# Genetic Basis of Biofilm Formation Genes *Ebp* and *Bph* (*phos*) among Multidrug Resistance *Enterococcus faecalis* Isolates, Iraq

**Muna Salman Attia, Ibtisam Habeeb Al-Azawi**

Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Al-Diwaniyah, Iraq



## Abstract

**Background:** Biofilm produced by *Enterococcus* spp. increase their inherent and acquired antibiotic resistance, posing a significant challenge to infection treatment, particularly in virulent strains. **Objective:** This study aimed to investigate some genes responsible for biofilm formation (*Bph* (*phos*) and *EbpB*) by polymerase chain reaction (PCR) technique. **Materials and Methods:** A total of 130 clinical samples were collected during this study, which were obtained from patients who were admitted to Feminine and Children Teaching Hospital in Al-Diwaniyah City during a period extending from (November 2022 to March 2023). All samples were subjected to culturing on different media (blood agar, MacConkey, Bile Esculin agar, and M-El chromogenic ager), after which catalase and oxidase tests were conducted biochemically. After cultivation, the VITEK-2 compact system was used to identify the samples. The isolates were investigated genotypically for harboring biofilm formation genes, including *EbpB* and *Bph* (*phos*), by molecular method (PCR). **Results:** The VITEK-2 compact system revealed that only 12 isolates were identified as *E. faecalis*. The most effective antibiotics against *E. faecalis* were (vancomycin, teicoplanin, linezolide, tigecycline, and ampicillin), and the highest resistance was against erythromycin (100%) and tetracycline (91.6%). *Enterococcus faecalis* isolates were investigated genotypically for harboring biofilm formation genes that include *EbpB* and *Bph* (*phos*) by molecular methods, PCR. The results showed that *EbpB* and *Bph* (*phos*) genes were positive for all isolates with a percentage of 100%. **Conclusions:** It was found that the presence of biofilm formation genes in *E. faecalis EbpB*, *and phos* (*Bph*) increase the pathogenicity of this pathogen. These genes showed a high percentage among *E. faecalis* isolates. Also, all isolates had the potential to form a biofilm, which complicates their treatment with antibiotics, confirming the critical need to develop novel antimicrobial agents that control the infection associated with the development.

**Keywords:** Antibiotic, biofilm, *Enterococcus faecalis*, virulence factors

## Introduction

Enterococci are primarily commensal residents in the intestines of humans and animals. The species *Enterococcus faecalis* is a common inhabitant of the human intestinal microflora and the genitourinary tract of men and women.[1] The species is recognized as an important causative agent of healthcare-associated infections, including urinary tract infections (UTIs), postsurgical wound infections, bacteremia, endocarditis, meningitis, and neonatal sepsis. *E. faecalis* was G+, facultatively anaerobic, catalase-negative, oxidase-negative, nonspore-forming bacteria belonging to the genus Enterococcus frequently live in the intestines of healthy people.[2] It typically appears in pairs or chains of various lengths.[3] In humans, *E. faecalis* is a common intestine bacterium that is, easily susceptible to developing antibiotic resistance, allowing it to serve as a biomarker of antimicrobial resistance.[4] Antibiotic resistances are a natural phenomenon that happens whenever antibiotics are in use; also, human behaviors contribute to rapid developments and spreads of bacterial

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_979_23

**Address for correspondence:** Mrs. Muna Salman Attia, Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Al-Diwaniyah, Iraq.
E-mail: muna2021salm@gmail.com

**Submission:** 13-Jul-2023 **Accepted:** 12-Aug-2023 **Published:** 24-Sep-2024



**How to cite this article:** Attia MS, Al-Azawi IH. Genetic basis of biofilm formation genes *Ebp* and *Bph* (*phos*) among multidrug resistance *Enterococcus faecalis* isolates, Iraq. Med J Babylon 2024;21:614-20.

antibiotic resistances.[5] In addition to their resistance to antibiotics, *E. faecalis* may also produce virulence factors that contribute to their pathogenicity.[6]

Biofilm is an aggregate of microorganisms such as bacteria and the attachment to the biotic surface, which is protected by an extracellular polymer matrix composed of polysaccharides and extracellular DNA; it has widespread implications in the medical field.[7]

One of the main mechanisms by which *E. faecalis* is resistant to antibiotics is its ability to form biofilms; *E. faecalis* can also cause persistent inflammation after forming a biofilm.[8] The endocarditis and biofilm-associated pilus minor *EbpB* and Biofilm association phosphatase *phos* (*Bph*). In addition to being necessary for the upregulation and synthesis of virulence determinants, it's also crucial for biofilm formation.[9]

Clinical management of bacterial infections has faced significant difficulties in recent years due to the advent and spread of multiple drug-resistant (MDR) bacteria.[10] Due to the extensive misuse of antimicrobial agents, treatment of infections associated with MDR enterococci is complicated. The inherent antibiotic resistance and dissemination of resistance genes through conjugative transposons and plasmids play an important role in the development of MDR enterococci.[11]

The aim of this study was to genetic detection of genes responsible for biofilm formation (*Bph* [*phos*] and *EbpB*) by polymerase chain reaction (PCR) technique.

## Materials and Methods

The current study was conducted in the Maternity and Children Teaching Hospital in Al-Diwanyah City during the period from November 2022 to March 2023. One hundred thirty samples were collected from two sites of infection (mid-stream urine and high vaginal swab), labeled with the patient's details, and then promptly delivered to the lab.

### Collection of urine specimens

Midstream urine samples (95 samples) were collected in sterile screw-cap containers (4–5 mL) from patients with UTI and immediately subjected to aerobic culture in the General Hospital laboratory. General urine examinations were conducted for each sample.

### Collection of high vaginal swap

High vaginal swabs were taken from women patients suffering from abnormal vaginal discharge, itching, burning, and lower abdominal pain. Gynecologists obtained swabs from 35 women. They had not received antibiotic therapy at least one week before sample collection. The target vaginal area smear has been observed by the gynecologists using a sterile unlubricated speculum. Vaginal sterile cotton-tipped swabs with Amies medium was used for culture.[12]

### Culture and identification

*Enterococcus faecalis* was identified according to its morphology with Gram stain and chains appearance or pairs for primary isolation after cultured on blood agar, MacConkey agar, Bile Esculin agar, m-EI chromogenic agar and incubating for 24–48 h at 37°C. Biochemical testing (oxidase and catalase tests) followed; additionally, as a last stage of confirmation, out of 130 samples, only 16 specimens were exposed to the VITEK-2 system.

### Test for antibiotic susceptibility

Drug sensitivity test results were evaluated using antibiotic susceptibility test (AST)-592 cards, and antibiotic susceptibility tests were conducted using the (VITEK-2) system on all bacterial isolates in the current investigation.

### Molecular characterization

#### *Bacterial genomic DNA extraction*

Extraction of the DNA was carried out for *E. faecalis* isolates employing a commercial kit, and it was conducted relying on the manufacturer's instructions (TRANS, China).

#### *Detection of biofilm formation genes by PCR*

The primer sequences used to amplify genes encoding biofilm formation are listed in Table 1. The primers were resuspended by dissolving the lyophilized product of primers and preparing the stock primer by adding PCR water (free nuclease water) according to instructions of the manufacturer, as shown in Table 2. The PCR tubes were positioned in the thermal cycler, and the conditions of the correct PCR cycling software parameters were changed according to each primer, as shown in Table 3.

#### *Agarose gel electrophoresis*

The PCR products were analyzed according to the manufacturer's instructions by agarose gel electrophoresis.

**Table 1: PCR primers for detection of biofilm formation genes**

| No | Gene | | Sequences of primer (5-3) | Product size of PCR (bp.) | Reference |
|---|---|---|---|---|---|
| 1 | *EbpB* | F | 5′-CGCAGCAACGAGATAAAGCC-3′ | 584 | NCBI |
| | | R | 5′-GACAAAAGACTTGCCGCCTG-3′ | | |
| 2 | *Bph* (*Phos*) | F | 5′-ACTCGTGAACCAGCCATTGT-3′ | 322 | NCBI |
| | | R | 5′-GACAAAAGACTTGCCGCCTG-3′ | | |

## Ethical approval

The present study has been managed according to the recommendations guide gained from the College of Medicine, University of Al-Qadisiyah, according to document number 30/4193 on November 14, 2022. The study did not include genetically modified creatures or prohibited biological substances. Each *E. faecalis* isolates included in the present study was obtained without any additional substances.

# Results

A total of 130 samples that collected from mid-stream urine (95 samples) and high vaginal swab (35 samples) that took from UTI and vaginal discharge patients admitting Maternity and Children Teaching Hospital in Al-Diwanyah City, during the period between (November 2022 to March 2023). Out of 130 samples, 25 (19.2%) isolates gave negative results for growth, and 105 (80.7%) isolates gave positive results for growth [Figure 1]. Out of 105 positive culturing samples, only 16 isolates (12.3%) were suspected to be *E. faecalis*.

*Enterococcus faecalis* was identified according to its morphology with Gram stain and chains appearance or pairs and biochemical tests (catalase, oxidase) for primary isolation after cultured on blood agar, MacConkey agar, Bile Esculin agar, m-EI chromogenic agar and incubating for 24–48 h at 37°C.

Colony morphology and culture characteristics were observed macroscopically; most isolates produce $\alpha$-hemolysis on blood agar, while others do not. All isolates grew on MacConkey agar and appeared as lactose fermenters with deep pink-magenta colored colonies. On bile esculin agar, all isolates convert the color of media to black coffee brown (due to the hydrolysis of esculin). The identification of *E. faecalis* was performed by direct inoculation on m-EI chromogenic agar (recovery and distinction of *E. faecalis*); the isolates were given blue colonies.

**Table 2: Components of the PCR mixture**

| No | Mixture contents | Volume (µl) |
|---|---|---|
| 1 | Master mix | 12.5 |
| 2 | Forward primer | 1.5 |
| 3 | Reveres primer | 1.5 |
| 4 | Template DNA | 5 |
| 5 | Distilled water | 4.5 |
| Total | | 25 |

## Diagnosis by VITEK-2 compact system

All 16 samples underwent a final confirmation phase using the VITEK-2 that confirmed the results. Table 4 explains the percentage of *E. faecalis* isolates from total samples, where only 12 (75%) isolates (according to the VITEK-2) were identified as *E. faecalis*. The percentage of *E. faecalis* isolated from midstream urine was (80%) while it was (66.6%) from high vaginal swabs.

## Antibiotic susceptibility profile of *Enterococcus faecalis*

The antibiotic susceptibility test in the present study was evaluated utilizing AST-P592 cards and an automated VITEK-2 system. *Enterococcus faecalis* antimicrobial susceptibility profile revealed that resistance was most frequently observed with erythromycin (100%), tetracycline (91.66%), streptomycin (58.33%), and gentamycin (50%) with a low resistance to ciprofloxacin (25%). The AST results indicated many available treatment options for the clinical management of *E. faecalis* infections since all the isolates tested were susceptible (100%) to antibiotics (vancomycin, teicoplanin, linezolide, tigecycline, and ampicillin). The antimicrobial susceptibility and percentage of isolates are shown in Figure 2.

## Multi drug resistance of *E. faecalis*

In the present study, the majority of *E. faecalis* isolates exhibited MDR to different antibiotics (75%, 9/12), as in Table 5. Multidrug resistance in the present study was less in urine isolates (62.5%) than vaginal isolates (100%).

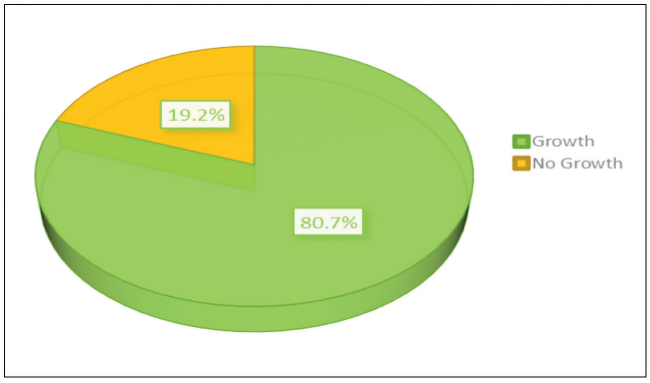


**Figure 1:** Percentage of isolates according to the growth

**Table 3: PCR thermo cycling conditions**

| Gene | Temperature (°C)/time (s) | | | | | Cycle no. |
|---|---|---|---|---|---|---|
| | Inetial denaturation | Cycling condition | | | Final extension | |
| | | Denaturation | Annealing | Extension | | |
| *EbpB* | 94 | 94/60 | 57/60 | 72/60 | 72 | 35 |
| *Bph (phos)* | 94 | 94/60 | 53/60 | 72/60 | 72 | |

## Molecular detection of biofilm formation genes *EbpB* and *Bph* (*phos*)

PCR was conducted for 12 isolates, using the *EbpB* and *Bph* (*phos*) using certain primers for amplifying such genes. The current findings made clear the existence of *the EbpB* gene was observed in all *E. faecalis* isolates (100%), where the bands showed up within the range of the gene's predicted size (584 bp) for all positive isolates, as shown in Figure 3. Also, 100% of isolates have the *Bph* (*phos*) gene, where the bands are seen within the gene's predicted size (322 bp), as illustrated in Figure 4.

**Table 4: Distribution of *E. faecalis* isolates according to the VITEK-2 system**

| Source samples | No of samples | Suspected *E. faecalis* (%) | No. of *E. faecalis* (%) by VITEK-2 |
|---|---|---|---|
| Urine | 95 | 10 (10.52%) | 8 (80%) |
| HSV swab | 35 | 6 (17.14%) | 4 (66.66%) |
| Total | 130 | 16 (12.3%) | 12 (75%) |
| Calculated $\chi^2$ | | 0.365 | |
| Calculated *P* value | | 0.551* | |

*No significant difference at $P > 0.05$

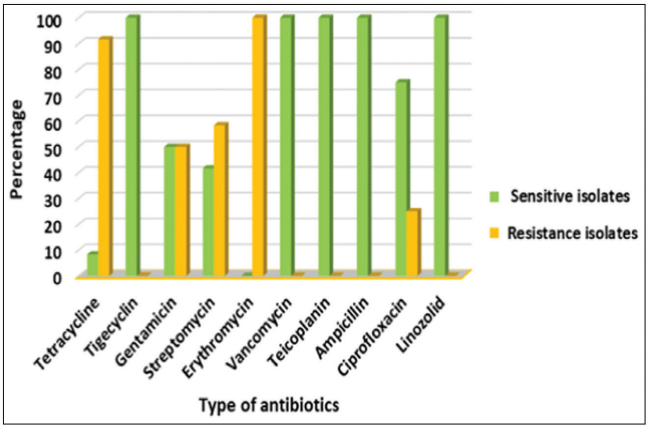


**Figure 2:** Antibiotic susceptibility profile of *E. faecalis*

## The relationship between virulence genes and antibiotic resistance

Results of the present study in Table 6 showed that *EbpB* and *phos*(*Bph*) genes were found in all *E. faecalis* isolates, so there was an association between these genes (biofilm formation genes) and (erythromycin, tetracycline, gentamycin, streptomycin, and ciprofloxacin) resistance.

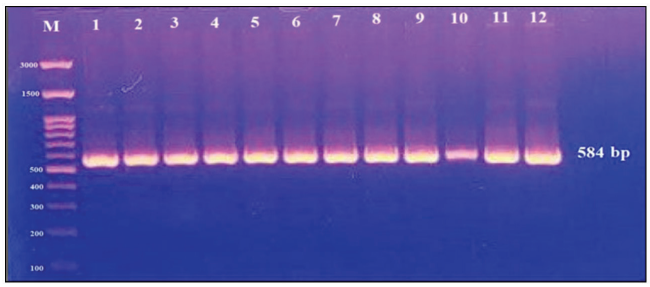


**Figure 3:** Shows a PCR-amplified *EbpB* gene (584 bp) electrophoresis on a 1.5% agarose gel after 60 min. Lane M (marker ladder 100-3000 bp). Lanes 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, and 12 isolate positively at 70 V

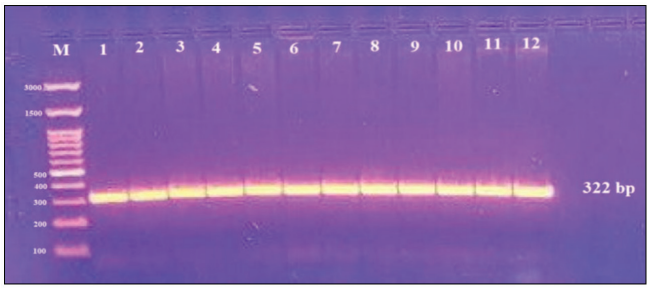


**Figure 4:** Shows a PCR-amplified *Bph* (*phos*) gene (322 bp) electrophoresis on a 1.5% agarose gel after 60 min. Lane M (marker ladder 100–3000 bp). Lanes 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, and 12 isolate positively at 70 V

**Table 5: Antimicrobial resistance profile of 12 *E. faecalis* isolates and resistance type**

| Isolate NO | Source of isolates | Antibiotics | | | | | | | | | | Type of resistance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AMB | LZD | E | CIP | GM | S | VA | TIC | T | TIG | |
| 1 | HVS | - | - | + | - | + | - | - | - | + | - | MDR |
| 2 | Urine | - | - | + | - | - | + | - | - | + | - | MDR |
| 3 | Urine | - | - | + | - | + | + | - | - | + | - | MDR |
| 4 | Urine | - | - | + | - | + | + | - | - | + | - | MDR |
| 5 | HVS | - | - | + | - | - | + | - | - | + | - | MDR |
| 6 | Urine | - | - | + | - | - | - | - | - | - | - | — |
| 7 | Urine | -- | - | + | - | - | - | - | - | + | - | — |
| 8 | Urine | - | - | + | - | - | - | - | - | + | - | — |
| 9 | HVS | - | - | + | - | + | - | - | - | + | - | MDR |
| 10 | HVS | - | - | + | + | - | + | - | - | + | - | MDR |
| 11 | Urine | - | - | + | + | + | + | - | - | + | - | MDR |
| 12 | Urine | - | - | + | + | + | + | - | - | + | - | MDR |

+: Presence of resistance gene, –: absence of resistance gene, S: streptomycin, T: tetracycline, E: erythromycin, CIP: ciprofloxacin, GM: gentamicin, AMB: ampicillin, TIG: tagicyclin, LZD: linezolid, VA: vancomycin, TCG: teicoplanin

**Table 6: Virulence genes and resistance patterns of *E. faecalis* isolates**

| Isolate no | Sample source | Genes | | Antibiotics | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Ebp* | *Bph (Phos)* | E | T | GM | S | CIP |
| 1 | HVS | + | + | + | + | + | - | - |
| 2 | Urine | + | + | + | + | - | + | - |
| 3 | Urine | + | + | + | + | + | + | - |
| 4 | Urine | + | + | + | + | + | + | - |
| 5 | HVS | + | + | + | + | - | + | - |
| 6 | Urine | + | + | + | - | - | - | - |
| 7 | Urine | + | + | + | + | - | - | - |
| 8 | Urine | + | + | + | + | - | - | - |
| 9 | HVS | + | + | + | + | + | - | - |
| 10 | HVS | + | + | + | + | - | + | + |
| 11 | Urine | + | + | + | + | + | + | + |
| 12 | Urine | + | + | + | + | + | + | + |

## Discussion

Although *E. faecalis* can live peacefully in the gastro intestinal tract (GIT) of the human, if it grows unchecked in the gut or gains access to extraintestinal sites, it can transform into an opportunistic pathogen. *Enterococcus faecalis* overgrowth in the GIT is often associated with antibiotic treatment and host inflammation, which can lead to subsequent translocation to other sites.[13] *Enterococcus faecalis* produces a biofilm that plays an important role in its virulence. It promotes bacterial adherence to the host cell surface, antibiotic resistance, and resistance to phagocytosis.[14]

Enterococci have an extraordinary ability to form biofilms, which is a remarkable pathogenesis strategy that allows their survival in adverse conditions and persistence at the site of infection. The formation of biofilm is a complex and multifactorial event but may be attributable in part to specific virulence factors, such as those associated with enterococci colonization/adhesion of the host.[15]

*Enterococcus faecalis* strains were able to survive within macrophages for up to 48 h; it seemed to be related to the ability of bacteria to synthesize extracellular polysaccharides (to form biofilm) induced by the presence of an additional carbohydrate source in the medium. Survival of *E. faecalis* within macrophages may contribute to pathogenesis by facilitating spread to distant sites after translocation through the intestinal barrier.[16]

The current results show that all isolates were very resistant to erythromycin (100%) and tetracyclin (91.66%), concurs with earlier research about the same issue.[17] And agreement with other studies carried out in India.[18,19] Previous studies[20,21] showed the majority of *E. faecalis* isolates were resistant to erythromycin, with 82.4% and 66%, respectively. Also, a study in Baghdad showed that 75% *of E. faecalis* isolates were resistant to erythromycin.[22] Another study about *E. faecalis* showed high susceptibility to tetracycline, linezolid, daptomycin, and tigecycline, whereas it showed a high resistance rate to levofloxacin, erythromycin, and clindamycin.[23]

In the present study, resistance was observed against streptomycin (58.33%) and gentamycin (50%); previous study[24] found (31.25%) and (43.75%) of *E. faecalis* isolates were to be resistant to gentamicin and streptomycin (both high level). Another study done by Szabó *et al.*[25] showed the highest resistance against streptomycin (67.7%) and gentamicin (59.3%).

This study is in the same line with the frequency of resistance to ciprofloxacin (20.6%) in urine specimens.[26] Another study[27] found that *E. faecalis* was highly resistant against ciprofloxacin (66.67%).

According to the current study, the highest resistance was related to erythromycin and tetracyclin, which are in concurrence with other studies.[28,29] Also, present results showed the prevalence of *EbpB and phos* (*Bph*) genes among *E. faecalis* isolates was high, with a rate of 100%, while the study by Kadhem[30] found that the most prevalence genes among *E. faecalis* isolates were *esp* gene 100%, and in another study which was performed in Tunis (2007) showed the most prevalence genes between *E. faecalis* isolates was *ace* gene 100%.[31]

In the present study, the majority of *E. faecalis* isolates exhibited MDR to different antibiotics (75%, 9/12). The rate of MDR of isolates in the current study was higher than in a study,[32] which reported that (57.1%) of *E. faecalis* showed MDR. A study[33] showed that multidrug resistance was more in urine isolates (55.7%) than vaginal isolates (53.6%), with many showing the same resistance patterns, while in the present study, multidrug resistance was less in urine isolates (62.5%) than vaginal isolates (100%).

There are at least three major reasons for the emergence of MDR enterococci: (i) baseline intrinsic resistance to several antimicrobial agents, (ii) acquired resistance via mobility of the resistance genes on plasmids and

transposons, and chromosomal exchange, and (iii) the transferability of resistance.[34]

*EbpB* gene is coding for Ebp production (the endocarditis and biofilm-associated pili minor), (Ebp) are surface-associated filamentous structures considered to play a pivotal role in *E. faecalis* virulence. Ebp pili contribute to biofilm formation, adherence to abiotic surfaces, and adherence to platelets, fibrinogen, and collagen of *E. faecalis*, thus supporting the establishment and persistence of this bacterium in clinically important infections and cause endocarditis and UTI. The presence of Ebp pili on the surface of *E. faecalis* cells likely has an important impact not only on colonization and adherence but also on bacterial pathogenicity and the spread of antibiotic resistance.[35] According to results from the present study, the *Ebp* gene was present in all *E. faecalis* (100%); a high incidence of this *Ebp* gene was reported in the previous study, with a percentage (90%) of all *E. faecalis* in urine.[36] The current study showed similarity to the result of another study,[37] with (95.9%) of the *EbpB* gene between *E. faecalis* isolates.

Recently, another virulence factor (biofilm association phosphatase) was described in *E. faecalis*, *Bph* (phos) is required for attachment to a relevant host surface. Expression of *Bph* from a pheromone-inducible complementation vector restored biofilm formation in all mutants. An increase in the number of attached biofilm cells was observed with the *Bph* strain expressing *Bph* from a plasmid. One explanation for reduced biofilm formation by *Bph* mutants is increased cell lysis due to cell envelope defects.[38-39]

## Financial support and sponsorship
Nil.

## Conflicts of interest
There are no conflicts of interest.

## References

1. AL-Khafaji JKT. Preparation of modified selective medium for isolation of *Enterococcus faecalis* in pure culture from heavy sources (rapid diagnosis). Med J Babylon 2021;18:340.
2. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev 1990;3:46-65.
3. Ch'ng J-H, Chong KK, Lam LN, Wong JJ, Kline KA. Biofilm-associated infection by enterococci. Nat Rev Microbiol 2019;17:82-94.
4. Jung RH, Kim M, Bhatt B, Choi JM, Roh JH. Identification of pathogenic bacteria from public libraries via proteomics analysis. Int J Environ Res Public Health 2019;16:912.
5. Jabbar AH, Al-Azawi IH, editors. The detection and investigation of tetracycline resistance associated genes among Shigella isolates using polymerase chain reaction and phylogenetic analysis methods in Al-Diwaniyah province, Iraq. J Phys Conf Ser 2020;1664:012123.
6. Abril AG, Quintela-Baluja M, Villa TG, Calo-Mata P, Barros-Velázquez J, Carrera M. Proteomic characterization of virulence factors and related proteins in enterococcus strains from dairy and fermented food products. Int J Mol Sci 2022;23:10971.
7. Hasson SO, Al-Hamadani AH, Al-Azawi IH. Occurrence of biofilm formation in *Serratia fonticola* and *Pantoea* sp. isolates among urinary catheterized patients. Nano Biomed Eng 2018;10:295-304.
8. Zeng X, She P, Zhou L, Li S, Hussain Z, Chen L, *et al*. Drug repurposing: Antimicrobial and antibiofilm effects of penfluridol against *Enterococcus faecalis*. Microbiologyopen 2021;10:e1148.
9. Willett JL, Dale JL, Kwiatkowski LM, Powers JL, Korir ML, Kohli R, *et al*. Comparative biofilm assays using *Enterococcus faecalis* OG1RF identify new determinants of biofilm formation. MBio 2021;12:1.
10. Abdulla AA, Almuttairi AA. Occurrence of Class 1, 2, and 3 integrons among multidrugresistant *Pseudomonas aeruginosa* in Babylon Province, Iraq. Med J Babylon 2023;20.
11. Haghi F, Lohrasbi V, Zeighami H. High incidence of virulence determinants, aminoglycoside and vancomycin resistance in enterococci isolated from hospitalized patients in Northwest Iran. BMC Infect Dis 2019;19:1-10.
12. Mondal S, Ghosh SK, Biswas SK, Pramanik JD, Das S. Profile of nonvenereal female genital dermatoses: A cross-sectional study from eastern India. J Low Genit Tract Dis 2022;26:276-82.
13. Kao PHN, Kline KAD. Jekyll and Mr. Hide: How *Enterococcus faecalis* subverts the host immune response to cause infection. J Mol Biol 2019;431:2932-45.
14. Popović N, Dinić M, Tolinački M, Mihajlović S, Terzić-Vidojević A, Bojić S, *et al*. New insight into biofilm formation ability, the presence of virulence genes and probiotic potential of Enterococcus sp. dairy isolates. Front Microbiol 2018;9:78.
15. Tibúrcio AA, Paiva AD, Pedrosa AL, Rodrigues WF, da Silva RB, Oliveira AG. Effect of sub-inhibitory concentrations of antibiotics on biofilm formation and expression of virulence genes in penicillin-resistant, ampicillin-susceptible *Enterococcus faecalis*. Heliyon 2022;8:e11154.
16. Ramos Y, Sansone S, Morales DK. Sugarcoating it: Enterococcal polysaccharides as key modulators of host–pathogen interactions. PLoS Pathog 2021;17:e1009822.
17. 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;2022:3129439.
18. Mathur P, Kapil A, Chandra R, Sharma P, Das B. Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India. Indian J Med Res 2003;118:25-8.
19. Agarwal J, Kalyan R, Singh M. High-level aminoglycoside resistance and β-lactamase production in enterococci at a tertiary care hospital in India. Jpn J Infect Dis 2009;62:158-9.
20. Lee T, Jordan D, Sahibzada S, Abraham R, Pang S, Coombs GW, *et al*. Antimicrobial resistance in porcine enterococci in Australia and the ramifications for human health. Appl Environ Microbiol 2021;87:e03037-20.
21. Klibi N, Gharbi S, Masmoudi A, Ben Slama K, Poeta P, Zarazaga M, *et al*. Antibiotic resistance and mechanisms implicated in clinical enterococci in a Tunisian hospital. J Chemother 2006;18:20-6.
22. AL-Marjani MF, Abdulrazaq RA. Macrolide–lincosamide-streptogramin b resistance in enterococcus spp. isolates in Baghdad. 2015.
23. Luty RS, Fadil AG, Najm JM, Abduljabbar HH, Kashmar SAA. Uropathogens antibiotic susceptibility as an indicator for the empirical therapy used for urinary tract infections: A retrospective observational study. Iran J Microbiol 2020;12:395-403.
24. Raj DHJ, Das DM, Mondal S. Prevalence of enterococcal infection and the antimicrobial susceptibility profile of the organism with special reference to vancomycin: A study in a rural Medical College Hospital in Eastern India. IOSR J Dent Med Sci 2019;18:9-15.
25. Szabó S, Feier B, Capatina D, Tertis M, Cristea C, Popa A. An overview of healthcare associated infections and their detection methods caused by pathogen bacteria in Romania and Europe. J Clin Med 2022;11:3204.
26. Ghalavand Z, Alebouyeh M, Ghanati K, Azimi L, Rashidan M. Genetic relatedness of the *Enterococcus faecalis* isolates in stool and

urine samples of patients with community-acquired urinary tract infection. Gut Pathog 2020;12:1-11.
27. Alzahrani MA, Ali MS, Anwar S. Bacteria causing urinary tract infections and its antibiotic susceptibility pattern at tertiary hospital in Al-Baha region, Saudi Arabia: A retrospective study. J Pharm Bioallied Sci 2020;12:449-56.
28. Seo Y, Lee G. Antimicrobial resistance pattern in *Enterococcus faecalis* strains isolated from expressed prostatic secretions of patients with chronic bacterial prostatitis. Korean J Urol 2013;54:477-81.
29. Gholizadeh P, Aghazadeh M, Ghotaslou R, Rezaee MA, Pirzadeh T, Cui L, *et al*. Role of CRISPR-Cas system on antibiotic resistance patterns of *Enterococcus faecalis*. Ann Clin Microbiol Antimicrob 2021;20:1-12.
30. Kadhem HS. Evaluation of virulence factors and detection of vanA, vanB and esp genes from clinical isolates of vancomycin-resistant *Enterococcus faecalis*. J Pure Appl Microbiol 2018;12.
31. Klibi N, Ben Slama K, Sáenz Y, Masmoudi A, Zanetti S, Sechi LA, *et al*. Detection of virulence factors in high-level gentamicin-resistant *Enterococcus faecalis* and Enterococcus faecium isolates from a Tunisian hospital. Can J Microbiol 2007;53:372-9.
32. Rana D, Sande S. Study of prevalence and antimicrobial susceptibility pattern of enterococci isolated from clinically relevant samples with special reference to high level aminoglycoside resistance (HLAR) in a rural tertiary care hospital. JEMDS 2020;9:2472-8.
33. Anyadoh-Nwadike SO, Okorondu SI, Obiajuru I, Nwadike PO, Nwaokorie F, Akerele J. Comparative study of the prevalence and antibiogram of bacterial isolates from the urinary and genital tracts of antenatal patients. IOSR J Pharm Biol Sci 2015;10: 15-9.
34. Oudah MA. Antibiotic Resistance Profile of Pathogenic Bacteria Isolated from Healthcare Rooms in the Mosul Government Hospital, Iraq. Med J Babylon 2024;17:S70-80.
35. La Rosa SL, Montealegre MC, Singh KV, Murray BE. *Enterococcus faecalis* Ebp pili are important for cell–cell aggregation and intraspecies gene transfer. Microbiology 2016;162:798-802.
36. Suchi SE, Shamsuzzaman S, Uddin BMM, Yusuf MA. Detection of virulence factors and antimicrobial resistance in enterococci isolated from urinary tract infection. Bangladesh J Infect Dis 2017; 4:30-4.
37. Gök SM, Kara F, Arslan U, Fındık D. Investigation of antibiotic resistance and virulence factors of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from clinical samples. Mikrobiyol Bul 2020;54:26-39.
38. Willett JL, Ji MM, Dunny GM. Exploiting biofilm phenotypes for functional characterization of hypothetical genes in *Enterococcus faecalis*. npj Biofilms Microbiomes 2019;5:23.
39. Alzaidi JR, Kareem AA. The Impact of Urogenital Tract Infectious Bacteria on Male Fertility. Med J Babylon 2024;21:476-80.