Polymorphism Of Virulence Genes Of Enterococcus Faecalis Associated With Aerobic Vaginitis

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Abstract

The aim of the current study is to determine the virulence genes of E. faecalis and polymorphism in these bacteria. A total of 300 females who visited the outpatient clinics in the Maternity and Pediatrics Teaching Hospital in Al-Diwaniya city, Iraq were enrolled. For twenty isolates of E. faecalis that showed dense growth when cultured on the specified culture media, then the sensitivity to antibiotics was tested, and then the virulence genes were determined for each isolate, where the results were shown there are statistically significant differences (P<0.05) in the presence of virulence genes in the tested bacterial isolates. (where the genetic analysis by PCR showed the presence of all virulence genes, except for isolate 3 of the gene esp (Enterococcal surface production protein), and isolates 11, 12, 15, 16 and 19 of the hly gene (haemolysin production). ERIC results showed the most of the isolates share a DNA segment of length 1500, 1000 bp, and 2000 pb. The curve also shows that most of the isolates lack the presence of the segment of length 500 pb. As for the pieces of 700, 600 and 400 bp, they showed a very high heterogeneity between the isolates. In conclusion, most genes responsible for virulence factors were positive, except for hly, which showed positive results for nearly more than half of the isolates, that the virulence factors of said bacteria are a major focus of infection in said bacteria, and if these genes are not available, they are unable to hit or stick to the surface of the vagina. There were two types of genetic polymorphism in strains, one of which is caused by horizontal transmission between strains via plasmids and other horizontal transmission methods, and the other is caused by vertical transmission through mutations that occur between generations, causing high resistance to some antibiotics. vitality.

Keywords: Aerobic Vaginitis (AV), Bacterial Vaginosis (BV), UTI, Enterococcus faecalis,

Introduction

Aerobic vaginitis is a common cause of vaginal discharge in reproductive-age women, increasing the risk of negative pregnancy outcomes such as premature delivery, abortion, premature rupture of membranes and stillbirth. However, the aetiology and pathogenesis of aerobic vaginitis causing negative pregnancy outcomes are still unclear, and there is no unified and standardized treatment method for aerobic vaginitis in the pregnancy period [1]. Aerobic vaginitis (AV) is an infectious-vaginal entity caused by excessive commensal aerobic microorganisms of intestinal origin and distinctly different from bacterial vaginosis, which is usually considered an internal infection [2]. It is one of the most common infections of the reproductive system in women during the childbearing period. AV was first identified by Belgian researcher Donders and colleagues in 2002 [3], and the main diagnosis for AV identification is currently observation of a wet film of vaginal secretion under a light microscope, along with clinical features [4]. Enterococcus faecalis is an opportunistic pathogen that resides in the human urogenital tracts [5]. While E. faecalis colonization is normally asymptomatic, certain populations are at risk for severe disease, including urinary tract
infections [6], pelvic inflammatory disease (PID), infective endocarditis, and adverse birth effects during pregnancy [7].

Enterococcal infections are often associated with the production of biofilms, assemblages of microbes enclosed in an extracellular polymeric matrix that exhibit cell-to-cell interactions [8]. These biofilms have been observed on catheters, resulting in severe infection. Treatment of enterococcal infections is becoming increasingly problematic due to their augmented ability to acquire mobile genetic elements [5].

The causes of AV that are responsible for inflammatory changes are: E. faecalis, Escherichia coli, group B streptococcus and Staphylococcus aureus [9]. The most common isolated pathogen of AV is E. faecalis in 32% [10]. The pathogenic effect of aerobic microorganisms such as E. faecalis has been shown to cause spontaneous abortion, premature birth, puerperal sepsis, abscesses, and urinary tract infections [1].

AV in pregnancy is associated with pregnancy complications, partially increases the risk of preterm birth, premature rupture of membranes and increases the risk of postpartum complications, is more common in cervical intraepithelial neoplasia (CIN) than in women with an orderly Pap test and is thought to contribute to the progression of cervical cancer [11].

A study of cervical-vaginal flora variations in Human papilloma virus (HPV)-positive women conducted in Peru in 2017 found a higher incidence of E. faecalis in high-risk HPV-positive women compared to low-risk HPV-positive women [12].

Certain risk factors are associated with the transition of E. faecalis from commensalism to pathogenicity, such as immune status, prolonged hospital stay, and the use of antibiotics. E. faecalis colonization and infection is often polymicrobial, and these interactions have been observed in the vaginal tract of healthy women [7]. The aim of the current study is to determine the virulence genes of E. faecalis and polymorphism in these bacteria.

2. Materials and Methods

2.1 Samples Collections

300 females, aged from 16-45 years, patients visited the outpatient clinics in the Maternity and Pediatrics Teaching Hospital, in addition to some private clinics in Al-Diwaniya city. The study was done during a period from November 2020 to April 2021.

2.2 Bacteria Diagnosis and Antibiotic Susceptibility

The bacteria were diagnosed with antibiotic Susceptibility were done using the Vitec device according to [13].

2.3. Detection Virulence Genes

DNA was extracted according to the instructions of the manufacturer of the kit used for extraction (Geneaid Biotech Ltd Kit).

Virulence genes detected using AccuPower PCR PreMix Kit. By using their tubes that had PCR needed components, DNA polymerase, dNTPs, KCl, Tris-HCl pH: 9.0, MgCl2, loading dye and Primers that showed in Table 1). After that, the tubes of the PCR were vortex centrifuged for three minutes at 3000 rpm. The tubes then were inserted into a thermocycler (BioRad-USA).

| Table 1 Primers that are used in current study to detect antibiotic resistance genes. |
|---------------------------------|-----------------|-----------------|
| **Gene** | **Sequences of primer (5’ – 3’)** | **Product size of PCR** |
| **ace** | 5’-ATGAAGGAAGCCACAGTTG-3’ | 100 |
| **R** | 5’-GTTGTCCTGTTCAGGAAAG-3’ |
| **efaA** | 5’-CCAATTGGGAGACCCCTC-3’ | 688 |
| **F** | 5’-CGCCTTCGGGTCTTCTGGGC-3’ |

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2.4. Enterococcus faecalis Phylogenetic

This assay was done by extraction of bacteria DNA then amplified using (ERIC-2 and ERIC-1R) primer thin gel electrophoresis to show the neighbor-joining phylogenetic tree (genetic relatedness) of E. faecalis isolates.

ERIC Primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>F</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERIC-2</td>
<td>5’-AAGTAAGTGACTGGGGTGAGCG-3’</td>
<td>No</td>
</tr>
<tr>
<td>ERIC-1R</td>
<td>5’-ATGTAAGCTCCTGGGTAGACGTC-3’</td>
<td>No</td>
</tr>
</tbody>
</table>

3. Result and Discussion

3.1. Detection Virulence Genes

For twenty samples of E. faecalis that showed dense growth when cultured on the specified culture media, then the sensitivity to antibiotics was tested, and then the virulence genes were determined for each isolate, where the results were shown there are statistically significant differences (P<0.05) in the presence of virulence genes in the tested bacterial isolates. (where the genetic analysis by polymerase chain reaction showed the presence of all virulence genes, except for isolate 3 of the gene esp (Enterococcal surface production protein), and isolates 11, 12, 15, 16 and 19 of the hly gene (haemolysin production), as shown in table 1 and figure 1.

Table (1) Result of polymerase chain reaction of virulence genes for 20 E. faecalis isolates.

<table>
<thead>
<tr>
<th>Isolate NO.</th>
<th>ace</th>
<th>AS</th>
<th>efa A</th>
<th>esp</th>
<th>gel E</th>
<th>hly</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>5</td>
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<td>+</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
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<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>No. (%)</td>
<td>20/20(10)</td>
<td>20/20(100)</td>
<td>20/20(100)</td>
<td>19/20(95)</td>
<td>20/20(100)</td>
<td>15/20(75)</td>
</tr>
<tr>
<td>X2</td>
<td>21.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P value</td>
<td>0.001*</td>
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<td></td>
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</tr>
</tbody>
</table>

Figure (1) Gel electrophoresis for A: ace gene that responsible Collagen binding protein. B: AS gene that responsible aggregation substance. C: efaA gen that responsibale endocarditis-associated antigen. D: esp that responsible Enterococcal surface protein production gene. E: gelE that responsible for gelatinase production. F: hly that responsible for haemolysin production gene, DNA was extracted from Enterococcus faecalis isolates that isolated women have vaginitis symptoms.

The gene responsible for Collagen binding protein(ace) was positive in all 20 isolates of E. faecalis. This is consistent with the findings of [14] where they revealed, through animal experiments, that ace is involved in urinary tract infection by E. faecalis.

[15] utilized qRT-PCR to elucidate the effect of SOTE on the biofilm-forming ability of the tested isolates at the molecular level. The studied biofilm-related genes in E. faecalis isolates were esp, cylL, cylA, agg, efaA, and ace, that is agreed with current study. The gene esp was reported to enhance biofilm formation by E. faecalis [16]. The genes agg, efaA, and ace contribute to the different steps of biofilm formation [17].

AS that responsible for producing aggregation substances was shown positive in all isolates, AS has found additional roles of this protein in enterococcal virulence [18]. A high incidence of this gene in E. faecalis has been reported in previous studies [19], [20]. By contrast, this gene was not found in E. faecalis isolates [21]. In the current study, AS were detected in 100% isolates.

efa A that responsible for producing endocarditis-associated antigen was positive in all E. faecalis isolates, that is agreed with [22], [23]. Previous studies by [24] showed that although E. faecalis strains derived from different sources such as endocarditis, urinary tract infections possessed distinct patterns of virulence factors ace, efaA, and gelE genes were found to be the most common virulence factors.

Enterococcal surface protein production gene was shown positive in 19 isolates from a total 20 isolates. The enterococcal surface protein, Esp, is a high-molecular-weight surface protein of unknown function whose frequency is significantly increased among infection-derived Enterococcus faecalis isolates [25]. In a previous study by [26] who found esp in (53%).

esp associated with adhesion, colonization and host immune evasion. Though previous reports suggest that esp is found in half percent, in current study, incidence of esp was 19/20 isolates of E. faecalis.
gelE gene responsible for gelatinase production appeared in all isolates. That is agreed with previous study by [27] who investigated the type of relationship of biofilm formation and gelE gene expression in enterococcus faecalis recovered from root canals in patients requiring endodontic retreatment. [28] described the Gelatinase (GelE), a secreted Zn-metalloprotease of E. faecalis. GelE are responsible for the instability of a number of Asc10 (aggregation substance) mutant proteins, implying that GelE functions to clear the bacterial cell surface of misfolded proteins. Disruption of GelE production led to increased cell chain length of E. faecalis, from a typical diplococcus morphology to chains of 5 to 10 cells. These functions attributed to GelE that it acts to increase the dissemination of E. faecalis in high-density environments.

3.2. Enterococcus faecalis Phylogenic

This assay was done by extraction of bacteria DNA then amplified using (ERIC-2 and ERIC-1R) primer thin gel electrophoresis to show the neighbor-joining phylogenetic tree (genetic relatedness) of E. faecalis isolates. The result showed all 20 isolates have bands with (3000, 1500) pb as shown in figure 1 and 2.

The study was conducted to characterize these isolates using the DNA genotyping method (ERIC-PCR). In this study, E. faecalis strains were isolated from different samples and the genetic similarity of the strains was examined by ERIC-PCR. The compound similarity pattern of E. faecalis isolates by ERIC-PCR showed the presence of 8 distinct groups. It is indicated that 100% of the isolates belong to a particular group, indicating that there may be horizontal transmission of the same strain, that is agreed with previous study by [29] who investigated about clonal spread of vancomycin resistance E. faecalis in an Iranian referral pediatrics center, they reached results similar to those of the current study.

Through the curve below, it was found that most of the isolates share a DNA segment of length 1500, 1000 bp, and 2000 pb. The curve also shows that most of the isolates lack the presence of the segment of length 500 pb. As for the pieces of 700, 600 and 400 bp, they showed a very high heterogeneity between the isolates. Half of the isolates possess these pieces and the other half do not, and this shows the extent of genetic mutations that occurred for those isolates to appear in this very high diversity. [30] also found no single genetic lineage was dominant among the E. faecalis.

ERIC-2 typing showed many major clusters, one of which had 2 strains(3and 16) of 100% similarity. The same results for isolates 9 and 17.

The longest band that appeared in the current study are 3000bp, which means the whole genome, as they appeared in all isolates, and this indicates that there are no differences in the length of the whole genome between the isolates. that is agreed with previous study by [31] who analysis Enterococcus phylogenetic, they found the average length of Enterococcus genomes was 3.20 Mb, also they found phylogenetic tree showed that habitat is very important in the evolution of Enterococcus. The genetic relationships were closer in strains that come from similar habitats.
Figure (1) Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR. By which determines the neighbor-joining phylogenetic tree (genetic relatedness) of E. faecalis isolates.

[32] investigated antibiotic resistance susceptibility profiles of E. faecalis from the human vagina, and genome analysis of the genetic basis of intrinsic and acquired resistances by using RAPD PCR technique that same ERIC PCR they found six strains among the E. faecalis isolates. Based on the phenotypic and genotypic results, five strains were selected for genome sequencing: E. faecalis VA02-2 (resistant to tetracycline and rifampicin), E. faecalis VA37-4 (resistant to clindamycin and quinupristin-dalfopristin).
Conclusion

In conclusion, most of the genes responsible for virulence factors were positive, except for the hly, which showed positive results for more than almost half of the samples. The reason may be due, as many studies mentioned above, that the virulence factors of the mentioned bacteria are a major focus of infection in the mentioned bacteria, and if these genes are lacking, they unable to hit or stick to the surface of the vagina.
Also the current study concluded that there are two types of genetic polymorphism in the strains, one of them resulting from horizontal transmission between strains through plasmids and other horizontal transmission methods, and the other resulting from vertical transmission through mutations that occur between generations, which caused high resistance to some antibiotics.

REFERENCES


