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Molecular Identification of Some Virulence Factors in *Enterococcus faecalis* Isolated from Vaginitis Patients

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ABSTRACT

Enterococcal bacteria have emerged as the main nosocomial pathogens. Virulence factors are important in enhancing the ability of *Enterococcus faecalis* to occur diseases in patients. Resistance genes only do not indicate the pathogenicity of bacteria and present them with virulence factors it can cause the strain to be dangerous. Ten *Enterococcus faecalis* were isolated from patients with vaginitis. All isolates were confirmed by biochemical tests and selective media and All isolates were completely resistant to gentamicin, 40% of isolates were resistant to chloramphenicol, and All isolates were highly susceptible to ampicillin, vancomycin, and nitrofurantoin. the virulence factors were detected by multiplex PCR. It was found that *Esp* and *ClyA* were observed in (100%), and (90%) In *E. faecalis* respectively. virulence factors commonly prevalent in *Enterococcus faecalis* isolated from vaginitis in this study.

1. Introduction

Enterococci is a commensal bacterium of the gastrointestinal tract, but it can also become an opportunistic pathogen. It may colonize the female genital tract and vaginal colonization rises in patients with aerobic vaginitis or after antibiotic therapy. *E. faecalis* is linked to a variety of illnesses, especially in immunocompromised individuals and when there is a change in the host microbiota. There is increasing evidence that links enterococci with bacterial vaginosis and aerobic vaginitis [1]. *Enterococcus* has two more frequent species: *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*), which have prevalences of around 90% and 10%, respectively, in nosocomial infections. Although cross-infection typically occurs in hospitalized patients, the majority of infections brought on by these bacteria are endogenous [2].

Invasion into the tissues of the host, manipulation of the host immune system, and the production of poisons and enzymes, which can intensify the infection, are all ways that virulence factors play a role in the pathogenesis of infection. In the environment of hospitals, a number of virulence factors in Enterococci, such as capsule formation, gelatinase [encoded by the chromosomal gelatinase (*gelE*)], aggregation substance, and enterococcal surface protein [encoded by the chromosomal enterococcal surface protein (*esp*)], are involved in bacterial adherence and/or the formation of biofilms [3].

Investigations reveal that *E. faecalis* produces biofilm, and its quorum-sensing system regulates biofilm formation [4]. A microbial biofilm is a mass of bacteria that forms on biotic and abiotic surfaces and causes the cells to produce extracellular polymers and an alginate matrix, causing the cells to adhere to the surfaces irreversibly [5]. It is thought that there is a connection between the growth of biofilm and bacterial resistance to antibiotics, which would likely result in serious issues in this field. In contrast to planktonic cells, bacteria participating in biofilms exhibit distinct behaviors [6]. In vitro, the extracellular surface protein (*esp*) improves bacterial biofilm formation and colonization, and it appears to be connected to the existence of biofilms in vivo [7].

There are very few studies in Iraq regarding the association of vaginitis with Enterococci isolates. This study was done to look for virulence factors in *Enterococcus faecalis* isolates from cases of vaginitis. The antimicrobial susceptibility pattern was also looked for in these cases.

2. Material and method

2.1 Bacterial isolation

In this study, vaginal discharge was collected with a sterile cotton swab from adult female patients suspected of having vaginitis were collected from teaching hospitals in Diwaniya, Iraq, between May and August 2020.

All specimens were cultivated using routine bacteriological methods on Blood agar, MacConkey agar, and bile esculin agar (Himedia, India). Culture characteristics and colony morphology was observed macroscopically. The genus *Enterococcus* was identified using gram staining, cultural characteristics, and biochemical tests, including L-pyrrolidinyl-β-naphthalyamide hydrolysis, bile esculin hydrolysis, and growth on 6.5% NaCl media at pH 9.6 [8].

2.2 Antimicrobial susceptibility testing

The antimicrobial susceptibility of the *E. faecalis* isolates was determined by disk diffusion method for the following antibacterial agents; ampicillin (AM, 25 mg), Gentamicin (GN, 10 mg), Nitrofurantoin (F, 300 mg), Chloramphenicol (C, 30 mg), and vancomycin (VA, 30 mg), (Bioanalyse, Turkey). Muller-Hinton agar plates were inoculated with 0.5 McFarland standard suspension of the strains, antimicrobial disks were placed into plates, and then were incubated at 37°C for 24 hours. Zone diameters were assessed according to the Clinical Laboratory Standard Institute guidelines [9].

2.3 DNA extraction

DNA was extracted using a genomic DNA Extraction Kit (Scientific Research Company, Iraq) according to the manufacturer's instructions.

2.4 Identifying the virulence genes by the multiplex-PCR method

To detect the virulence factors genes, including *Esp* and cytolysin (*CylA*) genes, PCR was used using appropriate primers. The employed PCR program, annealing temperature, and primer sequence are displayed in Tables 1 and 2 respectively. For PCR, a DNA thermal cycler was utilized (Master Cycler Gradient, Eppendorf, Germany). The amplicons were stained with ethidium bromide before being electrophoresed in 1.5 percent agarose gel at 80 V for 30 min. PCR results were examined and captured using UV doc gel documentation devices from Uvitec (UK). The PCR results were compared against a 100 bp DNA marker (Fermentas, Germany) [10].

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Table 1. The primers used in multiplex-PCR.

Primers	**Sequence** 5´ ⟶ 3´		**References**
Esp	**F**	**GATTTCATCTTTGATTCTTGG**	**[11]**
	R	**ATTTGATTCTTTAGCATCTGG**	
CylA	**F**	**ACTCGGGGATTGATAGGC**	**]12]**
	R	**GCTGCTAAAGCTGCGCTT**	

3. Results

Out of 100 vaginal samples, *E. faecalis* was detected in 10 samples (10%). All samples tested positive microbiologically were tested positive in a molecular study conducted using a specific primer (Table 1). All patients had vaginal discharge and itching.

3.1 Antibiotic resistance pattern

The antibiotic resistance of *Enterococcus faecalis* isolated from the vaginal is shown in Fig 1. All *Enterococcus faecalis* isolates were resistant to gentamicin (100%), followed by chloramphenicol (40%), All isolates were highly susceptible to vancomycin, ampicillin, and nitrofurantoin.

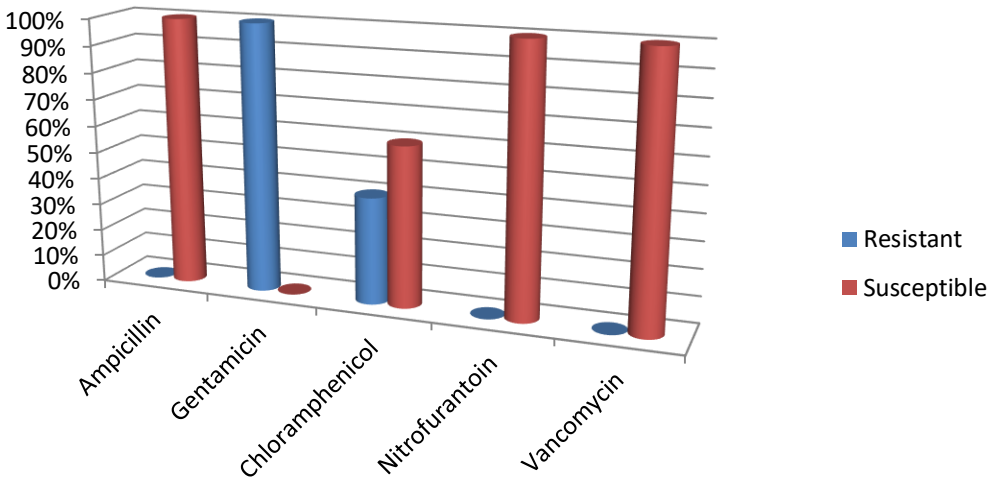

Fig.1: Antibacterial resistance patterns of *E. faecalis* isolated from vaginitis infections.

3.2 Virulence factors genes

According to multiplex PCR results, 11 (100%) had the *Esp* gene, and 10 (90%) had the *CylA* gene of *Enterococcus faecalis* isolates. Fig. 2.

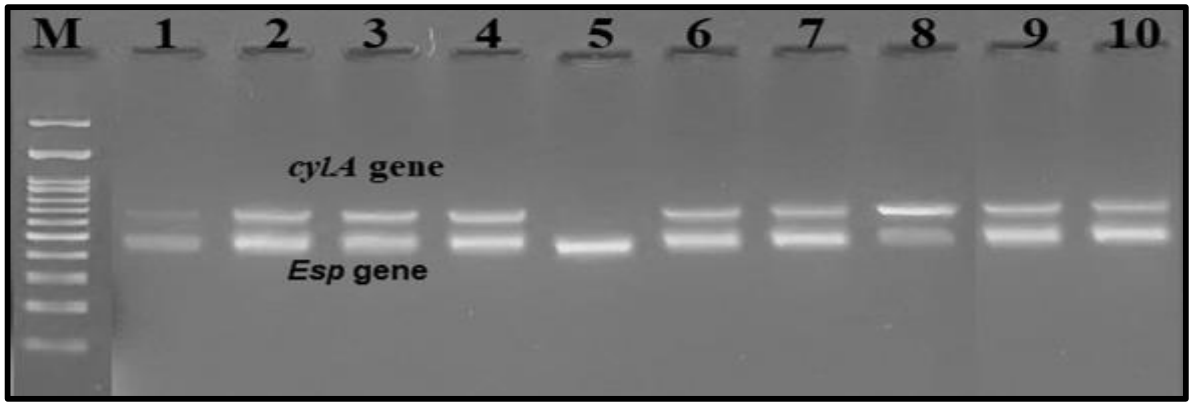

Fig.2: Gel electrophoresis (1.5%) of amplified products of *CylA* gene (700bp)., *Esp* gene (500bp). M: Size Marker 1000bp

4. DISCUSSION

Enterococcal infections are significantly influenced by biofilm, which also creates an environment that increases bacterial survival in the host [13]. Due to enterococci's contentious reputation, this study evaluated biofilm development and virulence genes, and antibiotic resistance in 10 clinical *E. faecalis* isolates. Based on the results, *E. faecalis* is the main cause of enterococcal vaginal infections. This is in accordance with [14]. who isolated enterococci from vaginitis patients in Baghdad. But it was different from the results of [15] in Al-Hilla.

Antibiotic resistance is a factor contributing to the pathogenesis of *E. faecalis* that can be acquired or found internally [16]. The highest resistance among all isolates was to gentamycin and chloramphenicol. A similar study by [17].

Additionally, it has been previously noted that gentamycin resistance is highly prevalent among Enterococci isolates. [18]. In this study, according to drug susceptibility testing, 100% of our isolates showed sensitivity to ampicillin, vancomycin, and nitrofurantoin and 60% of them had sensitivity to chloramphenicol. this study agrees with [19].

Fig. 2 displays the gene distributions for virulence components in *Enterococcus faecalis*. It was discovered that 10 *E. faecalis* isolates had 100% and 90% of the *Esp* and *ClyA* genes, respectively. This outcome is higher than those of other studies [17], [20]. Other research revealed that *Esp* genes were absent in every strain. The increased prevalence of *Esp* in enterococci may shed light on this gene's function in antibiotic resistance [21].

The *Esp* gene produces an extracellular surface protein that aids in immune system evasion, colonization, and adhesion. Additionally, this protein helps *E. faecalis* remain in the infection site longer and produce biofilms [22]. *Esp* gene is found in bacteria related to infections and hospital outbreaks [23]. *Esp* is a critical virulence factor in infections caused by either *E. faecium* or *E. faecalis* [24].

CylA can be found on a plasmid or a chromosome of bacteria. Negative haemolysis is correlated with *CylA* operon lack [25]. The *cylA* operon as a whole had to be present for cytolysin activity [26]. In a different investigation, *CylA* was linked to the development of biofilms in clinical infections [27]. [28] discovered that 16 percent of *E. faecalis* from bacteraemia produced cytolysin. According to our findings, there is no connection between isolates' capacity to form biofilms and the presence of *Esp* and *CylA* genes. Our results

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demonstrate that many virulence factors in antibiotic-resistant Enterococcus can be a problem. Additionally, the high incidence of the *Esp* gene among biofilm-producing clinical isolates raises the possibility that the *Esp* gene and biofilm development are related, where more research is necessary to determine the mechanism of biofilm inhibition.

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