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Molecular Detection of Virulence Factors and Antibiotic Resistance among Enterococcus Species Isolates from Kurdistan Region of Iraq

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ABSTRACT

Enterococcus spp., a part of the human normal flora, can acquire resistance to many antimicrobials. The existence of virulence determinants among the clinical isolates has led to the emergence of enterococci as potential pathogens in hospital settings. The study was performed to determine Enterococcus spp., the antibiogram, identify several virulence factors encoding genes, find the most prevalent genes from different sources, and determine the relationship of virulence factors with vancomycin resistance in both E. faecalis and E. faecium isolated from different clinical samples. Clinical isolates of enterococci were identified at the species level and then subjected to antibiotic susceptibility testing. The presence of seven virulence factors was investigated using the PCR technique. Various Enterococcal spp. have been identified among 116 clinical isolates; the two major species were E. faecalis (66.4%) and E. faecium (22.4%), and to a lesser extent, other Enterococcal spp. (11.2%). All the isolates were 100% resistant to tetracycline, and E. faecium isolates were significantly (P = 0.05) more resistant to ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, penicillin, meropenem, nitrofurantoin, and fosfomycin. All the virulence factor genes (gelE. asa1, hyl, esp, cylA, ace, and efaA) were detected in E. faecalis and E. faecium isolates. Hyl and cylA rates were significantly higher among E. faecium isolates, while ace gene was significantly higher among E. faecalis isolates. All E. faecalis isolates possess one or more VFs, while one E. faecium isolate is free of VFs. Only two E. faecium isolates possessed all the VFs. There wasn't an evident link between the VFs and clinical samples. In conclusion, Enterococcus spp. has an elevated frequency and heterogeneity of virulence genes. The high resistance rate and virulence factor results emphasize the importance of E. faecalis and E. faecium isolates in causing infections in Kurdistan region. Larger-scale species identification and genotypic studies are needed in the region.

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Keywords: Virulence factors, Enterococcus spp., E . faecalis, E. faecium

1. Introduction

Enterococcus spp. are natural environmental microorganisms and vital parts of the gut microbiome in healthy people and animals[1-2]. Enterococci are among the leading nosocomial pathogens; they can be transmitted from person to person, through contaminated food, and the environment causing soft tissue infections, bacteremia, endocarditis, and urinary tract infections[3-4]. They also isolated from patients with ventilator-associated pneumonia[5]. Most human enterococcus illnesses were caused by *E. faecalis* and *E. faecium*[1]. Enterococci are the 3rd most frequent hospital-acquired (HAI) pathogen, accounting for 14% of nosocomial infections in the USA between 2011 and 2014, a rise from 11% in 2007[6]. Enterococci are resistant to a wide range of antimicrobials used in the treatment of gram-positive bacterial infections, and their inherent resistance is shown to aminoglycosides, tetracyclin[7], clindamycin, and co-trimoxazole[8]. The appearance of multi-drug resistance strains, like vancomycin-resistance enterococcus (VRE), as an outcome of widespread use as well as abuse of antimicrobial agents in intensive farming and healthcare environments in the control of community-acquired infections, has grown to be an essential point of alarm around the world[9]. The presence of resistance genes in a strain alone does not make it pathogenic. Still, when paired with other virulence factors (VFs), it can make the strain life-threatening[10]. These VFs are categorized into two groups: secreted VFs including gelatinase (gelE), cytolysin (cylA), and hyaluronidase (hyl); and cell surface VFs such as enterococcal surface protein (esp), collagen-binding protein (ace), endocarditis antigen (efaA), and aggregation substances (asa1). These VFs play crucial roles in biofilm production, host cell attachment, invasion, and disease development[11]. Monitoring the incidence of VFs among Enterococcus isolates, in addition to antibiotic resistance is essential for understanding their clinical impact on a given location or healthcare facility and for considering measures of contro[12]. Understanding the pathogenic process of enterococci may be aided by knowledge of their VFs. As a result, this study was done to determine *Enterococcus* spp. in different

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age groups, antibiogram, identify several virulence factors encoding genes, find the most prevalent genes from various sources, and assess the relationship of virulence factors with vancomycin resistance in both *E. faecalis* and *E. faecium* isolated from various clinical samples.

2. Methods and Materials

2.1 Ethical approval

The Ethical approval letter was obtained from the scientific and ethical Committee of Publication Ethics at the College of Medicine and Duhok Health Directorate, Kurdistan region, Iraq, under reference no. 24102021-10-30 (dated October 24. 2021).

2.2 Period and place of the study

This cross-sectional study was done from October 2021 to June 2022. A total of 116 enterococcus isolates had been collected from specimens of patients (admitted and outpatients) from different age groups. The samples were collected from various teaching and specific hospitals, including Heevi Teaching Hospital, Emergency Teaching Hospital, Vin Private Hospital, Awny Labs in Duhok, Rizgary Teaching Hospital in Erbil, and San Medical Lab in Sulaimani. All the bacterial isolates were phenotypically investigated and molecularly characterized at the Duhok Medical Research Center/College of Medicine/University of Duhok.

2.3 Enterococcus spp. identification

The study included 116 clinical samples collected aseptically from various sources such as urine, blood, high vaginal swabs (HVS), semen, wound swabs, and bronchial washes. These samples were transported to the laboratory within 30 minutes to one hour for cultures. Bacteriological characterizations were conducted using different methods, including growing on blood agar, gram stain, and catalase test for species identification and characterization. The species were further verified using enterococcus differential agar and bile esculin agar. Subsequently, the specimens underwent confirmation using the Vitek 2 system.

2.4 Antibiotics sensitivity test

The antimicrobial sensitivity of enterococci isolates was evaluated against the following antibiotics (bioanalysis, Turkey): Erythromycin (E, 15 µg), Tetracycline (TE, 30 µg), Ampicillin (AMP, 10 µg), Nitrofurantoin (NIT, 100 µg), Ciprofloxacin (CIP, 5 µg), Penicillin (P, 10 µg), Vancomycin (VA, 30 µg), Teicoplanin (30 µg), Levofloxacin (5 µg), Trimethoprim-sulfamethoxazole (25 µg, 1.25/23.75), Gentamicin (10 µg), Meropenem (10 µg), Fosfomycin (200 µg), Linezolid (10 µg), and Tigecycline (15 µg) based on the Clinical and Laboratory Standards Institute (CLSI)[14]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were applied for antibiotics not on the CLSI list[15]. The disk diffusion technique of Kirby-Bauer was employed and confirmed by the VITEK-2 automated identification and susceptibility testing system according to the manufacturer's recommendations (BioMerieux, USA).

2.5 Bacterial DNA extraction

The bacterial genomic DNA was extracted using the boiling technique. Overnight-growth bacteria colonies were utilized. The colonies were placed in a test tube containing 400 µl of deionized water (dH2O), heated for 10 minutes in a water bath (100ºC), and centrifuged for 5 minutes at 2500 rpm. For the PCR, five microliters of supernatant were employed[16]. A NanoDrop Spectrophotometer at 260/280 nm (Thermo Scientific USA) was used to quantify the purity and concentration of isolated DNA specimens.

2.6 Enterococcus spp. identification using a Species-Specific primer.

PCR was used to identify the organism using the *ddl* genes, which encode D-alanine: D-alanine ligases unique to *E. faecalis* (*ddl E. faecalis*) and *E. faecium* (*ddl E. faecium*). Each PCR assay was performed in a 25 µl final reaction volume, which consisted of 12.5 µl of Hot StarTaq master mix (ADDBIO/Korea), 1 µl of (10 pmol/µl) of each prime (forward and reverse) (Macrogen/ Korea), and 5 µl of DNA template. The volume of the reaction was completed by adding of nuclease-free water (dH2O). the Amplification was performed using a thermal cycler (Biosan Ltd., U.A.). A molecular marker 100 bp (Jena Bioscience GmbH, Germany) was used to compare and determine the size of the amplicons. The resulting DNA fragments were visualized using a UV transilluminator at 302 nm and photographed using a digital camera (Bio-Rad). The optimization condition is shown in table (1).

2.7 Detection of virulence genes

Applying multiplex PCR, all Enterococci samples were tested for the existence of (7) virulence factors encoding genes (*gelE, asa1, hyl, esp, cylA, ace,* and *efaA*). Multiplex PCR is a widely used methodology for screening VFs genes in enterococci. Five primer pairs were employed to amplify the virulence genes *gelE, asa1, hyl*, *esp*, and *cylA*. Each 50 µl PCR mixture consisted of 5 µl of bacterial DNA, 0.5 µl (10 pmol/µl) from primers (Macrogen/ Korea) targeting *gelE, asa1, hyl*, *esp,* and *cylA* genes; and 25 µl of HotStarTaq master mixture (ADDBIO/Korea). The volume was completed by adding of dH2O. Regarding *ace* and *efaA* genes, multiplex PCR was also used; 25 µl PCR reaction consists of 5 µl DNA template, a 0.5 µl 10 pmol/µl of the specific primers (forward F and reverse R) for *ace* and *efa*A genes, and 12.5 µl of the HotStarTaq master mix, the volume completed with dH2O. 1% agarose gel electrophoresis at 95 volts for 45 minutes was used for the visualization of amplified DNA fragments. Amplicon size was determined by comparison with a 100-bp DNA marker. The primers' sequences, optimization, conditions, and references are shown in table (1).

2.8 Statistical analysis

The data was tabulated and analyzed on the statistical package for the social science "SPSS" version 25 (Chicago, IL, USA). Frequency and chi-square tests were utilized to assess the statistical significance of the data. A *P*-value < 0.05 was considered significant.

Tabel 1: Target genes, oligonucleotide primers, amplicon size, and PCR conditions used to detect genes in this study.

Gene	Sequence	Amplicons length (bp)	Optimization	Reference
ddI faecalis	F-ATCAAGTACAGTTAGTCTTTATTAG R-CGATTCAAAGCTAACTGAATCAGT	941	94 5 m 94 1 m 55 45 sec 72 1 m 72 10 m 30 cycle	41
ddI faecium	F-TTGAGGCAGACCAGATTGACG R-TATGACAGCGACTCCGATTCC	658	94 5 m 94 1 m 55 45 sec 72 1 m 72 10 m 30 cycle	41
asa1	F-GCACGCTATTACGAACTATGA R-TAAGAAAGAACATCACCACGA	375	95 15 m 94 1 m 56 1 sec 72 1 m 72 10 m 30 cycle	42
gelE	F-TATGACAATGCTTTTTGGGAT R-AGATGCACCCGAAATAATATA	213		43
cylA	F-ACTCGGGGATTGATAGGC R-GCTGCTAAAGCTGCGCTT	688		44
esp	F-AGATTTCATCTTTGATTCTTGG R-AATTGATTCTTTAGCATCTGG	510		45
hyl	F-ACAGAAGAGCTGCAGGAAATG R-GACTGACGTCCAAGTTTCCAA	276		36
ace	F:GGAATGACCGAGAACGATGGC R: GCTTGATGTTGGCCTGCTTCCG	616	95 10 m 94 1 m 58 1 sec 72 1 m 72 10 m 30 cycle	35
efaA	F: TGGGACAGACCCTCACGAATA R: CGCCTGTTTCTAAGTTCAAGCC	101		46

3. Results and Discussion

Recently, *Enterococcus* spp. has been of substantial interest because it tends to propagate quickly in hospital settings among healthcare personnel and critically ill patients[17,18]. In the Kurdistan region, few researchers have tackled the issue of antibiotic resistance associated with pathogenic enterococci, including the study done by Bahdeen Jalal and Husamuldeen Abdullah (2023)[7], Rashid and Omer (2018)[19], and Al-Naqshbandi, Chawsheen and Abdulqader (2019)[20]. The prevalence of the common virulence factors in clinical isolates hasn't been studied sufficiently in these regions, though their incidence in *Enterococcus* spp. varies depending on clinical origin and, in turn, affects their antibiogram profile. To our knowledge, this is the first study that included all these virulence factors. In addition, the study involved three Kurdistan provinces in one study regarding *Enterococcus* spp.

3.1 Demographic characteristics of patients

A total of 116 Enterococcus isolates were identified and included in the study. 45 (38.8%) enterococcus isolates were from Duhok city, followed by 44 (37.9%) from Sulaimani city and 27 (23.3%) from Erbil city. Among these, 77 (66.4%) were *E. faecalis,* 26 (22.4%) were *E. faecium*, and 13 (11.2%) were other *Enterococcus* spp. [5 (4.3%) *E. gallenarum*, 1 (0.86%) of each *E. avium* and *E. hirae* and 6 (5.2%) isolates were not being identified to the species level]. All *E. faecalis* and *E. faecium* bands have been detected after PCR amplification (Figure 1). In the current study, the culture-positive enterococci frequency in adult patients (17-75 years) was 93 (80.17%) (Mean =31, SD ±10.9) compared to 23 (19.83%) in child patients (less than 1–16 years) (Mean =3.5, SD ±4.2). These results follow the findings of Tedim *et al*.[21]. The commonness of *Enterococcus* spp. in adults might arise from variable changes in the environmental status with time as a consequence of alteration in nutrition, alteration in health

conditions, or antimicrobial treatment[22]. Likewise, enterococci-caused UTIs are known to be more common after 60 years of age, where obstructive uropathy is most common[23].

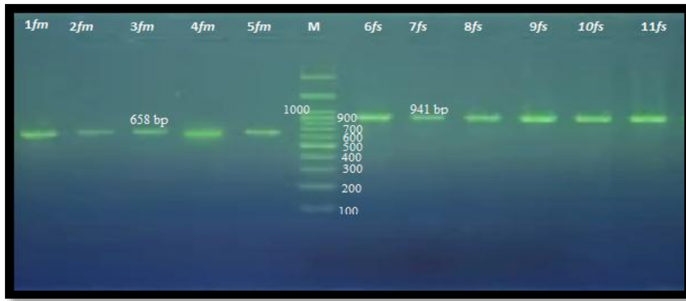

Figure 1: Gel electrophoresis of ddl faecalis and ddl faecium genes product PCR yields after being stained with safe dye and observed by ultraviolet light; fm: E. faecium, fs: E. faecalis; M:100 bp ladder; lanes 1,2,3,4,5 represent ddl E.faecium; lanes 6,7,8,9,1.

Enterococci showed a slight difference in the rates of Enterococcus spp. (E. faecalis, E. faecium, and others enterococci) among different age groups (Table 2). The association was statistically not significant (P = 0.952), which means the colonization rate is age-independent.

The samples were collected from urine (n = 60), HVS (n =15), wound swabs (n =11), blood (n = 9), semen (n =7), and only one sample from bronchial washes[24].

Tabel 2: Frequency and percentage of Enterococcus spp. among different age groups.

Age of patients	**Bacteria species** n (%)					
	Enterococcus faecalis **(n=77)**	***Enterococcus faecium*** **(n=26)**	**others enterococci (n=13)**	***P*** **(two-sided)**	**Range Mean (SD)**	**Total (n=116)**
Less than 1 year-16 years	15 (65.21)	5 (21.7)	3 (13.04)	0.952	1-14 3.5 (±4.2)	23 (19.82)
17-75 years	62 (66.66)	21 (22.58)	10 (10.75)		18-70 31 (±10.9)	93 (80.17)

3.2 Antibiotic resistance

The resistance rates of *E. faecalis*, *E. faecium*, and other enterococci for tetracycline were 100%. The resistance rats for other antibiotics were as follows: Gentamycin 81.9%, Ampicillin 84.5%, Erythromycin 86.2%, Trimethoprim-sulfamethoxazole 68.9%, Ciprofloxacin 68.1%, and Levofloxacin 53.4%. The lower resistance rate and most potent effects were observed for Penicillin 41.3%, Meropenem 31.9%, Nitrofurantoin 30.1%, Vancomycin 27.6%, Teicoplanin 14.6%, Fosfomycin (4.3%), Tigecycline (4.3%), and Linezolid (3.4%). The multi-drug resistant rate was 100% for all enterococcal isolates. The resistance rates to Ampicillin, Trimethoprim-sulfamethoxazole, Ciprofloxacin, Penicillin, Meropenem, Nitrofurantoin, and Fosfomycin antibiotics were significantly higher in *E. faecium* than in *E. faecalis* (P-value < 0.05). None of the strains tested were sensitive to all the antibiotics drugs used against enterococci[24].

3.3 Enterococcal virulence-associated genes

In the current study, the significance of the pathogenicity of the organisms was assessed by examining the virulence gene profile, which was the main goal of this study. Virulence factors were determined only for *E. faecium* and *E. faecalis* strains due to their importance in human infections in our locality. It effectively conveys that in this study, multiplex PCR has been applied to detect *gelE, asa1, hyl, esp, cylA, efaA,* and *ace* genes (Figure 2 and Figure 3), which showed a higher percentage of *gelE, asa1, hyl, esp,* and *cylA* genes among *E. faecium* isolates than their presence in *E. faecalis* isolates, with *efaA* and *ace* being the most prevalent among *E. faecalis* isolates (Table 3). This finding contrasts with many studies that reported a high prevalence of virulence factors (VFs) among *E. faecalis*[25–27]. *GelE, asa1, esp,* and *efaA* VFs genes are insignificantly associated with *E. faecalis* and *E. faecium* isolates, but the presence of these genes was high among isolates. High rates of *gelE* among both species (82.52%) enhance the ability of enterococci to hydrolyze host extracellular matrix proteins. In comparison, the presence of a high rates of *asa1* gene (95.14%) in the majority of this study isolates of *E. faecalis* (94.8%) and *E. faecium* strains (96.2%), can facilitate the exchange of resistance and virulence associated genes in community and hospital settings. The *esp* gene, detected at a high rate (80.58%) in enterococcal isolates, is reported to be highly associated with biofilm formation in enterococci[28]. In this study, high rates of this gene were detected in *E. faecalis* and *E. faecium* isolates (79.2% and 84.6%, respectively). These high rates in this study and other similar studies[29,30] could be due to the fact that strains containing this gene can acquire antimicrobial-resistant genes. These antibiotics resistant bacteria have long-lasting steadiness in the body[31]. In addition, it has also been illustrated

Tabel 3: Frequency of virulence factors genes in E. faecalis and E. faecium isolates from different clinical specimens.

Source	Virulence genes						
	gelE **Efs :Efm n(%)**	***asa1*** ***Efs:Efm n(%)***	***hyl*** ***Efs:Efm n(%)***	***esp*** ***Efs: Efm n(%)***	***cylA*** ***Efs: Efm n(%)***	***ace*** ***Efs:Efm n(%)***	***efaA*** ***Efs: Efm n(%)***
Blood	3 (3.89): 5(19.23)	4(5.19):5(19.23)	0(0.0):2(7.69)	2(2.59):5(19.23)	0(0.0):4(15.38)	2(2.59):3(11.53)	4(5.19):5(19.23)
Bronchial washes	0(0.0):1(3.84)	0(0.0):1(3.84)	0(0.0):0(0.0)	0(0.0):1(3.84)	0(0.0):1(3.84)	0(0.0): 1(3.84)	0(0.0):1(3.84)
HVS	10(12.98):2(7.69)	12(15.58):2(7.69)	1(1.29):0(0.0)	9(11.68):2(7.69)	5(6.49):0(0.0)	10(12.98):0(0.0)	13(16.88):2(7.69)
Semen	6(7.79):1(3.84)	6(7.79):1(3.84)	1(1.29):0(0.0)	6(7.79):1(3.84)	2(2.59):1(3.84)	5(6.49):1(3.84)	5(6.49):1(3.84)
Urine	37(48.05):12(46.15)	43(55.84):13(50.0)	2(2.59):3(11.53)	38(49.35):11(42.30)	22(28.57):10(38.46)	31(40.26):4(15.38)	42(54.54):10(38.46)
Wound swabs	7(9.09):1(3.84)	8(10.38):3(11.53)	1(1.29):0(0.0)	6(7.79):2(7.69)	3(3.89):3(11.53)	7(9.09):0(0.0)	8(10.38):2(7.69)

that this feature has a crucial role in the changing of antimicrobial resistance genes in between cells and in rising resistance to antimicrobials[32].

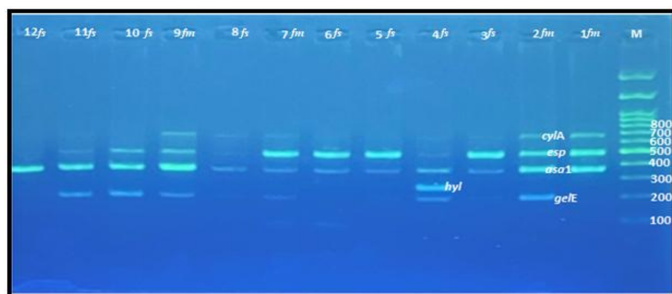

Figure 2: Gel electrophoresis of gelE, asa1, esp, hyl and cylA genes multiplex PCR yields after being stained with safe dye and observed by ultraviolet light; lanes 3,4,5,6,8,10,11,12 represent E. faecalis; lanes 1,2,7,9 represent E. faecium; M: molecular marker.

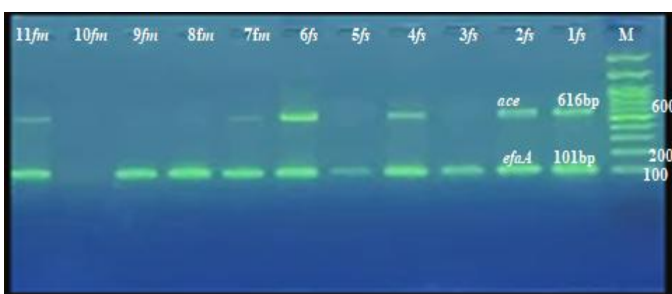

Figure 3: Gel electrophoresis of efaA and ace genes multiplex PCR products after being stained with safe dye and observed by ultraviolet light: lanes 1,2,3,4,5,6 indicate E. faecalis; lanes 7,8,9,10,11 indicate E. faecium; M: molecular marker of 100bp.

The *hyl* gene was found in a low percentage (9.7%), which is significantly higher in *E. faecium* isolate (P = 0.033). Their significance indicates that *E. faecium* isolates (19.23%) with a higher rate are more prone to acquire the gene than *E. faecalis* which has a lower rate of this gene (6.5%). *Hyl* gene was described earlier as restricted to *E. faecium* species[33], which is inconsistent with this study results obtained as the *hyl* gene was detected in both species. *CylA* gene was detected in 49.5% of isolates, and it was statistically significant (P = 0.005) higher among *E. faecium* (73.1%) isolates compared to *E. faecalis* isolates (41.5%) in the current study. This feature makes *E. faecium* able to hemolysis of blood cells and increase the infection rate more than *E. faecalis*.

Regarding *ace* gene, this gene was significantly higher among *E. faecalis* isolates (P= 0.001). The higher rate among *E. faecalis* isolates (71.4%) enhances the role of *E. faecalis* in infection and mediates the attachment of *E. faecalis* cells to host extracellular matrix proteins collagen and laminin, the first step of colonizing, then subsequently quickly invading the host cells. A high rate of *ace* (72%) was also detected in Duhok city in the study conducted by Khalid (2016) on *E. faecalis* isolated from urine[8]. Contrary to our results, a Bulgarian study reported the prevalence of these genes among *E. faecium* isolates (88.5%)[34]. The causes of the differences in the rates of virulence factors could be as follows: first, according to the regional origin, *Enterococcus* spp. are genetically different from each other depending on the life conditions in each region and environment; second, the nature of the sample used in the study as a sampling source, that is to say, some samples were taken from blood, while others used urine, HVS, wound swabs, and semen.

The co-existence of different VFs among isolates was assessed. There are various combinations of virulence genes in *E. faecalis* and *E. faecium*. Of *E. faecalis* isolates, 77 (100%) bear VFs; one isolate bears one gene and the others harbor three or more virulence genes simultaneously. Also, out of 26 *E. faecium* isolates; 25 (96.15%) possess VFs, one isolate from urine is free of genes, and 25 isolates harbor three or more virulence genes (Table 5). *E. faecium* that lacks virulence genes may be a commensal isolate that reaches the urethra because of the vicinity of the anal orifice to the urethra; such enterococci reside as commensals in the gastrointestinal tract. In *E. faecalis*, its most common combinations were *gelE, asa1, esp, cylA, efaA, ace* (20.77%) and *gelE, asa1, esp, efaA, ace* (18.18%), while in *E. faecium* it was *gelE, asa1, esp, cylA, efaA, ace* (19.23%) and *gelE, asa1, esp, cylA, efaA* (19.23%). None of the *E. faecalis* isolates possessed all of the virulence genes simultaneously, but two *E. faecium* isolates harbored all the tested VFs (one from blood and the other from urine). In the blood, *E. faecium* might express more virulence factors to evade or overpower the host's immune response, allowing them to establish and maintain an infection in the bloodstream. These findings contrast with a study that reported that isolates linked to bacteremia did not show any particular propensity for the carriage of virulence genes. In contrast, isolates from UTIs usually possessed two to four virulence traits[35]. In addition, in the urine one isolate of *E. faecium* has all the VFs genes. The presence of a high rates of virulence factors in *E. faecium* isolated from urine samples suggests a potential of severe infections or difficulties in treatment due to the bacterium's ability to resist antibiotics and persist in the urinary tract environment. The most common VFs combination in *E. faecalis* was *gelE, asa1, esp, cylA, efaA*, *ace* (n=16). This combination observed in urine, wound swabs, HVS, and semen samples following the study of Golob *et al.* who also found the most common combination to be *ace, asa1, cylA, efaA, esp, gelE* from urine, wound swabs, HVS, and ejaculate[26].

3.4 Distribution of virulence genes among clinical specimens

The results of the current study showed that *E. faecium* was isolated from the blood, harboring a high percentage of virulence genes, with equal percentages from *gelE*, *asa1*, *esp*, and *efaA* genes (19.23%) (Table 5). Also, as mentioned earlier, one sample from blood harbored all the VFs genes simultaneously, while in HVS and semen samples, a large proportion of VFs genes is present within *E. faecalis*. In urine samples (the most prevalent source), the percentage of *gelE*, *asa1*, *esp*, *ace*, and *efaA* genes is higher in *E. faecalis,* whilst *hyl*, *cylA* is higher in *E. faecium*. Further, *ace* and *hyl* are absent in wound swab isolates, while *gelE*, *ace*, and *efaA* are more common in *E. faecalis*. To our knowledge, there is little research involved in the association between VFs and different sample sources. A study done by Shokoohizadeh *et al*. (2018) indicates that there is no relationship between VFs and clinical specimens[27]. Also, it has been found no significant relationship between VFs and sample sources in our research. More VFs observed among *E. faecium* in the blood isolate, while VFs in *E. faecalis* are higher than those in *E.*

faecium in the genital area. Regarding the urine sample, there is a slight difference in the proportion of VFs in both species.

Table 4: Different combinations in Enterococcus faecalis and Enterococcus faecium isolates.

No. of VF in a combination	Genotype patterns	*Enterococcus faecalis* N=77	*Enterococcus faecium* N=26
0	*None*	----	1
1	*gelE*	1	----
3	*asa1,efaA, ace*	3	----
	gelE,asa1,efaA	3	----
	gelE,asa1,esp	2	----
	esp,efaA, ace	1	----
	asa1,efaA, ace	----	1
4	*gelE,asa1,efaA,ace*	4	----
	gelE,asa1,esp,efaA	7	2
	asa1, esp,efaA, ace	3	----
	gelE, esp,efaA, ace	2	----
	gelE.asa1,cylA,efaA	1	1
	gelE,asa1,esp,cylA	----	3
	asa1,cylA,efaA, ace	2	---
	gelE,asa1,hyl,cylA	----	1
	gelE,asa1,hyl,esp	1	----
	asa1,esp,cylA,efaA	1	1
5	*gelE,asa1,esp,efaA,ace*	14	1
	gelE,asa1,esp,cylA,efaA	7	5
	Asa1,esp,cylA,efaA,ace	4	1
	gelE,asa1,esp,cylA,ace	1	----
	gelE,asa1,cylA,efaA,ace	1	----
	gelE,asa1,hyl,esp,afaA	----	2
6	*gelE,asa1,esp,cylA,efaA,ace*	16	5
	gelE,asa1,hyl,esp,afaA,ace	3	----
7	*gelE,asa1,esp,hyl,cylA,efaA,ace*	----	2

3.5 Presence of virulence genes in vancomycin-sensitive enterococci and vancomycin-resistant enterococci

A correlation has been established between vancomycin-sensitive enterococci (VSE) and vancomycin-resistant enterococci (VRE) regarding the presence of VFs. The predominance of VFs in VSE was more significant in *E. faecalis* except for *hyl* gene (20%), which is significantly higher among VR*E. faecalis* compared to 1.75% of VS*E. faecalis* isolates (*P*-value 0.004). In contrast, *asa1*, *esp, cylA*, *ace*, and *efaA* were predominant in VR*E. faecium* isolates except in *gelE* (88.88%) and *hyl* (27.77%), which were insignificantly higher in VSE (P = 0.365 and 0.097, respectively). *Hyl*'s association with virulence, biofilm formation, and colonization ability make it a notable gene of interest, which can enhance the bacterium's persistence in the host, resistance to the immune system, and antimicrobial treatment. *Hyl* was not detected in VR*E faecium* but only among VS*E. faecium* (Table 6), which indicate that the presence or of *hyl* gene doesn't solely determine the the antibiotic resistance profile and the pathogenicity of the VRE strains. The complex relationship between genetic elements and VFs determines the overall behavior and clinical impact of VRE strains. Contrastly, *hyl* is a particular virulence gene in epidemic hospital-acquired strains of vancomycin-resistant *E. faecium* in the USA, Europe, and Australia[36]. In a study done by Duprè *et al*., it was shown that the prevalence of *hyl* (*P* = 0.04) genes was notably higher in VRE isolates (27.7%) than among VSE isolates (8.8%)[37]. The *esp* gene has been observed to be confined to vancomycin-resistant bacteria[38]. However, our findings revealed the presence of the *esp* gene in the two VSE and two VRE isolates. The detection of *esp* in isolates sensitive and resistant to vancomycin suggests that this feature evolved before the development of resistance, not just to vancomycin but also to additional medications used in health-care facilities[39]. On the other hand, in Turkey, the researchers discovered that VRS carries more virulence-related genes than VRE[40]. There were some limitations in the current study, including a small sample size and a short duration of sampling, which made us unable to detect different strains of *Enterococcus* spp.

Conclusion

Enterococcus spp. has an elevated frequency and heterogeneity of virulence genes. The present study revealed that the antimicrobial resistance and VFs genes are more common in *E. faecium* isolates than *E. faecalis* isolates. As a result, *E. faecium* is more pathogenic than *E. faecalis* isolates. The high resistance rate and virulence factor results emphasize the importance of *E.*

faecalis and *E. faecium* isolates in causing infections in the Kurdistan region. Larger-scale species identification and genotypic studies are needed in the area. Epidemiological studies on all *Enterococcus* spp. were required to detect and study the other important species that had not been studied well in this study because of low rates.

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Author contributions

Ahmed and Abdulrahman contributed to the conception and design of the study. Haliz and Abdulrahman did collect the samples. The practical part and the statistical analysis were done by Haliz. All authors contribute to article writing and final approval of the article.

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Conflict of interests

None.

Table 6: Combinations of VFs genes in VRE and VSE.

Genes	*E . faecalis*			*E. faecium*		
	VSE (n = 57)	VRE (n = 20)	*P*	VSE (n = 18)	VRE (n = 8)	*P*
gelE	47(82.45)	16(80)	0.806	16(88.88)	6(75)	0.365
asa1	55(96.49)	18(90)	0.260	17(94.44)	8(100.0)	0.497
hyl	1(1.75)	4(20)	0.004*	5(27.77)	0(0.0)	0.097
esp	47(82.45)	14(70)	0.237	14(77.77)	8(100.0)	0.147
cylA	25(43.85)	7(35)	0.489	12(66.66)	7(87.5)	0.269
ace	41(71.92)	14(70)	0.869	6(33.33)	3(37.5)	0.837
efaA	54(94.73)	18(90)	0.460	14(77.77)	7(87.5)	0.562

*significant association

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