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Frequency of detection of Gardnerella vaginalis in vaginal smears in the Upper Carniola region

Irena Grmek Košnik[1] ✉, Urška Dermota[1], Andrej Golle[1]

Abstract

Introduction: Bacterial vaginosis is of clinical interest because of its possible causal relationship with complications during pregnancy, postpartum, and complications after surgery.
Methods: Gram stain for clue cells and *Gardnerella vaginalis* culture methods were evaluated retrospectively in a microbiological medical laboratory for the first half of 2015. We were interested in the proportion of *G. vaginalis* bacteria isolated from genital samples, correlation with Gram-staining presence of clue cells, referral clinical diagnosis, and pregnancy.
Results: In the first half of 2015 we received 358 vaginal specimens; 82% of them had a referral clinical diagnosis of colpitis, cervicitis, or vaginal discharge; 40% were pregnant women. *G. vaginalis* was isolated from 14% of vaginal specimens, and 52% of these came from pregnant patients. Gram stain clue cells and isolation of *G. vaginalis* matched in 86%.
Conclusion: For diagnosing bacterial vaginosis in clinical practice, standard clinical criteria, Gram staining of vaginal discharge smear, and/or isolation of *G. vaginalis* are used. Isolation of *G. vaginalis* without clue cells is reported only in cases in which bacterial growth is predominant. The results of our studies confirm that isolating *G. vaginalis* helps confirm the diagnosis of bacterial vaginosis.

Keywords: *Gardnerella vaginalis*, vaginal smears, Gram staining, culture, bacterial vaginosis

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Introduction

Gardnerella vaginalis is the only species of the genus *Gardnerella*. It consists of facultatively anaerobic, oxidase- and catalase-negative, nonsporing, nonencapsulated, nonmotile, pleomorphic, Gram-variable rods (1). It grows slowly on standard cell culture medium and is difficult to distinguish from other bacteria in the vagina. It grows on sheet blood agar in the form of tiny colonies, either anaerobic or in 5% CO2. A suitable growth medium is Columbia Blood Agar Base, on which bacteria forms a beta hemolysis. *G. vaginalis* is found in the vagina of 15% to 69% of asymptomatic women and 13.5% of girls.

G. vaginalis is almost universally present in the vagina of women with bacterial vaginosis (BV), where it is found with mixed anaerobic flora (2). BV is a condition in the vagina in which the normally present lactobacilli are replaced by anaerobic bacteria. Patients with BV most often complain of odor and discharge, which tends to be gray and homogenous. Vulvovaginal irritation is usually not a prominent symptom, hence the use of term *vaginosis* rather than *vaginitis* (3). BV represents a serious public health problem because it is connected with premature births, premature rupture of membranes, chorioamnionitis and neonatal meningitis, endometritis, transmission of the human immunodeficiency virus (HIV) and other sexually transmitted diseases (4, 5). Bacterial vaginosis is triggered by sexual transmission of the bacteria *G. vaginalis*, which has virulent agents that enable attachment to epithelial cells of the host, creating a biofilm (4). Numerous researchers have found statistically significant links between BV and infection with the herpes simplex virus and also with infection with human papillomavirus (6, 7).

Due to broad diversity in selection of patients' material, methods, and criteria for diagnosis of BV, in various studies the isolation rate of *G. vaginalis* varies from 6 to 94% (8).

Direct examination of vaginal secretions is the gold standard for diagnosis of BV because a positive culture of *G. vaginalis* can also be recovered from healthy women. The typical smear of vaginal discharge from BV patients shows clue cells (bacteria covering epithelial cell margins) together with mixed flora consisting of large numbers of small rods and coccobacilli: gram-negative *Prevotella* and *Porphyromonas* spp. and gram-variable *G. vaginalis)* coccobacilli. Lactobacilli are almost always absent. It is recommended that a standardized Gram staining interpretative scheme be used in order to improve the reproducibility of this method (9).

Gram-stained vaginal smears are the least expensive and fastest among the laboratory methods. However, high intracenter variability has been shown using the Gram stain for diagnosis of BV (10).

This study compares two laboratory methods for detecting *G. vaginalis*: the Gram stain for clue cells and the *G. vaginalis* culture methods.

Patients and methods

We performed a retrospective analysis of the microbiological results of vaginal swabs sent in the first half of 2015 to the Kranj department of the microbiological laboratory at the National Laboratory of Health, Environment, and Food (NLZOH), which covers approximately one-tenth of the Slovenian population with its microbiological services.

The samples were sent from the gynecological clinics of healthcare centers in Upper Carniola and from the general hospital in Upper Carniola.

Microbiological analysis: *G. vaginalis* was detected using two tests. The first was the Gram stain, with which we were looking for epithelial clue cells. The second method was isolation of *G. vaginalis* on human blood agar incubated in an anaerobic or CO2 atmosphere. Mass spectrometry using MALDI TOF technology

[1]National Laboratory of Health, Environment, and Food, Maribor, Slovenia. ✉ **Corresponding author: irena.grmek.kosnik@nlzoh.si**

(Bruker, Billerica, MA) was used to identify *G. vaginalis*, the enterobacteria, *Streptococcus agalactiae* and *Candida* spp.

Statistical methods

Matching of the results of clue cells in the Gram stain and isolation of *G. vaginalis* was statistically analyzed for significance using a chi-square test. The analysis was performed by Microsoft Excel. $P < 0.05$ was taken as significant.

Results

Out of 358 patients included in this study, 148 (41%) had no pathogenic bacteria in the vaginal swab, 67 (19%) had yeasts, 46 (13%) had enterobacteria, 47 (13%) had *S. agalactiae* and other streptococcus, and 50 (14%) had isolates of *G. vaginalis*. The number of genital samples of pathogenic bacteria received, the number (%) of isolates of *G. vaginalis*, Gram stain matching with bacterial vaginosis (%), clinical diagnosis (%), and pregnancy (%) are presented in Table 1.

Matching of results of clue cells and isolation of *G. vaginalis* was 86% (Table 2). The difference between methods was statistically significant (chi-square; $p < 0.01$; Table 2). Using both methods, the detection rate of *G. vaginalis* increases from 50 to 57 out of 350 samples (from 14.3% to 16.3%).

The frequencies of bacteriological isolates in each clinical condition are presented in Table 1. We found no statistically significant differences between the proportions of written clinical diagnosis on the referral letters between smears positive and negative for *G. vaginalis*.

Discussion

The term *bacterial vaginosis* (BV) was introduced by a group of researchers from Washington University that established that non-specific vaginitis is connected with large changes in the vaginal flora, proving this through the molecular method of 16s RNA sequencing. This group also defined the clinical criteria for BV as follows: white milky secretion, pH of the vaginal excrement over 4.5, fishy smell after adding 10% KOH to the vaginal secretion, and at least 20% of vaginal epithelial cells covered with tiny coccobacilli (clue cells). Coccobacilli are best appreciated at the edges of the cell: when they abound, they partially obscure the nucleus. Not all cells in the specimens are clue cells, but some clue cells are seen in more than 90% of patients with BV (9). For a clinical diagnosis of BV, at least three of four criteria must be met (11).

Soon after the introduction of these criteria, Nugent et al. changed the Gram stain criteria. They proposed using a combination of most reliable morphotypes detected in the vaginal smear; namely, *Lactobacillus* spp. (Gram-positive bacilli), *G. vaginalis* (Gram-negative coccobacilli), and *Mobiluncus* spp. (Gram-negative bent bacilli). A weighted score of 0 to 3 is characteristic for normal flora (prevailing lactobacilli), and 7 to 10 for BV (absence of lactobacilli, two bacterial species prevailing). The weakness of this method is that it is time-consuming and demands trained staff (11, 12). Mota et al. found that both Amsel's and Nugent's methods have comparable diagnostic efficacy for diagnosing BV (13).

In our retrospective analysis, we identified the presence of *G. vaginalis* in 14% of vaginal swabs. We were aware that *G. vaginalis* can also be found in women without clinical signs of infection. It has to be taken into consideration that gynecologists decide on microbiological testing of the vaginal tract only in cases of clinical complaints. In our study, clinical data (clinical diagnosis, pregnancy) were obtained from referral letters. The difference between the results of the Gram stain and isolation of *G. vaginalis* was statistically significant. The most probable reason is that the Gram stain criteria are not uniform among our laboratory personnel.

Our data are fairly comparable with another Slovenian study, in which bacterial vaginosis was determined clinically and microbiologically in women in three hospital wards of the Ljubljana Gynecology Clinic. A diagnosis of BV was established in 5.5% of 75 pregnant women at the Pathological Pregnancy Clinic, in 14.0% of 100 women before therapeutic abortion at the Day Clinic, and in 23.0% of 13 women at the Sexually Transmitted Disease Clinic. A correlation was found between bacterial vaginosis and sexual behavior. Due to the small number of women investigated, a correlation could not be confirmed between bacterial vaginosis and premature birth (14).

At the Slovenian microbiological laboratory, we confirm BV by detection of clue cells and the absence of lactobacilli in direct Gram stain and with isolation of *G. vaginalis*. We do not use the Nugent score system. In our study, Gram-stained clue cells and isolation of *G. vaginalis* matched in 86% of samples. A significant association was found between clue cells and *G. vaginalis*, which was in line with earlier studies (15).

Kelsey et al. showed that isolation of *G. vaginalis* and anaerobes helps confirm the diagnosis of BV and distinguish it from other pathology. Compared to healthy women, the isolation of *G. vaginalis* was the most sensitive indicator of BV (100%), although

Table 1 | Number of genital samples for pathogenic bacteria received and number (%) of isolates of *G. vaginalis* in the first half of 2015 at the medical microbiology laboratory in Kranj, Slovenia.

	Genital samples analyzed for pathogenic bacteria	Isolates of *G. vaginalis* (%)	Isolates of *S. agalactiae* (%) and streptococci	Isolates of *C. albicans* (%) and other *Candida* spp.	Isolates of enterobacteria (%)	No pathogenic bacteria/yeast isolated (%)
No. of genital samples	358 (100%)	50 (14%)	47 (13%)	67 (19%)	46 (13%)	148 (41%)
Pregnancy	144 (40%)	26 (52%)	20 (42%)	29 (43%)	10 (22%)	77 (52%)
Diagnosed	295 (82%)	38 (76%)	40 (85%)	55 (82%)	39 (85%)	121 (82%)
Cervicitis	159 (44%)	14 (28%)	22 (47%)	36 (54%)	25 (54%)	65(44%)
Vaginal discharge	65 (18%)	12 (24%)	6 (13%)	11 (16%)	5 (11%)	31 (21%)
Vaginitis/vaginosis	37 (10%)	10 (20%)	8 (17%)	3 (4%)	7 (15%)	9 (6%)
Preterm labor	29 (8%)	2 (4%)	4 (9%)	5 (7%)	2 (4%)	16 (11%)
No diagnosis	68 (18%)	12 (24%)	7 (15%)	12 (18%)	7 (15%)	27 (18%)
Clue cells	50 (14%)	43 (86%)	1 (2%)	1(1%)	(0%)	5 (3%)

Table 2 | Matching of results of clue cells in Gram stain and isolation of *G. vaginalis* at the clinical microbiology department in Kranj, Slovenia. The difference between methods is statistically significant (chi-square; $p < 0.01$).

Clue cells	Isolation of *G. vaginalis*		
	Positive	Negative	Total
Positive	43	7	50
Negative	7	293	300
Total	50	300	350

it was not very specific (77.4%). Anaerobes were more specific (93.2%). Anaerobes in vaginal culture were a better predictor of BV (30.8%) than isolation of *G. vaginalis* (18.9%) (16).

Spiegel noted an inverse relationship between the quantity of the *Lactobacillus* morphotype and the *Gardnerella* morphotype on the Gram stain. When the *Lactobacillus* morphotype predominates (3 to 4+) with or without the *Gardnerella* morphotype, the Gram stain can be interpreted as normal. When the Gram stain shows mixed flora with few or no *Lactobacillus* morphotype (0 to 2+), the Gram stain is suspicious for BV (11).

Schwebke et al. studied the prevalence of *G. vaginalis* in healthy women. Vaginal specimens were self-collected daily for 30 days and analyzed by PCR. In half of the women, at least one specimen was positive for *G. vaginalis* (17).

Metronidazole is successfully used to treat bacterial vaginosis, highlighting the significance of anaerobic bacteria. Routine treatment of the sexual partner is not recommended. It is recommended to search for and treat bacterial vaginosis in women liable to premature birth, women before abortion, and women before hysterectomy (18).

With bacterial vaginosis, changes in the species of the lactobacilli can also be observed. *Lactobacillus iners* is present with bacterial vaginosis, and *Lactobacillus crispatus* prevails in the vaginal flora of women without BV symptoms. New laboratory methods allow more frequent identification of *G. vaginalis* and *Atopobium vaginae*, thus making it possible to identify pregnant women with BV and in this way provide therapy and prevent the risk of premature birth. Antibiotic treatment in preventing BV recurrence is not particularly effective because recurrences appear often. A better effect is expected with the use of new antibiotics (19).

Bacterial vaginosis is more common among homosexual women (20). Infections with *G. vaginalis* in children are rare. Invasive infections appear only in newborns (5).

Conclusion

Bacterial vaginosis affects a large number of women and has been associated with premature birth, chorioamnionitis, and postcesarean endometritis. Combining Gram-stained vaginal smears and isolation of *G. vaginalis* increases the diagnostic sensitivity for BV.

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