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作成者（著者）	福田, 麻実 / 牧田, 和也 / 山本, 泰弘 / 田岡, 英樹 / 浅川, 恭行 / 久布白, 兼行
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Age-Related Changes in the Vaginal Microbial Environment of Women with Abnormal Discharge or Vulvovaginal Pruritus

Mami Fukuda¹⁾ Kazuya Makita²⁾ Yasuhiro Yamamoto¹⁾
Hideki Taoka¹⁾ Yasuyuki Asakawa¹⁾ and Kaneyuki Kubushiro^{1)*}

¹⁾Department of Obstetrics and Gynecology (Ohashi), School of Medicine, Faculty of Medicine, Toho University

²⁾Makita Obstetrics and Gynecology Clinic

ABSTRACT

Background: To investigate age-related changes in the vaginal microbial environment, we analyzed specimens cultured from vaginal secretions of women with abnormal discharge or vulvovaginal pruritus.

Methods: The detection rates of *Lactobacillus*, *Candida*, and bacteria other than *Lactobacillus* and anaerobic bacteria were retrospectively compared among 3393 cultures of vaginal secretions from reproductive-age (18-44 years), middle-aged (45-55 years), and older (>56 years) women who presented with abnormal vaginal discharge or vulvovaginal pruritus. Pregnant women were excluded.

Results: The detection rates of bacteria (other than *Lactobacillus* and anaerobic bacteria) and/or *Candida* were significantly higher in middle-aged and older women than in those of reproductive-age (85.0% and 87.7% vs 78.6%, respectively). The detection rate of *Lactobacillus* was 52.3% in women of reproductive-age, 35.9% in middle-aged women, and 15.6% in older women. The detection rate of *Lactobacillus* significantly decreased with advancing age. The overall detection rate of *Candida* was 22.5%; the detection frequency of *Candida* was significantly lower in older women than in women of reproductive-age and middle-aged women (13.8% vs 24.9% and 23.9%, respectively). We also found that the constitution of bacteria and *Candida* significantly differed among the 3 age groups.

Conclusions: The species and frequency of bacteria and *Candida* in the vagina vary with age.

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KEYWORDS: aging, menopause, estrogen, vaginal microbial environment, culture

The Japanese population is rapidly aging, and the lifespan of women has increased by 10 years during the past 40 years. Therefore, middle-aged and older women have become increasingly health-conscious. Decreasing ovarian function is closely associated with quality of life among menopausal women and can cause abnormal menstruation, hot flashes, vulvovaginal pruritus, and pain as well as urinary incontinence. Vulvovaginal pruritus is

often caused by an abnormal vaginal milieu and is one of the most frequent reasons that women seek gynecologic consultations, both during and after the menopause. Lactobacilli, the predominant normal vaginal flora, metabolize glycogen generated by vaginal epithelial cells via the action of estrogen in premenopausal women. This process maintains vaginal acidity and prevents the growth of pathogens. The decline in estrogen during menopause de-

1) 2-17-6 Ohashi, Meguro, Tokyo 153-8515

2) 3-3-17 Atago, Niiza, Saitama 352-0021

*Corresponding Author: tel: 03 (3468) 1251

e-mail: kubushiro@oha.toho-u.ac.jp

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Table 1 Detection rates of bacteria (other than *Lactobacillus* and anaerobic bacteria) and/or *Candida*

	Culture-positive (%) 95%CI (%)	Total
Reproductive-age	1862 (78.57) 76.86-80.20	2370
Middle-age	277 (84.97) 80.62-88.67	326
Older	611 (87.66) 84.99-90.01	697

* Significant difference (Tukey's method)

CI: confidence interval

creases the ability for such self-regulation and increases the rate of bacterial infection. Therefore, age-related changes in the vaginal environment should be regarded as bacterial vaginosis (BV) and vulvovaginal candidiasis. However, only a few reports have specifically described age-related microbial changes in the vaginal milieu.¹⁻³⁾ We therefore analyzed vaginal secretions cultured from a large population of reproductive-age, middle-aged, and older women to compare the frequencies of a wide spectrum of organisms that can cause abnormal discharge and vulvovaginal pruritus.

Methods

1. Subjects

The Ethics Committee of Toho University School of Medicine, Ohashi Medical Center approved the retrospective study protocol. We cultured 3393 vaginal secretions from women who attended our outpatient clinic with abnormal vaginal discharge or vulvovaginal pruritus between November 2001 and December 2008. Pregnant women were excluded from the study.

2. Methods

Lesions caused by systemic diseases and tumors were removed and, using a speculum, the properties of vaginal secretions were visually examined for BV or vulvovaginal candidiasis. Vaginal secretions were collected from the posterior vaginal fornix using a sterile cotton swab. All specimens were Gram-stained and cultured in standard medium as required. *Lactobacillus* and anaerobic bacteria were examined by microscopy. We investigated and compared the species and detection rates of *Lactobacillus*, *Candida*, and bacteria other than *Lactobacillus* and anaerobic bacteria in vaginal samples obtained from reproductive-age (18-44 years, n=2370), middle-aged (45-55 years, n=326), and older (>56 years, n=697) women. We further classified

Table 2 Detection rates of *Lactobacillus*

	<i>Lactobacillus</i> -positive (%) 95%CI (%)	Total
Reproductive-age	1239 (52.28) 50.24-54.31	2370
Middle-age	117 (35.89) 30.68-41.36	326
Older	109 (15.64) 13.02-18.55	697

* Significant difference (Tukey's method)

CI: confidence interval

266 samples from middle-aged women as pre- or postmenopausal and compared the detection rate of bacteria other than *Lactobacillus* and anaerobic bacteria and *Candida* in these 2 subgroups.

3. Statistical analysis

Statistical analysis was performed using R version 2.15.1 for Windows (R Foundation for Statistical Computing, Vienna, Austria). First, the chi-square test was used to compare distributions. Then, Tukey's Wholly Significant Difference test was performed as a multiple comparison test to evaluate differences between groups. A P-value less than 0.05 was considered significant.

Results

1. Detection rates of bacteria and/or *Candida* in vaginal cultures

The detection rates of bacteria (other than *Lactobacillus* and anaerobic bacteria) and/or *Candida* were significantly higher in middle-aged [277 (85.0%) of 326; 95%CI: 80.62-88.67] and older women [611 (87.7%) of 697; 95%CI: 84.99-90.01] than in women of reproductive-age [1862 (78.6%) of 2370; 95%CI: 76.86-80.20; Table 1]. The detection rates of bacteria (other than *Lactobacillus* and anaerobic bacteria) and/or *Candida* did not significantly differ among cultures from premenopausal [141 (82.9%) of 170; 95%CI: 76.43-88.27] and postmenopausal [86 (89.6%) of 96; 95%CI: 81.68-94.89] middle-aged women (p=0.196).

2. Detection rates of *Lactobacillus* in vaginal cultures

The detection rate of *Lactobacillus* was 52.3% (1239 of 2370; 95%CI: 50.24-54.31) in women of reproductive-age, 35.9% (117 of 326; 95%CI: 30.68-41.36) in middle-aged women, and 15.6% (109 of 697; 95%CI: 13.02-18.55) in older women. The detection rate of *Lactobacillus* significantly decreased with advancing age (Table 2).

Table 3 Bacteria and *Candida* species detected in each group

3-1 Reproductive-age women		
	No.	(%)
1	<i>Gardnerella vaginalis</i>	621 33.35
2	<i>Candida albicans</i>	492 26.42
3	<i>Staphylococcus</i> sp.	396 21.27
4	Group B β -hemolytic <i>streptococcus</i>	388 20.84
5	<i>Enterococcus</i> sp.	254 13.64
6	<i>Escherichia coli</i>	187 10.04
7	<i>Corynebacterium</i> sp.	175 9.40
8	α -Hemolytic <i>streptococcus</i>	96 5.16
9	Methicillin-resistant coagulase-negative <i>Staphylococcus</i>	84 4.51
10	<i>Candida glabrata</i>	80 4.30
3-2 Middle-aged women		
	No.	(%)
1	Group B β -hemolytic <i>streptococcus</i>	63 22.74
2	<i>Gardnerella vaginalis</i>	58 20.94
3	<i>Escherichia coli</i>	50 18.05
3	<i>Candida albicans</i>	50 18.05
5	<i>Corynebacterium</i> sp.	44 15.88
6	<i>Staphylococcus</i> sp.	42 15.16
7	<i>Enterococcus</i> sp.	37 13.36
8	<i>Candida glabrata</i>	22 7.94
9	α -Hemolytic <i>streptococcus</i>	11 3.97
9	<i>Klebsiella</i> sp.	11 3.97
11	Methicillin-resistant coagulase-negative <i>Staphylococcus</i>	10 3.61
3-3 Older women		
	No.	(%)
1	<i>Escherichia coli</i>	188 30.77
2	<i>Enterococcus</i> sp.	143 23.40
3	<i>Corynebacterium</i> sp.	142 23.24
4	Group B β -hemolytic <i>streptococcus</i>	117 19.15
5	<i>Staphylococcus</i> sp.	82 13.42
6	α -Hemolytic <i>streptococcus</i>	49 8.02
7	<i>Candida albicans</i>	48 7.86
8	<i>Candida glabrata</i>	44 7.20
9	<i>Klebsiella</i> sp.	41 6.71
10	<i>Staphylococcus aureus</i>	37 6.06

3. Species of bacteria and *Candida* detected in each group

The constitution of bacteria and *Candida* significantly differed among the 3 age groups. The most frequently detected species in vaginal cultures from reproductive-age, middle-aged, and older women were *Gardnerella vaginalis* (33.4%), group B β -hemolytic *Streptococcus* (22.7%), and *Escherichia coli* (*E. coli*) (30.8%), respectively (Table 3-1, 2 and 3).

Table 4 Detection rate of *Candida*

	<i>Candida</i> -positive (%) 95%CI (%)	Total
Reproductive-age	591 (24.94) 23.21-26.73	2370
Middle-age	78 (23.93) 19.40-28.94	326
Older	96 (13.77) 11.30-16.56	697

* Significant difference (Tukey's method)

CI: confidence interval

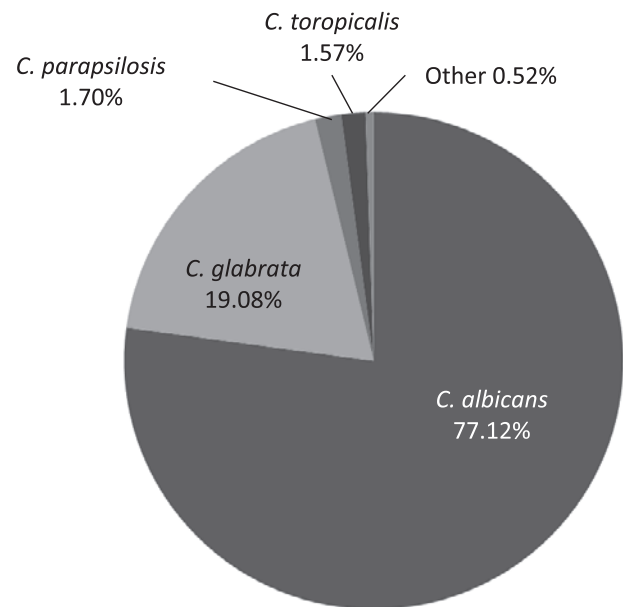
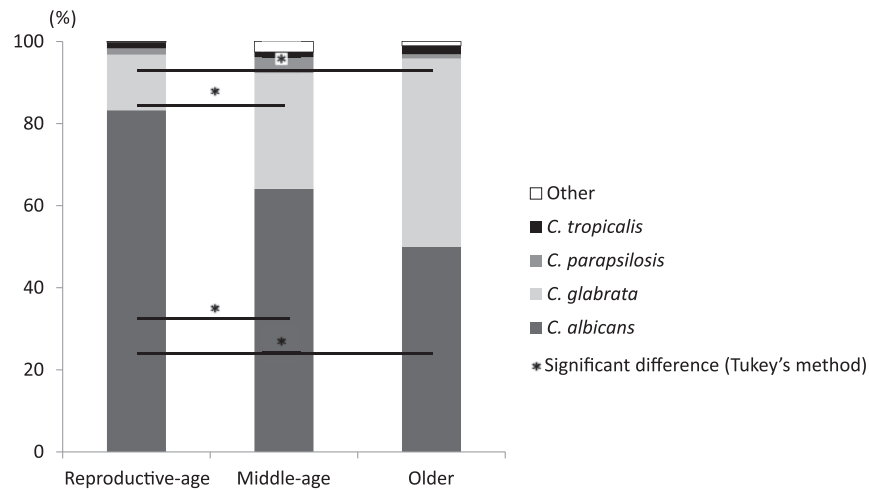


Fig. 1 Overall detection rates of *Candida* species. Together, *C. albicans* and *C. glabrata* were 96% of all *Candida* species identified.

C. albicans: *Candida albicans*, *C. glabrata*: *Candida glabrata*, *C. parapsilosis*: *Candida parapsilosis*, *C. tropicalis*: *Candida tropicalis*

4. Detection rates and species of *Candida* in each group

The overall detection rate of *Candida* was 22.5%. The detection rate of *Candida* was significantly lower among older women [96 (13.8%) of 697; 95%CI: 11.30-16.56] than in reproductive-age [591 (24.9%) of 2370; 95%CI: 23.21-26.73] and middle-aged women [78 (23.9%) of 326; 95%CI: 19.40-28.94; Table 4]. Fig. 1 shows the detection frequency and species of *Candida* in the 3 groups. Together, *Candida albicans* (*C. albicans*) and *Candida glabrata* (*C. glabrata*) made up 96% of all *Candida* species (spp.) identified. The detection frequencies of *C. albicans* and *C. glabrata* were significantly higher and lower, respectively, in women of reproductive-



	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	Other	Total
Reproductive-age	492	80	9	9	1	591
Middle-age	50	22	3	1	2	78
Older	48	44	1	2	1	96

Fig. 2 Detection frequency of *Candida* in reproductive-age, middle-aged and older women. The detection frequencies of *C. albicans* and *C. glabrata* were significantly higher and lower, respectively, in women of reproductive-age than in the other 2 groups.

C. albicans: *Candida albicans*, *C. glabrata*: *Candida glabrata*, *C. parapsilosis*: *Candida parapsilosis*, *C. tropicalis*: *Candida tropicalis*

age than in the other 2 groups (Fig. 2).

Discussion

The vagina can easily become contaminated during urination, defecation, and coitus, even in women of reproductive-age, and the balance of vaginal flora can be easily disrupted by factors such as antibiotics, steroids, stress, and diabetes. The present study found higher detection rates of bacteria (other than *Lactobacillus* and anaerobic bacteria) and/or *Candida* in middle-aged and older women than in women of reproductive-age. Middle-aged and older women often experience vaginal and genital itching and bacterial infection due to vaginal mucosal atrophy and decreased self-purification caused by reduced estrogen levels. In addition, women of reproductive-age often seek medical consultations because they misinterpret physiologic increases in cervical mucus associated with ovulation as an abnormal discharge. Thus, while examination rates were similar among the age groups, the detection rates of bacteria and *Candida* were very different. In this study, we did not compare detection rates at different phases of the menstrual cycle in women of reproductive-age; however, it is possible that physiologic increases in cervical mucus associated with ovulation may

cause differences in detection rates of bacteria and *Candida*. The detection rates did not significantly differ among cultures from pre- and postmenopausal middle-aged women. In this study, actual estrogen levels were not measured, but even in premenopausal women, self-purification may diminish due to the hormone imbalance caused by decreased ovarian function. Furthermore, cervical mucus secretion associated with ovulation decreases in middle-aged women. Thus, in comparison with women of reproductive-age, middle-aged women may be less likely to seek medical examination due to misinterpreting physiologic increases in cervical mucus as abnormal discharge.

Because of the action of estrogen, vaginal epithelial cells in women of reproductive-age contain glycogen. Döderlein bacilli such as lactobacilli and bifidobacteria metabolize glycogen from exfoliated vaginal epithelial cells and produce lactic acid, which maintains the acidity of the vagina (pH 4.4-4.6) and prevents infection by pathogenic bacteria (such as *E. coli*, which is mainly derived from the intestine). This process of self-purification diminishes during and after the menopause, due to reduced estrogen levels, which increases the risk of BV. We found that the detection rate of *Lactobacillus* significantly decreased with advancing age. The detection rate of *Lactobacillus* in older women was sig-

nificantly lower than that in middle-aged women; however, there was no significant difference in the detection rates of bacteria other than *Lactobacillus* and anaerobic bacteria and/or *Candida*. The quantity of bacteria may influence self-purification in the vagina, as may the presence or absence of *Lactobacillus*. Rates of detection of *E. coli* and *Enterococcus* sp. are also typically higher in elderly women, most likely because of the sustained decrease in self-purification associated with advancing age, which results in a vaginal environment that facilitates intestinal bacterial colonization and proliferation. Changes in intestinal bacterial composition associated with advanced age⁴⁾ might also affect intravaginal bacterial strains and detection rates.

A search of the English and Japanese literature did not identify comprehensive studies of the detection frequency of vaginal pathogens associated with aging, regardless of the presence or absence of symptoms. However, Yoshimura & Okamura⁵⁾ reported that the most common vaginal pathogens in 59 postmenopausal women with pruritus or vaginal discharge before hormone therapy (HT) were α -hemolytic *Streptococcus*, *E. coli*, *Streptococcus non-hemolyticus*, *Hemophilus vaginalis*, and *Corynebacterium* spp. Hillier & Lau⁶⁾ found that the most common vaginal pathogens among 73 postmenopausal women aged 55 to 79 years who did not have pruritus or vaginal discharge and who had never received HT were α -hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*, coryneforms, and *E. coli*. Accurate information on vaginal flora associated with BV, including the findings of molecular identification analysis,⁷⁻⁹⁾ is required because the actual bacterial flora of BV are probably more complex than those obtained by culturing swabbed specimens.

Estrogen promotes an increase in the incidence of vaginal *Candida*,^{10,11)} which is frequently detected in women of reproductive-age. We detected *Candida* in 22.5% of all women tested in the present study, and in 24.9, 23.9, and 13.8% of reproductive-age, middle-aged, and older women, respectively. The detection rate of *Candida* was significantly lower among older women than in the other 2 groups. Bauters et al.¹²⁾ reported that 20.1% of women have vaginal *Candida*, and Spinillo et al.¹³⁾ found rates of 34.1 and 13.5% in reproductive-age and menopausal women, respectively. Overall, the rates of infection with *C. albicans*, *C. glabrata*, and *C. parapsilosis* were 77.1, 19.1, and 1.7%, respectively, in the present study; Bauters et al.¹²⁾ reported rates of 68.3, 16.3, and 8.9%, respectively. The detection frequen-

cies of *C. albicans* and *C. glabrata* were significantly lower and higher, respectively, in women of reproductive-age than in the other 2 groups. Azole antifungal agents that are generally used to treat vaginal candidiasis are often ineffective against *C. glabrata*, and recurrence is frequent. Therefore, the frequency of refractory vaginal candidiasis caused by *C. glabrata* probably increases with age.

A common treatment for BV is antibacterial vaginal tablets. Only 2 antibacterial agents are presently covered by national health insurance in Japan. Because antibacterial agents kill the resident flora that is important for maintaining self-purification, repeated administration might increase bacterial resistance. Some reports have described the relevance of estrogen therapy to vaginal flora. Devilard et al.¹⁴⁾ found that the vaginal flora of menopausal women (n=19; age, 41-66 years) receiving estrogen plus progestogen therapy (EPT) was similar to those of premenopausal women but different from that of menopausal women who were not receiving HT. That study detected *Lactobacillus* in all women undergoing EPT. Yoshimura & Okamura⁵⁾ detected *Lactobacillus* in only 6 of 59 postmenopausal women (age 50-75 years) before treatment with oral estriol but in 27 women after treatment. These findings indicate that HT improves the microbial environment of the menopausal vagina.

Antonio et al.¹⁵⁾ reported that colonization of the vagina and/or rectum by lactobacilli that produce H₂O₂ is associated with BV prevalence. They found that 5% of women with lactobacilli in the vagina and rectum had BV, whereas 70% of women with lactobacilli at neither site had BV. Reid & Bruce¹⁶⁾ reported that daily oral intake of probiotic *Lactobacillus rhamnosus* (*L. rhamnosus*) GR-1 and *Lactobacillus fermentum* RC-14 caused some patients with asymptomatic BV to revert to a normal vaginal milieu dominated by lactobacilli, suggesting that an imbalance in intestinal flora—caused by factors such as aging, disease, drugs, and stress—can disrupt normal vaginal flora. In addition, BV sometimes causes urinary tract infection (UTI), and HT is effective not only in the treatment of BV but also in the prevention of UTI.¹⁷⁾ Moreover, several investigators have described the prophylactic effects of *Lactobacillus* strains. *L. rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 both inhibit the growth and virulence of *E. coli*, which is the most common pathogenic cause of UTI.¹⁸⁾ The vaginal use of probiotic *Lactobacillus* prevents UTI recurrence.^{16,19)} These findings indicate that not only antibiotics but also HT and probiotic *Lactobacillus* are useful for treating recurrent and

intractable UTI.

Vaginal pH-balanced gel was recently found to be effective for controlling atrophic vaginitis,²⁰⁾ and hyaluronic acid can be beneficial for atrophic epithelium.^{21, 22)} These might enhance treatment for BV in postmenopausal women. Thus, understanding the frequency of the wide spectrum of organisms present in vaginal secretions would be useful for clinical therapy.

Conclusions

We analyzed cultured vaginal secretions to determine the characteristics of vaginal flora that cause abnormal discharge and vulvovaginal pruritus. The species and frequency of bacteria and *Candida* in the vagina varied with age.

Because we did not examine the frequency of BV diagnosed based on specific criteria and anaerobic culture findings, we are unable to conclude that the detection of bacteria in the cultured vaginal secretions indicated BV. Further consideration of the effect of the vaginal microbial environment in relation to pessaries to treat uterine prolapse, urinary incontinence, and the actual value of estrogen is required. However, an imbalance in the vaginal microflora appears to increase the risk of BV and infection. Thus, the value of therapies such as probiotic *Lactobacillus* and HT should be investigated from the perspective of preventing BV and infection.

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腔分泌物培養検査からみた加齢と腔内菌叢の変化についての検討

福田 麻実¹⁾ 牧田 和也²⁾ 山本 泰弘¹⁾
田岡 英樹¹⁾ 浅川 恭行¹⁾ 久布白兼行¹⁾

¹⁾東邦大学医学部産科婦人科学講座 (大橋)

²⁾牧田産婦人科医院

要約

背景: 帯下の異常や外陰部・腔の搔痒の原因となり得る, 腔内細菌およびカンジダの検出頻度や検出菌種が, 加齢によりどのように変化するのかを検証するため, 腔分泌物培養検査の結果を分析した.

対象および方法: 医療センター大橋病院産婦人科外来にて帯下の異常や外陰部・腔の搔痒を訴える患者(妊婦を除く)に対して施行した腔分泌物培養検査 3393 検体を性成熟期世代 (18~44 歳), 更年期世代 (45~55 歳), 老年期世代 (56 歳以上) の 3 群に分け, それらにおける検出菌種と検出割合を後方視的に比較検討した.

結果: 各世代における全検体中の *Lactobacillus* および嫌気性菌を除いた細菌・カンジダが検出された割合は, 性成熟期世代 (78.6%) で更年期世代 (85.0%) と老年期世代 (87.7%) に比べ有意に低かった. *Lactobacillus* の検出割合は性成熟期世代 (52.3%), 更年期世代 (35.9%), 老年期世代 (15.6%) と年代が上がるにつれ有意に低くなっていた. 全世代におけるカンジダの検出割合は全検体中 22.5% であったが, 老年期世代 (13.8%) では性成熟期世代 (24.9%) および更年期世代 (23.9%) に比べ有意に検出割合が低かった. また, 検出された菌種とその割合は, 細菌・カンジダともに世代ごとに大きく異なっていた.

結論: 帯下の異常や外陰部・腔の搔痒の原因となり得る, 腔内細菌および真菌の検出頻度や菌種は, 世代が変わるごとに大きく変化することが確認された.

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1) 〒153-8515 東京都目黒区大橋 2-17-6

2) 〒352-0021 埼玉県新座市あたご 3-3-17

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