DETECTION OF ENTEROCOCCUS FAECALIS ISOLATED FROM WOMEN WITH VULVAVAGINITIS AND ITS RELATION WITH CRP, IL-1 Beta AND IL-23 IN IN SALAH AL-DIN GOVERNORATE

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ABSTRACT

Vulvovaginal infections (VVI) are the commonly reported microbiological syndrome affecting millions of women globally in all strata of society. This study was conducted to isolate and identify E. faecalis from vaginal swab of women in addition to other bacteria and some immunological parameters in sera of those women. A cross-sectional study conducted in Balad city from the period from 1/9/2020 to 1/2/2021. A total of 170 deep vaginal swabs were collected from women with vaginitis, their ages ranged from 15-45 years, and information was taken from each review, including the age of residence. The collected samples (vaginal swabs) were cultured directly on different microbiological media. The bacteria growing were diagnosed on the basis of color, shape, size, edge and height of the growing colonies, and further secondary culture were done for isolated E. faecalis for antibiotic sensitivity tests as well as different virulence factors included biofilm formation by different methods. All procedure were done according to manufacture instruction and standard operation procedures of microbiology laboratories. The study also includes collection of blood sample for determination of interleukin-1 beta and IL-23 and by enzyme linked immunosorbent assay C-reactive protein level by immunofluorescence technique. The study showed that 57.06% (97 of 170) of women with vaginitis have positive HVS culture comparing with 3.33% (1 of 30) of healthy control group (P value: 0.001). The study demonstrated that E. faecalis represented the most isolated bacteria from by HVS culture of women with vaginitis (n:60), E.coli, n=19, S. aureus n=18, K. pneumonia n=12 and E. faecium n=8. All E. faecalis isolated from women with vaginitis were sensitive toward cephalothin and imipenem (100%), 95% sensitive to carbenicillin, 91.67% sensitive to ampicillin, and 46.67% sensitive to chloramphenicol while all E. faecalis isolates were resistant to nalidixic acid and Clindamycin. The study demonstrated that the highest mean of CRP level was found among women with vaginitis infected with E. faecalis (15.23±2.11 mg/dl) comparing with the control group (4.53±1.12 mg/dl) with highly significant difference. The study demonstrated that the highest mean of IL-1 beta level was found among women with vaginitis infected with E. faecalis (2055.8±82.54 ng/ml) comparing with the control group (1722.6±62.17 ng/ml) with a significant difference between the two groups (P<0.05). The study demonstrated that the highest mean of IL-23 level was found among women with vaginitis infected with E. faecalis (219.52±173.16 ng/ml) comparing with the control group (173.16±7.18 ng/ml) with a significant difference between the two groups (P<0.05).

Keywords: Vulvovaginitis; IL-1 beta; CRP; IL-23; E. faecalis

I. INTRODUCTION

Vulvovaginal infections (VVI) are the commonly reported microbiological syndrome affecting millions of women globally in all strata of society. An abnormal vaginal discharge is the key trait and first sign of VVI that women seeking health care frequently complaint to gynecologist. Aerobic vaginitis (AV) is a state of abnormal vaginal flora that is distinct from the more common bacterial vaginosis (BV) (Bannister et al., 2006). AV is caused by a displacement of the healthy vaginal Lactobacillus species with aerobic pathogens such as Escherichia coli, Group B Streptococcus (GBS), Staphylococcus aureus, and Enterococcus faecalis that trigger a
localized vaginal inflammatory immune response (Paladine and Desai, 2018). Clinical signs and symptoms include vaginal inflammation, an itching or burning sensation, dyspareunia, yellowish discharge, and an increase in vaginal pH > 4.5, and inflammation with leukocyte infiltration (Kalia et al., 2019). Severe, persistent, or chronic forms of AV can also be referred to as desquamative inflammatory vaginitis (DIV) (Donders et al., 2017). *E. faecalis* normally lives harmlessly in human intestines. However, if it spreads to other parts of the body it can cause a more serious infection. The bacteria can get into blood, urine, or a wound during surgery. From there, it can spread to different sites causing more serious infections, including sepsis, endocarditis, and meningitis (Khdir, 2020). Enterococci are the second most common causative agent of urinary tract infections (UTIs) in hospitalized patients. Antimicrobial resistance and survival ability in various hospital environments have made them as serious problem in nosocomial infections due to the limited therapeutic options (Ben Braïek and Smaoui, 2019). The human vagina consists of multiple levels of protection in form of innate and adaptive immunity that is further compartmentalize into various components and is under strong hormonal control (Orababa et al., 2021). Inflammation signifies an essential immune mechanism that is meant to eliminate pathogens and repair damage caused by deleterious stimuli. Theoretically, inflammation is a process that involves four stages, including an activating system, a sensing mechanism, signal diffusion, and the effector cells activation (Kalia et al., 2019). In infectious diseases, the activating system is pathogen that has preserved biomolecular structures on its surface known as pathogen associated molecular patterns (PAMPs) (Renner and Hoppe, 2019). These PAMPs lead to the activation of quick and non-specific innate immunity that further signals for the activation of specific adaptive immunity, with the ultimate goal of eradicating the pathogens and repairing tissue damage elicited by the noxious stimuli (Stephen and Hajjar, 2018). This study was conducted to isolate and identify *E. faecalis* from vaginal swab of women in addition to other bacteria and study their virulence factors and biofilm properties and some immunological parameters in sera of those women.

### II. MATERIALS AND METHODS

A cross-sectional study conducted in Balad city from the period from 1/9/2020 to 1/2/2021. A total of 170 deep vaginal swabs were collected from women with vaginitis under medical supervision by the specialized doctor in the consultative clinic of Balad General Hospital in the microbiology laboratory, as well as 30 samples for uninfected women. The study also included married women. Non-pregnant women, their ages ranged from 15-45 years, and information was taken from each review, including the age of residence, the date of taking the swab, as well as the symptoms they feel, such as vaginal secretions, color and smell, and the presence or absence of vulvar itching and vaginal burning.

Collection of vaginal swabs included:

1- The swab package was partially opened.

2- Carefully the swab was inserted into vagina about 2 inches (5 cm) past the introitus and gently rotated for 10 to 30 seconds.

3- When the swab touched the vagina walls and moisture and absorbed the moisture, it was withdrawn without touching the skin.

4- Swabs was delivered to the laboratory within 1 hour of collection.

The collected samples (vaginal swabs) were cultured directly on solid blood agar medium and MacConkey's agar NO.2 , then they were cultured on the selective medium azid blood agar base and chromogenic agar medium , and the dishes were incubated at 37 °C for 24 hours. The bacteria growing on the media were diagnosed on the basis of color, shape, size, edge and height of the growing colonies, and the colonies were regrown more than once to obtain single pure cultures. Five ml of venous blood was collected from each subject by using sterile disposable syringe and transferred into sterile gell tubes, left to clot at room temperature for 20 minutes , then centrifuged at 3000 rpm for 15 minutes , sera were then removed and added in Eppendorf tubes and stored at -20°C for determination of IL-1 beta and IL-23 and by ELISA C-RP level by immunofluorescence technique (i-chroma II).
III. RESULTS.

Figure 1 shows that 57.06% (97 of 170) of women with vaginitis have positive HVS culture comparing with 3.33% (1 of 30) of healthy control group (P. value: 0.001). 

![Result of HVS culture](image)

\[X^2 = 49.66\quad P.\text{ value} = 0.001,\text{ Highly Significant}\]

Figure 1: Distribution of HVS culture in vaginitis women and the control group.

The study demonstrated that *E. faecalis* represented the most isolated bacteria from by HVS culture of women with vaginitis (n:60), *E. coli*, n=19, *S. aureus* n=18, *K. pneumonia* n=12 and *E. faecium* n=8, Table 1.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em></td>
<td>60</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>8</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>18</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>19</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1: Isolated bacteria by HVS culture

Figure 2 shows that all *E. faecalis* isolated from women with vaginitis were sensitive to toward cephalothin and imipenem (100%), 95% sensitive to carbenicillin, 91.67% sensitive to ampicillin, and 46.67% sensitive to chloramphenicol while all *E. faecalis* isolates were resistant to nalidixic acid and Clindamycin.

![E. faecalis](image)
The study demonstrated that the highest mean of CRP level was found among women with vaginitis infected with *E. faecalis* (15.23±2.11 mg/dl) comparing with the control group (4.53±1.12 mg/dl) with highly significant difference, Figure 3.

![Figure 3: Relation of C-reactive protein with *E. faecalis* infection in women with vaginitis](image)

The study demonstrated that the highest mean of IL-1 beta level was found among women with vaginitis infected with *E. faecalis* (2055.8±82.54 ng/ml) comparing with the control group (1722.6±62.17 ng/ml) with a significant difference between the two groups (P<0.05), Figure 4.

![Figure 4: Relation of IL-1 beta protein with *E. faecalis* infection in women with vaginitis](image)

The study demonstrated that the highest mean of IL-23 level was found among women with vaginitis infected with *E. faecalis* (219.52±173.16 ng/ml) comparing with the control group (173.16±7.18 ng/ml) with a significant difference between the two groups (P<0.05), Figure 4.24.
Figure 5: Relation of IL-23 with E. faecalis infection in women with vaginitis

IV. DISCUSSION

Vaginitis is the commonest RTI in sexually active women and is associated with a significant risk of morbidity. The management of vaginitis remains largely empirical, though establishing correct diagnosis is the most important factor for successful treatment (Mulu et al., 2015). In present study, the overall prevalence of vaginal infections (57.06%) was resemble to study done by Salih and Hassan, (2018) in Kirkuk city showed that 57% of women with recurrent abortion have positive high vaginal swab (HVS). Hussien et al., (2020) displayed that 48.7% of women in India had positive HVS culture. *E. faecalis* is associated with a wide spectrum of infections, particularly under immunocompromised states and during compositional shifts in the host microbiota. Our finding was comparable to a study of Shrestha et al., (2011) when showed that vaginitis symptoms have multiple etiologies. In agreement with our funding, study from Ethiopia by Mohammed et al., (2012) showed that the rate of *E. faecalis* causing vaginitis was 20.9%. In another study, Ali et al., (2019) showed that, the prevalence of *E. faecalis* among women with vaginitis was were 15.7%. Al-Kafajy et al., (2009) revealed that *Escherichia coli* were the most encountered frequency (37.2%) followed by *Staphylococcus aureus* with (27.9 %). *Enterococcus faecalis* (23.2%), *Gardnerella vaginalis* (16.2 %), and *Candida spp* (11.6 %) were less common. From a total of 602 vaginal swabs from pregnant women, Ghasemi et al., (2016) indicated that, 49 (8.14%) isolates were identified as enterococci. Predominant species were respectively, *E. faecalis* 44 (89.8%), *E. faecium* 3 (6.1%). This inconsistency might be associated with difference among study participants, varied etiologies studied and the detection techniques applied (Kiran et al., 2017).

*E. faecalis* colonization and infection is often polymicrobial, and these interactions have been observed in the intestine, bloodstream, and wounds (Donders et al., 2017). Furthermore, *E. faecalis* is frequently found in the vaginal tract of healthy women (Leyva-Gómez et al., 2019), and its prevalence is increased in women diagnosed with aerobic vaginitis (AV), an inflammatory response accompanied by depletion of commensal *Lactobacillus* sp. and increased presence of opportunistic pathogens, such as *E. faecalis*, group B *Streptococcus* (GBS), *Staphylococcus aureus*, and *Escherichia coli* (Kaambo et al., 2018).

In agreement with our finding, Ghasemi et al., (2016) indicated that, All of the *E. faecalis* isolates were found to be sensitive to ampicillin. In a review of urinary enterococci by Muratani and Matsumoto, (2004) conducted in Japan, it was shown that resistance to gentamicin in *E. faecalis* was 84.8%. Enterococci are believed to be difficult to treat because of their intrinsic resistance to antibiotics including beta – lactams and aminoglycosides which are frequently used to treat infections due to Gram-positive cocci (Gaca et al., 2012). Resistance to trimethoprim, gentamycin and vancomycin have also been reported (Cheng et al., 2014). Estimation of serum C-reactive protein has been proposed as a useful tool to characterize systemic inflammation, infection, and sepsis . Tang et al., (2018) found that the concentrations of serum CRP in patients with bacterial infection was higher than those with non-bacterial infection and in the G+ve bacterial infection group, a higher concentration of CRP was observed compared with fungus infection group. In agreement with the current findings, Wu et al., (2015) reported a significantly higher CRP levels in sera of patients with bacterial infection, Djordjevic et al., (2015)
study demonstrated significant elevation of CRP levels in patients with bloodstream infections caused by *E. coli*, *K. pneumoniae*, *S. epidermidis/S. aureus* and *E. faecalis*.

Immune activation in the female genital tract can be caused by infection, irritation or epithelial trauma, and results in increased or decreased expression of soluble immune proteins (Al-Bayati and Alazzawy, 2019). Evidence from several trials of ineffective or harmful microbiotics has shown that some candidate products can increase concentration of inflammatory immune proteins (Cummins et al., 2009). Interestingly, clinical studies are measuring soluble immune biomarkers to screen for product-induced mucosal toxicity/irritation in pre-clinical and clinical trials (Ensign et al., 2012). The most common soluble proteins evaluated in trials have been interleukin (IL)-1α, IL-1β, IL-1-receptor antagonist, IL-6, IL-8, tumour necrosis factor (TNF)-α and secretory leukocyte peptidase inhibitor (SLPI) (Arnold et al., 2016). In agreement with our finding, Ran et al., (2021) in recent study found that *E. faecalis* infection induce IL-1β secretion and referred to inflammatory response mechanism induced by *E. faecalis*. Yang et al., (2014) also showed that *E. faecalis* induced caspase-1 activation and IL-1 beta expression. Increased IL-1 beta levels patients with *E. faecalis* infection in have been detected by immunohistochemical staining and enzyme-linked immunosorbent assay by several studies done earlier (Wang et al., 2016; Ran et al., 2019). For an effective mucosal homeostasis, immune responses are tightly regulated to ensure protective immunity to the host, and it is of utmost importance that the adaptive and innate immune systems are able to recognize pathogenic organisms while ignoring the commensal flora (Ahern et al., 2008). IL-23 activates the adaptive and innate immune systems to produce IL-17A, IL-17F, IL-22, and TNF, all of which help to stimulate epithelial cells to produce antimicrobial factors. These properties are important in host defense against a number of infections as mentioned earlier, such as *Klebsiella pneumonia, Candida albicans* and *Toxoplasma gondii* (Tato and Cua, 2008).

**REFERENCE**