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Vaginal flora changes and reproducibility of interpretation of Gram-stained vaginal smears

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The vagina is characterized by a finely balanced microbial ecosystem [1] which can be altered by a number of factors, such as hormonal therapy and other

iatrogenic factors, e.g. antibiotic therapy, although in most cases of vaginal flora changes the cause is unknown. Lactobacilli are the predominant organisms in the vaginas of most women, with an important role in maintaining a stable ecosystem. The production of hydrogen peroxide by lactobacilli is proposed to be important for that balance. In vitro, lactobacilli can inhibit the growth of pathogenic microorganisms which may invade the vagina, including *Escherichia coli*, *Candida albicans*, *Gardnerella vaginalis* and *Mobiluncus* spp. Many other mechanisms have been proposed to explain the role of lactobacilli in protecting the vagina from invading pathogenic organisms, e.g. the low vaginal pH generated by lactic acid production, and competition of the lactobacilli with other pathogens for adherence to vaginal epithelial cells or cell receptors.

The aims of the present study were to compare the interpretations of Gram-stained smears by a trained observer and a trainee, and to confirm the relationship between the presence or absence of lactobacilli and various vaginal infections. The subjects were 746 women who had attended for contraceptive advice. Vaginal smears from all of the women were available, vaginal cultures were available from 696 (93.3%) and full data for analysis were available from 691 (92.6%). Vaginal fluid was collected from the mucosa on cotton swabs; these were then rolled over glass slides and the preparations were fixed with gentle heating. The smears were Gram-stained using carbol fuchsin as a counter-stain and examined under oil immersion ($\times 100$) by one venereologist (observer A) and one trainee in microbiology (observer B). Each observer was asked to interpret the slides according to the criteria of Spiegel et al [2].

Cultures for human papilloma virus (HPV), *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Gardnerella vaginalis* and anaerobic bacteria were made as described elsewhere [3].

The results showed excellent to good agreement between the two observers regarding the dominance or deficiency of lactobacilli, vaginal flora changes like those seen in bacterial vaginosis (BV) and the presence of clue cells (kappa index 0.80, 0.78, 0.54 and 0.56) (Table 1). The consistency of detection of a flora change was excellent to good when the two observers' results were compared (kappa index 0.79, 0.52, 0.69 and 0.40, respectively; Table 1).

Deficiency of lactobacilli, as detected by Gram-stained vaginal smears was significantly associated with a high frequency of HPV (OR=2.0, 95% CI=1.1-3.6). *G. vaginalis*, *M. hominis* and *U. urealyticum* occurred significantly more often in the women lacking lactobacilli than in those with lactobacilli (Table 2). The association of these organisms with deficiency of

Table 1 Vaginal flora changes in apparently healthy women ($n=746$) in Gram-stained vaginal smears examined by two independent observers (A and B)

	No. of smears (%)		Kappa index
	A	B	
Lactobacillus dominance	496 (66.7)	518 (69.4)	0.80
Lactobacillus deficiency	145 (19.4)	156 (20.9)	0.78
Bacterial vaginosis	133 (17.8)	158 (21.2)	0.54
Clue cells	81 (10.9)	91 (12.2)	0.56
Cocci	221 (29.6)	206 (27.6)	0.79
Gram-negative rods	154 (20.6)	237 (31.8)	0.52
Gram-positive cocci	238 (31.9)	280 (37.5)	0.69
Gram-variable rods	80 (10.7)	180 (24.1)	0.40

Table 2 Vaginal flora in women with no lactobacilli (Lacto-, $n=141$) as compared to those with lactobacilli in the vagina (Lacto+, $n=550$)

	Lacto+ (%)	Lacto- (%)	OR	95%CI
Human papillomavirus	35 (6.6)	17 (12.3)	2.0	1.1-3.6
<i>Gardnerella vaginalis</i>	74 (16.6)	76 (67.9)	10.6	6.7-17.2
<i>Mycoplasma hominis</i>	33 (6.1)	56 (40.3)	10.4	6.4-17.1
<i>Ureaplasma urealyticum</i>	321 (59.3)	100 (72.5)	1.8	1.2-2.7
<i>Mobiluncus</i> spp.	143 (27.1)	65 (47.8)	2.5	1.7-3.6
Anaerobic bacteria	86 (15.6)	89 (63.1)	9.3	6.2-14.1
Bacterial vaginosis	30 (5.7)	73 (53.7)	19.1	11.8-32.0

lactobacilli as detected by Gram-stained vaginal smears remained significant after exclusion of cases with BV defined by Amsel et al, i.e. when three out of the following four criteria are fulfilled: a homogeneous gray vaginal discharge, a vaginal pH ≥ 4.7 , a positive amine test and the presence of clue cells [4]. A mixed anaerobic flora, as detected by Gram-stained vaginal smears, occurred significantly more often in the lactobacillus-deficient women than in those with lactobacillus dominance (Table 2). This was the case both before and after exclusion of cases with BV.

Nugent et al [5] proposed a standardized protocol based on the Spiegel et al [2] criteria to be followed for interpreting Gram-stained vaginal smears which they found to be reproducible when read by microbiologists and technicians at different microbiological laboratories. However, we did not follow this protocol in our study. A high level of intra-observer and inter-observer agreement in the interpretation of Gram-stained vaginal smears in women with BV has also been reported [6]. Our results also show close agreement between trained and trainee observers.

A number of studies have evaluated the role of lactobacilli in maintaining a healthy vaginal environment and the association of lack of dominance of lactobacilli with disease [1]. We have described women who we designated as having the 'lactobacillus deficiency syndrome' [7]. Women with this syndrome (as defined by negative lactobacilli on culture) were older, more likely to have given birth and to have been pregnant more often and less likely to use oral contraceptives than the women who were culture positive for the organism [7]. The high prevalence of HPV in the lactobacillus-deficient women observed by us has, to the best of our knowledge, not been reported before. A higher frequency of cervical HPV infection has been found in women with BV [8]. After adjustment for sexual risk behavior such as younger age at first sexual intercourse, increased number of lifetime sexual partners and increased casual sex, the association of HPV and BV was no longer significant, but the association with a positive amine test remained significant. This might indicate that a changed vaginal milieu favors cervical HPV infection.

The association between lack of lactobacilli and BV is well established. More than 50% of the women deficient in lactobacilli in this study had BV according to our Gram-stained slides. The standard diagnosis of BV is at present based on clinical observations and some simple doctor's office tests [4]. When it became evident that BV is associated with a vaginal flora change, the use of Gram-stained vaginal smears alone to establish the diagnosis was proposed [2,9]. Dominance and deficiency of lactobacilli and the presence of clue cells as detected by Gram-stained vaginal smears showed a significant agreement between the two observers in the present study. Also, vaginal flora changes remained significantly more frequent in women lacking lactobacilli as detected by Gram-stained vaginal smears, even after exclusion of BV cases.

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