Molecular Detection of *Gardnerella vaginalis* 16S r DNA gene among Women of Reproductive Age in Riyadh, Saudi Arabia.

Tahany. I. Hamid , Hamedelnil . F.Y , Harish Khubnani

Department of Microbiology, Rabia hospital, Riyadh, Saudi Arabia.

Department of Microbiology, Sudan University of Science and Technology, Khartoum, Sudan.

Department of Microbiology , Prince Salman hospital, Riyadh, Saudi Arabia.

Corresponding author: Tahany .I. Hamid ,e.mail: tahanymo@hotmail.com

ABSTRACT

The aim of this study was to detect *Gardnerella vaginalis*, the main causative agent of bacterial vaginosis, more than other opportunistic anaerobic organisms that colonized in vagina.

Atotal of 74 isolates of *Gardnerella vaginalis*, were isolated from patients with bacterial vaginosis n=(75/150). Isolated *Gardnerella vaginalis* were examined for biotype (hippurate hydrolysis, catalase and oxidase activity) and genotype with molecular technique (PCR) Amplified ribosomal DNA (16S rDNA).

Out of 150 women that were visited the Obstetric and Gynecology Clinic in Rabia hospital, Riyadh- Saudi Arabia in the period between July 2011 to June 2012, who were included in this study (n=92/150) 61.3% were have vaginal infection. *Gardnerella vaginalis* was (n=74/92) 80.4% of women with bacterial vaginosis while *Candida species*, *Streptococcus species* and *Trichomomas vaginalis* were 16.3%,2.2% and 1.1 % respectively.

Keywords: *Gardnerella vaginalis*, 16S r DNA , bacterial vaginosis.

1.1 Introduction

From an ecological point of view, the vagina may be considered a complex anatomical site, where several bacterial species coexist and develop complex relationships .Over 50 species of microorganisms have already been isolated from the vagina, some species occupying a predominant position, guaranteeing their survival and contributing to the prevention of infectious diseases and health maintenance (Liven good, 2009).

The vagina is protection with the vaginal mucosa depends on the specific recognition of structures on the lactobacilli surface (adhesions) and the vaginal epithelium (receptors). This adhesion-receptor interaction results in the formation of a biofilm that exerts a protective local action against colonization by undesirable microorganisms (Boris *et al*.,1998) and sterile on birth. After a few days, when maternal estrogen raises the glycogen level of the epithelial cells, the baby's vagina is colonized by lactobacilli from the mother (Forsum *et al.*, 2005).

The etiology of bacterial vaginosis (BV) is probably multifactorial, and the condition is not regarded as an sexual transmitted infection (STI), though it is sexually associated. One factor is an increase in vaginal pH from the normal 3.5-4.5 to 7.0, which reduces the inhibitory effect of hydrogen peroxide on anaerobic growth (Martin *et al*., 2008).

This is associated with loss of *Lactobacilli* and an up to 1000-fold increase in the concentration of several organisms, most commonly *Gardnerella vaginalis*, *Bacteroides* (*Prevotella*) spp, *Mobiluncus* spp and *Mycoplasma hominis*. Hormonal changes and inoculation with organisms from a partner might be important (Phillip, 2002).

Bacterial vaginosis is the commonest cause of vaginal discharge among women of reproductive age. Symptoms and findings of bacterial vaginosis have been recognized for centuries. The condition has also been termed *Gardnerella vaginitis*, non-specific vaginitis and anaerobic colpitis (Hung *et al*., 2000) .

Bacterial vaginosis is a common condition affecting millions of women annually, and is associated with numerous health problems including preterm labor resulting in low birth weight, pelvic inflammatory disease (PID), and acquisition of the human immunodeficiency virus. Malodorous vaginal discharge may be the only symptom of bacterial vaginosis, and many affected women are asymptomatic (David *et al*, 2005).

The standard treatment for bacterial vaginosis is metronidazole 400 mg for 5 days, an alternative is a 2g single dose. The cure rate immediately after treatment with metronidazole is up to 95%, but after 4 weeks this falls to 80% in open-label studies and less than 70% in blinded studies. Topical treatments with intravaginal 2% clindamycin cream or 0.75% metronidazole gels are licensed for the treatment of bacterial vaginosis. They are more expensive than oral metronidazole, but have similar efficacy and can be useful when systemic treatment is not desirable.

2.2 Materials and methods

Study design and collection of the samples

This is descriptive cross sectional laboratory based study at women were attending Obstetrics and Gynecology clinic in Rabiah Hospital in, Riyadh Saudi Arabia. This study was carried out during the period from July 2011 to June 2012. Of 150 women who were included in the study (n=92/150) 61.3% were diagnosed bacterial vaginosis. *Gardnerella vaginalis* was (n=74/92) 80.4% of women with bacterial vaginosis caused by *Gardnerella vaginalis*.

High vaginal samples were collected by two sterile cotton swabs. One swab was returned to sterile tube (dry swab) for making smear(wet preparation and Gram stain) for the purpose of grading according Nugent scoring system. The other swab was placed into Amies transport media (comp, count) was used for anaerobic culture. The swabs were rotated against the vaginal wall at the mid portion of the vault and were carefully removed to prevent

contamination with vulva and introitus microflora Both swabs were processed in microbiology laboratory within 4 hours.

Dry swab was used for measuring pH on test strip ColorpHast pH 4.0-7.0.

A drop of normal saline on a clean slide was added then the swab was rotated to made suspension then covered with cover glass.

The preparation was examined under x 40 magnification to detect the clue cells. Swab containing vaginal secretion was placed in a test tube containing 0.5 cc of 10% KOH (Whiff test), fishy odor indicates possible bacterial vaginosis. These tests were carried out according to Amsel′s criteria.

Phenotypic Properties

Colony morphology obtained as tiny smooth, roundish colonies with zone of β-haemolytic on *Gardnerella* selective agar . Gram stain was done showed either Gram-negative or variable *cocco-bacilli*. Different biochemical reactions were used for initial identification(catalase test, oxidase test and hippurate test).

Genotypic Characterizations

Polymerase chain reaction was used to confirm the results of the conventional methods .

DNA Extraction

The extraction of DNA was made with Phenol-Chloroform Isoamyl Alcohol used as described by Barker, *et al* (1998).

The concentration of extracted DNA was read using the spectrophotometer (Bioeppendrof, Primer 125v, 500 mA, UK).

16Sr DNA was detected by PCR. The amplification was done by using (CONVERGYS® td peltier thermal cycle ,Germany). For amplification of the sequence 16S r DNA (1500 bp) internal transcribed spacer region, the oligonucleotide primers

5_TTCGATTCTGGCTCAGG-3_ [forward] and

5_CCATCCCAAAAGGGTTAGGC-3_ [reverse] was used.

Molecular analysis

The amplifications were performed in a final volume 20µl .The PCR mixture was subjected to initial denaturation step at 95°C for 3min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 1 min and extension at 72°C for 1 min, the last cycle had the extension step (72°C) for 5 min. These conditions were chosen after a series of preliminary experiments to optimize amplifications of *Gardnerella vaginalis* target sequences. A blank control tube containing no added nucleic acid was run with every set of reaction matures to control for the inadvertent introduction of exogenous nucleic acid. The presence and yield of specific PCR products were controlled by agarose (1% w/v) ethidium bromide gel electrophoresis in TAE. 7µl of PCR products of each samples was analyzed by electrophoresis apparatus to the power supply (Primer, 125v, 500 mA, UK). Then the electrophoresis was done in at 75 volts for 30 min, after that the gel was removed by gel holder and visualized by U.V transilluminater (Uvite –UK). And the gel was photographed by using the Polaroid film.

Data analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version-13.5 (SPSS Inc. Chicago). A descriptive analysis was used to describe the population enrolled in this study.

Result

Epidemiological Findings

Collection of Specimens

A total of one hundred fifty (n=150) High vaginal swabs were collected from patients attending Rabiah Hospital with vaginal infection at the period from July 2011 to June 2012 in Riyadh, from different ages. Out 92 samples (61.3%) samples gave a significant pure growth,

while the rest 58 samples (38.7%) non pathogenic organisms (normal flora). As shown in figure 1 bellow:

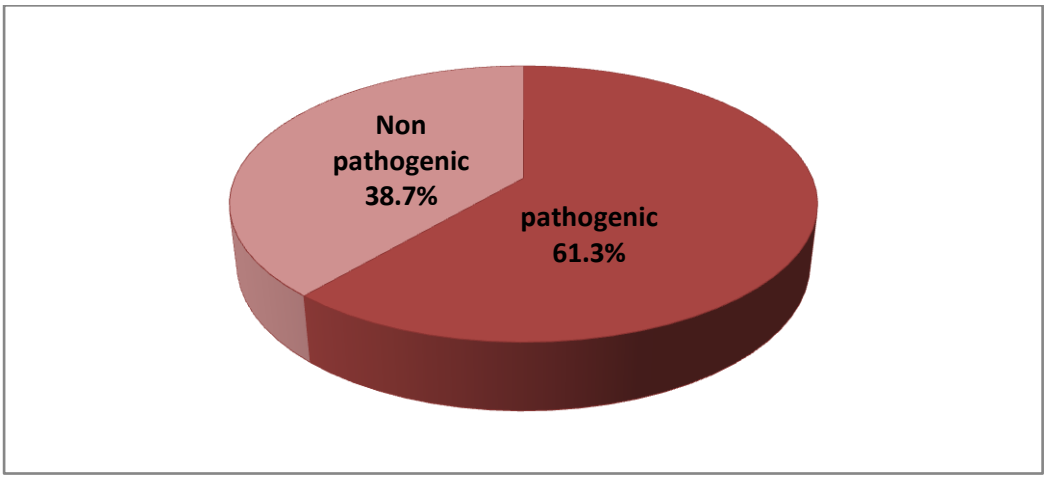


Figure (1):The distribution and percentage of bacterial growth

Bacteriological Findings

Frequency of the Isolate

The data obtained in this study confirmed clearly the existent of 92/150 (61.3%) pathogenic organisms 74/92 (80.4%) was *Gardnerella vaginalis*. On the other hand there were other organisms detected such as *Candida species* 15/92 (16.3%), *Streptcoccus species* 2/92 (2.2%) and *Trichomonas vaginalis* 1/92 (1.1%) as shown in Table1bellow

NO	Growth	Frequency	Percentage %
1	*G.vaginalis*	74	80.4%
2	*Candida species*	15	16.3%
3	*Streptcoccus species*	2	2.2%
4	*Trichomonas vaginalis*	1	1.1 %
Total		92	100%

Table (1): The frequency and percentage of isolated organisms.

Age group

All candidates were classified into four age groups; age group one less than 20 years old with lowest frequency of 4(4.4%) , age group two (20-30 years old) with the highest frequency of 48 (52.2%), age group three (31-40 years) with moderate frequency of 28 (30.4 %) and age group four with low frequency 12 (13.0%) as shown in the figure 2 bellow .

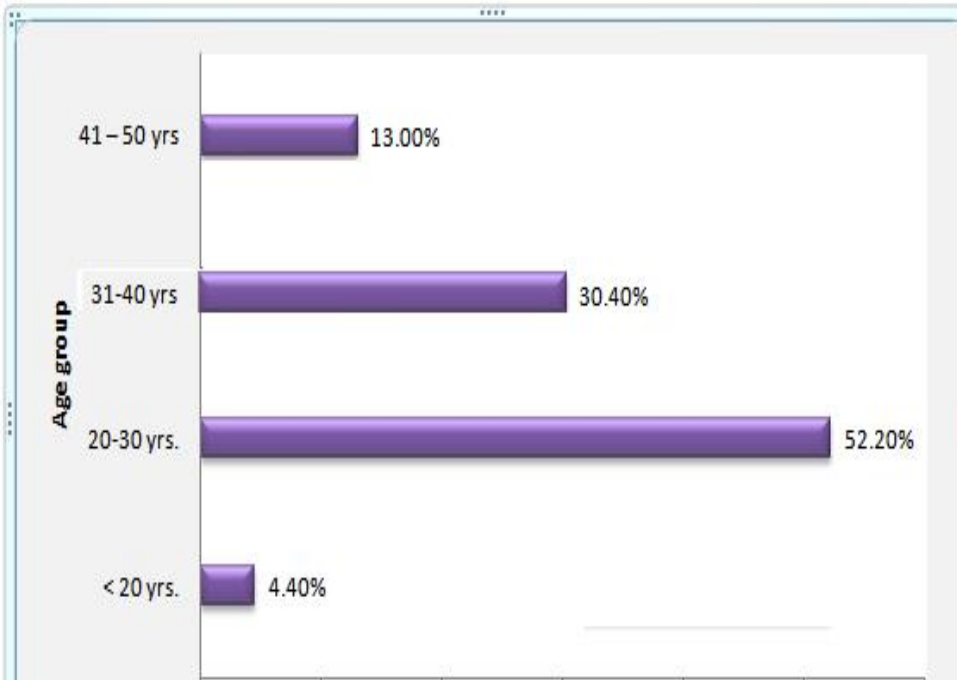


Figure (2) Distribution of samples among patients according to age groups.

Pregnancy

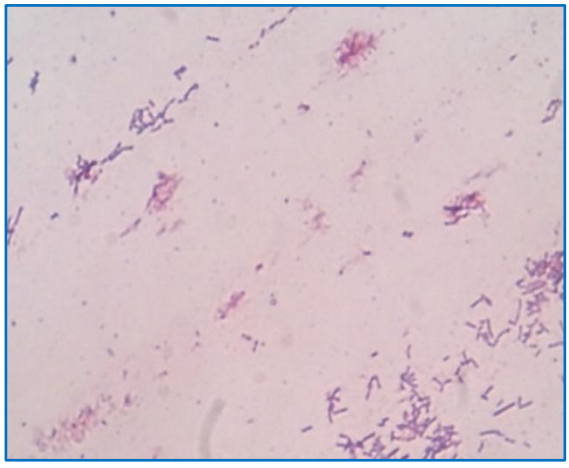
Among the study population 150 patients who were attending to Obstetrics and Gynecology clinic 35 were pregnant , 23(65.7 %) of them have vaginal infection *Gardnerella vaginalis* was17 (73.9%) and 6 others(26.1%). 115 were non-pregnant, 69(60.0%) of them have vaginal infection 57(82.6%)*Gardnerella vaginalis* and 12 (17.4%) others as shown in the table 2

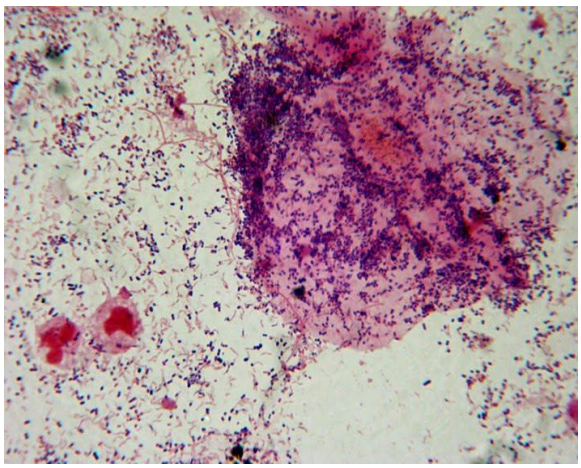
Isolated Organism	Pregnant		Non-pregnant	
	Freq	%	Freq	%
Gardnerella vaginalis	17	73.9	57	82.6
Other	6	26.1	12	17.4
Total	23	100	69	100

Table(2):Correlation between isolated organisms and pregnancy among the study population

Phenotypic Properties

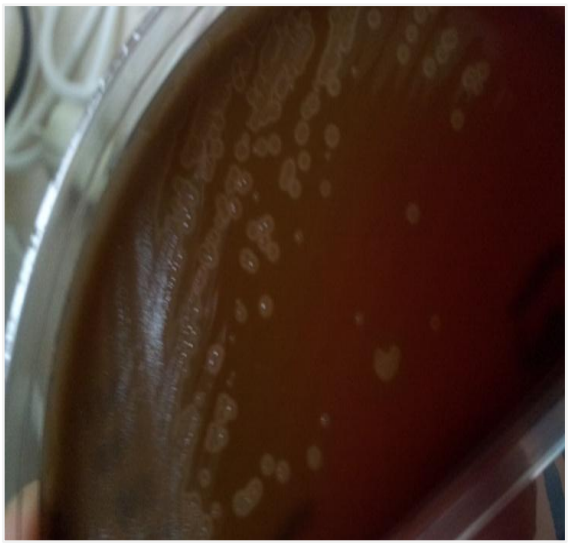
Gram stain, colony morphology and different biochemical reactions were used for initial identification. The results of these tests were listed in color plates as shown in figures bellow:

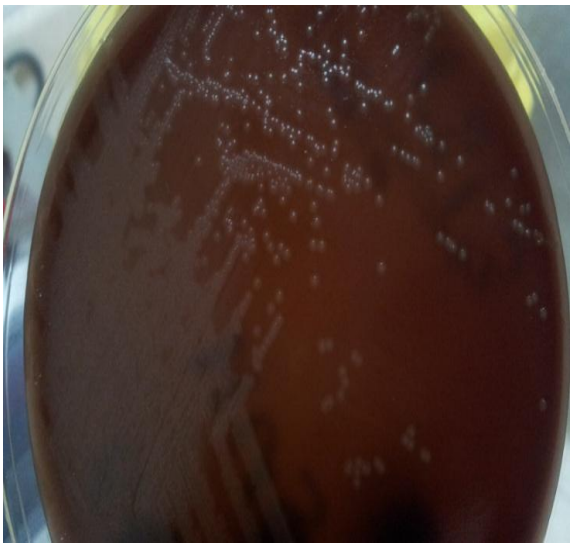




(3) (4)

Figures : (3) Gram stain of *Gardnerella vaginalis* (Gram variable *coccobacilli*). (4):Gram stain direct from the swab shown″ clue cell″.





(5) (6)

(7)

Figures (5&6): Colonial morphology of *Gardnerella vaginalis* on a *Gardnerella* selective agar. (7) *Candida spp*. isolated on Sabroued Dextrose Agar (SDA).

Genotypic detection of 16S r DNA

Out of 74 *Gardnerella vaginalis* isolates that gave positive result by phenotypic test only 70/74 (94.6%) isolates gave positive while there were 4 isolates gave negative result by PCR.

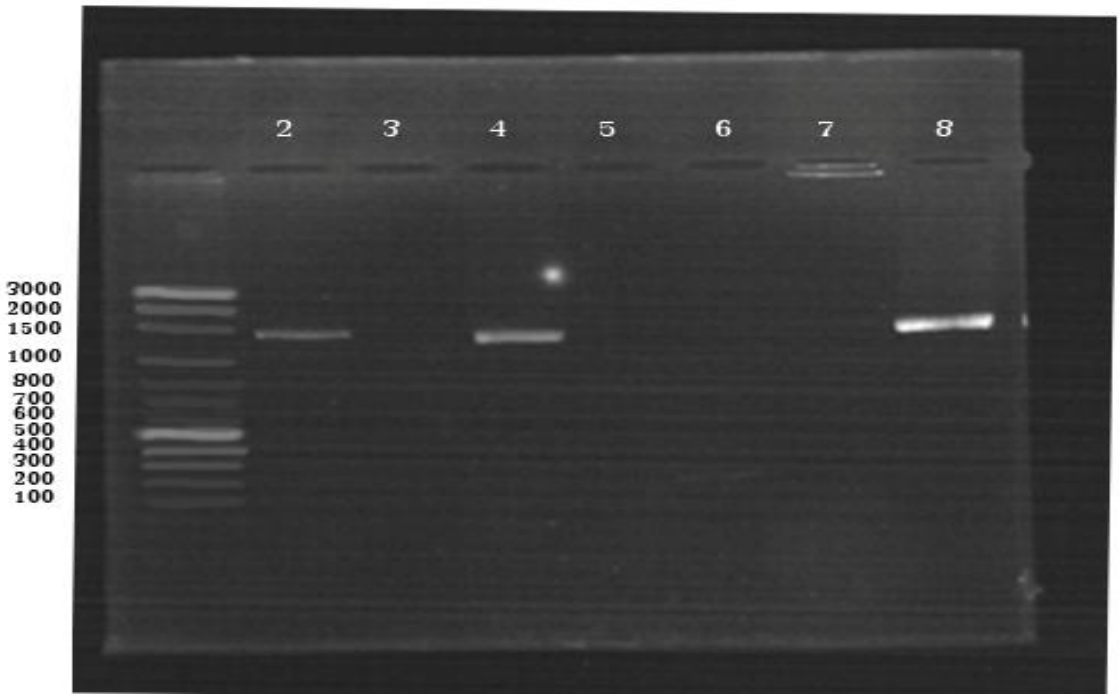


Figure (8): 1 % agarose gel electrophoresis of *Gardnerella vaginalis* isolated 16S r DNA gene .The lane one Marker M.W (100-3000 bp) fragments. The lane three is negative control. The lane 2,4,8 with aband typical in size (1500 bp) which are positive for 16S r DNA. The lane 5,6 and 7 they are negative isolates.

Discussion

Gardnerella vaginalis is the common cause of bacterial vaginosis . Further bacterial vaginosis is associated with a sizable burden of infectious complication, diagnosis relies on standardized clinic criteria (Amsel′s critaria) or on scoring bacterial cell morphotypes on a Gram-stained vaginal discharge smear.

But Amsel′s criteria may be misleading due to the appearance of vaginal secretion may be altered by factors such as douching or recent intercourse, both *Candidiasis* and *Trichomoniasis* can give a similar clinical appearance . Positive potassium hydroxide test and increased vaginal pH may be found during menstruation or in the presence of semen and detection of clue cells can confused with debris or degenerate cells.

In addition, some concern remains ,over the performance of the Nugent scoring system. First, it has been acknowledged that Nugent′s criteria are widely applied in the absence of standardized pre-analytical and analytical conditions. Emphasized the need for quality specifications in this respect as different sampling devices and procedures, different ways of spreading the vaginal specimen on the glass slide leading to differences in homogeneity of the sample and in the thickness of the smear, different fixation methods and time , and differences in the area of high power oil immersion filed at magnification ×100 all may affect Gram –stain interpretation.

The aimed of the present study was to detect, isolate and identify *Gardnerella vaginalis* by conventional and molecular methods and to evaluate the usefulness of PCR as rapid confirmatory test for bacterial vaginosis.

The percentage of *Gardnerella vaginalis* observed in this study (80.4%).

In England 203/294 (69%) were identified as *Gardnerella vaginalis* .

In Amrica of the 117 women visiting the gynecology clinic at Rush-Presbyterian –St,. luk′s Medical Center were 27.2% were found to have bacterial vaginosis . *Gardnerella vaginalis* was found (87.5%) .

In Canada(98.5%) *Gardnerella vaginalis* was isolated ,but in Nigeria (25.1%) was isolated.

In Thomas′s Hospital Medical School in London (83%) identified as *Gardnerella vaginalis* .

In this study out of 92/150 (61.3%) patient with vaginal infection ; *Gardnerella vaginalis* was 74/92 (80.4%) , *Candida species* 15/92 (16.3%) , *streptococcus species* 2/92 (2.2%) and 1/92 (1.1%) *Trichomonas vaginalis*. This increasing in prevalence and concentration of *Gardnerella vaginalis* among patient with this syndrome emphasize the fact that *Gardnerella vaginalis* plays a significant role in pathogenesis of bacterial vaginosis.

In this study the age group (20-30 years) was had the higher frequency of 48/74 (52.2%) then (31-40 years) (30.4%) ,(41-50 years) (13.0 %) and the lowest frequency is group(less than

20 years) (4.4%) that means distribution of the bacterial vaginosis caused by *Gardnerella vaginalis* .

Among the study pupulayion pregnant women were infected with *Gardnerella vaginalis* (65.7%) and non-pregnant were (73.9%).

PCR method has several potential advantages in studying the biology of bacterial vaginosis over the two most commonly used methods for diagnosed bacterial vaginosis, the Amsel′s criteria and the Gram stain.

Conclusion

Historically the literature regarding bacterial vaginosis largely focused on *Gardnerella vaginalis* in particular .This study confirms that *Gardnerella vaginalis* is most bacterial vaginosis causative agent in women of

reproductive ages in Riyadh –Saudi Arabia.

Recommendation

Primary and secondary prevention of bacterial vaginosis is hampered by a lack of information about the condition's natural history and the ecology of the vaginal flora, less than optimal treatment regimens and the lack of well designed studies evaluating the effectiveness of screening and

treatment in specific populations. Uncertainty about risks fuels controversial interpretations and inconsistent standards of care.

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