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Dental Root Canal Infections Caused by *Enterococcus faecalis* are Associated with Heart Valve Replacement Infections

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Abstract: A common oral pathogen *Enterococcus faecalis* causes periodontitis and dental infections. Biofilms, gelatinase, lipoteichoic acid, cytolysin toxin, surface adhesions, and hyaluronic acid are key mechanisms of virulence for dental *E. faecalis*. Study aims to investigate the association between dental root canal infection with *E. faecalis* and heart valve replacement infections. It is also examined its susceptibility to antibiotics. A total of 120 samples were collected, 80 from patients with valve replacements suffering from dental caries and 40 from healthy individuals with dental caries only. *16 srRNA* genes were used to identify *E. faecalis* and other species. A VITEK 2 system was used to test *E. faecalis* biochemical phenotyping and antimicrobial susceptibility. *E. faecalis* was the most prevalent species found in dental caries patients with valve replacements. *E. faecalis* isolates were 100% susceptible to ampicillin, while 66.6% to linolid and teicoplanin. *E. faecalis* strains are all resistant to vancomyin, but 33.3% to teicoplanin. Several infections related to heart valve replacement can be caused by the presence of *E. faecalis* in dental root canals. An antibiotic such as ampicillin is recommended for the treatment of a dental root infection caused by *E. faecalis*.

Keywords: *Enterococcus faecalis*, Dental Caries, Heart Valves, Polymerase Chain Reaction, Virulence genes.

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Introduction

The treatment of cardiovascular diseases, including heart failure, stroke, myocardial infarction, and others, still requires substantial advances. The prevalence of infarctions and myocarditis continues to increase. Among the many conditions that adversely affect the health of the public, peripheral vascular disease is one of the most significant. Cardiovascular disease (CVD) may be caused by a variety of infectious and non-infectious factors, and certain microbes have been linked to atherosclerosis, heart failure, and other vascular disorders (1). Approximately 70% of cases are caused by endocarditis, an inflammation of the inner lining of the heart (2). In addition to cardiovascular diseases, cardiac valve dysfunction is one of the leading causes of death worldwide. According to the

World Health Organization (WHO), 17.9 million deaths were caused by CVDs in 2019(3). Each year, cardiovascular disease (CVD) is one of the leading causes of mortality worldwide (4). Despite the fact that metabolic or unhealthy lifestyles, including low physical activity levels, obesity, alcohol consumption, and smoking, are the primary causes of CVDs, a lot of attention has been paid to the role gut microbiota plays in various pathogenic conditions.

Dental caries is a global epidemic that causes considerable morbidity (5). Although untreated dental caries directly affects oral health. Maintaining overall health and quality of life requires an understanding of the indirect link between dental caries and systemic health. Most research has focused on the relationship between systemic diseases and periodontitis, as well as the role of oral inflammation and microbiota in conditions such as Alzheimer's disease, diabetes mellitus, atherosclerosis, pneumonia, and chronic obstructive pulmonary disease (6). Several factors can contribute to localized tooth demineralization in dental caries, which can be regarded as a biofilm-mediated illness (7).

E. faecalis is a common pathogen associated with oral diseases such as periodontitis and dental infections. It lives in the oral biofilm layers and co-aggregates with saliva bacteria, resulting in the failure of endodontic therapy. The ability of *E. faecalis* to form biofilms allows it to survive in root canals for at least 12 months. There is an association between root canal frequent *E. faecalis* and salivary incidence. *E. faecalis* can spread through food or environmental surfaces during dental treatment. There was, however, no genetic connection between the root canal isolates and the normal gastrointestinal microflora discovered by the researchers. Systemic colonization and infection have also been linked to enterococci (8). There is evidence of oral *E. faecalis* -induced infective endocarditis and biofilms, aggregation material, gelatinase, lipoteichoic acid, cytolysin toxin, surface adhesions, and hyaluronic acid as key mechanisms of virulence. The virulence factor of *E. faecalis* plays a critical role in the persistence of root canal infections. Several virulence factors associated with *E. faecalis* have been shown to promote colonization, inflammation, and resistance (9). As a result of these virulence factors, *E. faecalis* becomes more capable of colonizing and exhibits greater phenotypic tolerance to a variety of disinfectants. Inflammatory reactions can be triggered by chemical or material substance (9). A number of species benefit from the ability of *E. faecalis* to adapt to root canals. This explains how it survives root canal infections, which are associated with very few nutrients and limited access to medications used during root canal therapy. Consequently, *E. faecalis* is identified as a pathogenic bacterium that may cause root canal therapy to fail.

Methods

The collection of samples

The study was approved by the Iraqi Ministry of Health's ethics committee. During the period between September 2023 and May 2024,

samples were taken from Iraqi patients at Ibn Al-Bitar Center for Cardiac Surgery, as well as from control. A total of (120) specimens were collected from patients who had valvular heart replacements with dental caries (80) samples and from healthy individuals with dental caries only (40) samples. Disposable swabs were used to collect dental caries samples from patients and control individuals using a transport medium. As soon as the samples were placed in transport media (containing nutrient agar), they were streaked onto a semi-selective medium Mitis Salivarius agar (MSA).

The isolation and identification of *Enterococcus faecalis* and other streptococci using conventional microbiological techniques

The bacterial swabs obtained from dental caries samples isolates from valvular heart replacement patients were streaked on blood agar medium and a selective medium (Mitis Salivaris Agar). A candle jar was used to incubate the plates at 37°C under anaerobic conditions for 48 hours. In order to obtain pure cultures of *E. faecalis*, this procedure was repeated numerous times. The morphological characteristics of bacterial growth on Nutrient, Mitis Salivarius agar and blood agar was studied in detail, including colony shape, color, texture, odor, and edges. After isolating and transferring a single colony to a microscopic slide, it was fixed and stained using Gram stain. Cell form, cell organization, and Gram reacting were all observed (10).

Identification of *Enterococcus faecalis* and other *streptococci* by molecular methods

DNA Extraction

Following the purification of the bacteria, single colony plates were prepared. Genomic DNA was extracted using FavorPrepTM Blood/Cultured Cells Genomic DNA Extraction Mini Kit from Korea Favorgen (thailand) according to the manufacturer's instructions.

DNA Quantitation by Qubit 4.0

Qubit™ dsDNA HS Assay Kit from Thermo Fisher® (USA) was used to quantify DNA purity and integrity in accordance with manufacturer instructions. With an initial sample concentration of 10 pg/L to 100 ng/L, the assay is highly selective for double-stranded DNA (dsDNA) over RNA. Assays are performed at room temperature, and the signal remains stable for three hours. There are no adverse effects from common contaminants such as salts, free nucleotides, solvents, detergents, or proteins in the assay (11).

Molecular identification of *Enterococcus faecalis* and other Streptococci using the 16S rRNA gene

Molecular identification of isolated bacteria was accomplished using *16 srRNA* genes to detect *E. faecalis* and other species isolated from dental root canals from valvular heart replacement patients. The Go Taq® Master Mix kit was homogenized using a vortex at room temperature. A conventional polymerase chain reaction (PCR) (Applied Biosystems, USA) is employed in the current study(12).

Table (1) shows primer sequences used in the present study The amplification procedure for these genes included the initial denaturation phase heated to 94°C for 3 minutes then, at 94°C, 35 cycles of denaturation for 30 seconds, annealing for 45 seconds at (48-49) °C, extension for 1 minute at 72°C, and final extension for 5 minutes at 72°C. The PCR product electrophoresed on a 2% Tris-acetate-EDTA (TAE) agarose gel dyed with RedSafe.

Table (1): The Specific primers of *16 srRNA* for Streptococci species detection.

Genus	Species	Primer sequence	TM(°C)	Product size(bp)
Streptococcus	*Mitis*	F (TTGTATTAGCTAGTTGGTGGG) R (ACAGCCTTTAACTTCAGACTT)	*48*	*378*
Streptococcus	*Salivarius*	F (GGGGATAACTATTGGAAACGA) R (ACAGCCTTTAACTTCAGACTT)	*48*	*479*
Streptocoous	*Mutans*	F (ACTACACTTTCGGGTGGCTTGG) R (CAGTATAAGCGCCAGTTTCATC	*48*	*469*
Streptocoous	*Sobrins*	F (TGCTATCTTTCCCTAGCATG) R (GGTATTCGGTTTGACTGC)	*48*	*1160*
Enterococcus	*Fecalis*	F (AGAGAAGAACAAGGACGTTAG) R (ACACTTAGCACTCATCGTTTA)	*49*	*390*

All primers used in this experiment was newly designed by Geneious prime software.

Biochemical phenotyping antimicrobial susceptibility testing for *E. faecalis* using VITEK 2 system

In accordance with the manufacturer's instructions, the VITEK 2 system (bioMérieux) was used to test the biochemical responses of the isolates. Each strain's pure colonies were grown in TSA and suspended in sterile saline (0.45% NaCl) to a McFarland turbidity range of 0.5-0.63. A gram-positive (GP) card was then loaded with bacteria (bioMérieux). Vitek 2 system is widely used for the identification of bacteria and the testing of their susceptibility to antimicrobial agents (13). The test uses eight antibiotics (Ampicillin, Ciprofloxacin, Erythromycin, Linezolid, Teicoplanin, Vancomycin, Tetracycline, and Tigecycline).

Statistical analysis

A combination of Microsoft Excel 2019 and R-4.4.2 Software was used to analyze the study's data. In order to compare two means, the unpaired t test was used, whereas a one-way analysis of variance (ANOVA) was used to compare more than two means. Furthermore, a Standard (LSD) and Post Hoc Tests (multiple comparison test) were used to compare more than two means. Note. P-value adjusted for comparing a family of 5(0.05).

Results

Isolation and identification of *Enterococcus faecalis.*

We collected 120 clinical specimens from patients undergoing valvular heart replacements who had dental caries. A total of 80 isolates were taken from patients and 40 isolates were taken from control. The patients ranged in age from 35 to 70 years.

Microbiological methods were used to identify *E. faecalis* isolates, including colony morphology on selective media Mitis Salivarius agar (MSA), and microscopic examination by Gram staining. The colony morphology was determined based on the form, shape, and color of bacterial colonies grown on MSA media. Blue-black, shiny, and slightly raised colonies form on the surface. *E. faecalis* can be alpha - hemolytic or beta-hemolytic (14). *E. faecalis* is a Gram-positive facultative anaerobic bacterium that grows in short and medium chains (15)

According to morphological and biochemical analysis, 66 bacterial isolates were identified as *E. faecalis*. There were 40 samples taken from patients and 26 samples taken from control subjects, Figure (1).

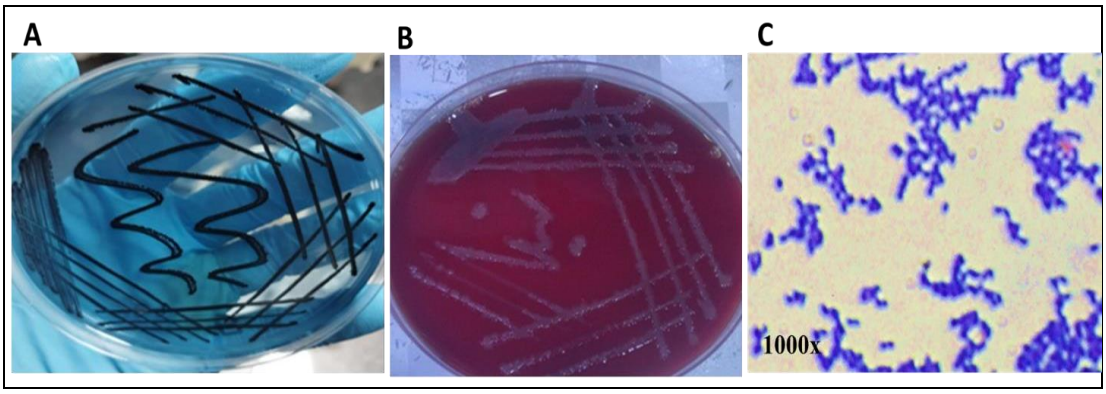

Figure (1): Colonies of *E. faecalis: (*A) Mitis Salivarius agar, (B) Blood agar, and (C) r representative images of *E. faecalis* stained with Gram-positive under a light microscope at a magnification of 1000x.

Detection of 16SrRNA gene in *E. faecalis* using conventional PCR.

In order to confirm the standard microbiological identification of the 66 isolates, the 16SrRNA gene fragment was amplified using a conventional PCR method. The *16S rRNA* gene analysis indicated that (23) samples out of (26) control samples were positive (88.46%) and (3) samples were negative (11.54%). In contrast, out of (40) patient samples, (5) were negative (12.50%) and the remaining isolates were positive (87.50%). Positive isolates were all identified as *E. faecalis* as shown in Figure 2. Below. The percentage and number of other species are demonstrated in (table 3) below. There were 44 positive *S. mitis* isolates from 52 samples, representing 84.61%, while there were only 8 negative of isolates. *S. salivarius* was detected in 35 positive isolates and 8 negative isolates. However, *S. sobrinus* was detected in five positive samples and 47 negative samples, whereas *S. mutans* was detected in one positive sample and 40 negative samples.

Table (2): Distribution of *E. faecalis* according to molecular detection by *16SrRNA* gene.

Samples	Control				Patient			
Streptococcus species	Positive No.	Percentage %	Negative No,	Percentage %	Positive No.	Percentage %	Negative No,	Percentage %
Enterococcus faecalis	23	88.46%	3	11.54%	35	87.50%	5	12.50%
Streptococcus mitis	14	100%	0	0%	44	84.61%	8	15.38%
Streptococcus salivarius	12	92.30%	1	7.69%	35	81.39%	8	18.60%
Streptococcus mutans	1	12.50%	7	87.50%	1	2.43%	40	97.56%
Streptococcus sobrinus	1	9.09%	10	90.90%	5	9.43%	47	88.67%

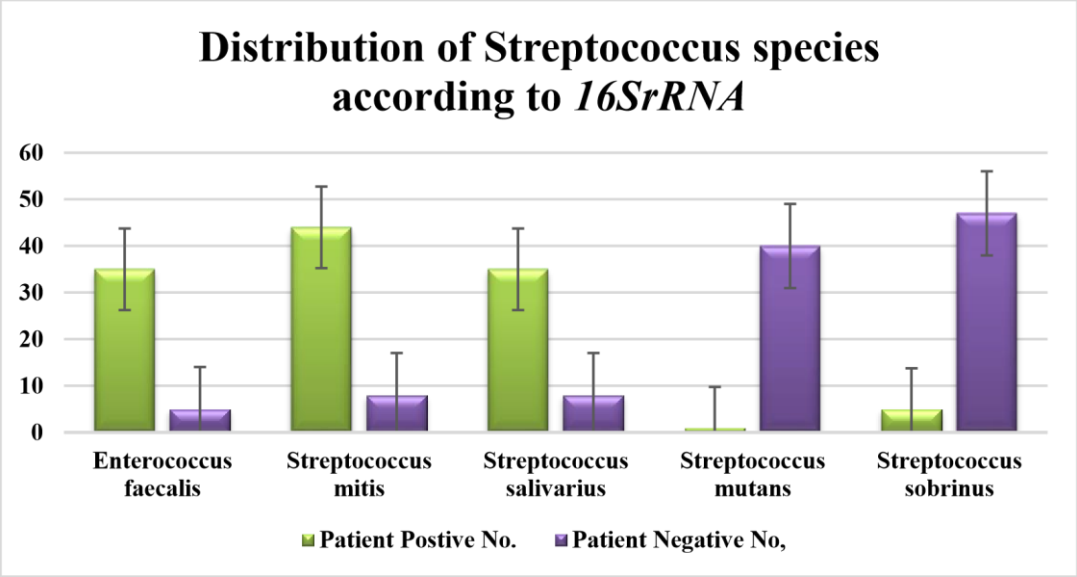

Figure (2): Distribution of *E. faecalis* and other Streptococcus species according to molecular detection by *16SrRNA* gene. This bar chart shows the number of positive and negative isolates from patient and control samples for each species x-axis represents the percentage of specis and y-axis represents the *streptococci* species.

Molecular detection by *16SrRNA* gene

A comparison of the molecular weights of the PCR products with a 100 base pair DNA ladder provided confirmation of the PCR results on 2% agarose gel electrophoresis (13). As mentioned previously (35) out of (40) isolates were identified as *E. faecalis* based on amplicons of 390 bp as shown in figure (3, A). In the same manner, PCR products from other isolates were applied an agarose gel electrophoresis of 2% agarose gels and a ladder of 100 base pairs of DNA. By analyzing the molecular weight of the PCR product amplicon, (44) isolates of *S. mitis* were detected with 378 base pairs Figure (3, B). A total of 35 isolates of *S.salivarius* were found at 479 bp, and six isolates of *S.sobrinus* were found at 1160 bp. Moreover, *S.mutants* were not detected in these isolates and the results were negative. The results are shown for each of the following figures (3 C and D).

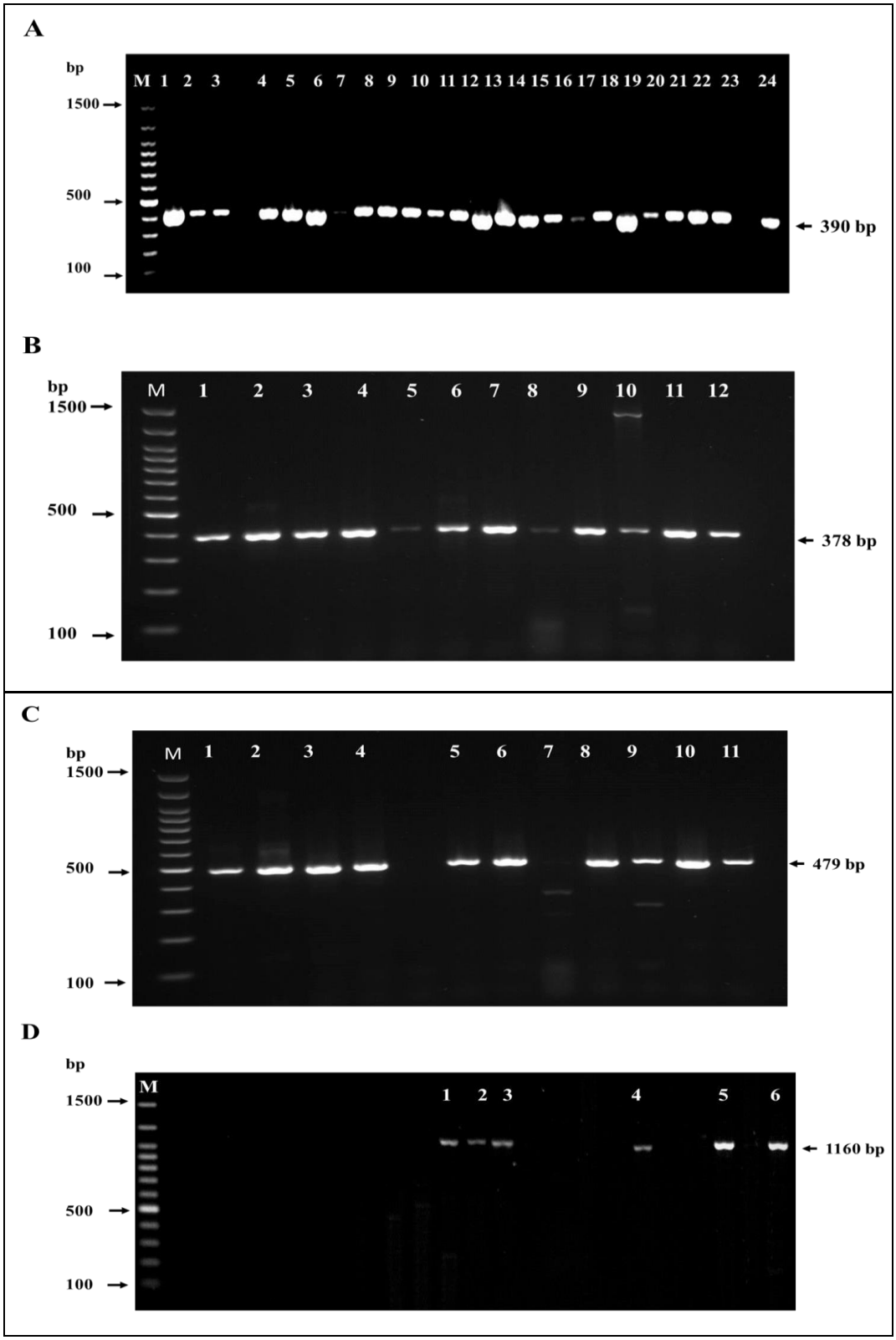

Figure (3): Polymerase chain reaction (PCR) of 16S rRNA gene of Streptococcus species. Lane M: 100 bp DNA ladder. (A): *E. faecalis*, Lanes 1-24 are positive samples, PCR product is 390 bp. (B): *S. mitis*, Lanes 1-12 are positive samples, PCR product amplicon is 378 bp. (C): *S. salivarius*, Lanes 1-11 are positive samples, PCR product amplicon is 479 bp. (D): *S. sobrinus*, Lanes 1-6 are positive samples, PCR product amplicon is 1160 bp.

This investigation utilized the GP card, which contains 43 substrates (biochemical tests) for Gram positive bacteria. Six isolates were selected for the Vitek2 susceptibility test. According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024), bacterial isolates were tested for susceptibility to antibiotic groups. These antibiotics are (Ampicillin, Ciprofloxacin, Erythromycin, Linezolid, Teicoplanin, Vancomycin, Tetracycline, Tigecycline).

Figure (4) shows that all isolates were susceptible to ampicillin and tigecycline (100%). In addition, they were resistant to ciprofloxacin, erythromycin, linezolid, teicoplanin, vancomycin, and tetracycline.

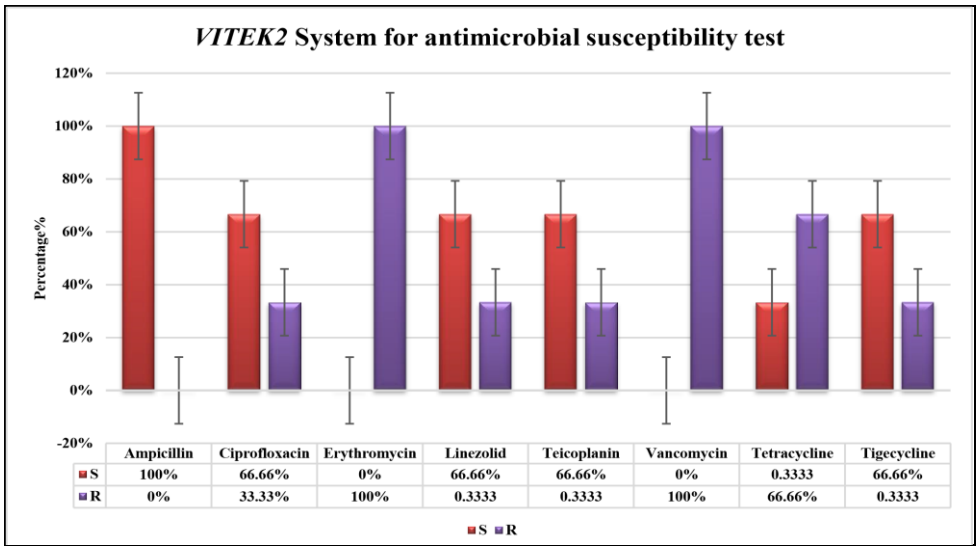

Figure (4): VITEK 2 System for identification *Enterococcus faecalis* and for Antimicrobial Susceptibility Testing.

Discussion

According to morphological and biochemical analysis, 66 isolates from valvular heart replacement patients with dental caries were identified as *E. faecalis.* There was a total of 40 samples of *E. faecalis* collected from patients and 26 samples collected from control subjects.

Based on the *16S rRNA* gene analysis, 23 samples out of 26 control samples were positive (88.46%) and 3 samples were negative (11.54%). Compared to (40) samples from patients, (5) were negative (12.50%), while the remaining isolates were positive (87.50%). Positive isolates were all identified as *E. faecalis*, which dominated the isolates. Our results are similar to those of (14). According to their study, 31 isolates out of 40 clinical samples were identified as *E. Faecalis* by conventional methods. The findings of another study which identified *E. Faecalis* as the predominant species isolated from the oral cavity indicated that 88.7% of clinical samples contained *E. Faecalis* (15). An additional study reported 126 enterococcal isolates from the oral cavity, 72% of which were classified as *E. Faecalis* (16).

As shown in Table (4), all isolates of *E. Faecalis* were susceptible to ampicillin and tigecycline. Additionally, they were resistant to ciprofloxacin, erythromycin, linezolid, teicoplanin, vancomycin, and tetracycline. The

majority of *E. faecalis* isolates showed 100% susceptibility to ampicillin, which is consistent with other studies. The susceptibility of *Faecalis* to antibiotics is due to the gelatinase (*gelE*) virulence gene, which plays a critical role in the pathogenesis of this organism, including autolysis, biofilm production, and antibiotic resistance. According to Conceiço *et al*., (17) study, they compare Etest with disk diffusion against broth dilution, Betalactam susceptibility testing was effective against penicillin-resistant and ampicillin-susceptible isolates of *E. faecalis*. According to Correa *et al.*, (18) a study published in 2022, isolates of *E. faecalis* exhibiting the unusual penicillin-resistant, but ampicillin-susceptible phenotype appeared to be restricted to hospital settings. A study conducted by Mousavi and colleagues in 2020 indicated that *E. faecalis* was also resistant to ampicillin. Our findings indicate that all isolates of *E. faecalis* were resistant to vancomycin (100%) while 66.6% were sensitive to Linzolid. This is in agreement with the findings of Manoil *et al*., (19). Results of their study revealed that most endodontic isolates were susceptible to amoxicillin and vancomycin, but that clindamycin exhibited varying levels of intrinsic resistance. The number of isolates showing resistance to linezolid (33.3%) corresponds to the number reported by Maliová et al, in 2021. According to their findings, 8% of the isolates of *E. faecalis* were resistant to linezolid. The results also indicated a 33.3% of *E. faecalis* were resistance to teicoplanin, which is in agreement with the findings of Kabhti et al., 2021. In their study, 43 (77%) *E. faecalis* isolates were tested, of which 5 (11.6%), 4 (9.3%), and 1 (2.3%) isolates showed resistance to linezolid, chloramphenicol, and teicoplanin respectively, while all isolates showed resistance to penicillin G and amoxicillin-clavulanic acid. Furthermore, *E. faecalis* isolates showed a 66.6 percent sensitivity to teicoplanin, which is consistent with the results of as with vancomycin, Teicoplanin has a similar antibacterial spectrum and is effective against most Gram-positive pathogens. As a bactericidal agent, teicoplanin is effective against sensitive staphylococci and most streptococci species, but weakly effective against enterococci and viridans streptococci. An in vitro and an in vivo pharmacological study was conducted to determine whether teicoplanin had any adverse effects on the central nervous system, cardiovascular system, or respiratory system.

Conclusion

Based on 16 srRNA analysis, *E. faecalis* was the most prevalent species isolated from patients with valvular heart replacement with dental caries. The majority of *E. faecalis* isolates were 100% susceptible to ampicillin, while 66.6% were susceptible to Linzolid and Teicoplanin.

Conversely, 100% of *E. faecalis* strains are resistant to vancomyin, and 33.3% to teicoplanin. Infections associated with the replacement of heart valves may be caused by *E. faecalis* in dental root canals. Ampicillin is recommended to treat a dental root infection caused by *E. faecalis*.

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Conflict of interest

The authors have no conflicts of interest to declare.

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