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The Impact of Lifestyle and Socioeconomic Factors on the Vaginal Microbiome in Üsküdar, Istanbul, Türkiye

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Abstract

The vaginal microbiome plays a critical role in women's health by maintaining vaginal acidity and preventing pathogenic overgrowth. This cross-sectional study investigated the impact of lifestyle and socioeconomic factors on vaginal microbiome composition among women in Kadıköy District, Istanbul, Türkiye. A total of 200 women were recruited from Central Kadıköy (urban), Üsküdar, and Beykoz (peri-urban/suburban districts). After excluding pregnant women and cases of non-bacterial infections, a final analytical cohort of 110 women (50 healthy, 60 with bacterial vaginosis) was analyzed using a polyphasic approach that included culture, phenotypic characterization, and multiplex PCR targeting *Lactobacillus* species. Vaginal infections were significantly more prevalent in peri-urban/suburban areas (70%) compared with the urban center (50%) ($p < 0.05$), with bacterial vaginosis accounting for 50% of diagnosed infections.

Molecular analysis of the 100 most vigorous isolates revealed that *Lactobacillus crispatus* (40%) was predominantly isolated from healthy women, whereas *Lactobacillus iners* (30%) was found exclusively in women with bacterial vaginosis. Multivariable analysis identified high psychological stress (AOR = 3.48), low monthly income (AOR = 2.82), and sharing of personal hygiene items (AOR = 1.98) as significant independent predictors of bacterial vaginosis. Regular probiotic yogurt consumption and physical activity were associated with lower BV prevalence in univariate analyses. These findings demonstrate that psychological, economic, and behavioral factors substantially influence vaginal microbial balance and dysbiosis, highlighting the need for integrated public health interventions that address mental health, economic constraints, and hygiene education to promote vaginal health in this population.

Keywords: Bacterial Vaginosis, *Lactobacillus*, mPCR, Psychological Stress

Introduction

Lactobacillus species play a fundamental role in maintaining vaginal health by producing lactic acid, hydrogen peroxide, and bacteriocins, which together form the primary defense mechanism against colonization by pathogenic microorganisms. These metabolic products help sustain a physiological vaginal pH ranging between 3.8 and 4.5, thereby inhibiting the overgrowth of harmful microbes (Witkin & Linhares 2017). However, the diversity and stability of *Lactobacillus* populations within the vaginal microbiome are strongly influenced by lifestyle and environmental factors (Sahib *et al.* 2024). Evidence indicates that a diet rich in probiotics, regular physical activity, and effective use of leisure time significantly enhance the abundance of beneficial *Lactobacillus* species. In contrast, microbial imbalance is commonly associated with diets high in sugars, sedentary lifestyles, and adverse socioeconomic conditions, including low income and limited access to hygiene and healthcare services (Huang *et al.* 2019; Abdul-Hussein *et al.* 2020) [10, 11]. *Lactobacillus* species differ in their protective capacities. *Lactobacillus crispatus* is considered one of the most beneficial vaginal commensals, as it effectively regulates vaginal pH through the production of high concentrations of hydrogen peroxide and D-lactic acid (Petrova *et al.* 2015) [13]. Conversely, *Lactobacillus iners* is regarded as a transitional species frequently detected during shifts toward bacterial vaginosis. It is associated with vaginal instability and provides limited protective effects (Macklaim *et al.* 2011) [12]. Therefore, its presence may reflect a state of microbial imbalance rather than effective disease prevention. Additionally, *Lactobacillus gasseri* contributes to vaginal health by producing lactic acid and maintaining pH balance, thereby suppressing the growth of pathogenic bacteria (Ravel *et*

al. 2011) [15]. The stability of the vaginal microbiome varies according to environmental exposures and individual behaviors. Factors such as antibiotic use, hormonal treatments, medical interventions, and lifestyle habits can substantially disrupt this microbial ecosystem (Gajer *et al.* 2012) [7]. Different *Lactobacillus* taxa, including *L. crispatus*, *L. iners*, and *L. jensenii*, influence the vaginal microbiome in distinct ways depending on host physiological and environmental conditions (Reid 2017) [16]. Disruption of this balance increases susceptibility to vaginal infections, including bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis (van de Wijgert & Jaspers 2017) [22]. These infections are frequently reported in Iraq, particularly in regions with limited healthcare access (Al-Khafaji & Al-Mosawi 2022) [2]. This study aimed to investigate the impact of lifestyle factors (diet, physical activity, and psychological stress) and socioeconomic status on the distribution and diversity of vaginal lactic acid bacteria among women in Najaf Governorate, Iraq. Specifically, The study compared women from three distinct areas—Central Kadıköy, Üsküdar, and Beykoz—to assess geographical variations in vaginal microbiome composition and their association with infection prevalence.

Material and Methods

A cross-sectional analytical study was conducted in Istanbul, Türkiye, encompassing the urban district of Kadıköy and its surrounding suburban districts, Üsküdar and Beykoz. The study aimed to evaluate the composition and diversity of the vaginal microbiome and to examine its association with sociodemographic, lifestyle, and clinical factors. The study aimed to evaluate the composition and diversity of the vaginal microbiome and to examine its association with sociodemographic, lifestyle, and clinical factors, an approach widely applied in population-based vaginal microbiome research (Gajer *et al.* 2012; Ravel *et al.* 2011) [7, 15]. Participant recruitment and data collection were carried out in outpatient obstetrics and gynecology clinics distributed across the three study areas to reflect varying socioeconomic conditions and varying levels of access to healthcare services, as recommended for epidemiological studies investigating vaginal microbiome variability across heterogeneous populations (van de Wijgert & Jaspers 2017) [22].

Study Population and Sample Selection A total of 200 women were initially recruited through purposive sampling from gynecology clinics in Kadıköy, Üsküdar, and Beykoz. To minimize confounding factors affecting vaginal microbial composition, a structured exclusion process was applied. Pregnant women ($n = 30$) were excluded due to pregnancy-associated hormonal and pH changes. Additionally, 60 participants diagnosed with nonbacterial vaginal infections—vulvovaginal candidiasis ($n = 42$) or trichomoniasis ($n = 18$)—were excluded (Table 1). Participants with chronic metabolic disorders or recent use of systemic antibiotics or corticosteroids were also excluded. Following these exclusions, 110 participants remained eligible. From these, 100 participants were systematically selected based on (1) possessing the most vigorous *Lactobacillus* growth in culture, and (2) clear and unambiguous phenotypic profiles to ensure high-quality DNA.

Table 1: Sample flow from enrollment to molecular analysis

Stage	Category	Enrolled (n)	Excluded (n)	Final PCR cohort (n)	Rationale
Married	Non-pregnant	100	0	100	Stable baseline microbiome
Unmarried	Single	70	60	10	Infection or protocol exclusion
Pregnant	Expecting	30	30	0	Hormonal confounding
Total		200	90	110	Final molecular cohort

Sample Collection and Storage

Vaginal swabs were collected aseptically from the lateral vaginal wall and mid-vagina using sterile rayontipped swabs, following standard protocols for vaginal microbiome sampling (Ravel *et al.* 2011; Srinivasan *et al.* 2014) [15, 21]. Samples were transported on ice at 4 °C and subsequently stored at -20 °C for short-term processing or at -80 °C for long-term preservation prior to DNA extraction, conditions shown to maintain microbial DNA integrity for downstream molecular analyses (Gajer *et al.* 2012) [7].

Polyphasic Identification of *Lactobacillus* Species A polyphasic approach combining conventional microbiological, biochemical, and molecular techniques was employed for the accurate identification of vaginal *Lactobacillus* species. Vaginal swabs were initially cultured on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid, UK) and incubated anaerobically at 37 °C for 48 h, a selective and widely accepted method for isolating lactobacilli from vaginal specimens (De Man *et al.* 1960; Reid *et al.* 2001) [6, 17]. Presumptive *Lactobacillus* isolates were identified based on colony morphology (small, round, creamy-white colonies), Gram-staining characteristics (Gram-positive rods), and conventional biochemical tests, including catalase negativity and non-motility, in accordance with standard taxonomic criteria (Holt *et al.* 1994) [9]. Further phenotypic differentiation was achieved through carbohydrate fermentation profiling and assessment of hydrogen peroxide (H₂O₂) production using TMB-plus agar. These functional characteristics provided preliminary phenotypic variation among isolates and were consistent with established identification frameworks for vaginal *Lactobacillus* species (Gaspar *et al.* 2019) [8]. For molecular confirmation, genomic DNA was extracted from the retained vaginal samples using the Presto™ Mini gDNA Bacterial Kit, following the manufacturer's instructions. Species-specific primers targeting conserved regions of the 16S rRNA gene were adopted from previously validated protocols (Gaspar *et al.* 2019) [8] and custom-synthesized by MacroGen Inc. (Seoul, South Korea). These primers (Table 2) were used for the multiplex PCR-based detection of clinically relevant vaginal *Lactobacillus* species, including *Lactobacillus crispatus*, *Lactobacillus iners*, and *Lactobacillus jensenii*. Multiplex polymerase chain reaction (m-PCR) was performed in a final reaction volume of 25 µL using an Applied Biosystems 2720 thermal cycler and a standardized 30–35 cycle amplification protocol. Positive and negative controls were included in each run to ensure analytical accuracy and to exclude contamination. The use of m-PCR enables the simultaneous and species-specific detection of multiple *Lactobacillus* taxa in a single reaction and has been widely validated for vaginal microbiome studies (Gaspar *et al.* 2019; Ravel *et al.* 2011) [8, 15]. PCR amplicons were separated by electrophoresis on a 2 % (w/v) agarose gel, stained with ethidium bromide or an equivalent fluorescent dye, and visualized under ultraviolet illumination. Fragment sizes were determined by comparison with a commercial 100-bp DNA ladder, following standard molecular biology

procedures (Sambrook & Russell 2001) [19]. To ensure comprehensive species-level classification, conventional biochemical assays were subsequently applied to isolates not targeted by the m-PCR primers. These complementary phenotypic analyses, including extended carbohydrate fermentation reactions and enzyme activity testing, enabled the accurate identification of additional *Lactobacillus* species such as *L. gasseri*, *L. acidophilus*, *L. fermentum*, *L. salivarius*, and *L. rhamnosus*. This integrated phenotypic-molecular strategy enhanced taxonomic resolution and ensured robust identification of vaginal *Lactobacillus* species. Molecular detection Preparations of Primer These primers synthesized by (Macrogen/Korea) (Table 2) Which were dissolved using sterile Double distilled water. The stock solution (100 μm) was prepared by adding ddH2O to the vial containing lyophilized primer while working solution of 10 μm was made by mixing 10 μl of the stock primer and 90 μl of ddH2O. the stock and working solution were stored in -20oC for reliable molecular identification. PCR Reaction Mixture Table 3 showed the details of the final 25 μL composition of the multiplex PCR master mix, including the ready-to-use enzyme mix (Master Mix), template DNA, forward and reverse primers, and nuclease-free water. The thermal cycling program used to amplify target genomic regions of vaginal *Lactobacillus* species via multiplex PCR is detailed in Table 4. This protocol begins with an initial denaturation step, followed by 35 cycles consisting of denaturation, annealing, and extension, and concludes with a final extension phase to ensure the complete amplification of all genomic products. The conditions were optimized based on the species specific primers as described by the sources.

Table 2: Species-specific primer sequences and expected product sizes for multiplex PCR detection of vaginal *Lactobacillus* species. Target species include *L. crispatus*, *L. jensenii*, and *L. iners*

Target species	Primer name	Primer sequence (5'-3')	Amplicon size (bp)	Reference
<i>L. jensenii</i>	Ljens	F: TGCTACAAAACCTGGTCCAG R: AGCCATGTTTGACTCGGTGC	967	
<i>L. iners</i>	Line	F: CGTCACTAATCATCGACCAGAA R: GCCGTGCTTTAATAGCAACTGCT	315	Gaspar et al. 2019
<i>L. crispatus</i>	Leri	F: TGTTAGTAATCACCTTCGCGCTA R: TTTGCCCTTCGACATAGCCA	474	

Results A total of 200 women were initially enrolled in this study from three geographical areas in Najaf Governorate: Kadıköy, Üsküdar, and Beykoz (periurban/rural). The study population was equally distributed geographically, with 100 participants recruited from the urban center and 100 from the surrounding districts. The mean age of participants was 32.5 ± 6.8 years. After applying the predefined exclusion criteria, including pregnancy (n = 30), vulvovaginal candidiasis (n = 42), trichomoniasis (n =18), chronic metabolic disorders, and recent antibiotic use, a final analytical cohort of 110 women was retained for molecular and statistical analyses. This cohort was divided into two clinical groups: a Healthy Group (n = 50) and a Bacterial Vaginosis (BV) Group (n = 60). The detailed participant

flow and group allocation are presented in Table 3.

Table 3: Participant attrition and retention throughout the study period

Stage	Group	n	Reason	Reason
Enrolled	Total assessed -	200	-----	-----
	Pregnant women			
Excluded	Vulvovaginal candidiasis	42	Excluded	Fungal infection
	Trichomoniasis	18	Excluded	Parasitic infection
Final cohort	Healthy (no infection)	50	Included	Control group
	BV-positive	60	Included	Case group
	Total analyzed	110	-----	-----

Table 4: Prevalence and distribution of vaginitis types categorized by geographical residence (N=200)

Residence Type	Total Participants (n)	Infected Cases (n)	Prevalence (%)	p-value
Urban (City Center)	100	50	50%	< 0.05*
Rural (Districts)	100	70	70%	
Total	200	120	60%	

Table 5: Distribution of Vaginitis Types Among Diagnosed Women (n=120)

Infection Type	Number (n)	Percentage (%)
Bacterial Vaginosis (BV)	60	50.0
Vulvovaginal Candidiasis(VVC)	42	35.0
Trichomoniasis	18	15.0
Total	120	100.0

Phenotypic and Biochemical Characterization of *Lactobacillus* Isolates Primary isolation of vaginal bacteria on de Man, Rogosa, and Sharpe (MRS) agar resulted in the recovery of colonies with morphological characteristics consistent with *Lactobacillus* species. After anaerobic incubation at 37 °C for 48 hours, colonies appeared small, circular, creamy white, and smooth-edged (Fig 1A). Microscopic examination following Gram staining confirmed that the isolates were Gram-positive, non-spore forming rods arranged singly, in pairs, or in short chains (Fig 1B). Phase-contrast microscopy further demonstrated the presence of viable rod-shaped bacterial cells without the need for staining (Fig 2). Biochemical assays revealed phenotypic variability among isolates, particularly in carbohydrate fermentation patterns and hydrogen peroxide (H₂O₂) production. Isolates recovered from clinically healthy women generally exhibited stronger H₂O₂ production compared with isolates obtained from BV-positive women. Molecular Identification of Predominant Culturable *Lactobacillus* Species Multiplex polymerase chain reaction (m-PCR) analysis was performed on 100 robustly growing *Lactobacillus* isolates selected from the final cohort to ensure reliable molecular identification. Species-specific amplification products corresponding to *Lactobacillus crispatus* (474 bp), *Lactobacillus iners* (315 bp), and *Lactobacillus jensenii* (967 bp) were successfully detected (Fig 3). Among the identified isolates, *L. crispatus* was the most prevalent species, accounting for 40%

(40/100) of isolates. Of these, 75% (30/40) were recovered from women in the Healthy Group. *L. iners* represented (30/100) of isolates and was detected exclusively in women diagnosed with BV. *L. jensenii* also accounted for 30% (30/100) of isolates and was distributed across both clinical groups, with 20 isolates from healthy women and 10 from BV-positive women.

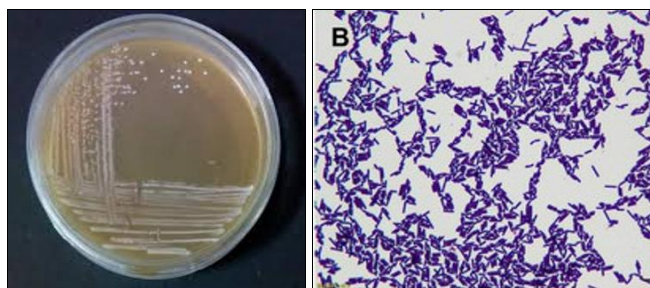


Fig 1: A. Isolation and Morphology of Vaginal Lactobacillus. A: Primary isolation. MRS agar streak plate after 48 h anaerobic incubation at 37°C. Arrow indicates a representative creamy-white, smooth colony selected for analysis. B. Gram stain. Isolate at 1000x magnification (oil immersion) displaying characteristic Gram-positive, non-spore-forming bacilli in single or short chains.

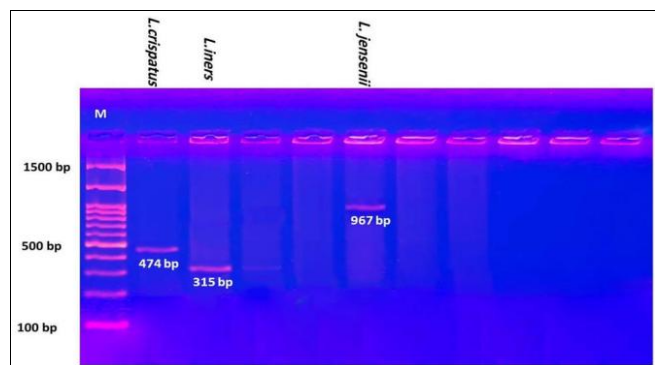


Fig 3: Multiplex PCR identification of Lactobacillus species. Agarose gel (2%) electrophoresis results. Lane M: 100 bp DNA ladder; Lane 1: *L. crispatus* (474 bp); Lane 2: *L. iners* (315 bp); Lane 5: *L. jensenii* (967 bp). Band sizes confirm species-specific differentiation

Discussion

This study provides a comprehensive, culture-supported molecular assessment of the vaginal microbiome and its association with clinical, socioeconomic, and lifestyle factors among women in Istanbul—Beşiktaş. By following a structured analytical sequence—from population characteristics and clinical diagnosis to phenotypic, molecular, and behavioral determinants—the findings demonstrate that vaginal microbial balance in this population is strongly shaped by both biological and social conditions. The observed geographical disparity in vaginitis prevalence, with significantly higher infection rates in peri-urban and rural districts compared with the urban center, reflects broader patterns of health inequality reported in low-resource settings (van de Wijgert & Jaspers 2017; Al-Khafaji & Al-Mosawi 2022) [22, 2]. Limited access to healthcare services, hygiene resources, and health education in peripheral areas likely contributes to delayed diagnosis and recurrent infections. The predominance of bacterial vaginosis among diagnosed cases further supports earlier regional observations indicating BV as the most common vaginal disorder among Iraqi women (Al-Khafaji & Al-

Mosawi 2022) [2]. At the microbiological level, the phenotypic and biochemical variability observed among Lactobacillus isolates underscores the functional heterogeneity of vaginal lactobacilli. Stronger hydrogen peroxide production among isolates from healthy women aligns with established evidence that H₂O₂-producing Lactobacillus species contribute to pathogen suppression and vaginal stability (Witkin & Linhares 2017; Petrova *et al.* 2015 [13]). These phenotypic findings were reinforced by molecular identification results, which revealed a clear species-level distinction between health-associated and dysbiosis-associated microbiota. The predominance of Lactobacillus crispatus among healthy women supports its role as a keystone species in maintaining vaginal homeostasis through lactic acid production and immune modulation (Ravel *et al.* 2011; Petrova *et al.* 2015) [15, 13]. In contrast, the exclusive association of Lactobacillus iners with bacterial vaginosis is consistent with its characterization as a transitional species adapted to unstable vaginal environments and frequently detected during dysbiosis (Macklaim *et al.* 2011; Petrova *et al.* 2017) [12, 14]. The presence of *L. jensenii* across both clinical groups further highlights the dynamic nature of vaginal microbial communities. Among the examined socioeconomic and lifestyle factors, psychological stress emerged as the strongest independent predictor of bacterial vaginosis. This finding strongly supports the proposed brain–vagina axis, whereby chronic stress and elevated cortisol levels disrupt estrogen mediated glycogen deposition in the vaginal epithelium, thereby reducing substrates essential for Lactobacillus dominance (Amabebe & Anumba 2018; Brotman *et al.* 2014) [3, 5]. In a region experiencing prolonged social and economic stressors, this association is particularly relevant. Low income and sharing of personal hygiene items were also significant predictors of BV, reflecting the role of economic constraints and hygiene practices in shaping vaginal health (Abdul-Hussein *et al.* 2020) [1]. Conversely, protective associations observed with physical activity and probiotic yogurt consumption align with evidence suggesting beneficial effects of lifestyle modification and probiotic exposure on vaginal microbial balance (Reid 2017) [16]. Although the cross-sectional design limits causal inference, the study's strength lies in its integrated polyphasic approach combining clinical assessment, classical microbiology, molecular identification, and behavioral analysis. Together, these findings emphasize that vaginal health in Istanbul—Beşiktaş is a biosocial condition requiring integrated public health strategies that address psychological well-being, socioeconomic inequality, and hygiene education to promote sustainable vaginal microbiome stability.

Conclusion and Public Health Implications

In conclusion, vaginal health in Istanbul—Beşiktaş is a biosocial condition. The dysbiotic state is not random but is significantly predicted by a triad of modern plagues: psychological distress, economic constraint, and high-risk hygiene practices. The microbial signature of this dysbiosis, dominated by *L. iners*, reflects a loss of the protective *L. crispatus*-driven ecosystem. These findings argue for a paradigm shift in public health interventions in Turkey and similar settings. Moving beyond syndromic treatment with antibiotics, effective strategies must integrate mental health support, economic empowerment initiatives, and

community-led hygiene education programs targeting specific high-risk behaviors. Empowering women with knowledge and resources to manage stress, nutrition, and hygiene may be key to fostering resilient vaginal microbiomes and breaking the cycle of recurrent infection.

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