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Original article

Prevalence of adherence factors and vancomycin resistant genes among *Enterococcus faecalis* isolated from women with urinary tract infection

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ABSTRACT

Background: *Enterococcus faecalis* is a facultative anaerobic gram-positive coccus, a saprophyte of the human gastrointestinal tract, and acts as an opportunistic pathogen that can cause a range of nosocomial infections, including urinary tract infections (UTIs). **Aim:** The current study was conducted for detection of the adherence genes and vancomycin-resistant genes in local *Enterococcus faecalis* isolated from women with UTIs. *E. faecalis is* thought to be one of the most significant causes of this infection recently in several hospitals in the province of Diyala, Iraq. **Methods**: One hundred and thirty-four urine samples were collected from actual cases of urinary tract infections during the period from September 2023 to January 2024 from women patients in Baqubah Teaching Hospital, Al-Batool Teaching Hospital for Maternity and Children, and the specialized consulting clinic in Baqubah City. **Results**: Twenty-four clinical isolates were diagnosed as *E. faecalis*; by percentage, it reached 17.9% based on microscopic examinations, morphological characteristics, and the molecular method of PCR, depending on the *ddl* gene. All isolates were *tested for the agg, ace, esp, pil, vanA, and vanB genes; the PCR results showed that these genes were found in 70.8%, 8.3%, 8.3%, 8.3%, 16.7%, and 0% of the samples, respectively.* Also, the results indicated that isolates that possess genes related to adhesion do not carry the vancomycin resistance gene, and the opposite is true. **Conclusion**: The high incidence of UTI caused by *E. faecalis* isolates that were vancomycin resistant may be due to reasons other than carrying *van*A genes. The study also found that *E. faecalis* local isolates do not have the vanB gene.

Introduction

Enterococcus faecalis is a facultative anaerobic gram-positive coccus, saprophyte of the human gastrointestinal tract and acts as opportunistic pathogens that can cause a range of nosocomial infections, including urinary tract infections (UTIs) [1]. Enterococci including commensal *E. faecalis* are colonized approximately 95% of people, and considered to offer a health benefit to the human host [2]. Although several strains of *E. faecalis* are able to cause community-acquired infections, as well as being considered opportunistic pathogens which causes hospital-acquired infections. It also causes many infections including endocarditis and bacteremia in addition urinary tract infections [3].

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E. faecalis has a low-GC genome. Enterococcus has an average GC content of 37.99% and a genomic length of 3.20 Mb [4]. In order to distinguish between the two main clinically significant species, *E. faecalis* and *E. faecium*, it was used a PCR assay to find internal segments of the gene encoding D-alanine-D-alanine ligase, or *ddl*. This gene was described as diagnostic for this species [5].

Adherence to host tissues, particularly UTIs, is a first crucial step in the pathogenesis of *E. faecalis* [2]. One of the virulence factors associated with adherence is an aggregation substance (Agg). It is a surface protein mediates the adherence of *E. faecalis* to renal epithelial cells, which is expressed in response to pheromone induction [6]. The adhesion factor (Ace) of *E. faecalis* is another surface protein that promotes bacterial adherence to collagen. Both Ace and Agg are essential for colonizing and adhering to host tissues. In addition, *E. faecalis* can form biofilms by permanently adhering [7]. These two genes are more prevalent in *E. faecalis* than in *E. faecium* [8].

The enterococcal surface protein (Esp) is a large protein includes 1,873 amino acids with an N-terminal domain (50-743 amino acids). In the database this protein without significant similarity to other proteins [9]. This extracellular surface protein encoded by *esp* gene, and contributes to the biofilm formation, so there is relationship between the *esp* gene and patterns of antibiotic susceptibility and biofilm development [10]. Several surface protein adhesins in bacteria bind to host ligands that have similar unique architecture of the Esp protein. Therefore, Esp might have same function as fimbriae in of *P. mirabilis* and *E. coli* that acting as a colonization factor that promotes adhesion to the uroepithelium [11]. In addition, *E. faecalis* have ability to cling to biotic and abiotic surfaces and biofilm-forming machinery by *pil* gene that gives it extra antimicrobial resistance. These diverse virulence factors allow *E. faecalis* to cause urinary tract infections [12]. Therefore, enterococci cause significant infections in hospital settings [13].

The ability of *E. faecalis* to resist the detrimental effects of our commonly utilized antibiotics contributed to an increase in the prevalence of enterococcus infections in humans [14]. According to epidemiological information, enterococci are significant conduits for the transmission of genes that confer resistance to antibiotic among different species of bacteria. Antimicrobial-resistant enterococci, especially resisting vancomycin *E. faecalis* (VREF), are therefore an eternal clinical issue on the global level [1].

Urinary tract infections (UTIs) associated with healthcare are rising rapidly due to vancomycin-resistant enterococci (VRE). In the USA, enterococci rank second overall, and account for 15% they usually linked to recurrent UTIs and are more prevalent in men [15]. In addition, aminoglycoside and glycopeptide resistance are usually at high level among *E. faecalis* and much higher when compare to other species belong to Enterococcus [16].

Human pathogens like *E. faecalis*, *E. faecium*, and *S. aureus* have clusters of genes that are resistant to vancomycin. The v*an*A and v*an*B two-component system triggers the of the van genes in response to extracellular glycopeptide antibiotics, and pathogenic bacteria exhibit two main forms of inducible vancomycin resistance [17]. The *van* gene, causing resistance to vancomycin, has subtypes A, B, D, C, and E. Transposon Tn1546 has classes A and B. High-grade vancomycin resistance can be caused by these two genes, which may be transmitted to conjugative plasmids in Enterococcal strains and other species like Staphylococcus. This could raise the likelihood of vancomycin resistance. On the contrary, genes D, C, and E are found on chromosome and cause low-grade resistance to vancomycin [10]. *E. faecalis* is now recognized as a major contributor to community- and hospital-acquired UTIs, which can result in life-threatening consequences such bacteremia [18].

Aims of the study: The current study was conducted for detection of the adherence genes and vancomycin-resistant genes in local *Enterococcus faecalis* isolated from women with UTIs. *E. faecalis* thought to be one of the most significant causes of this infection in recent years in several hospitals, province of Diyala, Iraq.

Methods and Materials

Sample collection and identification

From September 2023 to January 2024, 134 actual cases of urinary tract infections were compiled from women at two major hospitals in Baqubah City, province of Diyala. Baqubah Teaching Hospital and Al-Batool Teaching Hospital for Maternity and Children, in addition to the

specialized consulting clinic in Baqubah City. Midstream urine was collected and used for microscopy; the cultural characterization on MacConkey agar, blood agar, and Pfizer selective agar, together with an aerobic incubation at 37ºC for 24 hours, was used for the isolates' primary identification. Himedia/India was the source of all culture media orders.

DNA extraction and molecular identification of *E. faecalis*

The boiling method was used for genomic DNA extraction [19]. The concentrations and purity of the extracted DNA were estimated by nanodrop spectrophotometer at 260 and 280 nm [20]. A specific primer for the *dll* gene was used for identifying and classifying *E. faecalis* (Table 1).

PCR was performed via reaction components prepared by adding 5 µl of template, 12.5 µl of Go Taq® Green master mix (2X) from Promega, 1 µl from each forward and reverse *ddl* primer, and 4.5 µl of nuclease-free water to complete the volume to 25 µl. Table 2 illustrates PCR conditions. PCR products were detected by gel electrophoresis (1.5% agarose gel stained with ethidium bromide for 1 hr. at 60 V) and then visualized by transilluminator.

Detection of adherence and resistance genes

The spread of *agg, ace, pil, esp, van*A, and *van*B genes in local *E. faecalis* isolates was detected by using multiplex and uniplex PCR with specific primers and amplicon size (Table 1). The multiplex PCR was performed for (*agg, ace*) genes and (*van*A, *van*B) genes. Reaction components were prepared by adding 5 µl of template, 12.5 µl of Go Taq® Green master mix (2X) from Promega, 1 µl from each forward and reverse primer for both genes, and 2.5 µl of nuclease-free water was added for a completed volume of 25 µl. The uni-plex PCR was performed for *pil* and *esp* genes using the same reaction mixture used to detect the *ddl* gene and PCR conditions (Table 2). PCR products were detected by gel electrophoresis, which was mentioned previously.

Ethical approval

The inquiry was governed by the ethical standards outlined in the Declaration of Helsinki. Prior to the collection of the sample, the patient provided verbal and analytical consent. The study protocol, subject information, and permission form were reviewed and approved by a local ethics committee under document number 2030 (dated 20/8/2023).

Results and discussion

Identification of *E. faecalis*

Enterococcus faecalis isolates showed the ability to grow and tolerate bile salts on MacConkey agar medium; its colonies appeared as pale, colorless, and non-lactose fermenters. On the blood agar medium, they appeared as gray colonies with variable hemolysis ability; also, there is a clear growth on the Pfizer selective agar medium. All isolates were negative for the citrate and catalase. The isolate diagnosis was confirmed molecularly using *ddl* gene detection.

The results showed that all isolates carried the *ddl* gene, indicating that they are *E. faecalis*, as shown in Figure-1 for the electrophoresis of the PCR products, where the presence of bands with a length of 775 pb is evidence of a positive result. Twenty-four *E. faecalis* isolates were obtained from urine samples that were collected from UTI patients by a percentage of 17.9%. The most crucial method for identifying bacteria species that cause diseases quickly and more accurately is molecular techniques like PCR [20].

Detection of adherence genes

The *agg, ace, pil,* and *esp* genes were detected in the 24 *E. faecalis* isolates under study by PCR by use of specific primers (Table 1). All these genes are contributing to surface adhesion; therefore, it is considered involved in biofilm formation. Also, it participates in microcolony formation and dissemination. These genes (*agg, ace, esp,* and *pil*) play important roles in the formation of surface binding and the stability of biofilm structure. The results of DNA gel electrophoresis for multiplex PCR products, which are shown in Figure 2, show the presence of bands with a length of 413 bp and 615 bp, which is considereda positive result for *agg* and *ace* genes, respectively. In the current study, *agg* and *ace* genes were identified in 70% and 8% of *E. faecalis,* respectively.

The results were obtained from study which done by Hashem et al from Egypt [7], who found the most common gene in *E. faecalis* was agg with a prevalence reach 67%, this very similar to the results of our study. Ghazvinian et al [21] conducted a study in Iran on the prevalence of ace gene, the results indicated that its frequency in was 30% *E. faecalis* isolated from various sources.

The results of DNA gel electrophoresis for uni-plex PCR products to identify *esp* gene, the frequency of this gene among isolates under study was 8% by bands with length 932bp (Figure-3). Also, the frequency of *pil* gene among isolates under study was 8% by bands with length 620bp (Figure-4).

The results of a study done in Bangladesh showed 10% of *E. faecalis* isolates were carrying *pil* gene [22]. This is very close to the findings of the present study on the prevalence of this gene. A study conducted in India, among clinical isolates from different sources and commensal isolates of *E. faecalis* were 30% and 7% positive for the *esp* gene respectively. It was also found the few isolates that lack *esp* gene had ability to formation of biofilm, therefor may not be the only factor determining produced biofilm [23].

Detection of vancomycin genes

All of the *E. faecalis*-resistant isolates underwent multiplex PCR amplification; the amplified process revealed that 4 (16.7%) isolates contained the *van*A gene. and 0% do not have the *van*B gene. Positive results for the amplified *van*A and negative *van*B genes are displayed in Figure 5, with amplicon sizes of 223 bp without bands at 433 bp. In the previous study done by Azizi et al. in Iran [24], which aimed to detect *vanA* genes of *E. faecalis* isolated from clinical specimens in a hospital in Kermanshah, it was found that the *van*A gene was detected at a frequency of 2.3%.

Another study done by Moosavian et al. [25] from Iran found the vanA gene was detected in 91.5% of 59 vancomycin-resistant or semi-susceptible Enterococcus isolates, while these isolates lack the vanB gene. Generally, *van*A is mostly carried by *E. faecalis* and *E. faecium*; it is more widespread than other *van* genes. It is responsible for most of the human cases of VRE around the world [26].

The presence of genes in the isolates would be advantageous to determine the colonization and boost this pathogen's pathogenicity and the survival of the bacterium in the host. In the current study, the *agg* gene exhibited a greater proportion amongst *E. faecalis* isolated from UTI (Table 3).

These results indicate that the most prevalent pattern in local *E. faecalis* isolates is (*agg*) at 70.8%, followed by the pattern (*van*A) at 16.7%, the pattern (*esp),* and the pattern (*pil). The pattern* (*ace*) rate reached 8.3% of each, while there was a percentage of 4.2% that did not carry any pattern (Table 3). They also indicate that isolates that possess genes related to adhesion do not carry the vancomycin resistance gene and vice versa. The results of the current study showed that the isolates carrying the resistance gene *van*A are devoid of the studied virulence genes, indicating that isolates carrying the *van*A genopattern are definitely hospital-acquired because they do not carry the virulence genes that contribute to this infection.

The tree diagram showed the presence of three main genetic groups with a degree of similarity reaching 0%. The first group includes one isolate numbered (16) that did not carry any of the genes detected in the current study. The second group includes four isolates (10, 14, 20, and 21) that are 100% similar and all had the *vanA* genopattern (carry only *van*A). The third group includes nineteen isolates with similarity reaching 65% that were divided into two subgroups, A and B. Subgroup A includes seventeen isolates distributed according to genopatterns: pattern A1 in one isolate (8) carries *the agg and pil* genopatterns, pattern A2 in fourteen isolates (1, 2, 3, 4, 5, 9, 11, 12, 13, 15, 17, 22, 23, and 24) carries the *agg* genopattern, and pattern A3 in two isolates (6 and 19) with the genopattern *agg, esp*. Subgroup B includes two isolates with similarity reaching 66%; pattern B1 in one isolate (7) had the ace and *pil* genopatterns, and B2 in one isolate (18) had the ace and *esp* genopatterns.

Dendrogram showing the genetic relationships among 24 *E. faecalis* isolates depending on molecular detections. The scale indicates the Dice similarity coefficient (Figure 6).

Table-1**.** Primers and amplified PCR products used in study.

Primers	Primer sequences (5' 3')	size (bp)	Reference
ddl E. faecalis	F: GGCAGAAGTGAAGAGCACGA R: CATGCGCTGGGATTTGCATT	775	21
agg	F: TCTTGGACACGACCCATGAT R:AGAAAGAACATCACCACGAGC	413	15
ace	F: GAATGACCGAGAACGATGGC R:CTTGATGTTGGCCTGCTTCC	615	18
esp	F: TTGCTAATGCTAGTCCACGACC R: GCGTCAACACTTGCATTGCCGA	932	22
pil	F: GAAGAAACCAAAGCACCTAC R: CTACCTAAGAAAAGAAACGCG	620	23
vanA	F:CATGGCAAGTCAGGTGAAGA R:CCGGCTTAACAAAAACAGGA	223	24
vanB	F:GTGACAAACCGGAGGCGAGGA R: CCGCCATCCTCCTGCAAAAAA	433	24

Table-2. PCR conditions.

Amplified gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Elongation	Final extension
ddl E. faecalis	94ºC/ 1 min	35	94ºC/1 min	54ºC/1 min	72ºC/1 min	72ºC/2min
agg	94C/ 1 min	35	94ºC/1 min	58ºC/1 min	72ºC/1 min	72ºC/2min
ace	94C/ 1 min	35	94ºC/1 min	58ºC/1 min	72ºC/1 min	72ºC/2min
esp	94C/ 1 min	35	94ºC/1 min	61ºC/1 min	72ºC/1 min	72ºC/2min
pil	94C/ 1 min	35	94ºC/1 min	53ºC/1 min	72ºC/1 min	72ºC/2min
*van*A	95ºC/ 1 min	35	95ºC/1 sec	58ºC/1 min	72ºC/45 min	72ºC/2 min
*van*B	95ºC/ 1 min	35	95ºC/45 sec	58ºC/1 min	72ºC/45 min	72ºC/2 min

Table-3. PCR detection results of genes.

Isolates	*agg*	*ace*	*esp*	*pil*	*van*A	*van*B
1	+	-	-	-	-	-
2	+	-	-	-	-	-
3	+	-	-	-	-	-
4	+	-	-	-	-	-
5	+	-	-	-	-	-
6	+	-	+	-	-	-
7	-	+	-	+	-	-
8	+	-	-	+	-	-
9	+	-	-	-	-	-
10	-	-	-	-	+	-
11	+	-	-	-	-	-
12	+	-	-	-	-	-
13	+	-	-	-	-	-
14	-	-	-	-	+	-

15	+	-	-	-	-	-
16	-	-	-	-	-	-
17	+	-	-	-	-	-
18	-	+	-	-	-	-
19	+	-	+	-	-	-
20	-	-	-	-	+	-
21	-	-	-	-	+	-
22	+	-	-	-	-	-
23	+	-	-	-	-	-
24	+	-	-	-	-	-
%	**70.8%**	**8.3%**	**8.3%**	**8.3%**	**16.7%**	**0%**

Figure-1. Gel electrophoresis of amplified *ddl* gene with 775 bp product.

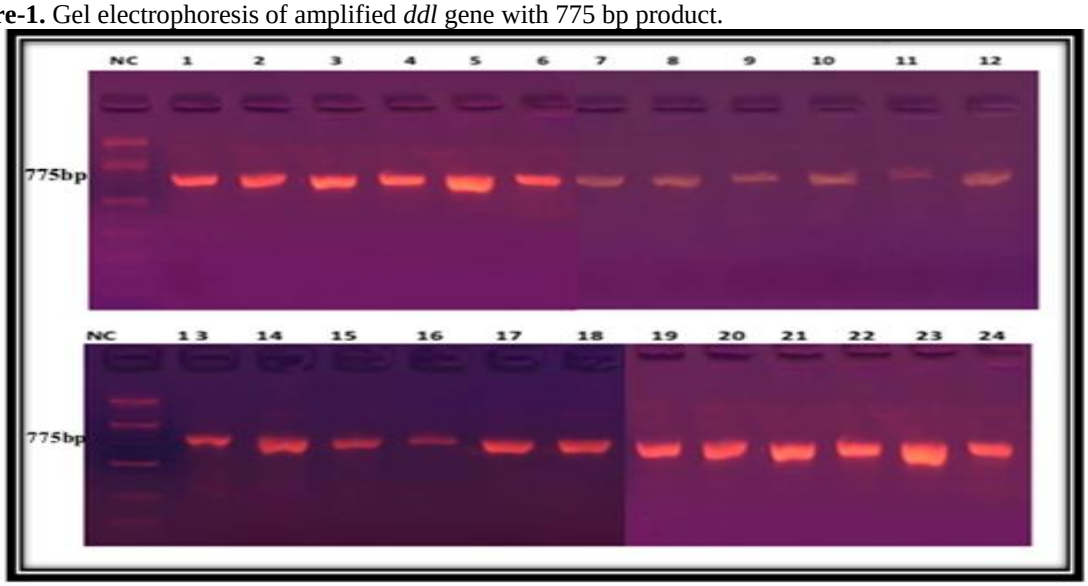


Figure-2. Gel electrophoresis of amplified *agg* and *ace* genes with 413pb and 615bp products.

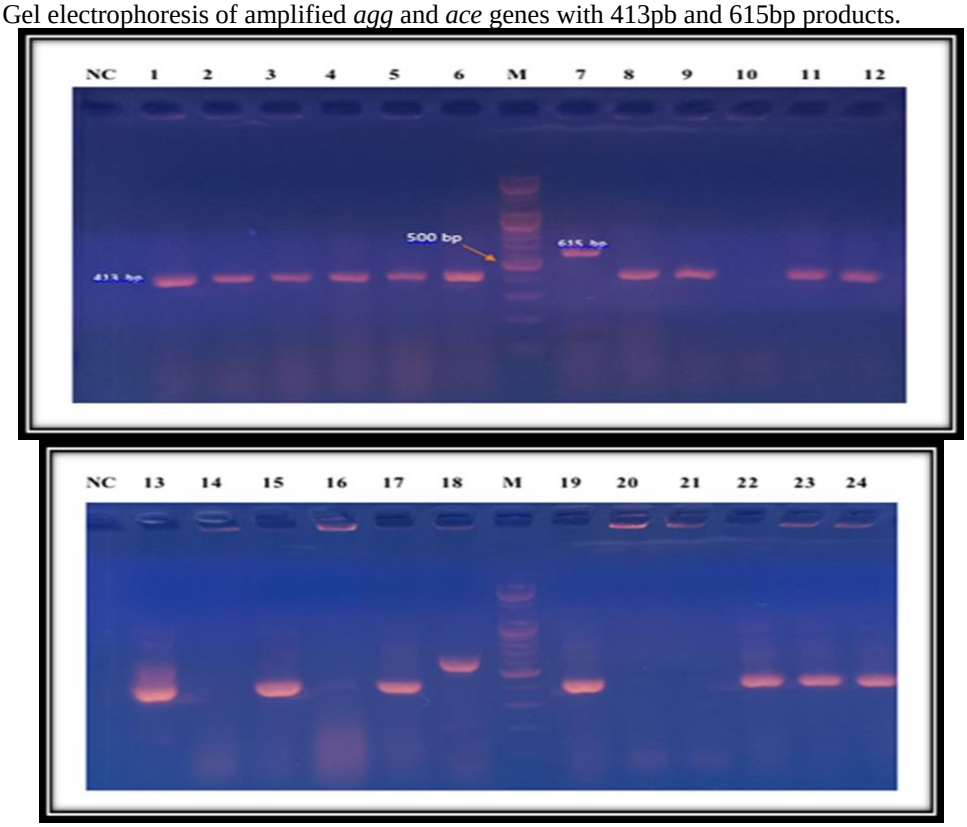


Figure-3. Gel electrophoresis of amplified *esp* gene with 932 bp product.

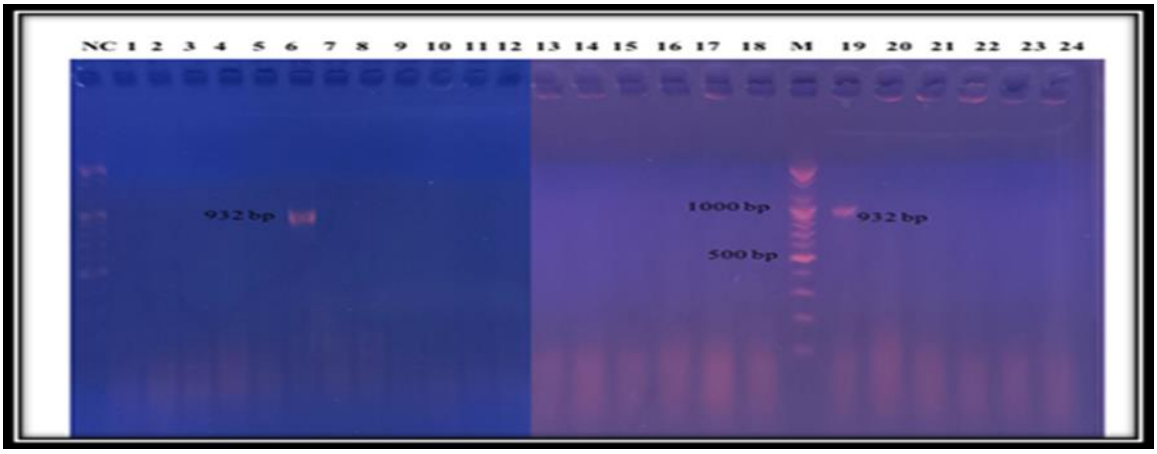


Figure 4. Gel electrophoresis of amplified *pil* gene with 620 bp product.

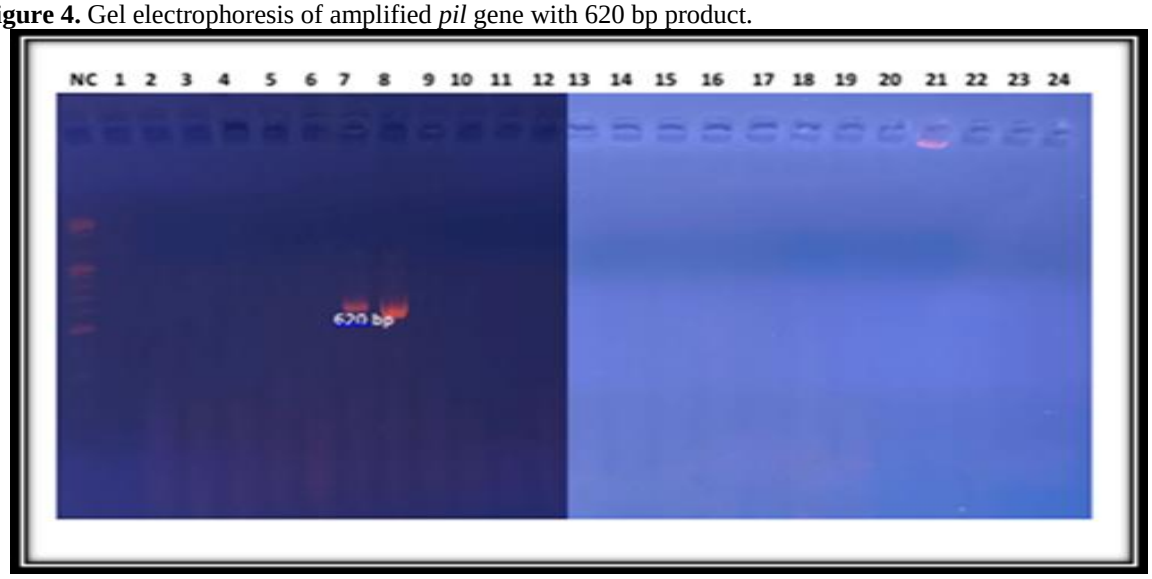
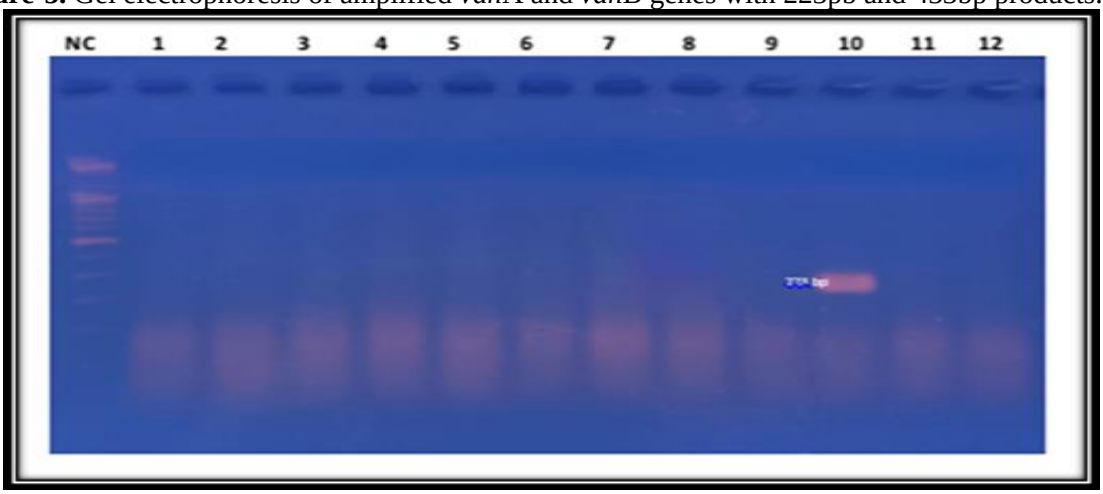


Figure-5. Gel electrophoresis of amplified *van*A and *van*B genes with 223pb and 433bp products.



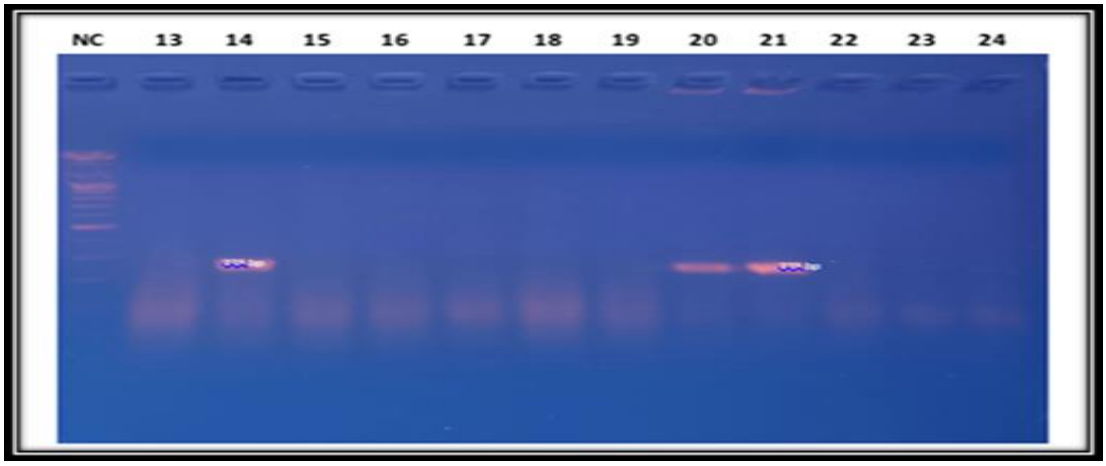
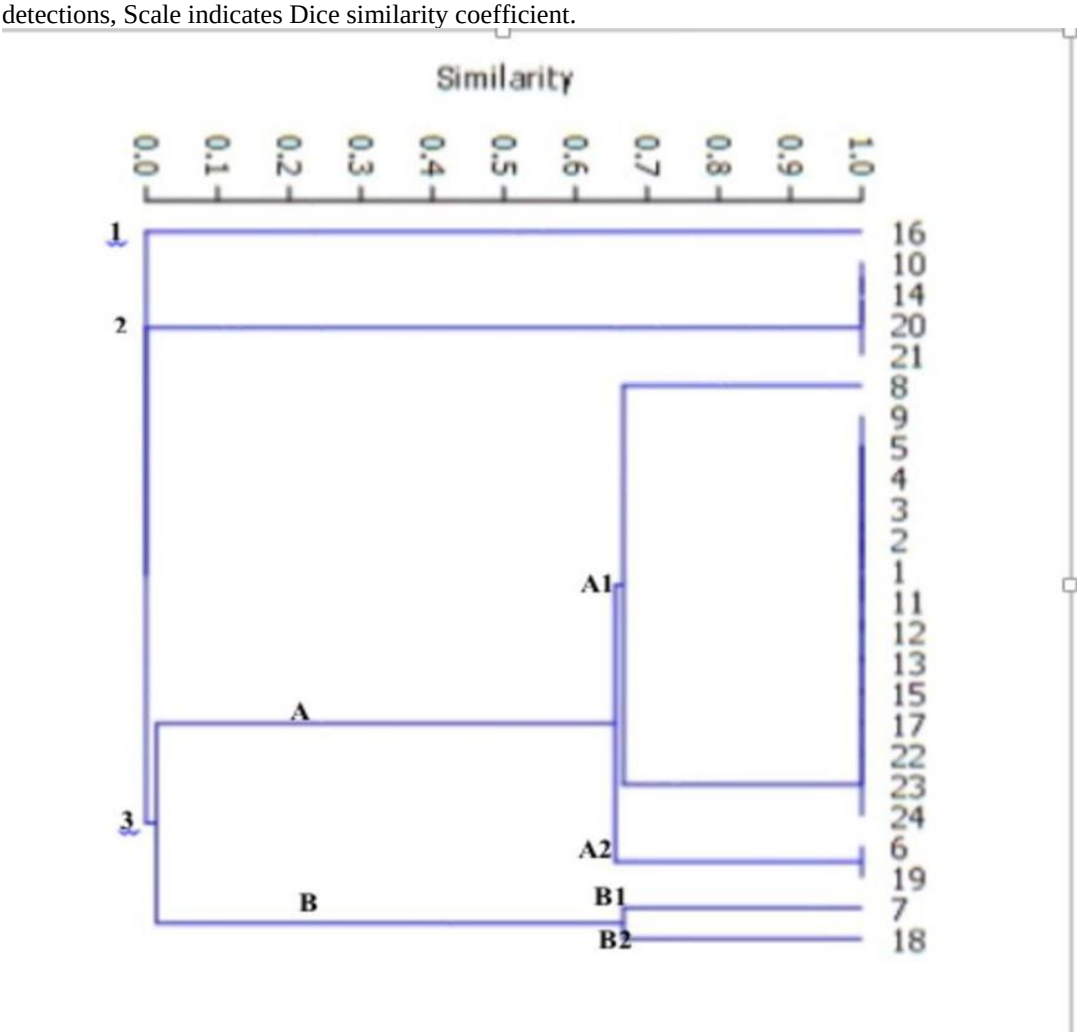


Figure 6. Dendogram showing the genetic relationships among 24 *E. faecalis* isolates depending on molecular detections, Scale indicates Dice similarity coefficient.



Conclusions

The incidence of urinary tract infections caused by *E. faecalis* is relatively high, which requires continuous monitoring. Also *E. faecalis* local isolates that possess vancomycin resistance genes do not have genes that related to adhesion. Vancomycin resistance may be due to reasons other than carrying *van*A genes. The study also found that *E. faecalis* local isolates do not have *van*B gene.

Conflict of interest

None declared.

Financial disclosure

None declared.

Data availability

All data generated or analyzed during this study are included in this puplished article.

Author's contribution

The two researchers conducted the experiments and wrote the manuscript

REFERENCES

1. Saleem AJ. Prevalence of vancomycin resistant genes among *Enterococcus faecium* isolated from patients with urinary tract infection. J. Pharm. Sci. & Res. 2020;12(7):1-1001.
2. AL-Hassani MA, Abdul-Lateef LA, Molecular detection of some virulence genes of *Enterococcus faecalis* isolated from different clinical samples in Babylon province/ Iraq. International Journal of Health Sciences. 2022;6(4):2377–2391.
3. Poh CH, Oh HM, Tan AL. Epidemiology and clinical outcome of Enterococcal bacteremia in an acute care hospital. J. Infect. 2006;52(5):383–386.
4. Zhong Z, Zhang, W, Song, Y et al. Comparative genomic analysis of the genus *Enterococcus*. Microbiological Research. 2017;196 (5):95-105.
5. Van Bambeke F, Chauvel M, Reynolds PE, Fraimow HS, Courvalin P. Vancomycin-dependent *Enterococcus faecalis* clinical isolates and revertant mutants. Antimicrob Agents Chemother. 1999;43(1):41-7.
6. Kreft B, Marre R, Schramm U, Wirth R. Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured renal tubular cells. Infect Immun. 1992;60(1):25-30.
7. 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. Infection and Drug Resistance.2021; 10(14):1713-1723.
8. Strateva AD, Savovb E, Petrovac G, Mitov I. Incidence of virulence determinants in clinical *Enterococcus faecalis* and Enterococcus faecium isolates collected in Bulgaria. Braz j infect dis .2016;20(2):127–133.
9. Toledo A, Valle J, Solano C, Igonlasa I. The Enterococcal Surface Protein, Esp, Is Involved in *Enterococcus faecalis* Biofilm Formation. Applied and Environmental microbiology. 2001;67(10):4538–4545.
10. Yang J, Li T, Yong Z et al. Molecular characterization of resistance, virulence and locality in vancomycin-resistant *Enterococcus faecium* and Enterococcus faecalis. Infect Genet Evol. 2015;33(9):253–260.
11. Shankar N, Lockatell V, Arto B et al. Role of *Enterococcus faecalis* Surface Protein Esp in the Pathogenesis of Ascending Urinary Tract Infection. Infection and Immunity .2001;79(7):4366–4372.
12. Afonina I, Lim X N, Tan R, Kline KA. Planktonic interference and biofilm alliance between aggregation substance and endocarditis- and biofilm-associated pili in *Enterococcus faecalis*. J Bacteriol .2018;200(4): 18-361.
13. Seguel N, Quezada M, Gonzalez OG, Bello H ,Sanchez G. Antibiotic resistance of *Enterococcus faecalis* from persistent endodontic infections. Int. J. Odontostomat. 2020;13(3):448-456.
14. Shobo O, Christiana O, Shobo DG et al. Genomic Snapshot of *Enterococcus faecalis* within Public Hospital Environments in South Africa. Global Health, Epidemiology and Genomics. 2023;39(6):10-11.
15. Karim FM, Abdullah RM. Detection of some virulence genes (*esp*, *agg*, *gelE*, *CylA*) in *Enterococcus faecalis* isolated from different clinical cases at Baghdad. Journal of Applied and Natural Science. 2023;15(3):1095–1101.
16. Lee T, Pang S, Abraham S, Coombs GW. Antimicrobial resistant CC17 *Enterococcus faecium*: The past, the present and the future. Journal of global antimicrobial resistance**.** 2019;16(10):36-47.
17. Jin X, Sun X, Wang Z, Dou J, Lin Z, Lu Q, Zhang T, Wen G, Shao H, Cheng G, Luo Q. Virulent Phage vB_EfaS_WH1 Removes *Enterococcus faecalis* Biofilm and Inhibits Its Growth on the Surface of Chicken Meat. Viruses. 2023;20;15(5):1208.
18. Hashem YA, Amin HM, Essam TM et al. Biofilm formation in enterococci: genotype-

phenotype correlations and inhibition by vancomycin. Scientific reports. 2017;7(1):5733.

19. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. Front Pediatr. 2018;6:285.
20. Saleem AJ. High Frequency of Hemolysin Associated Genes among *Staphylococcus aureus* Clinical Isolates in Iraq. Journal of Global Pharmacy Technology. 2017:9(12):308-314.
21. Ghazvinian M, Asgharzadeh Marghmalek S, Gholami M, Amir Gholami S, Amiri E, Goli HR. Antimicrobial resistance patterns, virulence genes, and biofilm formation in *enterococci* strains collected from different sources. BMC Infect Dis. 2024;24:274. doi: 10.1186/s12879-024-09117-2.
22. Ferdous FB, Islam MS, Ullah M et al. Antimicrobial Resistance Profiles, Virulence Determinants, and Biofilm Formation in *Enterococci* Isolated from Rhesus Macaques (Macaca mulatta): A Potential Threat for Wildlife in Bangladesh? Animals. 2023:13(226):10-33.
23. Upadhyaya GP, Lingadevaru UB, Lingegowda RK. Comparative study among clinical and commensal isolates of *Enterococcus faecalis* for presence of esp gene and biofilm production. J Infect Dev Ctries. 2011;28(5):365-369.
24. Azizi M, Motamedi H, Hossainpour H, Abiri R, Kashef M, Ahmadi K, et al. Rapid Detection of *vanA* Resistance Gene from *E. faecalis* Clinical Isolates Using Duplex Loop-Mediated Isothermal Amplification and Triplex PCR Assay. Biomed Res Int. 2022;115(10):1233-1254.
25. Moosavian M, Ghadri H, Samli Z. Molecular detection of vanA and vanB genes among vancomycin-resistant *enterococci* in ICU-hospitalized patients in Ahvaz in southwest of Iran. Infect Drug Resist. 2018;15(11):2269-2275.
26. Ahmed MO, Baptiste KE.Vancomycin-Resistant Enterococci: A Review of Antimicrobial Resistance Mechanisms and Perspectives of Human and Animal Health. For personal use only. 2018;24(5):132-146.