Bag and colleagues** found that 80% and 60% of *E. faecalis* isolates were positive for *fsrB* and *fsrC*, respectively [30].

The current study suggested that *gelE-sprE* genes were prevalent in the tested *E. faecalis* isolates by 72.9% and 89%, respectively **(Figure 3C & 3D)**. Similarly, Song and coauthers detected *gelE* in 88% of the *E. faecalis* isolates [31]. Another study also revealed that 89.9% of the *E. faecalis* isolates possessed the *sprE* gene [32].

Among the tested isolates, a total of ten patterns associated with the *fsr* QS genes were identified **(Table 2)**, with only one isolate lacking the *fsr* QS cluster, while 54% of the isolates possessed a complete *fsr* QS genes. These findings suggest a potential involvement of the *fsr* QS regulatory system in the virulence of the urinary tract *E. faecalis* isolates.

Aggregation substance is a surface protein encoded by the *asa1* gene that is essential for biofilm formation and adherence to host tissues [20].

The EbpA protein is a cell wall-anchored protein that is encoded by the *ebpA* gene. EbpA is a subunit of the endocarditis and biofilm associated pilus (Ebp), which is involved in the formation of biofilm and the development of endocarditis [33]. *E. faecalis* antigen A (EfaA) is a surface protein encoded by the *efaA* gene. It helps *E. faecalis* adhere to host cells and extracellular matrix proteins like collagen and fibrinogen, aiding in tissue colonization [34]. The enterococcal surface protein Esp encoded by *esp* gene is related to adhesion, colonization and host immune evasion [35]. Cytolysin is a pore-forming toxin that disrupts host cell membranes, leading to cell lysis, immune evasion, and tissue damage. Its production and regulation are controlled by the cytolysin operon, which includes genes like *cylA, cylB, cylM, cylLL, cylL2* and *cylI* [36].

It was found that the tested *E. faecalis* isolates were multi-virulent, with each isolate carrying two or more virulence genes. This result aligns with previous study conducted in India [37].

The prevalence of the *efaA* gene was observed with the highest frequency (100%) among the tested isolates, followed by *esp* (91.8%), *ebpA* (83.7%), *asa1* (64.8%) and *cylLL* was less frequently detected (48.6%) **(Figure 4)**. The highest prevalence of *efaA* was also investigated among *E. faecalis* isolates from UTIs in Iran [38]. **Bittencourt de Marques and Suzart** indicated that more than half of the *E. faecalis* isolates from different clinical sources in Brazil harbored *efaA* and *esp* gene markers [39]. Talebi and colleagues found *ebpA* gene among 86% of clinical *E. faecalis* isolates and 91% of environmental isolates from Iran [40]. Another study revealed that 69.6% and 47.1% of *E. faecalis* isolates from UTIs carried *asa1* and *esp* genes, respectively [41]. The prevalence of *cylLL* is

in accordance with a report by **Mei YiWen et al.,** who detected *cylLL* in 53.52% of *E. faecalis* isolates [42].

It was observed that uropathogenic *E. faecalis* isolates were characterized by the presence of two or more virulence genes, thereby contributing to bacterial colonization and pathogenesis. Nine virulence patterns were detected among the tested isolates, with the presence of all detected virulence genes (*efaA + esp + ebpA + asa1 + cylLL*) being the most prevalent virulence pattern **(Table 2)**. A previous study found that all *E. faecalis* strains from UTIs carried multiple virulence determinants [41].

The virulence traits and antimicrobial resistance in *E. faecalis* can enhance its ability to colonize tissues, invade, and adapt, improving its survival [43]. The results indicated a significant correlation between resistance to kanamycin and tetracycline. Additionally, there was an association observed between levofloxacin resistance and resistance to kanamycin and chloramphenicol **(Figure 5)**.

The study found significant associations between QS and virulence genes, including *fsrB* with *gelE* and *sprE* with *fsrC* and *gelE*. Additionally, the presence of *ebpA* was significantly linked to *fsrC, gelE* and *sprE* genes. Ciprofloxacin resistance was significantly related to *fsrB, fsrC, sprE* and *ebpA*. Previous research has shown that ciprofloxacin resistance is associated with risk factors like hospital-acquired UTI or prior treatment in a urological department [44].

Another significant correlation between antimicrobial resistance and virulence was observed between tetracycline and *asa1* and *ebpA* genes. Another study in Kenya also reported a connection between tetracycline resistance and *asa1* in clinical isolates [45].

Conclusion

This study provides important insights for managing *E. faecalis* UTIs. Understanding antimicrobial sensitivity patterns helps in selecting appropriate antibiotics, improving treatment outcomes and reducing resistance risks. Analyzing *fsr* QS and virulence genes reveals *E. faecalis* pathogenesis and virulence mechanisms, guiding targeted therapies and prevention strategies. This approach helps combat antibiotic resistance and develop effective UTI treatments, benefiting public health and patient care.

Declaration

Ethics approval and consent to participate

All specimens were collected using a protocol approved by the Research Ethics Committee of Faculty of Pharmacy, Mansoura University with the ethical codes 2024-72.

Disclosure statement

The authors declare that they have no competing interests.

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Authors' contributions

DA performed the practical work, analyzed the data, wrote the first draft of the manuscript and revised the final format. AM. A supervised the practical work, troubleshooted, revised the data analysis and revised the first manuscript. M.I.S suggested the research point, troubleshooted, revised the data analysis and revised the first and final manuscript. RH suggested the research point and revised the final manuscript.

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References

1- **Flores-Mireles AL, Walker JN, Caparon M, Hultgren S.** Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat. Rev. Microbiol 2015; 13(5):269-284.

2- **Codelia-Anjum A, Lerner LB, Elterman D, Zorn KC, Bhojani N, Chughtai B.** Enterococcal urinary tract infections: a review of the pathogenicity, epidemiology, and treatment. J. Antibiot. 2023; 12(4):778.

3- **Borgio JF, AlJindan R, Alghourab LH, Alquwaie R, Aldahhan R, Alhur NF, et al.** Genomic Landscape of multidrug resistance and virulence in Enterococcus faecalis IRMC827A from a long-term patient. J. Biol. 2023; 12(10):1296.

4- **Rao C, Dhawan B, Vishnubhatla S, Kapil A, Das B, Sood S.** Emergence of high-risk multidrug-resistant Enterococcus faecalis CC2 (ST181) and CC87 (ST28) causing healthcare-

associated infections in India. Infect. Genet. Evol. 2020; 85:104519.

5- **Hollenbeck BL, Rice LB.** Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence 2012; 3(5):421-569.

6- **Rotta IS, Rodrigues WF, Dos Santos CTB, Mantovani HC, De Oliveira AG, Machado ABF, et al.** Clinical isolates of E. faecalis and E. faecium harboring virulence genes show the concomitant presence of CRISPR loci and antibiotic resistance determinants. Microb. Pathog. 2022; 171:105715.

7- **Ali L, Goraya MU, Arafat Y, Ajmal M, Chen J-L, Yu DJI.** Molecular mechanism of quorum-sensing in Enterococcus faecalis: its role in virulence and therapeutic approaches. Int. J. Mol. Sci. 2017; 18(5):960.

8- **Gambarotto K, Ploy M-C, Turlure P, Grélaud C, Martin C, Bordessoule D, et al.** Prevalence of vancomycin-resistant enterococci in fecal samples from hospitalized patients and nonhospitalized controls in a cattle-rearing area of France. Clin. Microbiol. 2000; 38(2):620-624.

9- **Peykov SZ, Aleksandrova VD, Dimov SG.** Rapid identification of Enterococcus faecalis by species-specific primers based on the genes involved in the Entner–Doudoroff pathway. Mol. Biol. Rep. 2012; 39:7025-7030.

10- **Ran S, He Z, Liang J.** Survival of Enterococcus faecalis during alkaline stress: changes in morphology, ultrastructure, physiochemical properties of the cell wall and specific gene transcripts. Arch. Oral Biol. 2013; 58(11):1667-1676.

11- **CLSI.** Performance standards for antimicrobial susceptibility testing, 31st ed. CLSI supplement M100. Wayne, PA. Clinical and Laboratory Standards Institute 2021; 41(3):111-114.

12- **Bertelloni F, Salvadori C, Moni A, Cerri D, Mani P, Ebani V.** Antimicrobial resistance in Enterococcus spp. isolated from laying hens of backyard poultry flocks. AAEM 2015; 22(4).

13- **Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM.** Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait Med. J. 2009; 41(2):117-122.

14- **Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds).** PCR protocols: a guide to methods and applications. Academic Press 2012.

15- **Nakayama J, Tanaka E, Kariyama R, Nagata K, Nishiguchi K, Mitsuhata R, et al.** Siamycin attenuates fsr quorum sensing mediated by a gelatinase biosynthesis-activating pheromone in Enterococcus faecalis. Am. Soc. Microbiol 2007; 189(4).

16- **Selleck EM, Van Tyne D, Gilmore MS.** Pathogenicity of enterococci. Microbiol. Spectr. 2019; 7(4).

17- **Pourhajibagher M, Chiniforush N, Bahador A**. Antimicrobial action of photoactivated C-Phycocyanin against Enterococcus faecalis biofilms: Attenuation of quorum-sensing system. Photodiagnosis Photodyn. Ther. 2019; 28:286-291.

18- **Fan W, Huang Z, Fan B.** Effects of prolonged exposure to moderate static magnetic field and its synergistic effects with alkaline pH on Enterococcus faecalis. Microb. Pathog. 2018; 115:117-122.

19- **Shepard BD, Gilmore MS.** Differential expression of virulence-related genes in Enterococcus faecalis in response to biological cues in serum and urine. Infect. Immun. 2002; 70(8):4344-4352.

20- **Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, et al.** Development of a multiplex PCR for the

detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among european hospital isolates of Enterococcus faecium. J. Clin. Microbiol. 2004; 42(10):4473-4479.

21- **Kafil HS, Mobarez AM, Moghadam MF, sadat Hashemi Z, Yousefi M.** Gentamicin induces efaA expression and biofilm formation in Enterococcus faecalis. Microb. Pathog. 2016; 92:30-35.

22- **Gao P, Pinkston KL, Nallapareddy SR, van Hoof A, Murray BE, Harvey BR.** Enterococcus faecalis rnjB is required for pilin gene expression and biofilm formation. Bacteriol. 2010; 192(20):5489-5498.

23- **Barbosa J, Gibbs P, Teixeira P.** Virulence factors among enterococci isolated from traditional fermented meat products produced in the North of Portugal. Food Control 2010; 21(5):651-656.

24- **Hashem YA, Abdelrahman KA, Aziz RK.** Phenotype–genotype correlations and distribution of key virulence factors in Enterococcus faecalis isolated from patients with urinary tract infections. Infect. Drug Resist. 2021:1713-1723.

25- **Esmail MAM, Abdulghany HM, Khairy RM.** Prevalence of multidrug-resistant Enterococcus faecalis in hospital-acquired surgical wound infections and bacteremia: concomitant analysis of antimicrobial resistance genes. IJID 2019; 12: 1-6.

26- **Khalil MA, Alorabi JA, Al-Otaibi LM, Ali SS, Elsilk SE.** Antibiotic resistance and biofilm formation in Enterococcus spp. isolated from urinary tract infections. J. Pathog. 2022; 12(1):34.

27- **Al-Sa'ady AT.** Detection of vancomycin resistance in multidrug-resistant Enterococcus faecalis isolated from burn infections. Drug Invent. Today 2019; 11(11):2984-2989.

28- **ALbeloushi A, Elbehiry A, Marzouk E, Zahran R, Edrees HJ.** Highlight on multidrug resistance of Enterococcus faecalis recovered from diabetic foot patients. J. Pure Appl. Microbiol. 2019; 13(1):481-489.

29- **Said HS, Abdelmegeed ES.** Emergence of multidrug resistance and extensive drug resistance among enterococcal clinical isolates in Egypt. Infect. Drug Resist. 2019:1113-1125.

30- **Bag MAS, Arif M, Riaz S, Khan MSR, Islam MS, Punom SA, et al.** Antimicrobial resistance, virulence profiles, and public health significance of Enterococcus faecalis isolated from clinical mastitis of cattle in Bangladesh. Biomed Res. Int. 2022; 2022.

31- **Song H, Bae Y, Jeon E, Kwon Y, Joh SJ.** Multiplex PCR analysis of virulence genes and their influence on antibiotic resistance in Enterococcus spp. isolated from broiler **chicken. J. Vet. Sci. 2019; 20(3).**

32- **Li J, Yang L, Huang X, Wen Y, Zhao Q, Huang X, et al.** Molecular characterization of antimicrobial resistance and virulence factors of Enterococcus faecalis from ducks at slaughterhouses. Poult. Sci. 2022; 101(4):101646.

33- **Montealegre MC, La Rosa SL, Roh JH, Harvey BR, Murray BE.** The Enterococcus faecalis EbpA pilus protein: attenuation of expression, biofilm formation, and adherence to fibrinogen start with the rare initiation codon ATT. MBio 2015; 6(3):e00467-00415.

34- **Kafil HS, Mobarez AM, Moghadam MF, sadat Hashemi Z, Yousefi M.** Gentamicin induces efaA expression and biofilm formation in Enterococcus faecalis. Microb. Pathog. 2016; 92:30-35.

35- **Toledo-Arana A, Valle J, Solano C, Arrizubieta MaJs, Cucarella C, Lamata M, et al. The** enterococcal surface protein, Esp, is involved in Enterococcus faecalis biofilm formation. Appl. Environ. Microbiol. 2001; 67(10):4538-4545.

36- Van Tyne D, Martin MJ, Gilmore MS. Structure, function, and biology of the Enterococcus faecalis cytolysin. Toxins 2013; 5(5):895-911.

37- **Lata P, Ram S, Shanker R**. Multiplex PCR based genotypic characterization of pathogenic vancomycin resistant Enterococcus faecalis recovered from an indian river along a city landscape. Springerplus 2016; 5:1-11.

38- **Samani RJ, Tajbakhsh E, Momtaz H, Samani MK.** Prevalence of virulence genes and antibiotic resistance pattern in Enterococcus faecalis isolated from urinary tract infection in Shahrekord, Iran. Rep. Biochem Mol. Biol. 2021; 10(1):50.

39- **Bittencourt de Marques E, Suzart SJ.** Occurrence of virulence-associated genes in clinical Enterococcus faecalis strains isolated in Londrina, Brazil. J. Med. Microbiol. 2004; 53(11):1069-1073.

40- **Talebi M, Moghadam NA, Mamooii Z, Enayati M, Saifi M, Pourshafie MR.** Antibiotic resistance and biofilm formation of Enterococcus faecalis in patient and environmental samples. Jundishapur J. Microbiol. 2015; 8(10).

41- **Sharifi Y, Hasani A, Ghotaslou R, Naghili B, Aghazadeh M, Milani M, et al.** Virulence and antimicrobial resistance in enterococci isolated from urinary tract infections. Adv. Pharm. Bull. 2013; 3(1):197.

42- **Mei YiWen MY, Wei LinYu WL, Wang QunYing WQ.** Detection of virulence genes in and analysis of the drug resistance of Enterococcus faecalis infecting patients in gastrointestinal medicine in the Henan area. J. Pathog. Biology 2015.

43- **Yang J, Chen Y, Dong Z, Zhang W, Liu L, Meng W, et al.** Distribution and association of antimicrobial resistance and virulence characteristics in Enterococcus spp. isolates from captive asian elephants in China. Front. Microbiol. 2023; 14:1277221.

44- **Jovanović M, Velebit B, Tošić T, Maki G, Pavić S, Jovanović S, et al.** Comparative study of virulence factor genes, β-hemolysis and biofilm production in invasive and colonizing enterococci. Eur. J. Inflamm. 2023; 21.

45- **Georges M, Odoyo E, Matano D, Tiria F, Kyany'a C, Mbwika D, et al.** Determination of Enterococcus faecalis and Enterococcus faecium antimicrobial resistance and virulence factors and their association with clinical and demographic factors in Kenya. J. Pathog. 2022; 2022.