Comparing the effect of some plant extracts and commercial washes against some isolated Microorganisms from vagina of pregnant women

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ABSTRACT

The main role of the present study is to isolate and diagnose the main pathogens of female genital tract vaginal infections in pregnant women 3rd trimester months, as well as the inhibitory effect of commercial vaginal washes and the effect of a group of plant extracts on these bacteria to evaluate the possibility of using these extracts as alternatives to commercial or other treatments.

Thirty (30) vaginal samples High Vaginal Swab (HVS) were collected from pregnant women with different ages after the internal sonar trans vaginal US to women with clinical symptoms associated with vaginal and cervical infection. These samples were tested to achieve the objectives of this study.

The results of isolation and diagnosis showed the presence of (17) positive samples (56.66%) of bacterial culture belonging to Staphylococcus aureus bacteria. Four samples of Staphylococcus epidermidis (13.33%) while E. coli was detected in two samples (6.66%).

The study showed the prevalence of Candida either alone in (6) samples (20%) or in combination with the bacteria in (14) samples (46.66%).

By evaluating inhibitory activity of the commercial vaginal wash (Germex), a significant effect was found on the studied bacteria when compared with a number of water extracts. The water extract of honey had the most inhibitory effect on the bacteria followed by the extract of the apple vinegar, while olive oil and Chamomile (Matricaria chamomilla) showed a variation according to the type of bacteria and their resistance.

Keyword: Bacterial Vaginosis (BV), HVS, plant extracts, Staphylococcus aureus, E. coli, Candida, commercial vaginal wash, Germex


INTRODUCTION

Female genital infections are common health problems in developing and developed countries. These infections are known as vaginal infection and cervix as the pathogen replaces the existing natural flora.1 The female reproductive system is more susceptible to infections than the male reproductive system due to the short area of the urethra and closeness to the opening of the vagina, as well as vaginal secretions, menstrual cycle, the exposure of the female to the process of birth, scraping of the uterus, the occurrence of embolism in the lining of the uterus during the menstrual cycle, and finally the presence of natural cohabitation which may later be transformed by the effect of hormones, weak body resistance and vaginal acidity into opportunistic pathogens.2
Vaginal and uterine infections are the most serious threat to the fetus during pregnancy. These infections cause premature fetal immobilization, which is one of the causes of premature birth. It accounts for about 85% of the cases of childbirth and death. Bacterial Vaginosis (BV) is the most common cause of vaginal discharge among women of childbearing age and the main cause of UTI.\textsuperscript{3,4}

The inaccurate diagnosis of bacterial vaginosis and the rush to take treatment without sensitivity testing and the excessive and indiscriminate use of antibiotics lead to the emergence of antibiotic resistant strains.\textsuperscript{5} The increase in bacterial infection rates was not due to multiple resistance to antibiotics but also because the presence of many virulence factors that increase their severity.\textsuperscript{6} In addition to bacteria, Candida yeast (especially \textit{Candida albicans}) which is associated with vaginal infection, has increased its resistance to familiar fungicides, especially Azole Compounds.\textsuperscript{7} Therefore, the emergence and spread of antibiotic resistance between various bacteria has prompted researchers and those interested in the field of treatment to search for the discovery of therapeutic alternatives to these antibiotics. In recent years the use of commercial vaginal lotions has seen increased in usage due to their relatively easy use in maintaining the health of the vagina. The availability of these synthetic and semi-synthetics, although useful, have some side effects such as vaginal irritation during use or contain a high proportion of alcohol and vary in its effectiveness depending on the composition and manufactory origin.\textsuperscript{8}

Therefore, most countries in the world have recently resorted to medicinal plants and make them a major source of access to medical preparations as foods and natural effectiveness as well as availability, spread and accessibility, the natural materials used in treatment about 60% of the materials. Most of it is derived from plants with low side effects and high purity, as well as the inability of bacteria to find resistance that reduces the impact of these drugs.\textsuperscript{9}

The aim of this study is to compare the antimicrobial effect between plant extracts and commercial capsules towards the isolated bacteria from vaginal infections in pregnant women in recent months after the internal sonar.

**MATERIAL AND METHODS**

**Specimen:**

A total of 30 samples of high vaginal swabs from pregnant patients were collected 3\textsuperscript{rd} trimester months after the internal sonar, who attended the obstetric and gynecological consultation at Al-Khansa Hospital in Mosul. The sample was taken by permanent doctor /diagnostic radiology after conducting the internal sonar by swabbing a sterile container on a transport medium Tryptose soy broth to prevent dryness of the sample until it is transferred to the laboratory for tests in a period not exceeding three hours.\textsuperscript{10,11}

*Commercial vaginal wash :*

In this study we use Germax wash made in S.A.R

*Plants used in the study:*

1- Chamomile (\textit{Matricaria chamomilla})
2- Myrtle (\textit{Myrtus communis})
3- Apple Vinegar
4- Olive Oil
5- Honey
**ISOLATION & IDENTIFICATION**

Each sample was transferred to the laboratory, cultured on Mannitol Salt agar medium to investigate Staphylococcus and MacConkey's agar to investigate *E. coli* and Sabouraud, glucose agar (SGA) to investigate Candida yeast. All plates were incubated at 37 °C for 24-48 hours.

After the incubation period, characteristics of colonies such as colony morphology, color, size and microscopy were observed and recorded.

*Tube Coagulase Test*

This test was used to detect the ability of bacteria to produce free coagulase. The test was performed by adding 0.5 ml of human plasma + EDTA to the test tube and was inoculated by transferring a young bacterial colony to the tube and incubated at 37 °C for 2-4 hours. The positive result is the appearance of clotting or coagulation. The negative result was recorded only after incubation for 24 hours and at room temperature.

*Germ Tube Formation Test*

The test was conducted according to the described method (Forbes et al., 2007) to distinguish the yeast of *C. albicans* and a recently described yeast *C. dubliniensis* from the rest of the yeasts, by placing (0.5) milliliters of human serum in sterile test tubes and then inoculate each tube with a small part of the colony on the SDA medium and incubated the tubes at a temperature of 35-37 °C for a period of not more than three hours. After that, a drop of suspension was placed on a clean glass slide, covered with the slide cover and examined under the microscope at 40x for cells with resembling a germination hand mirror.

*Evaluation of the inhibitory effect of commercial Germax and plant water extracts.*

The Well Diffusion Assay Method was applied (Vadepitte and Balows, 2003). The bacterial isolates were inoculated in BHI broth and compared with the McFarland tube (0.5), which is equivalent to 10^8 cells / ml. The bacterial suspension was transferred to the Mueller- Hinton Agar and spread using cotton swab. After that, a 5 mm well was punched in the center of the medium using sterile sterilized perforation tubes. In each hole, (50) microliters of the plant extract bibone, (500 mg / ml), olive oil, apple cider vinegar directly and commercial Germax as per (Bactracin, Amkacin Ciproflaxcin) were compared and the plates were incubated at 37 °C for 24 h. After incubation, the inhibition zones were observed and their diameter was measured to determine the efficacy of the plant extract. Inhibition Zone diameter was measured for each tablet and the measurements were compared with standard tables approved by the World Health Organization.

**RESULT AND DISCUSSION**

The results of the study showed that the percentage of gram positive and negative bacterial vaginitis was significantly higher than the incidence of Candida yeast, where bacterial infections were (76.65%) followed by co-infection between bacteria and Candida yeast (46.66%). As compared to (20%) of candida infection alone as shown in Figure (1).
The presence of yeasts in the mucous membranes lining the mouth, the digestive tract and the genital canal is normal flora, but opportunistic, when the body’s immunity is weakened or antibiotics ingested, yeasts are ate and multiply to cause injuries. This is because *C. albicans* infections is a complex process, Transformation from commensalism condition to a pathological condition as a response to environmental changes in the surrounding that lead to the expression of the virulence factors. There is no single factor responsible for the yeast pathogenesis, as there is more than one agent.

Table (1) shows the percentage of Gram positive and Gram negative bacterial strains isolated and co-infections with Candida. The percentage of Gram positive bacteria causing bacterial vaginitis is significantly higher than that of the Gram negative bacteria isolated. The percentage of Gram positive bacteria *Staphylococcus aureus* was shown to be (56.66%), *S. epidermidis* (13.33%). While the percentage of Gram negative bacteria was very low (6.66%). These results agree with studies, while it disagrees with Jamali 2005 who found that the percentage of Gram negative bacteria causing vaginitis was higher than the Gram positive bacteria.

**Table (1): Number and percentages Gr+ & Gr- bacteria and Candida isolates.**

<table>
<thead>
<tr>
<th>Type bacteria</th>
<th>NO.</th>
<th>Percentage %</th>
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<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>17</td>
<td>56.66</td>
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<tr>
<td><em>S. epidermidis</em></td>
<td>4</td>
<td>13.33</td>
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<td><em>E. coli</em></td>
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<td>6.66</td>
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<tr>
<td><strong>Candida &amp; bacteria</strong></td>
<td>14</td>
<td>46.66</td>
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Many studies have investigated *S. aureus* and reveal its role in the infections of the female reproductive system. It was found that these bacteria have a role in the infection of bacterial vaginosis (BV) it ranked the first in vaginal swabs for pregnant and non-pregnant women in Babil, and was ranked first in bacterial vaginosis, accounting for 467% of the total number of 731 samples.

A study by Ali (2012) S. *epidermidis* was also isolated from vaginal swabs of healthy women and from urine of patients using urinary catheter tubes. Several studies have confirmed that *E. coli* is the most common factor in reproductive infections in pregnant and non-pregnant women, including a study.
by (Razzak et al., 2011) who isolated the bacteria from pregnant and non-pregnant women with bacterial vaginitis.

A study by Zaria et al., (2010) on the role of E. coli in the incidence of bacterial vaginitis and urinary tract infection isolated these bacteria from vaginal swabs and urine samples of pregnant women and non-pregnant women infected with BV associated with UTI and found that E. coli was prevalent in a high proportion among other bacteria causing infection. The results showed co-infections with bacteria and candida yeast at a percentage of 46.66% which agrees with a study by Lennox et al., 2013 who also indicated the presence of bacteria and yeast co-infections among fungal and bacterial infected women.

Results in table (1) shows that 50% of C. albicans were from candida yeast. Many studies found that the C. albicans is the main factor causing Vaginitis in women. A study by Ogunshe et al., (2009) isolated the C. albicans yeast from vaginal swabs (HVS) from women with vaginitis and C. albicans was the main pathogen. It is one of the most opportunistic yeast.

The results of our study showed that the second main cause of vaginitis is the yeast Candida albicans and was found individually or mixed with bacteria at a high incidence. The second cause of vaginitis is the yeast C. albicans, which was found at a high rate (19.8%) of the total (202) vaginal swabs.

Images (1), (2), (3), (4), (5) show some of the results of the culture, microscopic and biochemical tests of the bacterial isolates in the study.
Table (2) and (3) show the inhibition effect of commercial Germex and the aqueous extracts of Myrtle plant, Chamomile, Apple vinegar, Olive Oil and Honey on a number of Gram positive and negative bacteria, in addition to Candida albicans isolated in this study, as compared with the Amikacin antibiotic.

Results of well diffusion method showed variable sensitivity of all bacterial isolates depending on the type of organism and the extracts used. Honey showed high inhibition on most of bacteria studied. E. coli Staphylococcus epidermidis and Staphylococcus aureus showed susceptibility higher than Amikacin antibiotic in diameters as shown in image (1, 2, 3). This is due to the presence of oxidation and reduction enzymes in honey such as peroxidase, catalase, phosphatase and its role in killing of the bacteria, in addition to the lipase enzyme that breaks down lipids present in bacterial walls. Followed by inhibitory ability of commercial Germex wash water on bacteria. The inhibitory ability of myrtle plant and olive oil is equal on all bacteria studied, while the inhibitory effect of vinegar apple was high on some bacterial and weak on others. Chamomile does not appear to have any inhibition ability on the bacteria. Many studies have shown that bacteria that cause (BV) are resistant to antibiotics and have anti-inflammatory and resistance mechanisms, therefore the study of plant extracts is still important for new therapeutic sources in medicine.

Locally, many studies on the effect of plant extracts on microorganism have been carried out. Their inhibitory effect on the gram positive and gram negative bacteria shows that plant leaves possess many active substances that can be dissolved in water or alcohol varies in killing ability for bacterial.

The inhibitory activity of the myrtle plant extract against the studied bacterial due to effective substances content such as Kaempferol, Aglycone, Myricetin Glycosides, and phenolic substances especially group(Myrtucummulones) A and B and also plant extract contains volatile oils.
Table (2): The inhibitory effect of Germax commercial washes, plant extracts, apple vinegar, olive oil and honey against gram positive and gram negative.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Olive Oil</th>
<th>Apple Vinegar</th>
<th>Myrtle</th>
<th>Chamomile</th>
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Staphylococcus Aureus
Candida
Staphylococcus epidermidis
E.coli
plant extracts, apple vinegar, olive oil, and honey against Candida.

In 2012 Roby\(^28\) refers to that the inhibitory effect of alcohol plant extract of chamomile against bacteria such as *E. coli* and *S. aureus* and yeasts such *Candida albicans* and also refers to the important oils in chamomile which is consider as strong antibacterial, while another study\(^17\) refers to that the chamomile alcohol extract shows a high inhibitory effect against Gr-\&Gr+ bacteria.

Results of the table (3) show the highest inhibitory effect was demonstrated by commercial Garmax washes on all whitened Candida yeast, high inhibition activity of Garmax is due to a group of effective compounds like propylene, glygole, sodium lauryl, diamin ethanol, chlorine. Followed by apple vinegar and olive oil in the ability inhibition, while the effect of Myrtle and Chamomile extracts varied of on all the yeast. Honey is specific on kinds from the yeast only, just as in the image (4). Efficient inhibition ability of apple vinegar is due to the effective acids organic such as acetic acid, Oxalic acid, icteric acid and little from salts of the metals, amino acids and bactenats used in medication and treatments.\(^27\)

During the last decades the mycoses has increased despite the use of many antibiotics to treat the mycoses diseases surface and systematic, (Zacchin \textit{et al.}, 2003). This resistance is consider as a problem, and this growing problem makes the treatment by using antifungal unclear in the future.\(^29\) In the recent years, researchers focus to use medical plants having antifungal compounds.\(^30\)

Several studies have been conducted on the effect of vinegar on bacteria, including the study of \textit{Cefoloxime therapy}, the treatment with vinegar was faster than antibiotic treatment. A study by\(^21\) proved the vinegar effect of inhibiting the bacteria *Pseudomonas aeruginosa*.

This result agrees with result of Issazaden (2012)\(^32\) who found that the extract of Myrtle leaves against *C. albicans* the causing Vulvovaginal candidiasis is more effective than antibiotic Nystatin.
REFERENCES


