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Detection of Some Virulence Factors Genes in Clinical Isolates of *Enterococcus faecalis*

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Abstract: Nosocomial infections, caused by *Enterococcus faecalis* play a major role in causing a variety of infections, including endocarditis, urinary tract infections, and chronic root canal infections. *E. faecalis* has a selective virulence factors regulated by multiple genes such as hemolysin (*cylA*), gelatinase (*gel E*), andadhesion of collagen from *E. faecalis* (*ace*).The aim of current work was to study some of virulence factors in local clinical enterococcal isolates and detection for some genes at molecular level that encodes to these virulence traits,furthermore the correlation between these virulence factors phenotypically and genotypically was also studied. The obtained bacterial (65) isolates were subjected to some virulence factors tests like production of gelatinase, hemolysin and biofilm formation. Fifty (50) isolates out of (65) were gelatinase positive,44 isolates were beta hemolysis, 12 isolates were alpha hemolysis and 9 isolates were gamma hemolysis.The results of biofilm production showed that only 1 isolate non biofilm producer and the rest isolates(64) revealed different categories of biofilm production.The molecular detection assay for selected isolates (15) showed that 73.3% of selected isolates were *gelE*positive ,40% of selected isolates were *cylA* positive and 93.3% of selected isolates were *ace* positive. Besides the correlation between these virulence traits was also studied statistically.strong relationshipbetween biofilm (ace) phenotype and this genotypic factor (p value 0.002). While Hemolysin Gen. appears unrelated to hemolysin phenotype in this context (p value 0.241), Biofilm (ace) Gen. demonstrates a non-significant association with hemolysin phenotype (p value 0.875).It was observed that there was a distinct variation in virulence, taking into consideration the technique employed and the outcomes produced.

Keywords: *gelE ,cylA*, *Ace*, *E.faecalis*, gelatinase, hemolysin, biofilm, virulence factors

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Introduction

Enterococcus faecalis (E. faecalis) is a Gram-positive bacterium that is facultative anaerobic and does not generate spores. It can be seen as short chains, pairs, or single cells under the microscope. As normal commensals, they live in the human intestine. Healthy people aren't at risk from infection from this pathogen, but patients in intensive care units with serious illnesses or compromised immune systems are at risk. It is most commonly responsible for serious nosocomial infections such as urinary tract infections, endocarditis, bacteremia, intra-abdominal abscesses, and intra-pelvic abscesses, all of which are associated with the bacterium *E. faecalis*. (1), Cytolysin (hemolysin) and gelatinase, have been suggested as potential virulence factorso of *E. faecalis* strains, although their involvement in enterococcal pathogenicity is unknown. Cytolysin is hemolytic (lysing a wide range of cells, including human, horse, and rabbit erythrocytes) and bacteriolytic against

Gram-positive bacteria. Cytolysin boosts the pathogenicity of *E. faecalis* in animal models. Gelatinase is an extracellular metalloendopeptidase that can hydrolyze gelatin, collagen, casein, and other bioactive peptides, including *E. faecalis* sex pheromone-related peptides, implying that it may participate in inflammatory processes (2).Previous studies have shown that a number of virulence factors may be connected to the production of *E. faecalis* biofilms, and that some virulence factors are critical to the pathogenicity of *E. faecalis*. Gelatin, collagen, and hemoglobin are hydrolyzedby the extracellular metalloproteaseGelE. In addition to being implicated in the development of E. faecalis biofilms and bacterial adherence, GelE was found to be involved in the regulation of autolysis and the release of high-molecular-weight eDNA, which was crucial for the formation of these biofilms (3), Nevertheless, certain research teams have not shown any meaningful association between the development of *E. faecalis* biofilms and the presence of gelE(4).In addition to adhesion, *E. faecalis* secretes virulence factors that contribute to the severity of the infection. Cytolysin, a secreted toxin released in response to pheromones, adds to the pathogenicity of *E.faecalis* by causing blood hemolysis. The gelatinase enzyme (GelE) and hydrolyze gelatin and casein, respectively. Gelatinase's ability to degrade host tissues is vital in the spread of enterococci within their hosts. Gelatinase is also required for biofilm development. Hancock and Perego demonstrated that gelatinase increases the aggregation of cells in microcolonies, which forms the first step in biofilm development (5). The lytic action of cytolysin has been studied on several cell types, including its contribution to the virulence of *E. faecalis* in infections. The cylA gene of E. faecalis synthesizes a protein involved in the activation of cytolysin(3)CylA may be linked to the development of *E. faecalis*biofilm in urinary tract infections, according to a different study (3). Another surface protein with adhesive qualities is called *Ace* (Accessory colonization factor) also known as adhesion of collagen, from *E. faecalis*, and it has a molecular weight of roughly 74 kDa. Ace is the gene that codes for it. The protein was found to be present in *E. faecalis* strains that were recovered from both healthy carriers and enterococcal infection patients, suggesting that the species may be identified using this characteristic. Ace is involved in the process of colonization through binding to extracellular matrix (ECM) proteins, including type I and IV collagen (6). Ace is a surface protein that belongs to the family known as MSCRAMM (microbial surface component recognizing adhesive matrix molecules). Its high affinity and specificity for binding a ligand are attributed to its LPXTG (L - leucine, P - proline, X - any amino acids, T - threonine, G - glycine) sequence (6).adhesions and secreted virulence factors are among the several Enterococcus pathogenic factors that have been found.(7). An essential first stage in the pathophysiology of E. faecalis is adherence to host tissues, especially UTIs. (8). The aim of this study was to detect of some virulence factors in local clinical isolates of *E.faecalis* phenotypically and genotypically.

Material and method

Isolation and Identification of *E. faecalis*

Hundred and Twenty (120) clinical specimens were taken from the urine,

blood, tissue, and dental root canals of patients with endodontic infections, bacteremia, and urinary tract infections between October 2023 and January 2024, the specimens were collected from Educational Laboratories/Medical City, Al-Kadhimiya Hospital, and Al-Hareriy Teaching Hospital in Baghdad. After being streaked on Pfizer Selective Enterococcus, the collected specimens were cultured for 24 hours at 37°C. Based on standard biochemical and microbiological techniques, such as their morphologic appearance following Gram staining, their capacity to hydrolyze esculin in the presence of bile, and their ability to grow in the presence of 6.5% NaCl at 45°C (23) and pH 9.6 (24), isolates were identified down to the genus level. For the confirmation of *E. faecalis* isolates, VITEK 2 system was utilized \.

Virulence Factors of Enterococcus:

Gelatinase Activity:

Gelatinase production was assessed by the nutrient gelatin plate method, with slight adjustments. In summary, the isolates under investigation were streaked onto the surface of nutrient agar plates that were supplemented with 3% gelatin. These plates were then incubated at 37°C for a 24-h period. Following the incubation. The appearance of a clear zone surrounding the microbial growth served as an indicator of positive gelatinase activity (9).

Hemolytic Activity:

The hemolytic activity was assessed by cultivation of the isolates on blood agar base (Oxoid, UK) supplemented with 5% defibrinated blood. A clear zone around the colonies, observed after 24-hour incubation at 37 °C, indicated hemolytic activity (22).

Biofilm Formation:

Microtiter plateassay was used to measure the quantitative Biofilm generation by the bacterial isolatesThe bacterial isolates from overnight Brain heart infusion (BHI) broth established at 37°C was diluted to 1:100 in BHI with 2% glucose (w/v). A 200µl of The cell suspensions was placed in a 96-well micro titer plate with a U-bottom (BioTek,USA). For negative control, sterile BHI alone was used and then the plates were incubated aerobically at 37°C for 24 hours. The experiment was done in triplicates the micro titer wells were rinsed twice with phosphate-buffered saline (PBS), and 95% ethanol was used to fix adherent bacteria and 1% (w/v) crystal violet solution was used to stain them for 5 minutes. Each well's optical density at 570 nm (OD570) was measured using an automated Elisa reader after the microplates had been cleaned and air-dried. Taking into account that a low cutoff (O.D.) is indicated by a 3* SD increase in the mean values of the control wells, the isolates were described as follow: Non-producer (O. D ≤ O. DC), weakly-producer (O. DC < O.D ≤ 2*O. DC), moderate-producer (2*O. DC < O. D ≤ 4* O. DC) and strong-producer (4* O. DC < O.D) (10).

Molecular study

Fifteen *Enterococcus faecalis* isolates were utilized in this investigation in order to extract genomic DNA and complete molecular study.

DNA extraction from bacterial isolates

Easy pure® Genomic DNA Kit(Transgene®/China) was used inorder to extract genomic DNA from bacterial isolates.

Determination of DNA Concentration and Purity

DNA of the bacterial isolates was extracted as described Previously; the concentration of the extract DNA was determined by nanodrop. The

concentrations were ranged from 250 to 270 ng/µl, and the purity value was ranged from 1.75 to 1.8, the extract DNA was preserved in -20°C.

Primers design

A serial cloner 2-6-1 and Amplifx software were used for the primers' design (primers were design by DrHusam Sabah Auhim, University of Baghdad, College of Science, Department of Biology). Thermo Fisher Scientific's Tm Calculator (Allawi&SantaLucia, 1997) was used to calculate the annealing temperatures for primers. A list of the primer name, protein name, primer sequence, amplicon size, and annealing temperature is illustrated in Table (1).

Table (1): Primers used in this study,sequences,annealingtemparatures and product sizes .

Primer name	`5 -----Sequence -----3`	Annealing Temperature	Amplicon size (bp)
GelE-F	GGCGTTACTGTTGATTCAGATAATGTG	60	1147
GelE-R	CATCACGAGCATCACTGAATTGTGC		
Cyla-F	GGAACAACAGGTTATGCATCAGATCTC	61	755
Cyla-R	GTGCTTATATCACGCGACTCATTTCC		
Ace-F	TTGGTAATGATTCTACCAATTGCGG	60	420
Ace-R	TCCGAAATCCGTTTCTATCACATTC		

Amplification of genes by polymerase chain reaction (PCR)

Conventional PCR technique was used to amplify a fragment of three target genes (GelE, Cyla and Ace). The general reaction mixture and PCR conditions for Go Taq G2 Green Master Mix (Promega/USA) amplifications are shown in Table (2) and Table (3). Sterile PCR tubes were used for all PCR, which was carried out in an Applied Biosystems thermocycler.

Table (2): Composition of PCR Mixture and PCR conditions

Materials	Volume in µl
Go Taq G2 Green Master Mix (2X)	12.5
Forward Primer (10 µM/ µl)	1
Reverse Primer (10 µM/ µl)	1
Template DNA (25 ng/µl)	2
Nuclease-free water	8.5
Total reaction mixture	25

Table (3): PCR conditions of genes

Initial denaturation	30 reaction cycles			Final Extension	Hold
	Denaturation	Annealing	Extension		
5 min (95˚C)	30 s (95˚C)	30 s (X˚C) *	60 s (72˚C)	5 min (72˚C)	5min (4˚C)
* X= Tm used according to the primer pairs listed in Table xxx Annealing temp.for *GelE* and *Ace* 60˚C , for *Cyla* 61˚C					

Agarose gel electrophoresis

Agarose gel electrophoresis was used to analyze the PCR products, mostly using a 2% (w/v) agarose gel. Agarose gels were prepared by dissolving 2 g of agarose powder in 100 ml of 1X TAE buffer using a microwave, then supplemented with 4 µl ethidium bromide (10 mg/ml) after cooling to about 50-40 °C. Next, the gel was poured into a gel tray after sealing both ends and left for solidification at room temperature (20 -25°C). A 5 µl of sample was run against 3 µl of marker ladder (100 bp DNA ladder). Electrophoresis was performed at 100 V for 60 min. DNA bands were visualized with a UV viewer illuminator system (25).

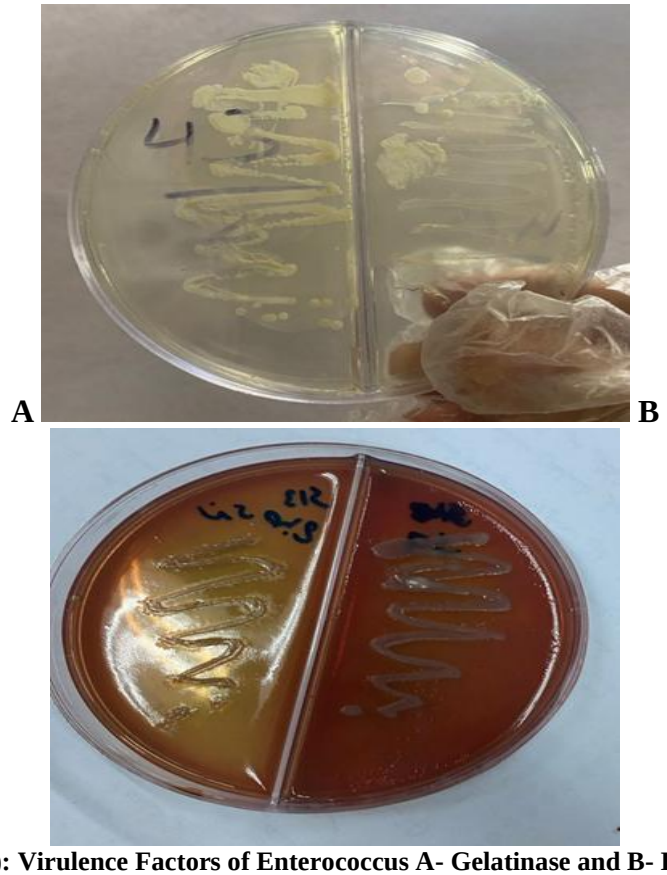
Statistical analysis

All features were appeared as frequencies and percentages, and Pearson-Chi-square test was detected to reveal significant differences in percentages. P≤0.05 was measured significant. Our data were analyzed using SPSS v. 23.0 statistical software.

Results and Discussion:

The results of current study showed that the total samples were (120), and the number of isolates was (65) with percentage of isolation for *Enterococcus faecalis*(54.16%).

Gelatinase positivity with percentage of (76.9%) produced in (50) out of 65 isolates with a significant difference ($p<0.01$)and hemolysin positivity in beta hemolysin (67.7%) producers in (44) out of 65 isolates with a significant difference ($p<0.01$) as shownin figure (1).



A **B**

Figure (1): Virulence Factors of Enterococcus A- Gelatinase and B- Hemolysin

Also, this study showed that 12 isolates were alpha hemolysis and 9 isolates were gamma hemolysis.Biofilm formation that showed non-adherent in one isolate (1.5%) and weak biofilm in (19) isolates (29.2%), moderate biofilm in (44) isolates (67.7%) and strongbiofilm in one isolate (1.5%) unpublished data.

The results of virulence factors genotypically

Only 15 isolates of *E.faecalis* from different sources were selected to study their molecular analysis.The molecular analysis showed that 11 out of 15 selected isolates of *E. feacalis* were positive for *gelE* gene with product size of 1147 bp, with a percentage of 73.3% , and only 4 isolates were negative (26.7%) as shown in Figure (2).

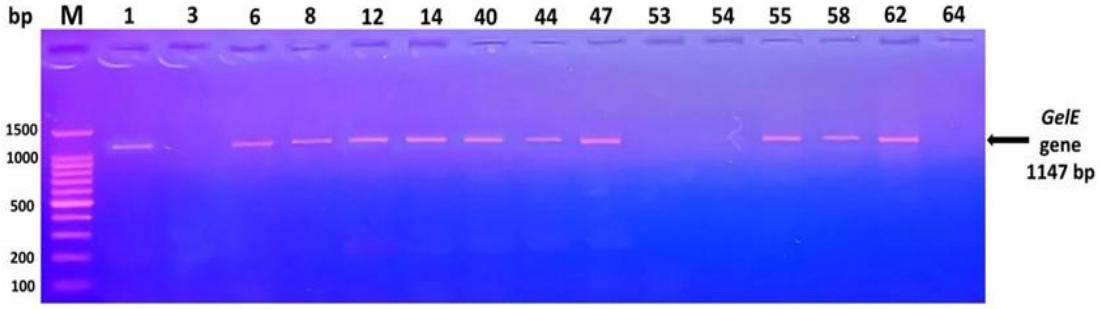


Figure (2): moleculare detection of GelE

This study found that (6) of 15 *E. faecalis* isolates were positive for *cylA* gene (755bp) with percentage of 40%, while 9 samples were negative with percentage of 60% as shown in Figure (3).

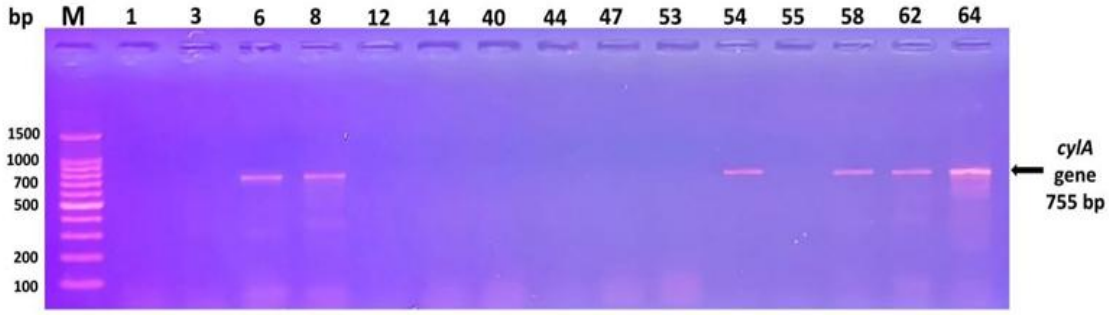


Figure (3): moleculare detection CylA

The results of the molecular study showed that 14 isolates from 15 isolates of E. faecalis were positive (93.3%) for *Ace* gene with product size of 420bp, while only one isolate was negative as shown in Figure (4).

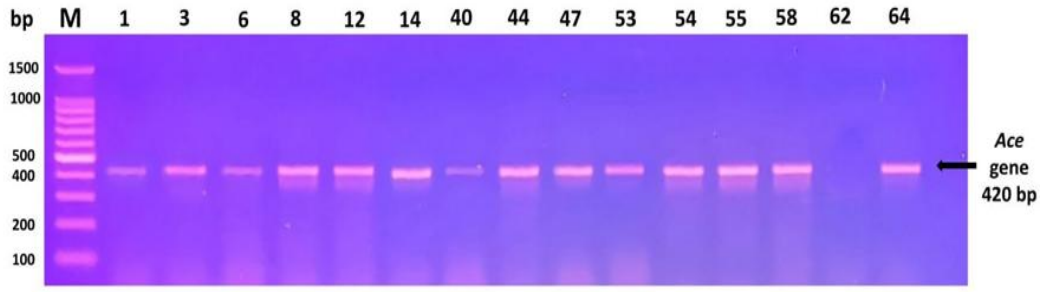


Figure (4): Molecular Detection Adhesion to collagen of E. faeclis (Ace)

Findings of the conducted study showed that one isolate of *Enterococcus faecalis* that showed non-adherence and another was strong biofilm, each producing gelatinase (100% and 100%) and beta hemolysin producers (100% and 100%) with a significant difference (p<0.05), Alfa hemolysin producer (22.7%) was moderate biofilm with significant difference (P<0.05), where Gama hemolysin producer (15.8%) was weak biofilmwith significant difference (P>0.05) (table 4). Most E. *faecalis* isolates were moderate biofilm producers and gelatinase positive, with a percentage of 84.1%, while 57.9% of isolates were weak biofilm producers and gelatinase positive.

Table (4): comparative of biofilm with virulence factors of Enterococcus faecalis

			Biofilm				Total	P value
			Non-adherent	Weak	Moderate	Strong		
Gelatinase	Positive	n	1	11	37	1	50	p<0.01**
		%	100.0%	57.9%	84.1%	100.0%	76.9%	
	Negative	n	0	8	7	0	15	p>0.05
		%	0.0%	42.1%	15.9%	0.0%	23.1%	
P value			1.00	p>0.05	p<0.01**	1.00	p<0.01**	
Hemolysin	α	n	0	2	10	0	12	P<0.05*
		%	0.0%	10.5%	22.7%	0.0%	18.5%	
	B	n	1	14	28	1	44	p<0.01**
		%	100.0%	73.7%	63.6%	100.0%	67.7%	
	Y	n	0	3	6	0	9	p>0.05
		%	0.0%	15.8%	13.6%	0.0%	13.8%	
P value			1.00	p<0.01**	P<0.05*	1.00	p<0.01**	

Outcomes of conducted study showed the most *Enterococcus faecalis* isolates that produce gelatinase was also produce beta hemolysin (70.0%) followed α hemolysin (20.0%) and Y hemolysin (10.0%) with significant difference (p<0.05) table (5).

Table (5): comparative between gelatinase and hemolysin of Enterococcus *faecalis*

			Gelatinase		Total	P value
			Positive	Negative		
Hemolysin	α	n	10	2	12	P<0.05*
		%	20.0%	13.3%	18.5%	
	B	n	35	9	44	p<0.01**
		%	70.0%	60.0%	67.7%	
	Y	n	5	4	9	p>0.05
		%	10.0%	26.7%	13.8%	
P value			p<0.01**	p<0.01**	P<0.05*	

Results of present study showed no significant differences (p>0.05) between positivity of gelatinase that produced by *Enterococcus faecalis* and study groups (genotype and phenotype).as shown in Table (6).

Table (6): Comparative of genotypic *gelE* and phenotypic characters of gelatinase in Enterococcus faecalis

			Study groups		Total	P value
			Genotype (n=15)	Phenotype (n=15)		
Gelatinase	Positive	n	11	9	20	p>0.05
		%	73.3%	60.0%	66.7%	
	Negative	n	4	6	10	p>0.05
		%	26.7%	40.0%	33.3%	
P value			p<0.01**	p>0.05	p>0.05	

The table presents the results of a chi-squared test examining the association between Hemolysin Phenotype (Hemolysin Ph.) and two genotypic factors: Hemolysin Gen *cylA*. and Biofilm (ace) Gen. The chi-squared test statistics and associated p-values are provided to assess the significance of the associations. For Hemolysin Gen., the chi-squared value is 2.847 with a p-value of 0.241, indicating a non-significant association (NS) between hemolysin phenotype and this genotypic factor. Similarly, for Biofilm (ace) Gen., the chi-squared value is 0.268 with a p-value of 0.875, also indicating a non-significant association (NS).In summary, based on the p-values, there is no significant association between hemolysin phenotype and either Hemolysin Gen. or Biofilm (ace) Gen. (NS), suggesting that these genotypic factors may not be strongly predictive of hemolysin phenotype in this context Table (7).

Table (7): The association between Hemolysin Ph. and Genotypic

Chi squared Test		Hemolysin (cylA) Gen.		Biofilm (ace) Gen.	
		-	+	-	+
Hemolysin ph.	**Y**	0	1	0	1
	α	2	0	0	2
	β	7	5	1	11
Total		9	6	1	14
Chi-squared		2.847		0.268	
P value		0.241		0.875	
S / NS		**NS**		**NS**	

NS: Non-significant association between groups (p value > 0.05)
S: significant association between groups (p value < 0.05)

Table (8) illustrates the results of a chi-squared test exploring the relationship between Hemolysin Phenotype (Hemolysin Ph.) and two genotypic factors: Hemolysin Gen. and Biofilm (ace) Gen. Organized into rows by different biofilm (ace) phenotypes (Moderate, Non-adherent, Strong, Weak) and columns indicating the presence (+) or absence (-) of the two genotypic factors, the table displays frequency counts of individuals across various combinations of hemolysin phenotype and genotypic factors. For example, there are 7 individuals with a Moderate biofilm phenotype and α-hemolysin genotypic factor. Chi-squared test statistics and corresponding p-values are provided to assess the significance of associations. For Hemolysin Gen., a chi-squared value of 5.394 and a p-value of 0.145 suggest a non-significant association (NS), indicating that hemolysin phenotype and this genotypic factor may not be significantly related. Conversely, for Biofilm (ace) Gen., a chi-squared value of 15.00 and a p-value of 0.002 indicate a significant association (S), suggesting a strong relationship between biofilm (ace) phenotype and this genotypic factor. While Hemolysin Gen. appears unrelated to hemolysin phenotype in this context, Biofilm (ace) Gen. demonstrates a non-significant association with hemolysin phenotype.

Table (8):The association between Biofilm and Hemolysin Phenotypic and Genotypic

Chi squared Test		Hemolysin Gen.		Biofilm (ace) Gen.	
		-	+	-	+
Biofilm (ace) Ph.	Moderate	7	2	0	9
	Non-adherant	0	1	1	0
	Strong	1	0	0	1
	Weak	1	3	0	4
Total		9	6	1	14
Chi-squared		5.394		15.00	
P value		0.145		0.002	
S / NS		NS		S	

NS: Non-significant association between groups (p value > 0.05)
S: significant association between groups (p value < 0.05)

Sixty- five clinical isolates producers all three virulence factors. Phenotypic assays were used to evaluate gelatinase and cytolytic activity of isolate,also biofilm formation was studied previously, (50) isolates (76.9%) generated gelatinase phenotypically and (44) isolates were beta hemolysin ,(12) were alfa hemolysin, and (9) isolates gamma hemolysin .

In the current study, the gelatinase was present in (50) of the isolates (76.9%). High frequency of gelatinase enterococcal isolates. Despite this high frequency of detection of *gelE*, only (60.0%) of the isolates expressed a phenotypic gelatinase activity. In this study by using molecular analysis to detect E. faecalis of virulence factors of gelatinasegelE (73.3%)in (11) isolates out of (15) positive. phenotypically, 9 samples were gelatinase positive with a percentage of 60%, and 6 (40%) isolates were negative and hemolysincyle (40.0%) in (6) from (15) isolates positive the results of hemolysin production showed that 12 from 15 were beta hemolysis, two alpha hemolysis, and one gamma hemolysis based on the p-values, there is no

significant association between hemolysin phenotype and either Hemolysin Gen. or Biofilm (ace) Gen. (NS), This lack of phenotypic/genotypic expression of cytolysin may indicate the missing genes in the cyl operon (11).Studies targeting the E. faecalis gene were also detected and revealed that 31 (48.43%) isolates from urine samples were positive for gen acyl (12)

Our results may be in some accordance with those of (13) who reported 99% of their samples possessed the gelE gene. (14) demonstrated that 41% of Enterococci carried the cylA gene, and hemolytic activity was identified in 38% of cylA positive isolates, supporting the conclusions of this investigation.

Another study of (15) in which gelatinase activity was observed in 10% of isolates while hemolysis was observed in 16.7%. (16)reported hemolysis and gelatinase activity in 36.3% of the sample. Whereas (17)reported gelatinase activity in 77% of his samples his finding was completely inaccordance to our finding.

In this study, *E feacalis isolates* were collected from patients with diffrent infections and identified to the species level, where most of the isolates were *E. faecalis* (n = 65).The biofilm formation all isolates produce biofilm except (1) isolates non-adherent .Weak biofilm was detected in 19 isolates (29.2%) and moderate biofilm was formation in 44 isolates (67.7%) and strong biofilm formation in 1 isolate (1.5%) and non -adherent biofilm detected in 1 isolate (1.5%) Similar reports of E. faecalis infection prevalence have been made globally and in Egypt. Just four of the examined *E. faecalis* isolates were unable to produce biofilms, whereas the majority (96%) were capable of doing so to varying degrees. Comparable findings on E. faecalis's capacity to build biofilms are accessible. As per earlier research, 72% of the isolates developed biofilms with a moderate intensity phenotypically (9) .

It was interesting to see if enhanced biofilm generation capacity was connected with virulence features reported in the same isolates (16) Overall, a link was discovered between E. faecalis isolates' ability to form biofilms and the presence of the virulence determinants cylA, , and gelE. In the same vein a positive correlation between biofilm formation and acyl, gel virulence factors of E. faecalis strains was shown. They also found that the expression of the collagen-related protein Ace is associated with increased adhesion (18).

In this work, the presence of gelE, acyl, and ace genes was found to be substantially linked with biofilm formation strength. The result of (ace) 14 isolates (93.3%) in genotpic Biofilm (ace) Gen. demonstrates a significant association with hemolysin phenotype **(p value < 0.05).** in phenotypic biofilm formation: 9 isolates were moderate, 4 isolates were weak, one sample was non-adherent, and one sample was strong (19) among others, have previously reported on the role of gelE in biofilm development. However, (20) as well as (21), concluded that the presence of the gelE gene had no influence on biofilm development (5).

In this work, the combination of cyl, gelE genes demonstrated a significant correlation with biofilm formation ($p>0.05$ and $p>0.05$, respectively) in *E. faecalis*. Other studies suggest that gelatinase was not required for biofilm formation and biofilm producer of E. faecalis which was statistically significant (.006, .032 respectively) (11). All biofilm-producing E. faecalis isolates in this study contained

numerous biofilm-forming genes. In contrast, another study found that a single biofilm-forming gene was connected with biofilm formation. Biofilm production is a complex process that depends on several parameters in Enterococcus strains (11).

A comparative study among the different clinical isolates with respect to production of the three virulence factors shows that biofilm production is very high among isolates grown from different sources (22). but virulence mechanism and related genes in biofilm formation are not well understood(18).

Conclusion:

It was feasible to ascertain that *E. faecalis* was capable of forming biofilms and their effect on the substrates by looking at the phenotypic and genetic patterns. The criteria that were gathered and presented indicated that hemolysin and gelatinase had an impact on the formation of biofilms. It was observed that there was a distinct variation in virulence.

References

1. Hassoun, B. H. and Mohammed, G. J. (2022). Molecular genotyping of enterococcus faecalis isolated from clinical sources by BOX-PCR and ERIC-PCR. International Journal of Health Sciences.
2. Archimbaud, C.; Shankar, N; Forestier, C.; Baghdayan, A; Gilmore, M. S; Charbonné, F. and Joly, B. (2002). In vitro adhesive properties and virulence factors of Enterococcus faecalis strains. Research in Microbiology, 153(2), 75–80.
3. Zheng, J.-X.; Wu, Y; Lin, Z.-W; Pu, Z.-Y; Yao, W.-M; Chen, Z; Li, D.-Y; Deng, Q.-W; Qu, D. and Yu, Z.-J. (2017). Characteristics of and Virulence Factors Associated with Biofilm Formation in Clinical *Enterococcus faecalis* Isolates in China. Frontiers in Microbiology, 8, 2338.
4. Momotaz, T.; Afroz, F; Chowdhury, S; Islam, N; Sarwar, M. T; Khan, R. R. and Sattar, A. N. I. (2023a). Phenotypic and genotypic detection of the virulence factors and their association with antibiotic resistance in *Enterococcus* species [Preprint]. Microbiology.
5. Hashem, Y. A; Abdelrahman, K. A. and Aziz, R. K. (2021). Phenotype–Genotype Correlations and Distribution of Key Virulence Factors in Enterococcus faecalis Isolated from Patients with Urinary Tract Infections. Infection and Drug Resistance, Volume 14, 1713–1723.
6. Too, E. and Masila, E. (2024). The Interconnection between Virulence Factors, Biofilm Formation, and Horizontal Gene Transfer in Enterococcus: A Review. In Infectious Diseases (Vol. 0). IntechOpen.
7. Al-Halaby, A. H; Al-Hashimy,B; Al-Kadmy, I.andAl_Ajeeli, I. E. (2017). Molecular Detection of Enterococcus Surface Protein (Esp) Gene in E.faecalis and E.faecium and their Role in Biofilm Formation. Iraqi Journal of Biotechnology, 2017, Vol. 16, No. 1 , 80-91
8. Hamel, H. A; GHree, A. M. and Shareef, S. A. (2023) Detection of FsrB Quorum-Sensing Gene and Biofilm Production in Enterococcus faecalis Isolated from UTI Women. Iraqi Journal of Biotechnology, Vol. 22, No. 1, 245-251
9. Abdelrahman, K. A; Kashef, M. T; Aziz, R. K; and Hashem, Y. A. (2024). Genotype–phenotype correlation of fecal Streptococcus regulator (fsr) locus with gelatinase activity and biofilm formation intensity in clinical E. faecalis isolates. Future Journal of Pharmaceutical Sciences, 10(1), 37.
10. Nallapareddy, S. R; Singh, K. V; Sillanpaa, J; Garsin, D. A; Hook, M; Erlandsen, S. L; and Murray, B. E. (2006). Endocarditis and biofilm-associated pili of Enterococcus faecalis. Journal of Clinical Investigation, 116(10), 2799–2807.
11. Momotaz, T; Afroz, F; Chowdhury, S; Islam, N; Sarwar, M. T; Khan, R. R. and Sattar, A. N. I. (2023b). Phenotypic and genotypic detection of the virulence factors and their association with antibiotic resistance in Enterococcus species [Preprint]. Microbiology.
12. Al-shawi, D. A. N., & Al-Quraishi, G. M. S. (2023). Distribution of Some Virulence Genes among Enterococcus Faecalis Isolates from Urine Samples. The Egyptian Journal of Hospital Medicine, 90(1), 166–171. https://doi.org/10.21608/ejhm.2023.279255
13. Anderson, A. C; Al-Ahmad, A; Elamin, F; Jonas, D; Mirghani, Y; Schilhabel, M; Karygianni, L; Hellwig, E. and Rehman,

A. (2013). Comparison of the Bacterial Composition and Structure in Symptomatic and Asymptomatic Endodontic Infections Associated with Root-Filled Teeth Using Pyrosequencing. PLoS ONE, 8(12), e84960.

14. Saffari, F; Dalfardi, M. S; Mansouri, S. and Ahmadrajabi, R. (2017). Survey for Correlation between Biofilm Formation and Virulence Determinants in a Collection of Pathogenic and Fecal Enterococcus faecalis Isolates. Infection & Chemotherapy, 49(3), 176.
15. Dahlén, G; Blomqvist, S; Almståhl, A. and Carlén, A. (2012). Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosal and deep infections. Journal of Oral Microbiology, 4(1), 10855.
16. Sedgley, C. M; Lennan, S. L; and Clewell, D. B. (2004). Prevalence, phenotype and genotype of oral enterococci. Oral Microbiology and Immunology, 19(2), 95–101.
17. Akbari Aghdam, M.; Soroush Barhaghi, M. H; Aghazadeh, M; Jafari, F; Beomide Hagh, M; Haghdoost, M; Memar, M. Y; Ahangarzadeh Rezaee, M; and Samadi Kafil, H. (2017). Virulence genes in biofilm producer Enterococcus faecalis isolates from root canal infections. *Cellular and Molecular Biology*, *63*(5), 55–59.
18. Duggan, J. and Sedgley, C. (2007). Biofilm Formation of Oral and Endodontic Enterococcus faecalis. *Journal of Endodontics*, *33*(7), 815–818.
19. Hancock, L. E. and Perego, M. (2004). The *Enterococcus faecalis fsr* Two-Component System Controls Biofilm Development through Production of Gelatinase. *Journal of Bacteriology*, *186*(17), 5629–5639.
20. Kafil, H. S. and Mobarez, A. M. (2015). Assessment of biofilm formation by enterococci isolates from urinary tract infections with different virulence profiles. *Journal of King Saud University - Science*, *27*(4), 312–317.
21. Kart, D. and KuştiMur, A. S. (2019). Investigation of Gelatinase Gene Expression and Growth of *Enterococcus faecalis* Clinical Isolates in Biofilm Models. *Turkish Journal of Pharmaceutical Sciences*, *16*(3), 356–361.
22. M.; G. U. P; L.; U. B. and L., R. K. (2010). Comparative Study for the Presence of Enterococcal Virulence Factors Gelatinase, Hemolysin and Biofilm Among Clinical and Commensal Isolates of Enterococcus Faecalis. *Journal of Laboratory Physicians*, *2*(02), 100–104.
23. Badr, E. A., Nagy, Y. I., Sayed, R. M., & Kashef, M. T. (2024). Development of a transcription factor decoy-nanocarrier system as a successful inhibitor of Enterococcus faecalis virulence in vitro and in vivo. Microbial Pathogenesis, 193, 106762.https://doi.org/10.1016/j.micpath.2024.106762
24. Morello, J. A.; E., M. H. and P.A., G. 2006 Laboratory Manual and Work Book in Microbiology. 8th ed., New YorkMcGraw-Hill Higher Education.
25. Collee, J. G.; Fraser, A. G., Marmison, B. P. and Simmon, S. A. 1996. Mackie and MaCathneys. Practical Medical Microbiology. 14th ed., U.K., Churchill Livingstone.
26. Husam, S A.2019. Structure, function and engineering of biotechnologically important proteins. PhD thesis, Cardiff University. Cardiff. UK