Molecular Detection of Gardnerella Vaginalis Isolated from Preterm Labor Patients and Study of Some Factors on Biofilm Formation in Al-Hilla City, Iraq

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Abstract

Objectives: the present study aims to isolation and identification of G.vaginalis from preterm labor, detection of bio film formation of G.vaginalis and effect of some factor on bacterial adherence. Material and methods: One hundred fifty clinical sample were collected from preterm labor patients with (bacterial vaginosis, urinary tract infection and aborted women) 80, 15, 55 respectively, admitted to Babylon Maternity and pediatric hospital and Al-Hilla Teaching Hospital, at the period from February to October 2016. These high vaginal samples were subjected to different methods of identification of G.vaginalis. Results and conclusion: It was found that 6 (4%) G.vaginalis isolates were recovered by using selective media where 30 (20%) isolates were recovered dependent on direct extraction to high vaginal swab on molecular level. We were detection and found that (4) isolates were gave strong biofilm While the other Gave moderate then subjected to some factors to reduce , The effect (Alum ,sodium bicarbonate and sodium chloride) at concentration (50%) which gave results (100%) very effective to eradication bio film ,the antibacterial activity of thymol plant extract (carvacrol) at concentration (50%) were investigated the results proved that is effective on disrupted biofilm in (83.4%). The used of metronidazole were very effective on biofilm (100%) as well as in Alum ,sodium chloride and sodium bicarbonate The results showed that all factors below have the ability to destruction G.vaginalis biofilm .

Keywords: G. vaginalis, cpn60, 16sRNA, bio film, Preterm labor.

Introduction

Preterm labor (PTL) is labor which occurs before 37 completed weeks of gestation and can lead to preterm birth (PTB). PTB causes most of neonatal deaths and different forms of neonatal morbidities [1], the causes of PTB in most cases have not been established although several risk factors have been identified [2].

Because many of these infections are asymptomatic, underestimation of their importance may have been occurred furthermore, few studies focusing on these infections, and they investigated only one infection in relation to PTB, such as chlamydia, bacterial vaginosis, or urinary tract infection [3,4].Gardnerella vaginalis : It is Fastidious , facultative anaerobic, little, pleomorphic poles that are non-motile and don't have flagella, endospores, or regular cases In vaginal discharge varied into Gram response of G. vaginalis may shift from positive to negative[5].The second diagnostic feature of a Gram positive cell wall variety due of di-amino pimelic acid and lipopolysaccharide disappear in the cell wall [6]. Many studies concentrated and focused on this genetic primer and found that G. vaginalis detection could be achieved with varying degree of success with primers specific for 16sr RNA-encoding genes.

Universal cpn60 target that ameliorate evolvement of the vaginal microbiota including G. vaginalis was applied in a prospective study of the vaginal microbiota of women with preterm premature rupture of membranes (PPROM) [7]. Bio film formation a complex aggregation of bacteria which growing on a solid surface. Biofilms are usually found on solid substance submerged in or exposed to some aqueous solution [8].
They are found in a wide range of natural and artificial environments and provide their constituent microbial cells with plethora of protected dynamic microenvironment [9]. Microorganisms produce a biofilm in many variables way, which may incorporate cell acknowledgment of particular connection locales on a surface, wholesome signs, or now and again by presentation of planktonic to sub-inhibitory convergences of antibiotics [10].

Materials and Methods

Sample Collection

A total of 100 samples only 20 isolates of preterm labor were recovered from clinical samples (high vaginal swabs), this include6 isolates were isolated from patients. All samples or individual were admitted to Al-Hilla surgical teaching hospital and Maternities and pediatric hospital in Al-Hilla city/ Iraq.

Cultural Characterstrastic

Isolates have been cultured on Colombia agar supplemented with 5%fresh blood with the addition (Naldixic acid-Gentamycin and Nystatin) diagnosed according to [11].

DNA Extraction

DNA was extracted from bacterial isolate according to the kit (genaid U.S.A.)

Detection of Specific Gene Markers by PCR

Table 1: The primers were used to amplify 16sRNA, Cpn60 listed in

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5’-3’)</th>
<th>Size (bp)</th>
<th>PCR condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>F-5’GACGACGGCGAATGGCAGA 3’  R-5’ AGTCGCACTCCGCGCAAGTC 3’</td>
<td>331</td>
<td>94°C 10min 1x 94°C 2min 52°C 1min 40x 72°C 1min 72°C 10min 1x</td>
<td>12</td>
</tr>
<tr>
<td>Cpn60</td>
<td>F-5’CGCATCTGCTAAGGATGTTG 3’  R-5’CAGCAATCTTTTTCGCCAAGCT 3’</td>
<td>615</td>
<td>94°C 10min 1x 94°C 1min 62-66C1min 35x 72°C 1min 72°C 10min 1x</td>
<td>In this study procedure designed by Optimize Protocol Writer online</td>
</tr>
</tbody>
</table>

Primer and PCR conditions were used to detect gene of G. vaginalis are present in Table (1). However, each 25μl of PCR consist of each upstream and downstream primer (2.5 μl), free nuclease water (2.5 μl), DNA extraction in concentration 0.1μg/ml (5μl), and master mix (12.5 μl). The polymerase chain reaction amplicon was detected by gel electrophoresis on 1.5% agarose gels for 40 min at 70 V.

Bio Film Production

Tissue culture plate method (TCP) assay (also called semi quantitative microtiter plate test (biofilm assay) described by (13)) was considered as standard test for detection of biofilm and classified as described in Table (2)

Table 2: Classification of bacterial adherence and biofilm formation by TCB method by (14)

<table>
<thead>
<tr>
<th>Mean of O.D. value at 630 nm</th>
<th>Adherence</th>
<th>Biofilm formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.120</td>
<td>Non</td>
<td>Non</td>
</tr>
<tr>
<td>0.120 – 0.240</td>
<td>Moderately</td>
<td>Moderate</td>
</tr>
<tr>
<td>&gt; 0.240</td>
<td>Strong</td>
<td>High</td>
</tr>
</tbody>
</table>

Effect of Some Factors on Bio Film Formation

The same procedure described in (tissue culture plate method for detection biofilm formation) was done with modification. (Metronidazole-sodium bicarbonate-sodium chloride-Thymol extract –alum) were added to samples in wells of sterile polystyrene 96 well-flat bottom tissue culture plate, after fixed with sodium acetate for an hour and all steps done as the same steps that described previously.

Results and Discussion

Depending on (culture characteristic, biochemical test) detection 6 isolates of bacteria were obtained during this study belonging to different clinical manufactures as shown in Figure (1) and confirmed by molecular identification. This results showed from 15patients only one isolate were
obtained which gave (10%) percentage belong to urinary tract infections. The high rate of aborted women are belong to estrogen abnormality during the pregnancy period which convert the Glycogen to lactic acid the result was showed that from 55 patients only 2 isolates were obtained which gave (37%) percentage among aborted women. On the other hand, the result was showed that from 80 patients with bacterial vaginosis only 3 isolates were obtained which gave a percentage (53%).

Figure 1: Distribution of patients according to clinical diagnosis

*Positive results obtained only from 6 patients (3 bacterial vaginosis, 1 UTI and 2 aborted women).

Then these bacteria were diagnosed as *G. vaginalis* after amplification and gave a specific band 331 bp depending on 16SRNA as shown in Figure (2).

For conformational identification the Chaperonin protein 60 was used gave 613 bp with comparison with the allelic ladder as showed in Figure (3).
Bio Film Formation of G. vaginalis

Bio film formation on polymeric surfaces was tested in the semi quantitative micro titer plate test (bio film assay) using (TSB) supplemented with 1% glucose.

Table 3: Production of bio film in G.vaginalis

<table>
<thead>
<tr>
<th>Bacterial isolate No.</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
</tr>
<tr>
<td>G.vaginalis (6)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4: Effect of some factor and Antibiotics on Biofilm formation of G.vaginalis: (n=6)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
</tr>
<tr>
<td>Biofilm</td>
<td>3 (50.0%)</td>
</tr>
<tr>
<td>Allium</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Thymol</td>
<td>5 (83.4%)</td>
</tr>
<tr>
<td>Na2co3</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

G. vaginalis was isolated from different type of infection may lead to preterm labor from Bacterial vaginosis the percentage is similar to that results obtained by [14] which referred that the percentage to isolated this bacteria was (40.8%) in Nigeria whereas the reproductive age play role when it isolated in America and Europe as mentioned by [15] this variation in isolation rate may be due to nature and size of the habit to the area that sample were taken, or due to the antibiotic usage by the patient before the result of this study were agreement with these obtained by [16] which said that bacteria adhere to the epithelial cell in urine with large quantity in cohesive shape especially at pregnancy period.

The high isolation rate of this bacteria from aborted women are belong to estrogen abnormality during the pregnancy period which convert the Glycogen to lactic acid made vagina environment acidic that give chance to G.vaginalis to grow this acidity are responsible to prevent lactobacillus spp. grow and produced hydrogen peroxide, this bacteria adhere and evade the uterine endothelial made it evade the embryo tissue or chorioamnionic membrane area which responsible for tropical secretion of phospholipase A2 and prostaglandin release which accountable on early delivery, abortion and broken immature tissue [17].

Bio film facilitates the adherence of microorganisms to biomedical surfaces and protects them from host immune response and antimicrobial therapy [18]. In addition, the production of bio film may promote the colonization and lead to increased rate of vaginitis and such infections may be difficult to treat as they exhibit multidrug resistance [19]. In vagina, infections associated with bio films include vaginitis, bacterial vaginosis the results showed that [4] isolates were gave a strong Bio film whereas the two isolates gave moderate bio film these results were in agreement with those obtained by [20]. Moreover, bio film formation seems to be a trait associated with vaginal infections an indicating that bio film might be important for persistent colonization of the surfaces [21].

Effect of some factor and Antibiotics on Bio Film Formation of G. vaginalis

In this study were used more than one factor to reduced bio film Regarding to effect of sodium chloride (50%) on bio film formation, the result of this study demonstrate its effectively reduces the viability of bio film-forming bacteria, such as G.vaginalis. It reduces the bio film formation from moderate weak.

These findings suggest that although sodium chloride caused disruption of bio film-forming cells (100%), the constituents derived from disrupted cells were maintained in the bio films, which sustained their external structures. Moreover, the residual bio films could serve as a scaffold for the formation of new bio films. To date, sodium bicarbonate was gave result (100%) been one of the most effective antimicrobial agents for the adjunctive control of vaginitis and vaginosis. However, its broad spectrum of activity and adverse effect profile limits its prolonged use as a topical vaginal antimicrobial agent by the all the women this affective by washing.
activity and reduced the hydrogen peroxides. The effect of some plant extracts on bio film formation was studied. The results showed that using of these extracts at (50 %) concentrations have some effect on bio film formation. The bio film formation affected by using thymol extract (carvacol) with concentration (50%), According to the effects of thymol (50%), the percentage of bio film formation changed from (100%) to (50%). The presence of active compounds exhibiting antivaginal activity in the plant extracts may be useful for the development of anti-infective drugs such as the high levels of eugenol contained in thymol essential oil are responsible for its strong biological and antimicrobial activities. It is well known thymol essential oil phenolic compounds can denature proteins and react with cell membrane phospholipids changing their permeability and inhibiting a great number of Gram-negative and Gram-positive bacteria as well as different types of yeast [22].

The use of Alum was being more effective and useful in eradication the G.vaginalis bio films changed it from (100%) bio film producer to (0%). Formulating these into dosage forms that can be used for the treatment of all vaginal infections that persist due to bio film formation may be useful alternatives to antibiotic therapy [23, 24]. Found that 50% of Alum gives inhibition zone reach to 40mm in diameter against different types of G+ and G- isolated from skin and otitis media. Also used the metronidazole were more capable on reduction bio film this results were in agreement with those obtained by [25] described the major action of metronidazole is a rapid inhibition of DNA replication by causing DNA strand destruction at concentrations easily attainable during routine drug administration, but which only occurs after reduction of the nitro group. Also it is an antibiotic to which susceptible anaerobes have yet to develop clinical resistance; therefore it has been shown that there is excellent activity of metronidazole against G.vaginalis [26]. One major disadvantage of using metronidazole is that it has a very broad spectrum [27].

Conclusion

all isolates are capable to adhere and produce bio film in different amount , used of sodium chloride , sodium bicarbonate, Alum and thymol extract have a strong ability to reduced bio film in addition of Metronidazole.

Ethical Approval

A valid consent was achieved from hospitals administration and from each female (patients and controls) before their inclusion in the study. For every female or her followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects.

Acknowledgement

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References


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