

Prevalence of Lactobacilli in Normal Women and Women with Bacterial Vaginosis

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Objective: To study the prevalence and the number of lactobacilli in normal subjects and in women with bacterial vaginosis (BV).

Material and Method: The studied subjects consisted of 60 normal and 60 BV women. The diagnosis of BV was based on at least three of five indicators of Chandeying criteria. The vaginal specimens were collected and cultured on MRS plates for determination of lactobacilli counts.

Results: The number of lactobacilli was highly significant ($p < 0.001$) in the women with diagnosis of BV, and the mean count of lactobacilli in BV was markedly decreased (5.3 ± 1.8 log colony-forming unit - CFU/ml), compared with normal women (8.8 ± 2.9 log CFU/ml). Among the treated BV group, follow-up vaginal specimen was encountered in 47 of 60 cases (78.3%). The proportion of lactobacilli isolation was slightly increased from 12 (25.5%) to 15 (31.9%) in 47 cases. Concordance with quantitative settlement of lactobacilli in BV was increased from 1.4 ± 2.6 log CFU/ml, to 1.8 ± 2.9 log CFU/ml in treated BV. Treated BV, the lactobacilli had not restored significance ($p = 0.5831$), as well as the total bacteria.

Conclusion: Lactobacilli dominantly occur in healthy women, and markedly decline in BV. However, the vaginal ecosystem is dynamically changed in the lactobacilli of either normal or BV women.

Keywords: Prevalence, Lactobacilli, Bacterial vaginosis

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Bacterial vaginosis (BV) is the most common abnormal vaginal condition and is the leading cause of abnormal vaginal discharge (AVD) accounting for up to 48% of the cases⁽¹⁻⁵⁾. Though BV is common in most populations, the prevalence of BV is dependent on use of varying diagnostic criteria. Normal vaginal flora has a predominance of *Lactobacillus* species that constitutes up to 96% of the total bacterial flora, and most women with *Lactobacillus* dominant flora have H₂O₂-producing lactobacilli. In contrast to normal women, one-third of the women with BV have no lactobacilli, and the remainder has lower concentrations of non-H₂O₂-producing lactobacilli. Lactobacilli are present at concentrations of 10⁵ to 10⁸ colony-forming

units (CFU)/ml of vaginal secretions in normal women, and H₂O₂ produced by certain strains of lactobacilli is inhibitory for certain bacteria in the vagina, particularly catalase-negative bacteria that do not have the enzyme that detoxifies H₂O₂^(6,7).

Material and Method

The study protocol was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University. The vaginal samples were collected by swab via speculscopy from normal and BV, attending out gynecologic clinic who voluntarily participated in the investigation. The diagnosis of BV was based on at least three of five indicators of Chandeying criteria⁽⁸⁾, 1) the vaginal pH >4.5, 2) homogeneous and thin discharge (milky discharge), 3) positive sniff/amine test, 4) clue cell >20% of total vaginal epithelial cells, and 5) scanty or absence lactobacilli.

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A number of specimens were also picked up at the follow-up visit, one to two weeks after complete medication of metronidazole, 1 g daily for seven days.

The swab was kept into 1 ml of reduced transport media, which was thorough shaken. The mixture was spread on selective Rogosa medium and incubated anaerobically for 24 to 48 hours. The colonies were tentatively identified as *Lactobacillus* based on their growth on Rogosa agar. The colonial morphology and gram staining showed gram-positive bacilli that were catalase negative. The colonies were counted and presented as log CFU/ml.

Results

The *Lactobacillus* species and total bacteria in log CFU/ml in the vagina of normal women (60 cases) and women with BV (60 cases) were demonstrated in Table 1. The mean age (SD) of normal and BV women were 42±8.3 and 44±9.4 years, respectively.

No lactobacilli determination was highly significant ($p < 0.001$) in the diagnosis of BV. In addition, the quantitative of lactobacilli determination in BV (5.3±1.8 log CFU/ml) was markedly decreased compared with normal women (8.8±2.9 log CFU/ml). In contrast, the quantitative settlement of total bacteria in BV was increased of 13.1±3.2 log CFU/ml, compared with 8.8±2.9 log CFU/ml in normal women.

Of 60 women with BV at the initial visit, 47 cases (78.3%) had complete follow-up, determining the reduplication of lactobacilli. Shortly after complete medication of one to two weeks, the proportion of lactobacilli isolation was slightly increased from

12 (25.5%) to 15 (31.9%) of 47 cases. Concordance with quantitative settlement of lactobacilli in BV was increased from 1.4±2.6 log CFU/ml to 1.8±2.9 log CFU/ml in treated BV. The quantitative sequence of total bacteria in BV was decreased from 12.9±3.4 log CFU/ml to 10.9±4.1 log CFU/ml in treated BV.

The distribution of quantitative of *Lactobacillus* species and total bacteria in vagina of normal and BV women were demonstrated in Table 2. The lactobacilli, of 0 to 4 log CFU/ml, were highly significant ($p < 0.001$) in BV, while the total bacteria was not. After treated BV for one to two weeks, the reduplication of lactobacilli, of 5 to 14 log CFU/ml, had not restored significance ($p = 0.5831$). It did not restore significance for the total bacteria either.

Discussion

Lactobacilli colonize the genital tract, making it an integrate part of the vaginal micro flora. In the present study, the lactobacilli determination in BV was 28.0%, compared with 70.0% of normal women. While a study reported that *Lactobacillus* species, hydrogen peroxide-producing, were isolated in only 6% of BV, compared to 96% of non-BV⁽⁷⁾. It is likely that the absence of lactobacilli establishes the overgrowth by certain organisms of BV organism, or lactobacilli may prevent the development of BV in some circumstances.

The present study has some limitations. First, the follow-up isolation of lactobacilli was performed with a single specimen, a short period after completing medication. However, the reduplication of lactobacilli might need a study collecting serial samples. Second, other BV organisms should be explored to represent

Table 1. Qualitative and/or quantitative of *Lactobacillus* species, and total bacteria in vagina of normal/BV women

Initial isolated (n = 60)	n (%)		p-value	log CFU/ml	
	Normal	BV		Normal	BV
Lactobacilli	42 (70.0)	17 (28.0)	-	8.8±2.9	5.3±1.8*
No Lactobacilli	18 (30.0)	43 (72.0)	<0.001	-	-
Total bacteria (n = 60, 60)	-	-	-	11.5±3.6	13.1±3.2
Initial isolated (n = 47)	n (%)		Follow-up isolated (n = 47)	n (%)	
	Normal	BV		Normal	Treated BV
Lactobacilli	-	12 (25.5)	-	-	15 (31.9)
No Lactobacilli	-	35 (74.4)	-	-	32 (68.0)
Initial isolated (n = 47)	log CFU/ml		Follow-up isolated (n = 47)	log CFU/ml	
	Normal	BV		Normal	Treated BV
Lactobacilli	-	1.4±2.6	-	-	1.8±2.9
Total bacteria	-	12.9±3.4	-	-	10.9±4.1

* Statistical significance, $p < 0.05$

Table 2. Distribution of quantitative of *Lactobacillus* species, and total bacteria in vagina of normal/BV women

Initial isolated (n = 60)	Lactobacilli (log CFU/ml)		p-value	Total bacteria (log CFU/ml)		p-value
	0 to 4	5 to 14		0 to 4	5 to 14	
Normal, n (%)	23 (28.3)	37 (61.6)	-	2 (3.3)	58 (96.6)	-
BV, n (%)	50 (83.3)	10 (16.6)	<0.001	2 (3.3)	58 (96.6)	>0.99
Isolated (n = 47)	log CFU/ml			log CFU/ml		
	0 to 4	5 to 14		0 to 4	5 to 14	
BV, n (%)	40 (85.1)	7 (14.8)	-	1 (2.1)	46 (97.8)	-
Treated BV, n (%)	38 (80.8)	9 (19.1)	0.5831	1 (2.1)	46 (97.8)	>0.99

all the bacteria and cover vaginal bacterial dynamics. The microorganisms most frequently recovered from women with BV included *Gardnerella vaginalis*, *Prevotella bivia/disians*, *Bacteroides ureolyticus*, *Prevotella corporis/Bacteroides levii*, *Fusobacterium nucleatum*, *Mobiluncus species*, *Peptostreptococcus prevotii*, *Peptostreptococcus tetradius*, *Peptostreptococcus anaerobius*, viridans streptococci, *Ureaplasma urealyticum*, and *Mycoplasma hominis* (p<0.05 for each). The presence of all but three of these organisms was inversely related to vaginal colonization by H₂O₂-producing lactobacilli. The exceptions were *B. ureolyticus*, *F. nucleatum*, and *P. prevotii*⁽⁶⁾.

Third, all specimens were collected from a single clinical service and may not represent the variety of reproductive women. Fourth, the small number or absence of lactobacilli varies at different ages. By Nugent score, the absence of lactobacilli was encountered in 2.1% of fertile women, 11.4% of perimenopausal women, and 44.1/6.9% postmenopausal women without/with hormonal therapy⁽⁹⁾.

In conclusion, lactobacilli dominantly occur in healthy women, and markedly decline in BV. However, the vaginal ecosystem is dynamically changed in the lactobacilli of either normal or BV women.

Potential conflicts of interest

None.

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อุบัติการณ์ของชนิดสกุลแลคโตแบซิลัสในสตรีปกติและภาวะช่องคลอดอักเสบแบบที่เรียกผสม

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วัตถุประสงค์: เพื่อศึกษาอุบัติการณ์และจำนวนแลคโตแบซิลัสในสตรีปกติ และภาวะช่องคลอดอักเสบแบบที่เรียกผสม

วัสดุและวิธีการ: กลุ่มประชากรศึกษาประกอบด้วยสตรีปกติ/ภาวะช่องคลอดอักเสบแบบที่เรียกผสมจำนวน 60/60 ราย การวินิจฉัยช่องคลอดอักเสบแบบที่เรียกผสมอิงกับเกณฑ์จันทร์ดียิ่ง ด้วยตัวบ่งชี้อย่างน้อย 3 ใน 5 เกือบตัวอย่างสารคัดหลั่งช่องคลอด และเพาะเชื้อบน MRS plate เพื่อระบุหาจำนวนนับแลคโตแบซิลัส

ผลการศึกษา: จำนวนแลคโตแบซิลัสมีนัยสำคัญอย่างสูง (ค่า p น้อยกว่า 0.001) ในการวินิจฉัยสตรีภาวะช่องคลอดอักเสบแบบที่เรียกผสม และค่าเฉลี่ยแลคโตแบซิลัสในภาวะช่องคลอดอักเสบแบบที่เรียกผสมลดลงอย่างชัดเจน ($5.3 \pm 1.8 \log \text{CFU/ml}$) เปรียบเทียบกับสตรีปกติ ($8.8 \pm 2.9 \log \text{CFU/ml}$) สำหรับกลุ่มรักษาภาวะช่องคลอดอักเสบแบบที่เรียกผสมติดตามเก็บตัวอย่างได้ 47 ใน 60 ราย (ร้อยละ 78.3) สัดส่วนของการเพาะเชื้อแลคโตแบซิลัสเพิ่มขึ้นเล็กน้อยจาก 12 ใน 47 ราย (ร้อยละ 25.5) เป็น 15 ใน 47 ราย (ร้อยละ 31.9) ไปด้วยกันกับปริมาณแลคโตแบซิลัสเพิ่มขึ้นจาก $1.4 \pm 2.6 \log \text{CFU/ml}$ เป็น $1.8 \pm 2.9 \log \text{CFU/ml}$ ในกลุ่มรักษาภาวะช่องคลอดอักเสบแบบที่เรียกผสม และกลุ่มรักษาภาวะช่องคลอดอักเสบมีแลคโตแบซิลัสเพิ่มทวีเป็น 5 ถึง 14 $\log \text{CFU/ml}$ ไม่พบบนพื้นที่ที่ทดสอบอย่างมีนัยสำคัญ ($p = 0.5831$) เช่นเดียวกับปริมาณแบคทีเรียทั้งหมด

สรุป: แลคโตแบซิลัสพบเด่นในสตรีสุขภาพดี และลดลงชัดเจนในภาวะช่องคลอดอักเสบแบบที่เรียกผสม อย่างไรก็ตามการเปลี่ยนแปลงบริเวณช่องคลอดเป็นการเปลี่ยนแปลงของแลคโตแบซิลัสแบบพลวัต ทั้งสตรีปกติและภาวะช่องคลอดอักเสบแบบที่เรียกผสม
