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**Molecular analysis of antibiotic resistance genes
and biofilms formation in *Enterococcus* spp
isolated from Al Muthanna province , Iraq**

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Abstract

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In the recent years, *Enterococcus* species have clinical significance due to the causes of many infections, such as urinary tract infections, dental root caries, and infections of wounds and burns. This risk increased with rising antibiotic resistance, especially vancomycin, among *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*), which are the most common and the most dangerous, so the present study aimed to isolate and diagnose pathogenic *enterococci* from different clinical samples, evaluating the antibiotic sensitivity of bacterial isolates to a number of antibiotics and phenotypic detection of virulence genes, hemolysis, and biofilm, in addition to investigating some resistance genes and their gene expression and knowing the genetic closeness of local isolates to the most genetically close of global isolates.

In the present study, 170 clinical samples were collected from different sources, including urine, inflamed dental root canals, and inflamed burns, in addition to vaginitis samples from the Women and Children Hospital and Al-Hussein Teaching Hospital in Al-Muthanna Governorate during period September 2023 to October 2023. Sixty-three (77.8%) of *E. faecalis* strains and 18 (22.2%) *E. faecium* isolates were confirmed by cultural diagnostic and biochemical testing; the majority of infections occurred in young people aged 21–30 and the majority of infections occurs in females (71.6%). Furthermore, these infections were widespread in rural regions. Antibiotic sensitivity testing was performed for nine antibiotics based on the Kirby-Bauer disk diffusion method; the rates of antibiotic resistance against *E. faecalis* were as follows: 100% with meropenem, tetracycline 65%, ampicillin 63.4%, 12.6% for azithromycin, 42.8% ciprofloxacin, 17.4% AMC, vancomycin, and nitrofurantoin (15.8%, 7.9%) respectively. The resistance profiles of *E. faecium*

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were 94.4% to meropenem, 66.6% to tetracycline, 61.1% to ampicillin, 77.8% to azithromycin, 44.4% to ciprofloxacin, 22.2% to AMC, 33.3% to vancomycin, teicoplanin, and nitrofurantoin (16.6% and 5.5%), respectively.

Subsequently, molecular diagnosis, isolates were confirmed; 55/63 (87.3%) isolates were found to be *E. faecalis*, and only 10/18 (55.6%) were found to be *E. faecium* using Taqman real-time PCR. Of the thirteen VRE isolates, genes for *vanB* and *bla_{TEM}* were found to be prevalent, with nine isolates carrying every gene. while only one isolate is carrying the *vanA* gene. Moreover, the isolate harboring the *vanA* gene exhibited elevated gene expression subsequent to vancomycin induction, as evidenced by a folding value of 1.62. Conversely, the *vanB* gene folding values varied between 0.007 and 7.82, while the *bla_{TEM}* gene folding value ranged from 0.132 to 6.062. The phylogenetic trees of a chosen gene (16S rRNA, *vanB* *vanA*, and *bla_{TEM}* genes) revealed genetic relation with isolates that were registered in the NCBI.