Iraqi Journal of Biotechnology, 2023, Vol. 22, No. 1, 245-251

# Detection of *FsrB* Quorum-Sensing Gene and Biofilm Production in *Enterococcus faecalis* Isolated from UTI Women

[1]Hawraa A. Hamel, [1] Abdulameer M. Ghareeb,[2] Saja A. Shareef

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad
[2]Assistant Lecture, Department of Molecular and Medical Biotechnology, College of Biotechnology, AL-Nahrain University

**Received: May 7, 2023/ Accepted: May 28, 2023/Published: June 4, 2023**

**Abstract:** *Enterococcus faecalis* are Gram-positive responsible for a variety of infections in susceptible hosts, including endocarditis, bacteremia, urinary tract infections (UTI), and mouth infections, The adhesion of *E. faecalis* to host tissues, notably urinary tract infections, is a crucial phase in the pathogenesis of the organism. The aim of the study Detection of a *FsrB* Gene and Biofilm Production in *Enterococcus faecalis* Isolated. The patients with various age groups who had urinary tract infections were included in this study for collection 150 urine samples from (Baghdad Hospital of Medical City and teaching laboratories of Medical City) in Baghdad Between November 2022 and February 2023. Urine samples were cultured on differential culture media and the isolated bacteria were identical according to cultural characteristics, biochemical test and VITEK- 2 system also molecular method was used in the identification of bacterial growth. The results revealed 32 isolates were belonging to *E. faecalis* and this was confirmed through amplification of species specific gene (*23SrRNA*). All 32 *E. faecalis* isolates were subjected to further molecular analysis to check for the presence of the diagnostic biomarker *FsrB* gene, which is linked to the quorum-sensing system that regulates the production of gelatinas to control biofilm growth. The results showed that 26 (81.2%) *E. faecalis* possess *fsrB* gene. Biofilm producers was detected in *E. faecalis* isolates using microtiter plate method (MIC) which that the results indicated that 9 (28.12%) of bacterial isolates were strong biofilm production, 11 (34.375%) as moderate and 12 (37.5%) as week biofilm production. Antibiotic susceptibility of the bacterial isolates was examined against 22 types of antibiotics. Isolates showed resistance to Cefpodoxime, Eryrthomycin and Ceftriaxone (96.875%), while the lowest resistance (12.5%) was against Amikacin. According to our study, the spread of *Enterococcus faecalis* began to increase significantly compared to previous years. The study proved that *Enterococcus faecalis* were highly resistant to Cefpodoxime, Eryrthomycin and Ceftriaxone and highly sensitive to Amikacin. The study proved that all isolates were biofilm producers. Also The study proved that all isolates contain the *23SrRNA* gene The current *in vitro* study demonstrats the presence of *FsrB* quorum-sensing gene.

**Keywords:** *Enterococcus faecalis,* biofilm production, **FsrB** gene, Antibiotic susceptibility.

**Corresponding author:** (Email: hawraa.abbas2100m@ige.uobaghdad.edu.iq).

## Introduction

One of the most common bacterial diseases that call for antibiotic treatment is urinary tract infections (UTIs) (1). Endocarditis, bacteremia, oral infections, and urinary tract infections (UTI) are only a few of the illnesses caused by *Enterococcus faecalis* in susceptible hosts (2). In the gut of many creatures, *enterococcus* bacteria are facultative anaerobes and non-motile normal flora (3). If their growth is unregulated, however, they have the potential to develop into an opportunistic pathogen and affect further intestinal locations in the host as well as hospitals (4). Adherence to host tissues, particularly UTIs, is a crucial first step in E. faecalis pathogenesis (5). *E. faecalis* have several virulence factors may be related to colonization of the host, adhesion, resistance to the

host's defense mechanisms, tissue invasion, and the production of pathological changes both directly through the production of toxins and indirectly through the induction of inflammation including hylauronidase, lipotecichoic acid, and sex pheromones (6), Antibiotic resistance, Gelatinase, Cytolysin toxins, Extracellular superoxide production, Aggregation substance, Enterococcal surface protein (7) and Biofilm development (8). Quorum-sensing system in *Enterococcus faecalis* (QS) is a dynamic process that controls gene expression in relation to cell density that controls a number of bacterial functions, including the production of biofilms, pathogenicity, and antibiotic resistance. (9). The Fsr regulator gene, which controls the production of both gelatinase and serine protease, is a key component of E. faecalis' quorum sensing system. Gelatinas are produced under the direction of the Fsr quorum-sensing system, which also governs the development of biofilms (10). The transmembrane protein FsrB, which is a member of the accessory gene regulator protein B family, is produced by the fsrB gene (11). A diagnostic biomarker for the gelatinase activity is the fsrB. (10). Microorganisms adhered to a surface that form a biofilm and are shielded from the outside environment by an extracellular polymeric material matrix (EPS) (12). *E. faecalis* has the capacity to generate biofilms during chronic infections (13). Gelatinase is crucial for the development of biofilms by controlling the production of gelatinase the Fsr quorum-sensing system regulates the development of biofilms (10). The resistance of *Enterococcus spp* enabled them to live in heavily used antimicrobial environment and it has been discovered that about 24% of nosocomial infections are difficult to treat which related to intrinsic resistance to many groups of antibiotics in addition to acquired vancomycin resistance, this represents a serious threat to people around the world (14). Aim of study: Detection of a *FsrB* Gene and Biofilm Production in *Enterococcus faecalis* Isolated.

## Material and methods

One hundred fifty urine samples collected from patients with various age groups (25-67 years), during the period of study from beginning November 2022 to end of February 2023, urine samples were taken at the Baghdad Hospital of Medical City and Teaching laboratories of Medical City. All specimens were cultured on Blood agar and HiCrome™ UTI Agar). The positive growth was identified based on colony morphological (Shape, size, and color of colonies), micro scopic Examination, biochemical tests and VITEK-2 system also we used molecular analysis in the identification of bacterial growth.

### Biofilm formation assay

Quantitative determination of biofilm was determined by Tissue Culture Plate (TCP) method (also called microtiter plate assay) in accordance with some modifications. The isolates of *Enterococcus Faecalis* were cultivated on blood agar, then added to 10 ml of trypticase soy broth (TSB) with 1% glucose, and assessed using a 0.5 McFarland scale. Incubation took place for 18 hours with the inoculated broth at 37°C. The overnight-incubated bacterial suspension was added to 9900 ml of fresh TSB medium and diluted to 1:100 after 18 hours of incubation then, 200 µl of the diluted prepared bacterial suspension was placed in each of the 96 separate wells of a sterile polystyrene tissue culture plate. The microtiter plates were then incubated at 37°C for 24 hours. After incubation, the wells were twice rinsed with 200 µl of phosphate buffer saline (PBS) to get rid of bacteria that were floating around. After 15 to 20 minutes of ethanol addition, a micro- ELISA reader (at 570 nm wave length) was used to read the

dye associated with the adhering biofilm.

**Table (1): Classification of bacterial adherence and biofilm formation by microtiter plate assay method (15)**

| Mean OD values | Adherence | Biofilm formation |
|---|---|---|
| **(OD ≤ 0.5)** | None | None |
| **(OD > 0.5 but <1)** | Weak | Weak |
| **(OD > 1 but <2)** | Moderately | Moderate |
| **(OD > 2)** | Strong | High |

## Antimicrobial susceptibility test

Disc diffusion method or Kirby-Bauer technique was used to conduct the susceptibility testing for 22 different antibiotics was executed depended on recommendations given by the Clinical Laboratory Standards Institute (CSLI) and also we used VITEK-2 system in the Antimicrobial susceptibility test.

## Molecular methods

## DNA extraction

Genomic DNA Extraction Mini Kit (Favorgen®, Korea), was used to extract DNA from *E. faecalis* isolates, purity and concentration of DNA isolated were calculated, primer sequence of *FsrB* and *23SrRNA* gene their size product are shown in table (2).

**Table (2): The primers, sequence for detection of genes**

| Target gene | Nucleotide sequence (5'—3') | Product Size/ bp | Reference |
|---|---|---|---|
| ***FsrB*** | F-TACAGGGAGTATCATCAGACC | 144 | Designed |
| | R-CAGCCATCTGATTTAAACGTG | | |
| ***23S rRNA*** | F-GAAGGGGAGTGAAATAGATCC | 177 | Designed |
| | R-GACTACATACTCATTTGCCCT | | |

## Results and discussion

## Isolation and identification

Under a microscope, all bacterial isolates were recognized as Gram-positive cocci with ovoid or spherical forms that may be seen alone, in pairs, or in short chains.. On HiCrome™ UTI Agar differential medium, the colonies were spherical, small, with a blue colony, while colonies of *E. faecalis* on the blood agar were in a white color and surrounded by a transparent halo, which indicates they were able to lyse blood cells and produced beta hemolysis (β-hemolysis) (16) as shown in (Figure 1).

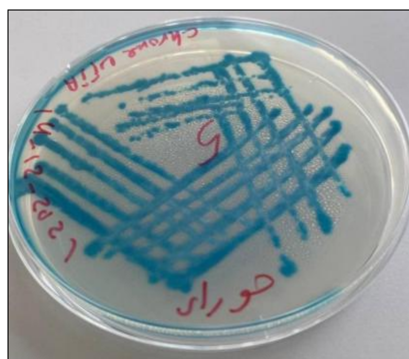

**Figure (1): *Enterococcus faecalis* growth on culture media HiCrome™ UTI Agar**

According to the biochemical properties, isolates of *E. faecalis* that identified in this study; were positive for the Voges-Proskauer But they were negative to the catalase, oxidase test, Methyl red, citrate, urease test and indole test, according to the study that stated the same results about this bacteria (17) who reported that all isolated identified by using biochemical test.

This study's findings revealed that 100% of isolates were able to produce biofilm but in different level which ranged from weak to moderate then strong. Isolates were more common 12 (37.5%) among weak biofilm formers, while 11 (34.375%) isolates were moderate and 9 (28.12%) were strong biofilm formers. A local study conducted by Aya (18), there are dissimilarities with current study as they revealed that 44% of isolates were strong biofilm former, 50% moderate and 6% weak biofilm former.

**Antibiotic Susceptibility**

The 32 isolates were completely showed very high resistant (96.875%) to Cefpodoxime, , Eryrthomycin and Ceftriaxone, while some isolates showed high resistance to Cefixime and Cefoxitin (93.75%), Cefepime and Ceftazidime (90.625%).Moderate resistance to Tetracycline (68.75%), Levofloxacin (62.5%), and show low resistance to Vancomycin (46.875%), Gentamicin (43.75%), Teicoplanin (37.5%), Linezolid (28.125%), Ampicillin(25%), Daptomycin (18.75 %), and Amikacin (12.5%), The sensitivity test results percentage which show as in (Table 2).

**Table (2): Antibiotic Susceptibility of 32 *Enterococcus faecalis* isolates.**

| Antibiotic | Sensitive (%) | Resistance (%) | P-value |
|---|---|---|---|
| **Tigecycline** | 26 (81.25) | 6 (18.75) | 0.0074 ** |
| **Tetracycline** | 10 (31.25) | 22 (68.75) | 0.037 * |
| **Minocycline** | 5 (15.625) | 27 (84.37) | 0.0009 ** |
| **Doxycycline** | 8 (25) | 24 (75) | 0.0026 ** |
| **Vancomycin** | 17 ( 53.125) | 15 ( 46.875) | 0.724 NS |
| **Teicoplanin** | 20 ( 62.5) | 12 ( 37.5) | 0.046 * |
| **Daptomycin** | 26 ( 81.25) | 6 ( 18.75) | 0.0074 ** |
| **Linezolid** | 23 (71.875) | 9 ( 28.125) | 0.027 * |
| **Eryrthomycin** | 1 ( 3.125) | 31 ( 96.875) | 0.0001 ** |
| **Norfloxacin** | 8 ( 25) | 24 ( 75) | 0.0026 ** |
| **Levofloxacin** | 12 ( 37.5) | 20 ( 62.5) | 0.046 * |
| **Ciprofloxacin** | 6 ( 18.75) | 26 ( 81.25) | 0.0003 ** |
| **Amikacin** | 28 ( 87.5) | 4 ( 12.5) | 0.0001 ** |
| **Streptomycin** | 4 ( 12.5) | 28 ( 87.5) | 0.0001 ** |
| **Gentamicin** | 18 ( 56.250) | 14 ( 43.75) | 0.307 NS |
| **Cefepime** | 3 ( 9.375) | 29 ( 90.625) | 0.0001 ** |
| **Ceftriaxone** | 1 ( 3.125) | 31 ( 96.875) | 0.0001 ** |
| **Ceftazidime** | 3 ( 9.375) | 29 ( 90.625) | 0.0001 ** |
| **Cefpodoxime** | 1 ( 3.125) | 31 ( 96.875) | 0.0001 ** |
| **Cefixime** | 2 ( 6.25) | 30 ( 93.75) | 0.0001 ** |
| **Cefoxitin** | 2 ( 6.25) | 30 ( 93.75) | 0.0001 ** |
| **Ampicillin** | 24 ( 75) | 8 ( 25) | 0.0026 ** |
| **P-value** | 0.0001 ** | 0.0001 ** | --- |
| *** (P≤0.05), ** (P≤0.01).** | | | |

The *E. faecalis* isolates showed that 25% were resistant to Ampicillin, this result was similar the result of (19) who showed that 25 % of *Enterococcus faecalis* were resistant to Ampicillin. *Enterococcus faecalis* isolates showed that 81.25 % were resistant to Ciprofloxacin, 18.75% Tigecycline, 43.75% Gentamicin, 37.5% Teicoplanin, this result was contradicts the result of (20) who showed that 4 % of *Enterococcus faecalis* were resistant to Ciprofloxacin, Tigecycline and Gentamicin. Result of present study showed that 46.87 % were resistant to vancomycin this result was close to

result (20) that showed that 53% were resistant to vancomycin. This study showed that isolates were resistant to Ampicillin and Tetracycline this result is identical to (20) but with a slight difference in proportions. *E.faecalis* isolates showed that 96.875% were resistant to Erythromycin, which is similar to the studies (21) who exhibited that 100% and 96% of E. *faecalis* isolates were resistant to Erythromycin.

**Molecular methods for identification of *Enterococcus faecalis* isolates**

To detect *23S rRNA* gene for diagnosing of E. *faecalis*, thirty two isolates of E. *faecalis* were tested using the PCR method.. All the tested *E. faecalis* clinical contained *23S rRNA* gene (177bp). With 2% agarose gel electrophoresis stained with Redsafe, electrophoresed at 70 volts for 60 minutes, and photographed using an ultraviolet (UV) Trans illuminator, the favorable *23S rRNA* gene result was confirmed in (Figure 2).

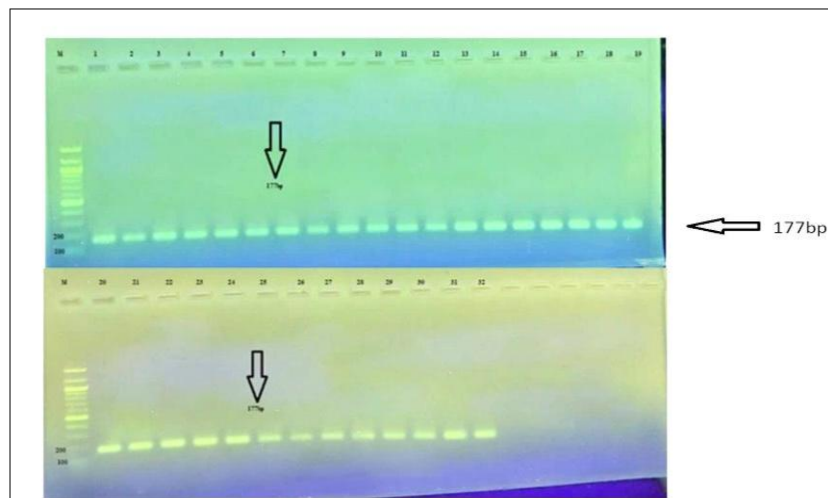


**Figure (2): Agarose gel electrophoresis of conventional PCR amplification products of *23SrRNA*gene in *E. faecalis* isolates. M: marker (100 bp ladder); lanes 1-23: PCR amplicons of target genes. (Expected size 177bp; 2% agarose, 70 Vol / 1 hour)**

According to (figure 2), the *23S rRNA* gene is present in all *E. faecalis* isolates isolated from urine samples. The current study's findings were comparable to those of the previous one (22).

**Molecular detection of *FsrB* Gene**

*FsrB* gene had been detected in *E. faecalis* isolates by PCR technique, *FsrB* positively confirmed by agarose gel electrophoresis in 2% agarose stained with Redsafe and then electrophoreses in 70 V for 60 min and then photographed under the transilluminator as shown in figure 3). The result display that *FsrB* gene band was detected at 144bp region, 26(81.2%) of *E. faecalis* isolates have *FsrB* gene. The findings of the current investigation were comparable to those of a previous study (23), which found that the *FsrB* gene was present in 80% of *E. faecalis* isolates.

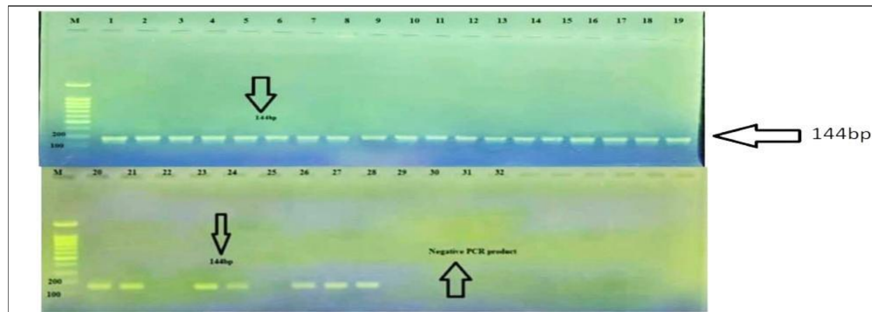


**Figure (3): Agarose gel electrophoresis of conventional PCR amplification products of *FsrB* gene in *E. faecalis* isolates. M: marker (100 bp ladder); lanes 1-23: PCR amplicons of target genes. (Expected size 144 bp; 2% agarose, 70 Vol / 1 hour.**

The results of another investigation conducted by (24) who demonstrated that 100% of *E. faecalis* isolates have the *FsrB* gene were in disagreement with our findings. The results of another investigation demonstrated that 11.8% of *E. faecalis* isolates possess the *FsrB* gene, were in disagreement with our findings (25).

## Conclusion

According to our study, the spread of *Enterococcus faecalis* began to increase significantly compared to previous years. The study proved that *Enterococcus faecalis* were highly resistant to Cefpodoxime, Eryrthomycin and Ceftriaxone and highly sensitive to Amikacin. The study proved that all isolates were biofilm producers. Also The study proved that all isolates contain the *23SrRNA* gene The current *in vitro* study demonstrats the presence of *FsrB* quorum-sensing gene.

## References


1. Hamad, S. T. (2022). Prevalence of Carbapenemase Genes in *Klebsiella pneumoniae* Isolates from Patients with Urinary Tract Infections in Baghdad Hospitals. Iraqi Journal of Biotechnology, 21(1):102-114
2. Turjeman, A; Babich,T; Pujol, M; Carratalà, J; Shaw E, Gomila-Grange, A; Vuong, C; Addy, I; Wiegand, I; Grier, S; MacGowan, A; Vank, C; Cuperus, N; van den Heuvel, L; Leibovici, L; Eliakim-Raz, N. (2021). Combacte magnet wp5 rescuing Study Group and Study Sites. Risk factors for *enterococcal* urinary tract infections: a multinational, retrospective cohort study. European Journal of Clinical Microbiology and Infectious Disease, 40(9): 2005-2010.
3. Georges, M; Odoyo, E; Matano, D; Tiria, F; Kyany'a, C, Mbwika, D; ... and Musila, L. (2022). Determination of *Enterococcus faecalis* and *Enterococcus faecium* Antimicrobial Resistance and Virulence Factors and Their Association with Clinical and Demographic Factors in Kenya. Journal of Pathogens 43(9):1-9.
4. Kao, P. H. N; Kline, K. A. Dr; Jekyll and Mr. Hide. (2019). How *Enterococcus faecalis* subverts the Host Immune Response to cause infection. Journal of Molecular Biology, 431(16), 2932-2945
5. Jett, BD; Huycke, MM. and Gilmore, MS.(1994). Virulence of *enterococci*. Clinical Microbiology Reviews.7(4):462–478.
6. Kayaoglu, G. and Ørstavik, D. (2004). Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. Critical Reviews in Oral Biology & Medicine .15(5):308-320.
7. Mundy, L.M.; Sham, D.F. and Gilmor, M. (2000). Focus relationships between *Enterococcal* virulence and antimicrobial resistance. Clinical Microbiology Reviews. Rev. 13(4):513-522.
8. Necidova, L; Janstova, B; Karpiskova, S; Cupakova, S; Duskova, M. and Karpiskova, R. (2009). Importance of *Enterococcus spp.* for forming a biofilm. Czech Journal of Food Sciences. 27:354-356.
9. Saied, E; Eid A.M; Hassan S.E.-D; Salem S.S., Radwan A.A., Halawa M., Saleh F.M., Saad H.A., Saied E.M., Fouda A.(2021). The Catalytic Activity of Biosynthesized Magnesium Oxide Nanoparticles (MgO-NPs) for Inhibiting the Growth of Pathogenic Microbes,

Tanning Effluent Treatment, and Chromium Ion Removal. Catalysts 11, 821.

10. 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. 1713-1723.
11. Ali, L; Goraya, MU; Arafat, Y; Ajmal, M. and Chen JL, Yu D.(2017). Molecular Mechanism of Quorum-Sensing in *Enterococcus faecalis*: It's Role in Virulence and Therapeutic Approaches. International Journal of Molecular Sciences. 3; 18(5):960.
12. Ozma, M. A; Khodadadi, E; Rezaee, M. A; Kamounah, F. S; Asgharzadeh, M; Ganbarov, K; Aghazadeh, M; Yousefi, M; Pirzadeh, T. and Kafil, H. S. (2021). Induction of proteome changes involved in biofilm formation of *Enterococcus faecalis* in response to gentamicin. Microbial Pathogenesis, 157, 105003.
13. Narenji, H; Teymournejad, O; Rezaee, M. A; Taghizadeh, S; Mehramuz, B; Aghazadeh, M; Asgharzadeh, M; Madhi, M; Gholizadeh, P; Ganbarov, K; Yousefi, M; Pakravan, A; Dal, T; Ahmadi, R. and Samadi Kafil, H. (2020). Antisense peptide nucleic acids againstfts Z and efaA genes inhibit growth and biofilm formation of *Enterococcus faecalis*. Microbial pathogenesis, 10(4):139, 103907.
14. Lebreton, F; van Schaik, W; McGuire, A.M.; Godfrey, P; Griggs, A; Mazumdar, V; Corander, J; Cheng, L; Saif, S; Young, S; Zeng, Q; Wortman, J; Birren, B; Willems, RJ; Earl, AM. and Gilmore, MS.(2013). Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. American Society for Microbiology. 20; 4(4):e00534-13.
15. Sengupta, M; Sarkar, S; SenGupta, M; Ghosh, S; Sarkar, R. and Banerjee, P. (2021). Biofilm Producing Enterococcus Isolates from Vaginal Microbiota. Antibiotics, 10(9), 1082-1091.
16. Kandela, N. J; Aziz, G M. and Khanaka, H. H. (2012). A Genetic Study on Enterocin – Production from *Enterococcus faecalis* isolated from different clinical sources , Iraqi Journal of Biotechnology. 11 (2):503-518.
17. Daniels, A. O; and Temikotan, T. (2023). Antibiotic Resistance and Plasmid Profile of *Enterococcus Feacalis* Isolated from Human Anal Swab. International Journal of Innovative Research in Sciences and Engineering Studies, 3: (2583-1658).
18. Aya H. Al-Halaby; Ashwak B. Al-Hashimy; Israa, Al-Kadmy. and Emad Al_Ajeeli . (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, 16(1): 80-91.
19. Salaam K. Muslem, Othman T. Qasim Sarah R. Rasool and Muataz M. Al-Taee. (2021). Antibiotic resistance markers in *Enterococcus sp.* Isolates, Al-Nisour Journal for Medical Sciences, 3(1) 2021.
20. Molechan, C; Amoako, DG; Abia ALK, Somboro, AM; Bester, LA. and Essack, SY.(2019). Molecular Epidemiology of antibiotic-resistant *Enterococcus spp.* from the farm-to-fork continuum in intensive poultry production in KwaZulu-Natal, South Africa Sciences. 20(692):868-878.
21. Esmail M. A. M; Abdulghany H. M. and Khairy R. M. (2019). Prevalence of multidrug-resistant *Enterococcus faecalis* in Hospitalacquired surgical wound infections and bacteremia: concomitant analysis of antimicrobial resistance genes. Infectious Diseases: Research and Treatment, 12:1-6.
22. Kafil, HS; Mobarez, AM; Moghadam, MF; Hashemi, ZS. and Yousefi, M.(2016). Gentamicin induces efaA expression and biofilm formation in *Enterococcus faecalis*. Microbial Pathogenesis. 92:30-35.
23. Bag, M; Sattar, A; Arif, M; Riaz, S; Khan, M; Rahman, S. and Hassan, J. (2022). Antimicrobial resistance, virulence profiles, and public health significance of *Enterococcus faecalis* isolated from clinical mastitis of cattle in Bangladesh, Hindawi BioMed Research International. 15(5): 1-8.
24. Stępień-Pyśniak, D; Hauschild, T; Kosikowska, U; Dec, M. and Urban-Chmiel, R. (2019). Biofilm formation capacity and presence of virulence factors among commensal *Enterococcus* spp. from wild birds of Sciences. Scientific Report, 9(3): 1–7.
25. Gök, Ş. M; Kara, F; Arslan, U, and Fındık, D. (2020). Investigation of antibiotic resistance and virulence factors of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from clinical samples. Mikrobiyoloji bulteni, *54*(1), 26-39.