Prevalence of Multidrug-Resistant *Enterococcus faecalis* in Bacterial Vaginosis: Molecular Detection of Virulence and ESBL Genes

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Abstract

This study examined the prevalence and antibiotic resistance of *Enterococcus faecalis* in women with vaginitis during 2024. Among 100 specimens, 65 strains were isolated, with 75% of patients presenting vaginal discharge and 62% experiencing irritation. The findings revealed significant associations between aerobic vaginitis and Enterococcus infections.

The isolated strains demonstrated multi-drug resistance (MDR) to erythromycin, ampicillin, chloramphenicol, doxycycline, and minocycline. However, newer antibiotics, including linezolid, tedizolid, vancomycin, teicoplanin, and tigecycline, showed high efficacy, while ciprofloxacin and levofloxacin demonstrated moderate resistance.

Molecular analysis of ten strains revealed consistent presence of virulence and resistance genes: the cytolysin gene (*cylA*) was found in all isolates, bla_{SHV} in 70%, bla_{TEM} in 100%, and *ermB* in all strains. A significant correlation was observed between biofilm formation and antibiotic resistance, highlighting biofilm's role in enhancing pathogenicity.

These findings underscore the escalating global trend of antibiotic resistance in Enterococcus species and emphasize the critical need for improved diagnostic methodologies, comprehensive surveillance systems, and adapted therapeutic strategies to effectively manage vaginitis caused by these increasingly resistant pathogens.

Keywords: vaginitis, antibiotic resistance, biofilm, virulence genes, MDR

Introduction

Vaginitis is a group of diseases that can make the vagina or vulva itch, burn, hurt, smell foul, or leak [1]. Women used to ignore problems with their vaginas, but now they are more likely to go to the doctor. The vaginal flora that lives there naturally helps keep the pH levels in the vagina where they should be and keeps infections and pathogens from settling there [2]. The ecosystem of the vagina includes multiple microbes, some of which are mutualistic, while others are considered to be opportunistic pathogens [3]. The medical condition in question has increased in importance among pregnant women and those of childbearing age. Although some individuals may be asymptomatic, they can still experience BV complications in the future. The case of BV is troubling, and treatment options are limited. The presence of lactobacilli and an array of other microflora is pivotal in keeping the harmful flora and infection from taking root [4].

An imbalanced vaginal microbiome can result in various infections such as bacterial vaginosis, trichomoniasis, and vulvovaginal candidiasis. Recent investigations have begun to develop an understanding of the complexities of vaginal dysbiosis and what microbiome-targeted therapeutic options exist and can be developed further [5]. If there is no treatment for chronic vaginal infections, it is much more common for childbearing-aged women to face issues such as premature delivery as well as infertility [6]. Various situational factors, such as menstruation, pregnancy, sexual intercourse, and vaginal douching, may predispose one to infection, as might the overuse of antibiotics. Gardnerella vaginalis, Treponema pallidum, Neisseria gonorrhoeae, and Chlamydia trachomatis are the most common pathogens responsible for vaginal infections [7].

Enterococcus exhibits the unique dual trait of natural and acquired resistance to a variety of antibiotics. This trait is exhibited while they simultaneously perform the unique trait of catalyzing energy anaerobically. As *Enterococcus* species gained resistance, the microbes incrementally changed their nucleic acid sequences or acquired additional higher-order polynucleotide sequences through alternative mechanisms of genomic transfer. The mechanism of acquired resistance, coupled with a length of time, leads microbes to fall out of susceptibility to numerous antibiotics, including, but not limited to, tetracycline, macrolides, fluoroquinolones, and vancomycin [8]. It is crucial to take the necessary precautions to refine the limits and track biofilm and antibiotic-resistant infections to avoid complications further down the line [9].

The formation of biofilm is central to understanding chronic bacterial disease processes because of how biofilm surrounding cells protects them against antibiotics [10]. The commensal bacterium *Enterococcus* can sometimes turn into an opportunistic threat. However, vaginal colonization increases with antibiotic therapy or in women with aerobic vaginitis, which suggests that it may live in the female genital system. More studies are showing that enterococci are linked to bacterial vaginosis and aerobic vaginitis [11]. Researchers have also looked at antimicrobial resistance and whole-genome sequencing of new *Enterococcus* strains. This approach has helped to understand how biofilm helps bacteria resist drugs [12]. Consequently, examining certain virulence factors, such as cytolysin (encoded by the *cylA* gene), and significant antibiotic resistance markers, including the extended-spectrum β-lactamase genes *(bla_{TEM}*,

bla$_{SHV,}$ and *ermB*), is essential for comprehending the complete pathogenic capacity of *E. faecalis* in vaginal infections [13].

2. Materials and Methods

The Biology Department at Basrah University conducted a prospective study from January 2024 to December 2024. The research may proceed if the Institutional Ethics Committee gives its approval. The study encompassed adult female patients diagnosed with vaginitis. The study did not include patients who had vaginal ulcers. After patients gave their informed consent, we used a pro forma to record their clinical data as shown in Table 3.

We used standard bacteriological procedures to culture vaginal discharge samples collected with sterile cotton swabs from 100 women. For this reason, the research only included samples that had grown *Enterococcus* species. The initial step in identifying enterococci was using a Gram stain and non-fastidious growth. we used the VITEK 2 Compact automated identification system to help us figure out what kind of organism it was.

2.1. Antimicrobial Susceptibility Test

The growing isolates were further tested for antimicrobial susceptibility to different antimicrobial agents, including Ampicillin (10mg), Norfloxacin (10mg), Chloramphenicol (30 µg), and Ciprofloxacin (5 µg), using the Kirby-Bauer disc diffusion method and standard microbiological techniques on Mueller-Hinton agar plates. The minimum inhibitory concentration (MIC) was tested using the VITEK 2 Compact (BioMerieux Inc., France) for Doxycycline, Minocycline, Nitrofurantoin, Tetracycline, Levofloxacin, Vancomycin, Tigecycline, Teicoplanin, Linezolid, Tedizolid, and Erythromycin. All interpretation of susceptibility patterns was performed according to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines, and interpretation was also guided by the EUCAST guidelines version 2016 [14].

2.2. Detection of Biofilm

Congo Red Agar (CRA) is one of the procedures used to identify biofilm formation. 40 g of blood agar base, 0.8 g of Congo red, and 50 g of sucrose in 1 liter of distilled water were prepared. The mixture was then heated on a hot plate until it was completely mixed, the pH was corrected to 8, and it was autoclaved at 121°C for 15 minutes. the bacterial isolates were cultivated on the prepared media and incubated at 37 °C for 24 hours [15].

2.3. Molecular Methods:

2.3.1: DNA Extraction:

The Wizard® Genomic DNA purification kit (Promega, USA) was used to extract the genomic DNA from *E. faecalis*

2.3.2: Polymerase chain reaction PCR:

A Nanodrop spectrophotometer was then used to evaluate the concentration and purity of the DNA samples before proceeding to PCR. The DNA samples are stored at -20°C until the PCR assay. A PCR assay was then conducted to examine certain virulence factors, such as cytolysin (encoded by the *cylA* gene), and significant antibiotic resistance markers, including the extended-spectrum β-lactamase genes (bla_{TEM}, bla_{SHV}) and the Macrolides gene, like the *ermB* gene, using specific primers as outlined in Table 1. The PCR mixture volume for 16S rRNA amplification was 25 µL, comprising 13 µL of premaster mix (Bioneer, Korea), 1 µL of each primer (10 pmol), 2 µL of genomic DNA (5-50 ng/mL), and the remainder filled with nuclease-free water. The PCR thermocycler conditions were conducted as in the reference, as shown in Table 2 [16]. The PCR products were analyzed via electrophoresis on a 1.5% agarose gel for 45 minutes, followed by ethidium bromide staining and visualization under a UV transilluminator [17], as shown in Figure 4,5,6,7

Table 1: Oligonucleotide primer's sequences and PCR products.

Primers	Sequence of Primers	Product size(bp)	References
***cylA* F**	5-ACTCGGGGATTGATAGGC-3	688 bp	[18]
***cylA* R**	5-GCTGCTAAAGCTGCGCTT-3		
***ermB* F**	5- GAAAAGGTACTCAACCAAATA-3	640 bp	[19]
***ermB* R**	5-AGTAACGGTACTTAAATTGTTTAC-3		
***bla* $_{TEM}$ F**	5- CATTTCCGTTGTCGCCCTTATTC-3	800 bp	[20]
***bla* $_{TEM}$ R**	5- CGTTCATCCATAGTTGCCTGAC-3		
***bla*$_{SHV}$ F**	5-AGCCGCTTGAGCAAATTAAAC-3'	713 bp	[21]
***bla*$_{SHV}$ R**	5- ATCCCCGCAGATAAATCACCAC-3		

Table 2: Amplification conditions of all primer genes

Steps	Temperature	Time	No. of cycles
Initial Denaturation	95C	5 min	1
Denaturation	95C	30sec	
Annealing	52,54,55,60 C°	30sec	35 cycles
Extension	72C	1min	
Final extension	72C	5min	1
Hold	4C	Forever	-

2.4. Ethical approval:

The ethical statement received approval from the ethics committee of the College of Science, University of Basrah. Moreover, implicit assent from each participant was obtained. Information obtained from the patient will be maintained with absolute confidentiality, encompassing all enquiries related to socio-demographic, reproductive, and sexual histories, behavioral characteristics, and clinical features.

3. Results

During the period (from January 2024 to December 2024), we collected 100 specimens, of which 65 isolates were diagnosed as *Enterococcus* spp. obtained from the cases of vaginitis. The clinical profiles of the patients are shown in Table 3. 91% of the patients were in the reproductive age group (18-50 years). 75% of the patients experienced vaginal discharge, and 62% of patients reported itching; 15% of women were pregnant.

Table 3: Clinical and demographic characteristics of the patients

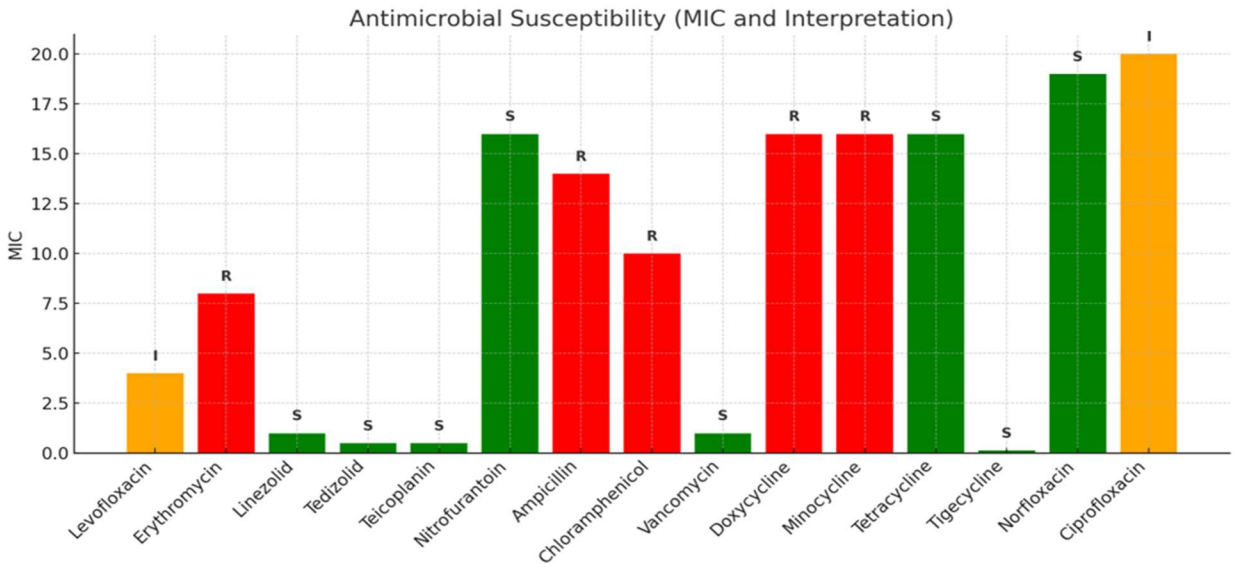
Characteristics	Present (%)
Age (18-50 years)	91(91%)
Vaginal discharge	75 (75 %)
Itching	62 (62 %)
Pregnancy	15 (15 %)

Antibiotic susceptibility

Among 100 specimens, 65 isolates of *Enterococcus faecalis* were isolated. The antibiotic susceptibility assay results showed that all isolates were highly susceptible to Vancomycin, Teicoplanin, Linezolid, Tedizolid, Nitrofurantoin, Tetracycline, Tigecycline, and Norfloxacin. There was only one isolate of vancomycin-resistant *Enterococcus*, which was resistant to both vancomycin and teicoplanin. Whereas they were resistant to Erythromycin, Ampicillin, Chloramphenicol, Doxycycline, and Minocycline. An Intermediate resistance was recorded to Levofloxacin and Ciprofloxacin. Among the 65 isolates, 45 (69.23%) were multidrug resistant, which, resistant to three different classes of antibiotics, as shown in Table 4and Figure 1

Table 4: shows the susceptibility of the *Enterococcus faecalis* isolates to the different antimicrobial agents

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Levofloxacin	4	I	Vancomycin	1	S
Erythromycin	>=8	R	+Doxycycline	>=16	R
Linezolid	1	S	+Minocycline	>=16	R
Tedizolid	<=0.5	S	Tetracycline	>=16	S
Teicoplanin	<=0.5	S	Tigecycline	<0.12	S
Nitrofurantoin	<=16	S	Norfloxacin	19	S
Ampicillin	<=14	R	Ciprofloxacin	20	I
Chloramphenicol	<=10	R			



- **Green** = Susceptible (S) **Orange** = Intermediate (I) **Red** = Resistant (R)

Figure 1: Antimicrobial Susceptibility (MIC and interpretation)

Biofilm formation

The results of the biofilm production assay showed that 13 (20%) of *E. faecalis* isolates were strong biofilm producers, and 48(73.8 %) were moderate biofilm producers. There were 7(10.7%) no-biofilm producer isolates as shown in Figure 2 and 3.

Figure 2 shows the results of the biofilm formation of *E. faecalis* isolates on Congo Red Agar (CRA). black colonies with dry crystalline indicated slime production, whereas the dark-pink colonies are intermediate (Weak slime producer), and pink-white colonies are considered negative (non-slime producer).

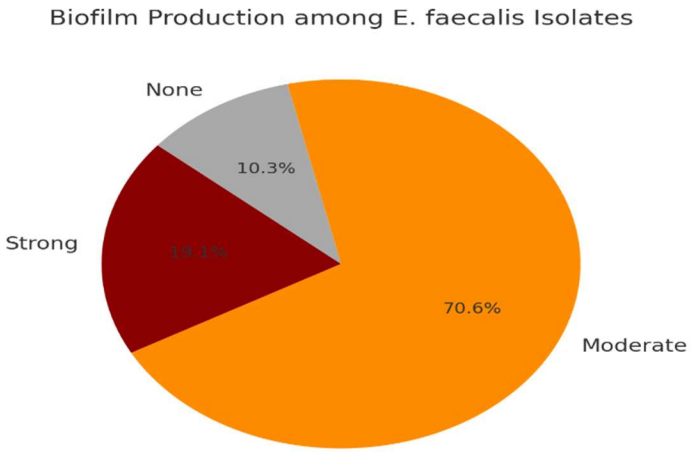


Figure 3: Distribution of biofilm formation levels among *E. faecalis* isolates

Identification of Virulence and Resistance Genes

The molecular examination of the ten chosen *E. faecalis* isolates revealed a notable presence of virulence and antibiotic resistance genes. The cytolysin virulence gene, *cylA*, was identified in all 10 isolates (100%), as illustrated in Figure 4. The prevalence of ESBL resistance genes was significant. The bla_{TEM} gene was identified in 10 out of 10 isolates (100%). The bla_{SHV} gene was identified in 7 out of 10 isolates (70%), as shown in Figures 5 and 6, and the *erm B* gene in 10(100%), as shown in Figure 7.

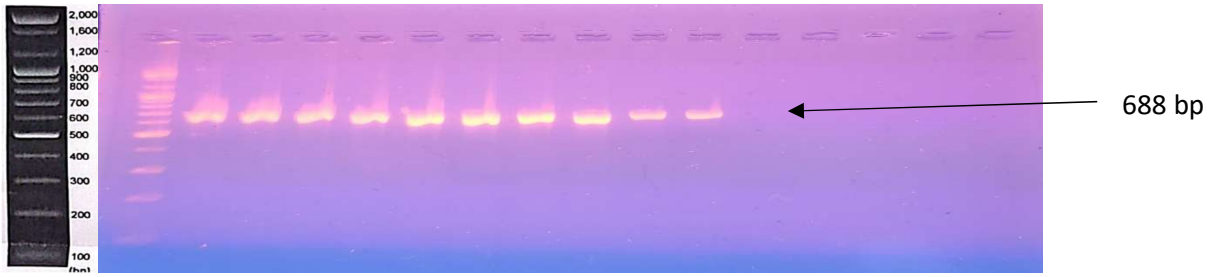


Figure 4: Agarose gel electrophoresis image that showed PCR product analysis for the ***cylA*** gene (Marker ladder 2000-100bp)

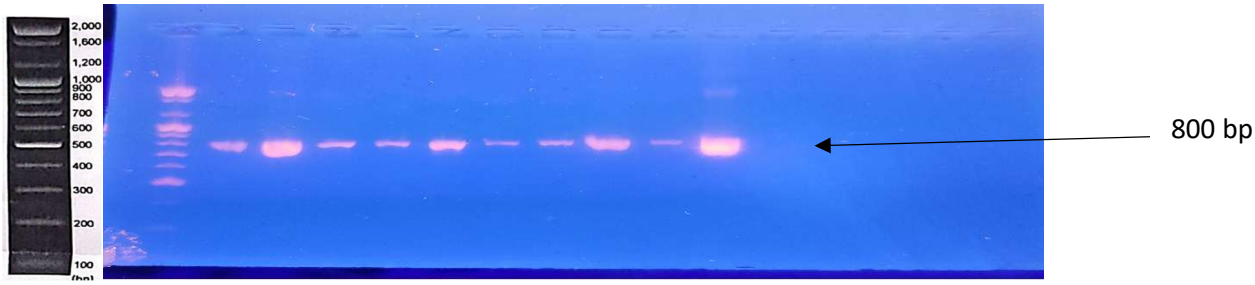


Figure 5: Agarose gel electrophoresis image that showed PCR product analysis for the bla_{TEM} gene

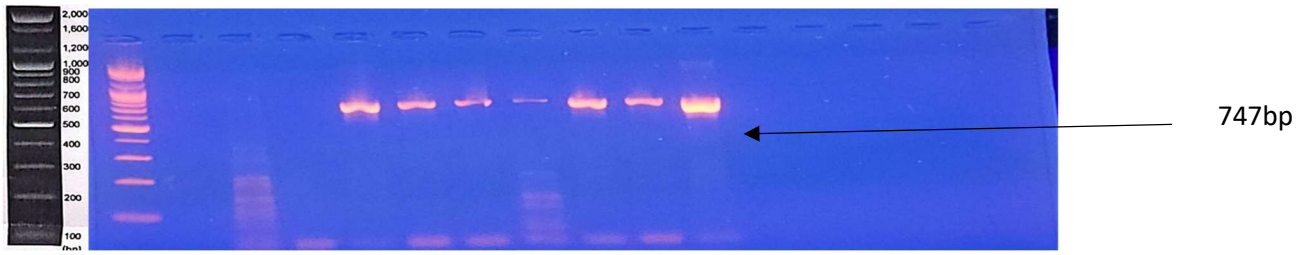


Figure 6: Agarose gel electrophoresis image that showed PCR product analysis for the bla_{SHV} gene

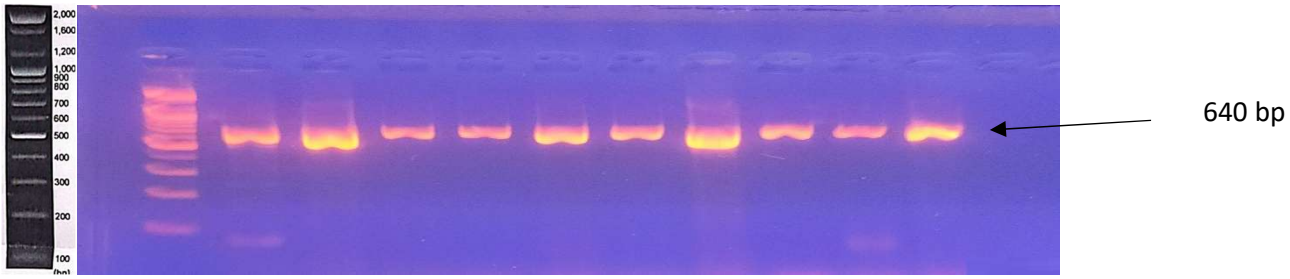


Figure 7: Agarose gel electrophoresis image that showed PCR product analysis for the *ermB* gene

4. Discussion

Bacterial vaginosis is associated with a shift in the vaginal tract's ecology, characterized by a decrease in the quantity and concentration of facultative lactobacilli. Some studies, such as that of Al-Zaidi *et al*., point out that some strains of *Enterococcus faecalis* can reduce the count of lactobacilli in the intestines, which may contribute to the development of bacterial vaginosis [22]. This research indicated that 65 isolates of *Enterococcus faecalis* were isolated from vaginal discharge. The research also indicated that 91% of women of reproductive age were the most affected during those years. This kind of information is critical for stopping, finding, and treating diseases. However, due to the limited number of pregnancies in this cohort, additional research is necessary to comprehensively elucidate the clinical ramifications of this finding. Significantly, 91% of the women in this cohort are of childbearing age, which corresponds with the prevailing findings concerning gynecological and urological disorders [23]. *Corynebacterium*, *Mobiluncus*, and *Lactobacillus*, the predominant members of the vaginal microflora, are also present. Anaerobic bacteria may be present, as well as rare strains of Staphylococcus and Streptococcus. These organisms play a vital role in maintaining the vagina's low pH and preventing other harmful bacteria from colonizing it [24].

The clinical profiles observed in this study (75% vaginal discharge and 62% itching) match with the symptoms reported in the most recent literature. When someone has vaginitis, they often have vaginal discharge and itching. This is true even when *Enterococcus* spp. is involved. The fact that 91% of the people in this study were of reproductive age fits with what most people think: that vaginitis is common in this age group [25]. These results show that *Enterococcus* spp. is very common in cases of vaginitis, especially in women of childbearing age. Common symptoms include vaginal discharge and itching [26].

There were 65 *Enterococcus faecalis* isolates in a study that looked at all clinical samples (vaginal discharge). The Vitek 2 automated system was able to identify types of Enterococci in a study, just like standard biochemical tests. The current study continues to demonstrate that *Enterococcus faecalis* causes and maintains aerobic vaginitis (AV), a condition characterized by dysbiosis of the vaginal flora, which leads to pathologic vaginal secretions, inflammation, and the presence of aerobes. Enterococcus faecalis is likely to predominate in vaginal secretions due to three factors, of which the most pivotal is vaginal dysbiosis, dysregulation of the vaginal microbiome, as well as enhanced survival mechanisms, which include antimicrobial resistance and biofilm formation. Enterococcus utilizes the gastrointestinal system as its principal reservoir, from which it can be easily transmitted to the vaginal system due to its close anatomical proximity, allowing effortless translocation, rapid colonization, and persistence as a source of pathologic sequelae [27].

E. faecalis was the most common species found, and *Enterococcus* has become resistant to glycopeptides. This study examined the prevalence of multidrug-resistant *E. faecalis*. This is like what Al-Dulaimi found, where 63% of 200 clinical isolates of *Enterococcus* were found to be multidrug resistant. [28].

This study shows that *E. faecalis* strains are more resistant to multiple drugs (MDR) than the strains in the 2020 study by Sirichoat et al. In that trial, antibiotics like erythromycin and ampicillin worked, but they don't work in your case anymore. This difference shows how important it is to test for sensitivity in each case in order to find the best treatment. It also shows how quickly and steadily enterococci are becoming resistant to antibiotics [29].

An intermediate (I) interpretation is given to levofloxacin with an MIC of 4 and to ciprofloxacin with an MIC of 20. Recent studies have shown that *E. faecalis* can exhibit different degrees of resistance to fluoroquinolones. Case in point: research by Al-Bayati *et al* [30] reported ciprofloxacin resistance as high as 59.7% in *E. faecalis*. A different study by IWA Publishing (2023) found that 65% of *E. faecalis* and *E. faecium* isolates were resistant to ciprofloxacin. The original study's intermediate susceptibility suggests that the bacteria are becoming more resistant or that the population is becoming less susceptible. This fits with the global trend of increasing fluoroquinolone resistance [31].
Erythromycin is resistant (R) if the MIC is (8-10). It is common for *Enterococcus* species to have high levels of erythromycin resistance. BMC Microbiology (2019) found that all *E. faecalis* isolates were resistant to erythromycin. This study's high resistance is in line with the fact that *Enterococcus* is resistant to a lot of macrolides [32].
Tedizolid (MIC <=0.5, S) and Linezolid (MIC 1, S) both show susceptibility. Most people think that linezolid and tedizolid work against *Enterococcus* species, even those that are resistant to vancomycin. According to Al-Bayati *et al*. (2024), *E. faecalis* was not resistant to linezolid at all. Infectious Disease Advisor (2023) also points out linezolid as a new antimicrobial agent that works against enterococci. The study's finding of susceptibility is a positive one, as it shows that these drugs are still viable treatment options.
Teicoplanin (MIC <= 0.5, S) and vancomycin (MIC 1, S) are both sensitive. Vancomycin resistance in *Enterococcus* (VRE) is a big problem, especially in E. faecium. *E. f*aecalis, on the other hand, usually has lower rates of vancomycin resistance than E. faecium. LWW (2023) did a study and found that none of the *E. faecalis* isolates were resistant to vancomycin. The study's findings about the *E. faecalis* isolates' resistance to vancomycin and teicoplanin are good news. This means that these important last-line antibiotics are still effective against these bacteria in this case [33].

Nitrofurantoin is sensitive (S) if its MIC is 16 or less. People often take nitrofurantoin to get rid of urinary tract infections caused by *Enterococcus.* The first search didn't identify any recent information on how *E. faecalis* reacts to nitrofurantoin in cases of vaginitis, but it is generally believed that it does for uncomplicated UTIs. We need to do more research to find out how well it works for vaginitis [34].

Ampicillin is resistant (R) with a minimum inhibitory concentration (MIC) of 14. *E. faecalis* is becoming more resistant to ampicillin, but it is usually more sensitive to the drug than *E. faecium*. Al-Bayati *et al*. (2024) found that 11.8% of *E. faecalis* were resistant to ampicillin. The study found a lot of resistance, which means that ampicillin may not be a viable empirical treatment for this group of people. The high resistance to ampicillin in the sample is likely due to the bacteria's acquisition of a plasmid carrying the beta-lactamase enzyme gene, facilitated by the widespread use of ampicillin, and mutations in penicillin-binding proteins (PBPs), which further contribute to the resistance.

Chloramphenicol is resistant (R) and has a MIC of less than or equal to 10. Recent studies have focused less on *Enterococcus* resistance to chloramphenicol than on other antibiotics, but it can occur. The resistance seen in the first study suggests that chloramphenicol might not be a favorable choice. [35].

Doxycycline (MIC >=16, R) and minocycline (MIC >=16, R) are both resistant (R). Nature's 2023 study found that 45% of *Enterococcus* isolates were resistant to tetracycline. This study's resistance to doxycycline and minocycline fits with this trend, which makes them less useful [36].

Tetracycline is susceptible (S) when the MIC is 16 or higher. This result goes against what we saw with doxycycline and minocycline resistance, as well as the general pattern of tetracycline resistance. There could be a specific way to interpret the breakpoint or a unique feature of these isolates. But because of the resistance to other tetracyclines, one should be careful [37].

Tigecycline has an MIC of less than 0.12, making it susceptible (S). Tigecycline is a glycylcycline that is frequently used to treat *Enterococcus* and other bacteria that are resistant to multiple drugs. In general, it exhibits good activity against species of *Enterococcus*. According to a 2015 ScienceDirect study, tigecycline's MIC50 and MIC90 against *E. faecalis* were low. This study's susceptibility is favorable and anticipated [38].

Norfloxacin is susceptible (S) with an MIC of 19. Another fluoroquinolone is norfloxacin. It is more susceptible here than ciprofloxacin, which is in the middle. This could mean that the resistance mechanisms or breakpoints are different. But it's important to note that the MIC value of 19 is high for a susceptible interpretation because it is close to or above the resistance breakpoints for other fluoroquinolones.

Enterococcus faecalis makes biofilms, which makes it resistant to some antimicrobial drugs. *Enterococcus faecalis*, which comes from chronic infections, is a bacterium that makes biofilms as a way to make itself more dangerous [39]. In this study, biofilm-forming *Enterococcus faecalis* strains were harder to kill than non-biofilm-forming strains. This conclusion parallels those of Anna Sienko, who identified a strong association between biofilm production and antimicrobial resistance. An additional investigation determined that *Enterococcus* isolates that are biofilm producers were also more likely to be resistant to antibiotics. Anna Sienko identified a strong correlation between biofilm production and antimicrobial resistance. Biofilm producers were found to be more resistant to antibiotics after analyzing *Enterococcus* isolates [40].

The study also discusses *Enterococcus* isolates' drug resistance and responses. You should consider this information when making plans for treatment.

The study demonstrates that biofilm production is a critical virulence factor, particularly in isolates exhibiting multidrug resistance. This is a key part of figuring out why Enterococcus infections last so long and are so difficult to treat.

This study demonstrated a significant correlation between biofilm formation and multidrug resistance. The molecular examination of *Enterococcus faecalis* isolates uncovers concerning information about their ability to cause disease, focusing on the genetic factors that contribute to virulence and resistance. The cytolysin gene (*cylA*) was present in all isolates, demonstrating active pathogenicity instead of mere commensalism. This virulence factor causes tissue damage and inflammation, which is linked to adverse clinical outcomes [41]. The prevalence of CylA most likely accounts for the major symptoms detected in the concerned individuals. The genetic makeup shows a significant association between certain genes and the phenotypes of resistance observed in the different strains of enterococci [42]. The ermB gene was found in all of the samples, which means that they are very resistant to erythromycin. This is because it's code for a methylase that changes the ribosomal binding site, making macrolides useless [43]. This supports findings showing ermB as the most prevalent contributor to erythromycin resistance. Moreover, there exists a concerning high frequency of extended-spectrum beta-lactamase (ESBL) genes with bla_{TEM} present in 100%, and 70% of the studied isolates carried bla_{SHV}. The increasing occurrence of beta-lactamase production from *E. faecalis*, which had not been reported previously, represents a significant clinical threat, as these enzymes are capable of hydrolyzing many beta-lactam antibiotics that are crucial to therapy. The presence of bla_{TEM} as the most prevalent enzyme supports phenotypic resistance, as this enzyme is known to hydrolyze ampicillin and other structurally related antibiotics, and the high frequency of bla_{SHV} increases the potential for clinically concerning resistance. The findings indicate a high prevalence of bla_{TEM} in clinical isolates, suggesting ampicillin and related antibiotics are unsuitable for treating enterococcal infections, especially bacteria with inherent resistance. Concomitantly, the high prevalence of bla_{SHV} (70%) increases the risk of producing a significant multiple resistance phenotype and increases the difficulty of treatment [44]. For this reason, *E. faecalis* may be in an especially good position to carry ESBL genes, which would enhance the dissemination of resistance to other infections. There is an urgent need to assess the antibiotic recommendations and revise the strategies to curb treatment failure and the dissemination of resistance genes [45].

5. Conclusions

This research shows the large prevalence of *E. faecalis* in people who suffer from vaginitis. Also of concern is the species' ability to tolerate various antibiotics and create biofilms. Rates of multidrug resistance of erythromycin and ampicillin make the probability of treatment success even lower and enhance the chances of a case becoming chronic. The good news is that newer antibiotics such as linezolid and vancomycin still have a good effect. The case is still concerning, though, due to moderate resistance to ciprofloxacin and a lack of good surveillance of it. Furthermore, *E. faecalis* biofilms are protective and augment the ability of the pathogen to cause disease, making such infections very difficult to treat. The growing challenge of antibiotic resistance is important and calls for even more proactive and responsive approaches on the regulatory and administrative sides. Treating vaginitis caused by *E. faecalis* should

involve continuous monitoring of resistance trends, rapid responsive actions, adjustments of the antibiotics being used, and providing a basis for guidelines that take resistance to biofilms into consideration. The earlier biofilms and resistance are targeted, the lower the impact of the infections on reproductive health will be. The molecular data highlight an important concern with *E. faecalis* from this patient group; high levels of virulence and resistance to most antibiotics are observed.

The fully characterized cytolysin gene (*cylA*), along with comprehensive macrolide resistance and ermB gene expression, and significant β-lactam resistance through the mediation of the genes bla_{TEM} and bla_{SHV}, represents a publicly funded health concern with narrowing treatment options and the risk of enduring infections. These findings indicate an immediate necessity for a comprehensive risk assessment of the molecular surveillance of the monoclonal isolates.

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