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Detection of Gardnerellavaginalis among Women at Reproductive Age in Al- Medina Al-Munawwarah, KSA by Polymerase Chain Reaction

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Abstract:

Background*: Bacterial vaginosis is a popular condition, influencing a large number of women yearly and related with various wellbeing issues including preterm labor leading to low birth weight, pelvic inflammatory disease and procurement of the human immunodeficiency infection. The main manifestation of bacterial vaginosis is foul vaginal discharges and numerous influenced females are asymptomatic.*

Objective*: This study aimed to detect G.vaginalis among women in the reproductive age in Al-Madinah Al-Munawwarah, KSA.*

Patients and Methods: *A cross-sectional laboratory based study was performed on 119 HVS collected from patients who attended Maternity and Children Hospital and were clinically suspected with bacterial vaginosis, during the period from September 2016 to April 2017. Specimens were processed with conventional tests and positive results were confirmed by PCR.*

Results: *20 samples out of 119 (16.8%) were found positive for G. vaginalis, 31 samples (26%) for candida spp., 17 samples (14.3%) for streptococcus spp. In addition, the remaining 43 samples (36.13%) were found free from infection.*

Conclusion: *In the target population, the prevalence of bacterial vaginosis caused by G. vaginalis appeared with a significant percentage (17%). G. vaginalis infection was more prevalent among the age group 20-30 years (the reproductive age). In non-pregnant women, prevalence of G. vaginalis (43.3%) was significantly higher than in pregnant women (15.3%). PCR was found as a rapid and an accurate technique that can be useful in detection of G. vaginalis.*

Keywords: *Bacterial vaginosis, G.vaginalis,Vaginal flora,Al-Madinah Al-Munawwarah, KSA.*

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I. Introduction

The normal flora of the vagina transcendently comprises of *Lactobacillus* spp., with *Lactobacillus crispatus*, *Lactobacillus jensenii* and *Lactobacillus iners*being the most predominant species[1,2,3]. The ecologic dynamics related to the vaginal microbiota shift during Bacterial vaginosis (BV) has not been fully understood . [4,5] It is believed that, with few exceptions, all BV- associated microbial species exist in low concentrations in the vaginal ecosystem of healthy women [4] .Risk factors that have been associated with BV include frequent vaginal douching,Ethenic group, having multiple sex partners, a new male sex partner, and sex with a woman [6,7] .Incontrast, hormonal contraceptive use, male circumcision, andsteady condom utilization reduce the rate of BV [6,8,9]. Bacterial vaginosis is a popular condition, influencing a large number of ladies yearly [10] and related with various wellbeing issues including preterm labor leading to low birth weight [11,12] , pelvic inflammatory disease[13,14]and procurement of the human immunodeficiency infection[15,16]. Symptoms of BV include thin, whitish-gray discharge with an unpleasant odorand numerous influenced females are asymptomatic [17] . This condition is typically associated with a shift in the vaginal flora from a homogeneous, lactobacillus-dominated state to a heterogeneous state containing a complex population of anaerobic and microaerophilic organisms, for example, *Gardnerellavaginalis* and *Prevotella, Peptostreptococcus* and *Bacteroides* spp. [4,18,19,20] .

One of few microorganisms that are found in females identified with bacterial vaginosis is *G.vaginalis*.They are found in humanbeing and some kinds of animals like mares, and horses.Also found in the urinary tract and the bladder, in the endometrium, fetal membranes, and newborn infants and are caused by

maternal infections, neonatal infections, and suppurative lesions. It can also be transmitted by sex [21] . *G.vaginalis*, in the pastknown as *Haemophilusvaginalis* and *Corynebacteriumvaginale*, is a facultative anaerobic, non-motile, pleomorphic gram-negative to gram-variable rod bacteria. It is makescolonization on the female genital tract and survives high pH [22] . It also survives poorly in human urine at 37º C (23). High prenatal mortality in pregnant women that resulting from premature labor and preterm delivery that caused by BV [11,24,25,26] .BV-associated microorganisms and their toxins causes brain injury for fetuses. BV is considered a risk factor for long-term neurological consequences in children, such as hyperactivity, academic difficulties in school and severe handicaps such as cerebral palsy and periventricular leucomalacia[27,28,29] . The most important method in molecular techniques that have been developed rapidly and widely in the clinical diagnosis laboratory is PCR method, that depend on the amplification of nucleic acid and that opened a new world in more and more accurate detection of microorganisms [30].

This study aimed to detect of *G.vaginalis* among women in the reproductive age in Al-Madinah Al-Munawwarah, KSA and to determine the usefulness of molecular methods as a tool of diagnosis.

II. Patients And Methods

This is a descriptive cross-sectional laboratory based study was carried out during the period from September 2013 to April 2014 in Al-Madinah Al-Munawwarah, KSA. The study targeted women (One hundred and nineteen (119) women of reproductive age (75 pregnant and 44 non -pregnant)) attending Obstetrics and Gynecology clinic and emergency unit in Maternity and Children Hospital in Al-Madinah Al-Munawwarah, Saudi Arabia. Sampling was taken as follows: we usenon –lubrificated speculum, one sterile cotton swab was inserted into the vaginal vault. The swab was rotated against the vaginal wall at the mid portion of the vault and was carefully removed to prevent contamination with vulva and introitusmicroflora. The swab was placed into Amies transport media (comp, count) which was used for anaerobic culture and making smear (wet preparation and Gram stain). The swab sent to the Microbiology Laboratory throw4 hours.

Conventional Method for Identification

Different biochemical tests including: Whiff test, Catalase test Oxidase test, HVS Culture on 5% Human blood agar and Gram's stain were used to identify target organisms.

DNA Extraction

Simple boiling method was used for extraction of the DNA. The primers used in amplification were:
5_GGGCGGGCTAGAGTGCA-3_ forward and
5_GAACCCGTGGAATGGGCC-3_ reverses.

PCR Reaction

100 pmol/ µl from forward primer was dissolved in 320µl of distilled water.
100 pmol/ µl from reverse primer was dissolved in 320µl of distilled water.
A master mix reagent was prepared for 50 reactions as following:
125ul of 10 x PCR buffer, 125ul MgCl2, 50ul from each dNTP (100mM), 10ul of each primer, 25ul Taqpolymerase (5u/lug) and 855ul distilled water. The master mix was distributed in PCR tube (24µl tube) and 1µl of template DNA from each sample was added to separate tubes.

The PCR assay was carried out in a total volume of 25 µL of mixture containing 2 µLMaxime PCR Premix containing 1X PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTP, and 1 U Taq DNA polymerase, 1 µL from 0.2mM forward primer and 1 µL from 0.2mM reverse primer was added (2 µL), 5 µL of template DNA and 18 µL of (nuclease free water). the contents of master mix was vortexed after addition of each item . In negative control 5µl of sterile distilled water was added, while DNA extracted was used as positive control .The amplification conditions included three steps: initial denaturation step at 95°C 10 minutes for one cycle followed by repeating cycles of denaturation (30 seconds at 95°C), annealing (30 seconds at 58°C) and extension (30 seconds at 72°C) for 40 cycles, followed by a 7 minutes final extension step at 72°C.

The gel casting tray was put into the electrophoresis, tank flooded with 1x TBE buffer just to cover the gel surface, then 7µl of PCR products were mixed with 3 µl of promo phenol blue stain and one sample was put into each well. Then to the first well of casting tray 5µl of DNA ladder (marker) were injected in each run. The electrophoresis was performed at 90 volts for 30 min, after that the gel was removed by gel holder and visualized using U.V transilluminater and the gel was photographed using Gel documentation system.A positive result of *Gardnerellavaginalis* will produce a band of (210 bp).

III. Results

Identification Scheme

Direct and indirect gram's stain from high vaginal swab, different bio chemical tests and culture media were used to identify the target isolates as illustrated in Figures 1 (A, B, C and D).

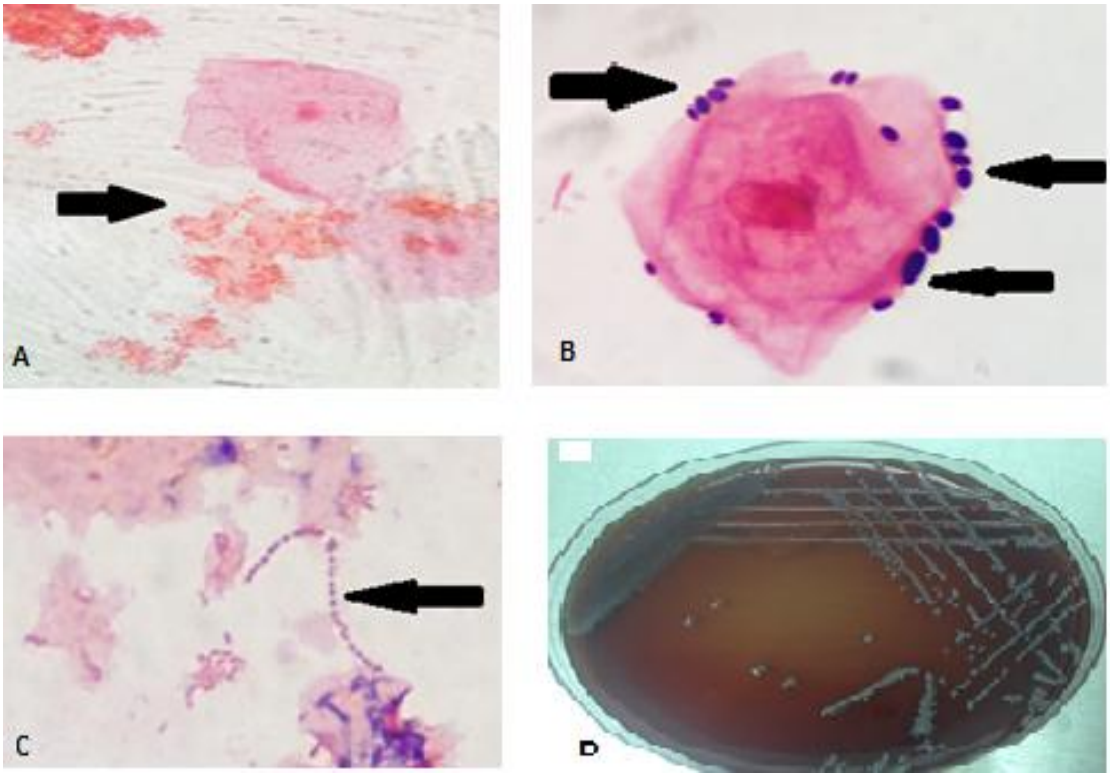

Figure 1. A: Direct Gram's stain from high vaginal swab of*G.vaginalis* showing Clue cells; B: *C. albicans* surrounded epithelium cell; C: *Streptococcus* spp. neighboring to the epithelium cell; D: 24 hours growth of *G. vaginalis* on Blood agar showing white-creamy colonies.The results reflected low ratio of*G.vaginalis* among enrolled subjects 20/119 (17%) as shown in figure 2.

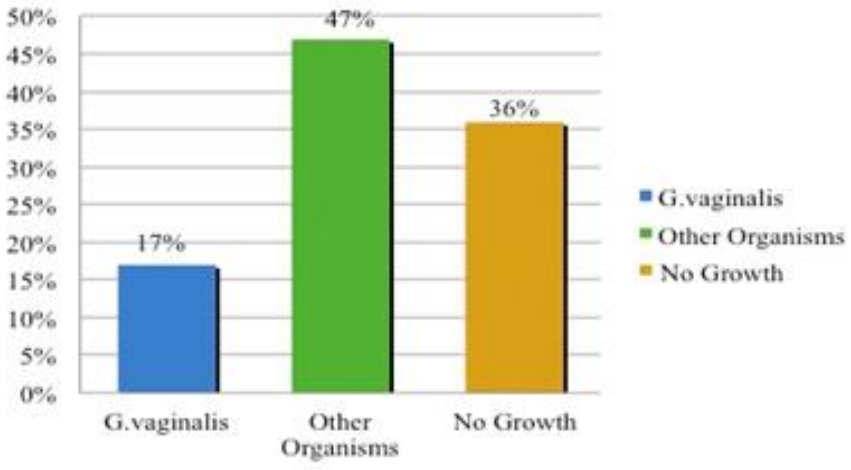

Figure 2. Distribution of bacterial growth among enrolled subjects

Frequency of the Isolates

The data obtained in this study confirmed clearly the existence of vaginal infection among 76/119 (64.7%) subjects, 20/76 (26.3%) was *G.vaginalis* out of them eight as co-infection with other organism. On the other hand organisms other than *G. vaginalis* were detected in this study these include: *Candidaalbicans*35/76 (46.1%) out of them four as co-infection with *Streptococcus* spp., 21/76 (27.6%) out of them four as co-infection with *Candida albicans* as shown in Figure 3.

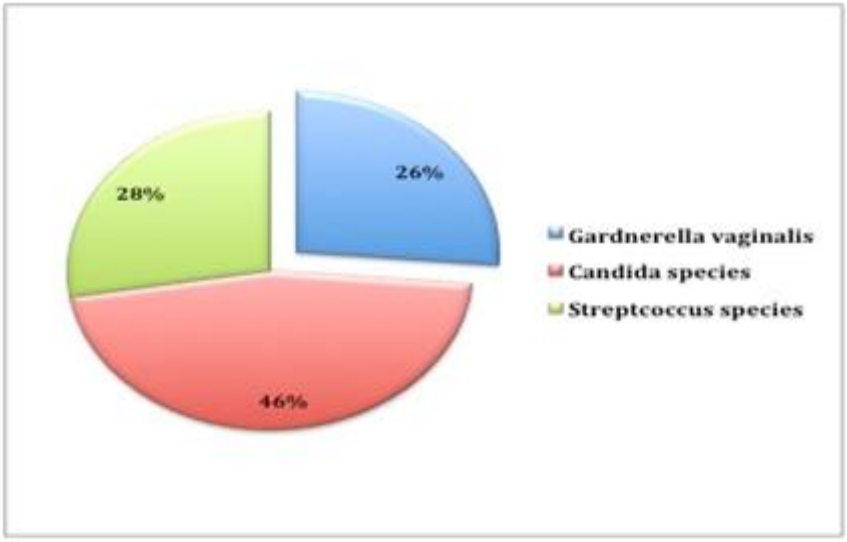

Figure 3. The Frequency of Isolated Organisms among Enrolled Subjects.

PCR Results

From the 20 specimens which were tentatively identified as *G. vaginalis* were directly subjected to PCR. 20/20 (100.0%) showed a band typical in size (210bp) to the target *gene* as indicated by the standard DNA marker (Figure 4).

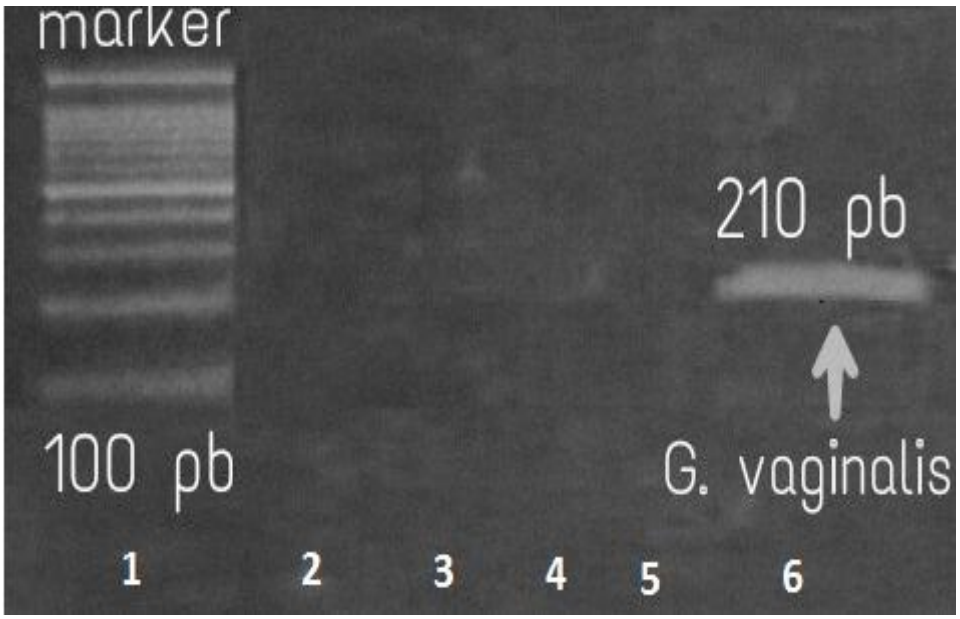

Figure 4.2% agarose gel electrophoresis of PCR products: lane 1: DNA marker (1000bp); lane 2: negative control; lanes (3, 4, 5) samples showing negative results; lane6: sample showing positive result for *G. vaginalis* (210 pb).

Epidemiological Findings

The results confirmed the existence of*G.vaginalis* among enrolled subjects with a significant ratio. All candidates with *G. vaginalis* were classified into four age groups as illustrated in Figure 5.

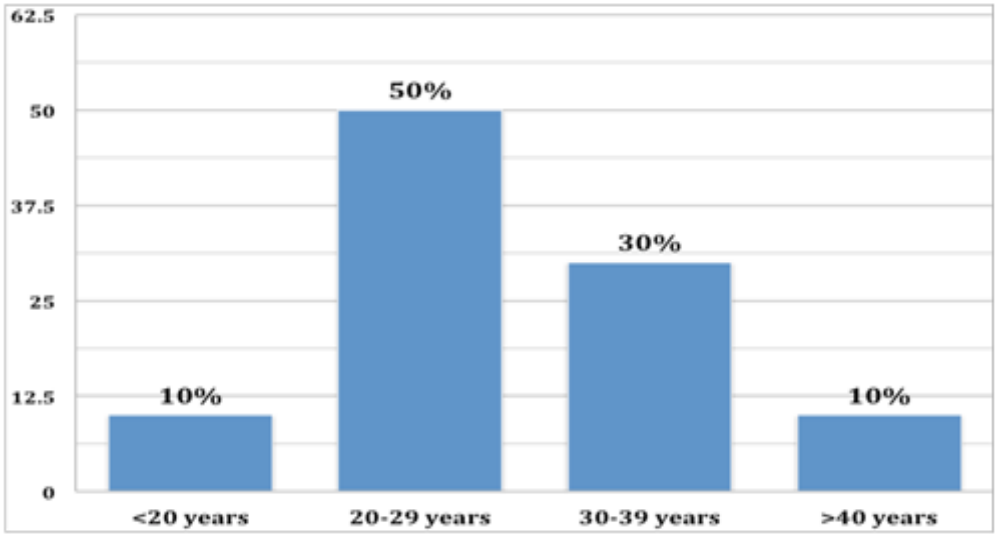

Figure 5. Distribution of *G. vaginalis* among patients according to age groups.

Frequency of Vaginal Infection among Pregnant Women

In this study 75 pregnant women were included (75/119, 63.0%) as shown in Figure 6 and 7.

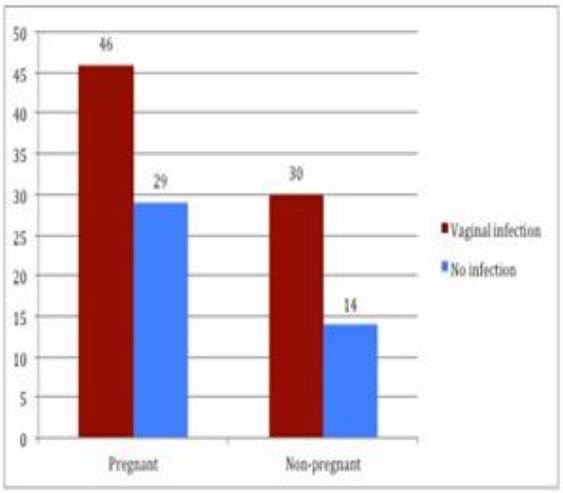

Figure 6. Frequency of vaginal infection among pregnant women.

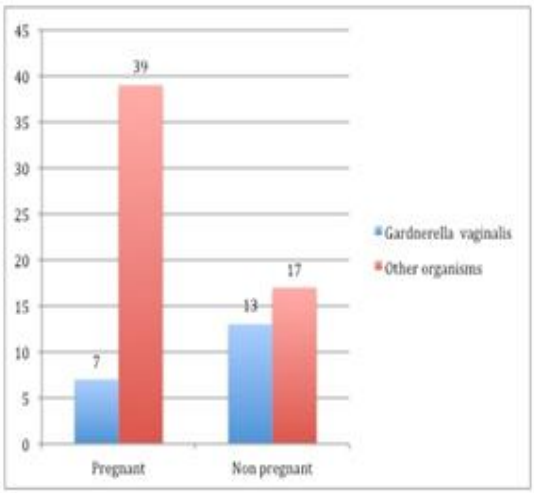

Figure 7. Frequency of *G. vaginalis* among pregnant women.

IV. Discussion

This study aimed to aimed to provide evidences aboutthe prevalence of *G. vaginalis* among women in reproductive age with sings of bacterial vaginosis attending the Maternity and Children Hospital in Al-Madinah Al-Munawwarah, KSA. And to phenotypically as well as genotypically identify the causative agent(s) through PCR technique.

The results confirm the existence of vaginal infection in 63.8% of the study population, similar results were obtained in Riyadh, KSA byTahany, et al .,2013[31] who reported 61.3% among enrolled women and this is an alarming of increased incidence of vaginal infection in KSA. Also, similar results were reported in Ghana byGloria and Daniel, 2013 [32] as they confirmed that 66% of student populations were presented with vaginal infection.

The results of *G. vaginalis* confirmed that (26.3%) of women included in the study. In Riyadh, KSA, Tahany,.,2013[31] reported that 80.4% of enrolled women were infected with *G. vaginalis*. In England (69%) of infection with bacterial vaginosis was reported byJolly, 1983 [33]and in America (87%) were reported byAlla, et al., 2001 [34]. Similar results were listed in Ghana byGloria and Daniel, 2013 [32], (28%) and in

Bangladesh (25.5%) byBegum, et al., 2011 [35], moderate to slightly high results were reported in Portugal by Debora,2014 [36], who found an infection rate of (36.9%).

In this study, the results of *candida* spp. were (46.1%). Lower percentage was obtained in Riyadh, KSA byTahany, et al., 2013 [31] which was (16.3%) and in Ghana by Gloria and Daniel, 2013[32] was (16%). However, in Tanzania, identical results (45%) were reported byNamkinga, et al., 2005 [37]. This result shows the high prevalence of vaginal candidiasis in women of Al-Madinah Al-Munawwarah, KSA.

Streptococcus spp. was reported in (27.6%) of this in the study population. This disagree with both the low percentage (2.2%) found in Riyadh, KSA byTahany, et al., 2013 [31]and the high percentage (58.7%) which was reported from Greece byTansarli, et al., 2013 [38].

In this study, the age group (20-29 years) was the one with the highest percentage of infection (50%) then (30-39 years) was with the moderate percentage (30%), and the lowest percentage was observed in the older group (more than 40 years, 10%) and younger group (less than 20 years), (10%). That means age could be considered as a risk factor in the spread of the bacterial vaginosis caused by *Gardnerellavaginalias*. A similar result was by Tahany et al., 2013 [31]that the age group (21-30 years) had the highest percentage (52.2%) and in age group (31-40) had the moderate percentage (30.4%) but differs in age groups (less than 20 years and more than 40 years), which had the percentages (4.4% and 13%) respectively.

In this study bacterial vaginosis caused by *Gardnerellavaginalis* in pregnant women represent (15.3%) and in non-pregnant women represent (44.3%). Identical finding was reported in Bulgaria byRaina, et al., 2013 [39]who showed that in non-pregnant women the percentage was (44.3%) but it was different in pregnant women where the percentage was (41.1%). That means there are increase of BV in non-pregnant women more than pregnant women.

V. Conclusion

Whilst the prevalence of bacterial vaginosis caused by *G. vaginalis* was relatively high (26.3%) PCR technique is more efficient over conventional methods in diagnosis of *G. vaginalis*, especially from direct HVS samples as it revealed rapid, accurate, specific and sensitive results.

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