
Incidence and Type of the Vaginal Cavity Infections with Referring To the Role in the Potential of Hydrogen Changes during the Infection Period, Taif, KSA

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Abstract: This paper work was done for the incidence and type of the vaginal cavity (VC) infections (VCIs) with referring to the role in the potential of Hydrogen (pH) changes during the infection period (IP), Taif, KSA. Normal pH of VC is (3.5-4.5) for women in reproductive-age (RA). High vaginal swabs (HVSs) were collected from healthy women in RA, which complained of suspected VCIs. Incidence of micro-organisms (MOs) detection in the total HVS suspected specimens were resulted in (55.6 and 44.4%) for (MOs positive and negative specimens). Incidence of MOs Spp. were detected in the positive HVS specimens, the results indicated for (bacteria, yeast and protozoa) as (40.3, 13.4 and 1.9% was in related to the total HVS) and (72.5, 24 and 3.5% was in related to the positive HVS). The predominant MOs isolated were in BV included (G-positive and G-negative), yeast *Candida albicans* (CA) and protozoa *Trichomonas vaginalis* (TV). Incidence of the mean pH of VC during the IP under the treatments, VC-pH were for bacteria, yeast and protozoa at 1st day of infection as (7.2, 5.5 and 7.1). As well VC-pH were going to the normal degree with the treatments, as well at 9th day of IP were as (3.9, 3.6 and 4.1) for (bacteria, yeast and protozoa). VC-pH were returned to the normal after the cure of MOs infections. This paper concluded that, the incidence of VCIs and the role of MOs to change VC-pH during the VCIs period, that changing VC-pH help MOs in the replication and elongation of the microbial IP for the women.

Keywords: Vaginal Cavity (VC), Vaginal Cavity Infections (VCIs), Potential of Hydrogen (pH), Infection Period (IP), Reproductive-age (RA), High Vaginal Swabs (HVSs), Micro-organisms (MOs), Species (Spp.), Bacterial Vaginosis (BV), *Candida albicans* (CA), *Trichomonas vaginalis* (TV).

1. INTRODUCTION

Women in RA has VC-pH (3.5-4.5), that due to the primary colonizing bacteria of a healthy individual of the genus *Lactobacillus*^[1]. It has been generally considered as the gate-keepers of the VC-ecosystem. *Lactobacilli* had been shown to inhibit in-vitro growth of pathogenic MOs. Its achieved through the action of lactic acid primarily^[2], *lactobacilli* ensure long-term colonization of the VC through adherence to VC-epithelial cells, blocking the adherence of bacterial pathogens^[3]. Next to lactic acid production and competition for adherence, other antagonistic mechanisms consisted of hydrogen peroxide (H₂O₂), (broad-spectrum anti-microbial) and bacteriocin (target specific anti-microbial) production^[4]. Low VC-pH was generally accepted to be the main mechanism controlling the composition of the VC-micro-flora. Although the lactic acid produced by *lactobacilli* contributes to the VC-acidity, it was still not proven to be the primary source of low VC-pH, but the fact remains that most *lactobacilli* thrive best at a pH < 4.5^[5]. Changes in VC-pH by VCIs as BV was a poly-microbial disorder characterized by an increase in the VC-pH over 4.5, a reduction in or absence of *lactobacillus* colonization, and over-growth of several facultative and obligatory anaerobic bacteria, that clear the loss of acidity, to control bacterial growth, the VC was normally slightly acidic with a pH of (3.5-4.5)^[6]. It was higher in developing countries as (12-25%) of RA women in routine clinic populations^[7]. It caused by a complex alteration in the VC-microbial-flora with an up to 1.000 fold increased in *Gardnerella vaginalis* and a decreased in *lactobacilli*^[8]. It was presented with no-symptoms but could be accompanied by VC-irritation, discharges and fish-like odour^[9]. It was a disordering of the chemical and biological balance of the VC-normal-flora^[10], a syndrome characterized by a change in VC-ecology, where the normal-flora of *Lactobacillus* was replaced by a mixed MOs flora consisting of anaerobes and an increased in the VC-pH over 4.5; it was often originated as a result of a reduction in, or in the absence of, *Lactobacillus* colonization and

overgrowth of several facultative and obligate anaerobic bacteria^[11]. It was considered as the most common form of disease among women in RA as (19-24%)^[12]. A change in VC-normal-flora including the reduction of *Lactobacilli*, which may be due to VC-pH imbalance, allows more resistant bacteria to gain a foothold and multiply. One of the most direct causes of BV was douching, which altered the VC-flora and predisposes women to developing the BV^[13]. VCI's often had multiple causes varies between countries in (20-40%)^[14]. Another criteria for diagnosis of BV was Nugent's score^[15]. Vaginitis was the commonest Reproductive Tract Infections (RTIs) in RA women. RTIs were the major public health problems among women in RA especially in the developing countries^[16]. Diagnosis was suspected based on the symptom and may be verified by testing the VC-discharge and finding a higher than normal VC-pH and large numbers of bacteria^[17]. BV was caused by an imbalance of the naturally occurring bacteria in the VC. There was a change in the most common type of bacteria and a hundred to thousand fold increase in total numbers of VC-normal bacteria present^[18]. Prevalence of MOs in VCI's; BV, candidiasis and Trichomoniasis were responsible for 90% of VCI's cases. Vulvovaginitis, characterized by VC-discharge and/or vulvar itching, VC-irritation and odour were the most frequent and common reasons for women visiting Obstetrics/Gynecology (Ob/Gyne) clinics^[19]. CA was the most commonly implicated fungi infection of the vagina and vulva characterized by severe itching, burning sensation, soreness, irritation and whitish-grey cottage cheese-like discharge often with a curd-like appearance^[20]. TV was (17.6-20%)^[21]. Group B *Strept.* was a common MOs to colonize the VC. Enterobacteriaceae group were also isolated from culture of VC-discharge^[22]. TV was a sexually transmitted parasite causing vulvovaginitis characterized by intense frothy yellow-greenish VC-discharges, irritation and pain in the vulva, perineum and thighs, and dyspareunia and dysuria^[23]. VCI's accounts for 90% were cases in RA women was represented by the triad vaginitis: Candidiasis caused by CA. Bacterial vaginosis caused by bacteria *Spp.*, and some parasites, notably TV^[24]. Vulvovaginal candidiasis (VVC) affected 75% of women in RA at least once, nearly half will experience recurrences, and (5-8%) had multiple episodes/year^[25]. TV ranges were (0.4-27.4%) in RA women, the characteristic frothy, purulent discharge, punctate haemorrhagic areas called "strawberry cervix", lower abdominal pain and dyspareunia^[26]. Vaginitis was usually characterized by a VC-discharge and/or vulvar itching and irritation. The three diseases most frequently associated with VC-discharge were BV, replacement of the normal VC-flora by an overgrowth of anaerobic bacteria, TV and CA^[27]. Even in the modern advances in medicine, there was a rise in the incidence of fungal infections especially those due to *Candida Spp*^[28]. The three main kinds of vaginitis are BV, vaginal candidiasis, and Trichomoniasis. A woman may had any combination of VC-infections at one time^[29]. TV were (16.6-29%)^[30], *Candida Spp.* were the VC-normal-flora, under certain conditions, it may became pathogenic and caused candidiasis. The majority of these infections were caused by CA^[31]. BV was the most common of VCI's in RA women. The women affected in (5-70%)^[32]. BV, *Candida Spp.*, *E. coli*, G-positive Cocci and TV were (47.31, 29.75, 11.98, 3.41 and 1.21%). The age group which was most prone to the infection were (26-35 years, and 36-45 years) as (41.68 and 13.11%). The most common pathogens in VCI's were (BV, and *Candida Spp.*) as (47.3 and 29.8%). The distribution of *Candida Spp.* among different age groups showed the highest incidence in RA women group of (26-35 years)^[33]. Also genital infections were included in (26.5 and 1.2%) for (CA, and TV)^[34].

The aim: It was a study to determine the prevalence of MOs among women in RA and complained of VCI's by the symptoms. It was carried-up to detect the prevalence of infected MOs types and the changes in VC-pH by the presence of infected MOs. It was also investigated the contributions of VC-pH in the prevalence of the different MOs types infections and the role of MOs causing VCI's in changing of the VC-pH. As well follow-up the returning back of VC-pH to the normal degree during the IP of the women under the ideal treatments.

2. MATERIALS AND METHODS

Study-area Preparation: It was a great random collection of women patient suspected cases (WPSCs) for VCI's, so it was selected a suitable Ob/Gyne clinics in a private hospitals. This work was started after the agreements of the Hospital Owners, Doctors and WPSCs for this search work only and without any reminding of the personal information.

Patients Selection: WPSCs under-study were selected by Ob/Gyne Doctors, in a suspicion of VCI's. They were in RA (35±10 years), complained of VCI's by the symptoms, non-pregnant, healthy, and did not use any medications or contraceptive methods. The data were recorded of all WPSCs and they were asked in a standard questionnaire for their symptoms (VC-discharge, vulvovaginal itching,

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burning sensation, dysuria and dyspareunia). The amount, colour, character and smell of VC-discharge were all noted.

Specimens Collection: WPSCs must be at the 1st (10 days) of menses period, and they did not have any marry relation for at least 3 days. HVSs specimens were collected under aseptic condition and were labeled. The collected HVS specimens were 6 in number. Sterile HVSs were used for the collection of VC-discharge from VC lateral and posterior walls, for each WPSCs and were considered as (1st specimens group) at the 1st day of VCIs. The HVSs collected were transferred to Micro. Lab. within (30-60 min.), for Lab. detection tests. Moreover HVSs were collected again from MOs positive WPSCs at (3rd, 5th, 7th and 9th) days of VCIs, they were in IP and under the treatments were prescribed from Ob/Gyne doctors.

Lab. Detection Tests: It were included:

-1st: PH Measure Test: It was done for HVS on the glass slide, by use of Colour Litmus Strips with a pH range of (3.0-7.0), (Merck). Repeated pH Measure Test for MOs positive cases every other day at (3rd, 5th, 7th and 9th) day.

-2nd: Whiff-Amine Test: A drop of 10% KOH was added to HVS taken on a clean glass slide and fishy odour was noticed. A characteristic fishy odor was considered a positive suggestive of BV. It was identified using two different methods; Whiff-Amine Test and G-staining. Whiff-Amine Test was used to test for the production of a fishy ammonia-Cal smell and G-staining was used for microscopical identification of BV through VC-flora typing. The VC-flora were divided and labeled into 5 different types: (I) Only G-positive bacilli = normal VC-flora; (II) G-positive bacilli and few other bacteria; (III) Few G-positive bacilli and many other bacteria = BV; (IV) No G-positive MOs = BV; and (V) Presence of Clue cells, confirmed by: (Wet Mount Test: Placing a drop of sodium chloride solution on a slide containing HVS. Clue cells can be visualized under the microscope, as epithelial cells were coated with bacteria). Presence of 3 out of 5 criteria was necessary for diagnosis of BV^[35].

-3rd: Wet Mount Test: HVS was taken on a clean glass slide and a drop of normal saline added, mounted with a cover slip and counted pus cells and Clue cells. CA was identified as highly retractile, round or oval budding yeast cells, was identified through a step-wise process, and examined for budding yeast cells and pseudo hyphae using (Germ Tube Test).

-4th: G-staining Test: Smears were prepared by HVS specimen and was fixed by flaming, was stained by G-Method and was examined under microscope for detection of G-positive budding yeast cells with or without pseudo hyphae and any bacteria.

-5th: Protozoa Detection Test: HVSs were kept in tube containing 3 ml sterile Phosphate Buffered Saline (PBS), pH: 7.2, for Wet Mount Microscopy and Giemsa staining. The wet preparation was made immediately using a clean glass slide with cover and examined microscopically for motile TV. For Giemsa stain, HVSs smear were applied on a slide allowed to air-dry before fixing it with methanol for about 1 minute, and then stained with 20% Giemsa to be screened for TV trophozoites.

-6th: Isolation and Identification Test: HVSs were cultured on Blood agar (BA), MacConkey agar (MA), and Sabouraud dextrose agar (SDA), then were incubated. Identification of bacteria were done based on colony morphology, G-stain and biochemical reaction. CA was confirmed by (Germ Tube Test), as in (few colonies + human sera), which were subsequently viewed microscopical for the identification of tube-like yeast cells^[36].

Data Analysis: The all data were recorded and entered into Microsoft Excel Sheet, then summarized and analyzed in tables and graphs^[37].

3. RESULTS AND DISCUSSION

Table and graph1. Incidence of *MOs detection in total *HVS suspected specimens

Total examined *HVS specimens	*MOs positive specimens		*MOs negative specimens	
	*No	*%	*No	*%
Total *No=620	345	345/620 55.6%	275	275/620 44.4%

*MOs: Micro-organisms, *HVS: High Vaginal Swab, *%: percentage, *No: Number

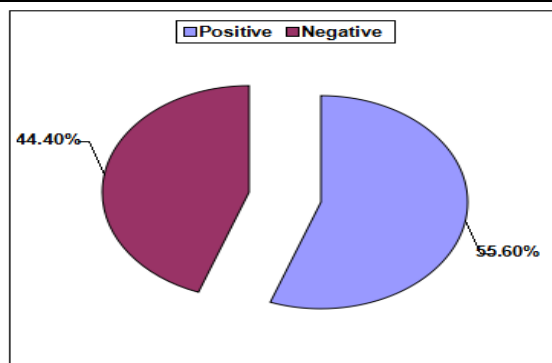


Table and graph 1 showed the incidence of MOs detection in total HVS suspected specimens, total specimens were (No=620), which resulted in (55.6 and 44.4%) for (MOs positive and negative) respectively. The results indicated the present of MOs in positive higher than negative from the suspected specimens. That it was cleared the VCIs play role in RA for women and with high prevalence of infection percentage as more than half of complained and suspected women. It was higher in developing countries, found in (12-25%) of RA women^[7]. It was the most common form of disease among RA women as (19-24%)^[12]. VCIs often had in countries (20-40%)^[14]. MOs were in VCIs, for about 90%^[19], in RA women^[24]. The half of RA women had experience recurrences, and (5-8%) had multiple episodes/year^[25].

Table 2 and graph2. Incidence of *MOs *Spp. detected in positive *HVS specimens

Isolates	Total isolates *No=345	*%	
		To total *HVS *No=620	To positive *HVS *No=345
Bacteria	250	250/620 40.3%	250/345 72.5%
Yeast	83	83/620 13.4%	83/345 24%
Protozoa	12	12/620 1.9%	12/345 3.5%

*MOs: Micro-organisms, *Spp.: species, *HVS: High Vaginal Swab, *No: Number, *%: percentage

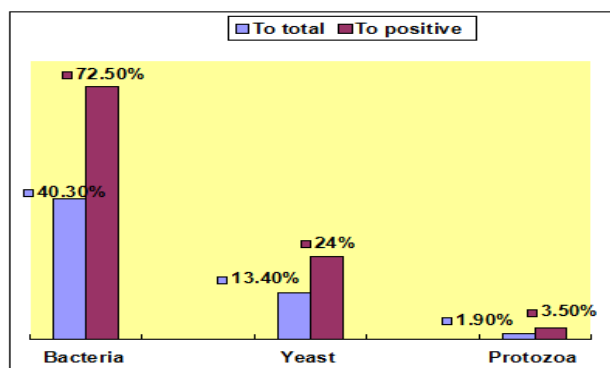


Table 2 and graph 2 showed the incidence of MOs Spp. detected in positive HVS specimens, the results indicated for (bacteria, yeast and protozoa) as (40.3, 13.4 and 1.9% in related to total HVS) and (72.5, 24 and 3.5% in related to positive HVS) respectively. BV in VCIs was higher than yeast and protozoa, it was more than 3 times of yeast and 20 times than protozoa. That it was cleared for the predominant of BV in VCIs and it had a role in change pH of VC, also yeast and protozoa had role in changing pH although with even low infection percentage of VCIs. The predominant MOs isolated were in BV included (G-positive and G-negative), yeast (CA) and protozoa (TV). Prevalence of MOs in VCIs; BV, candidiasis and Trichomoniasis were 90%^[19]. CA was the most commonly implicated fungi infection of the VC and vulva^[20]. TV was reported (17.6-20%)^[21]. Group B *Strept.* was a common MOs to colonize the VC. Enterobacteriaceae group were also isolated from culture of VC-discharge^[22]. TV was a sexually transmitted parasite causing vulvovaginitis^[23]. VCIs accounts for 90% were cases in RA women was represented by the triad vaginitis: Candidiasis caused by CA. Bacterial vaginosis caused by bacteria Spp., and some parasites, notably TV^[24]. VVC affected up to 75% of women in RA at least once, nearly half will experience recurrences, and (5-8%) had multiple epi-

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sodes/year^[25]. *TV* ranges were (0.4-27.4%) in RA women. ^[26]. The three diseases most frequently associated with VC-discharge were BV, replacement of the normal VC-flora by an overgrowth of anaerobic bacteria, *TV* and *CA*^[27]. Even in the modern advances in medicine, there was a rise in the incidence of fungal infections especially those due to *Candida Spp.*^[28]. The three main kinds of vaginitis are BV, vaginal candidiasis, and Trichomoniasis. A woman may had any combination of vaginal infections at one time^[29]. *TV* were (16.6-29%)^[30], *Candida Spp.* were the VC-normal-flora, under certain conditions, *Candida* may became pathogenic and caused candidiasis. The majority of these infections were caused by *CA*^[31]. BV was the most common of VCIs in RA women as (5-70%)^[32]. BV, *Candida Spp.*, *E. coli*, G-positive Cocci and *TV* were (47.31, 29.75, 11.98, 3.41 and 1.21%). The age group which was most prone to the infection were (26-35 years, and 36-45 years) as (41.68 and 13.11%). The most common pathogens in VCIs were (BV, and *Candida Spp.*) as (47.3 and 29.8%). Distribution of *Candida Spp.* among different age groups showed the highest incidence in RA group of (26-35 years)^[33]. Also genital infections were included (26.5 and 1.2%) for (*CA*, and *TV*)^[34].

Table3 and graph 3. Incidence of the mean *pH of *VC during the *IP under treatment

Items	Mean *pH at days of the infection period under treatment				
	1 st day	3 rd day	5 th day	7 th day	9 th day
Bacteria	7.2	6.5	5.8	4.7	3.9
Yeast	5.5	5.3	4.9	4.2	3.6
Protozoa	7.1	6.6	6	5.1	4.1

pH: Potential of Hydrogen, *VC: Vaginal Cavity, *IP: Infection Period, *MOs: Micro-organisms

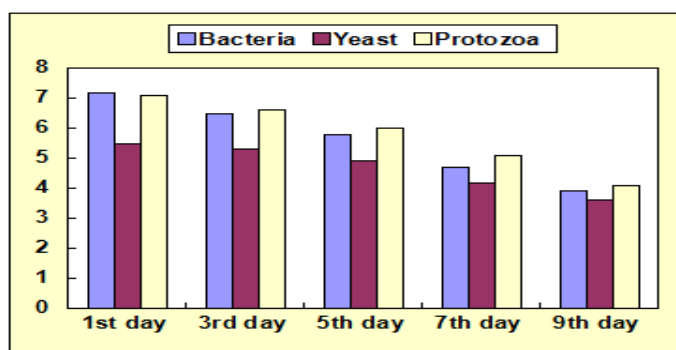


Table 3 and graph 3 showed the incidence of the mean pH of VC during the IP and under treatment, pH were returned to normal of VC during the IP with the treatments. pH was for bacteria, yeast and protozoa at 1st day of infection were as (7.2, 5.5 and 7.1) according the type of infected MOs. pH was different and special according to the type of infected MOs for replication and stay long time to cause more infections. As well pH were going to the normal VC-pH, at 9th day of IP were as (3.9, 3.6 and 4.1) for bacteria, yeast and protozoa respectively. That indicated the treatments were killed the infected MOs so there were in the end of IP the VC-pH was steady in normal range of (3.5-4.5). The pH were returned to the normal VC-pH after the cure of MOs infections. Women in RA has VC-pH (3.5-4.5), that due to the primary colonizing bacteria of a healthy individual of the genus *Lactobacillus*^[1]. It has been generally considered as the gate-keepers of the VC-ecosystem. *Lactobacilli* had been shown to inhibit in-vitro growth of pathogenic MOs. Its achieved through the action of lactic acid primarily^[2], moreover, *lactobacilli* ensure long-term colonization of the VC through adherence to VC-epithelial cells, blocking the adherence of bacterial pathogens^[3]. Next to lactic acid production and competition for adherence, other antagonistic mechanisms consisted of hydrogen peroxide (H₂O₂), (broad-spectrum anti-microbial) and bacteriocin (target specific anti-microbial) production^[4]. Low VC-pH was generally accepted to be the main mechanism controlling the composition of the VC-micro-flora. Although the lactic acid produced by *lactobacilli* contributes to the VC-acidity, it was still not proven to be the primary source of low VC-pH, but the fact remains that most *lactobacilli* thrive best at a pH < 4.5^[5]. Changes in VC-pH by VCIs as BV was a polymicrobial disorder characterized by an increase in the VC-pH over 4.5, a reduction in or absence of *lactobacillus* colonization, and over-growth of several facultative and obligatory anaerobic bacteria. That clear the loss of acidity, to control bacterial growth, the VC was normally slightly acidic with a pH of (3.5-4.5)^[6]. It was higher in developing countries, moreover, the disease had been found in (12-

25%) of RA women in routine clinic populations^[7]. It was a synergistic infection caused by a complex alteration in the VC-microbial-flora with an up to 1.000 fold increase in *Gardnerella vaginalis* and a decrease in *Lactobacilli*^[8]. It was presented with no-symptoms but could be accompanied by VC-irritation, discharges and fish-like odour^[9], it was a disordering of the chemical and biological balance of the VC-normal-flora^[10], a syndrome characterized by a change in VC-ecology, where the normal-flora of *Lactobacillus* was replaced by a mixed MOs flora consisting of anaerobes and an increased in the VC-pH over 4.5; it was often originated as a result of a reduction in, or in the absence of, *Lactobacillus* colonization and overgrowth of several facultative and obligate anaerobic bacteria^[11]. It was considered as the most common form of disease among women in RA^[12]. A change in VC-normal-flora including the reduction of *Lactobacilli*, which may be due to VC-pH imbalance, allows more resistant bacteria to gain a foothold and multiply. One of the most direct causes of BV was douching, which altered the VC-flora and predisposes women to developing BV^[13]. Vaginitis was the commonest RTIs in RA women. RTIs were the major public health problems among women in RA especially in the developing countries^[16]. Diagnosis was suspected based on the symptom and may be verified by testing the VC-discharge and finding a higher than normal VC-pH and large numbers of bacteria^[17]. BV was caused by an imbalance of the naturally occurring bacteria in the VC. There was a change in the most common type of bacteria and a hundred to thousand fold increase in total numbers of VC-normal bacteria present^[18].

4. CONCLUSIONS

This work concluded that, the incidence of MOs infection for the VC and the most predisposing and helping factors of MOs to change VC-pH during the microbial IP to help the MOs in the replication and elongation the microbial IP for the women in RA.

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