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A Comparative study of Vaginal Microbiome Composition in Premenopausal and Postmenopausal Women

Hawraa Nasser Hamza[1,*], Dhay Ali Azeez[1] and Hanaa Ali Aziz[1]

[1]Department of Biology, College of Science, Al Muthanna University.

Corresponding Author: hawraanass@mu.edu.iq

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Abstract: Vaginal microbiome has as an essential part in maintaining female reproductive health, through the prevalence of *Lactobacillus* species, which preserving the vaginal milieu homeostasis. After menopause the hormonal alternations could contribute in reducing microbiota and making the vaginal dysbiosis take place, that's may increase the risk of urogenital complications. Objective of this study is investigation about the shifts in female's vaginal microbiome through menopausal transition and their role in the emergence of urogenital symptoms that are related with Genitourinary Syndrome (GSM). The duration of the study was from September to December 2024. Included 100 participants, 75 women above 45 years old with no longer menstruation and at different time intervals, 25 women with regular menstrual cycle under forties. Clinical checkup before obtain the sample, chromoagar, and biochemical test were utilized to identify the microbial isolates. A significant difference in vaginal bacterial growth, are showed 12.0% of bacterial growth was diverse in pre-menopausal participants compared with 92.0% in post-menopausal participants. Microbial profiles of the vaginal microbiota characterized by low *Lactobacillus acidophilus* in post-menopausal 24.0% participants in compared with pre-menopausal 52.0% participants. The most pathogenic bacteria that were isolated is *Enterococcus faecalis* 60.0% in post-menopausal participants respectively in compared with pre-menopausal 12.0% participants. In post-menopausal group, there was a significant association between urogenital symptoms and bacterial growth ($P > 0.05$), in contrast to pre-menopausal group which show non-significant correlation ($p < 0.05$). The diverse bacterial growth that's more predominant in post-menopause participants compared to pre-menopause women, indicating that a hormonal fluctuations at menopause stage which resulting from ovarian dysfunction, may contribute in reducing the existence of *Lactobacillus* species. Thu, vaginal microbial diversity increase specially *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, and Candida spp., which were the most frequent pathogen that isolated from menopausal group. a significant correlation between a bacterial growth was found in post-menopausal groups and the emerging urogenital symptoms, supporting an association between the altered vaginal microbiota and Genitourinary Syndrome of Menopause (GSM).

***Keywords*:** Vaginal microbiome, Menopause, GSM, *Lactobacillus*, *Enterococcus faecalis.*

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1. Introduction

Female reproductive tract dividing into the upper FRT (endocervix, uterus, and oviduct) which is in somewhat sterile, and the lower FRT (vagina and ectocervix) which contains a great microbial presence [1]. Vagina colonized by a diverse range of microorganisms that make up the mycobiota and microbiota. *Lactobacillus* is the most frequent microorganism that isolated from healthy human vagina, which also known as lactic acid bacteria (LAB). These vaginal lactobacilli have been hailed for their ability to prevent pathogens invasion by maintaining a healthy population, by producing lactic acid and secreting a various antimicrobial ingredients such as H_2O_2, cytokines, and surfactants [2]. However, disrupting the vaginal ecosystem promotes pathogens overgrowth. This known as dysbiosis associated with severe vaginal diseases such as bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and sexually transmitted infection (STI) [3]. Predisposing variables such as menstruation, pregnancy, sexual practice, uncontrolled antibiotic use, and menopause can change the microbial inhabitance [4].

Classifying of the vaginal microorganisms is done according to their an oxygen instance to aerobic and anaerobic or pathogenicity, some are endogenous species that female genital tract is their normal inhabitant, whereas some are exogenous that are acquired from an extrinsic source [5]. Vaginal microbiota structure and abundance are influenced by estrogen and progesterone, that are starting at puberty and continue through a reproductive years in a dynamic equilibrium with some variations [6]. Estrogen promote the proliferation of a vaginal epithelial cells and rise in glycogen storage, while a progesterone lyses the epithelial cells of vagina, thus facilitating release of glycogen to maintain the normal pH [7, 8].

Women in menopause loss of ovarian reproductive activity, either naturally or as a result of other disorder [9]. However, female life stages classified to pre-menopausal phase is considered the reproductive phase which the ovulation is regular and the ovaries completely functional and its starting at puberty, a peri-menopause is a transitional phase when female hormones levels especially estrogen start to decline leading to irregular period and reduction in ovaries hormones role [10]. Menopause phase represent a 12 months without amenorrhea and has two types a natural menopause and sudden or induced menopause due to surgeries or medical treatments [11]. A postmenopausal phase following the menopause and last to the rest of women life which undergo several changes in reproductive hormones, comprising reducing in estrogen and rising in follicle stimulating hormone. Wherefore, there is a notable increase in anaerobic bacteria and vaginosis related bacteria [12].

Pathogenic microorganism can break vaginal micro flora by multiple pathways through disrupting host physiology, consuming nutrients of the vaginal microenvironment, disrupting the barrier of vagina by a hydrolytic enzymes (e.g., prolidase, sialic acidase), and promotes the production of an inflammatory chemokines such as (IL-1β, IL-8), which enhancing the recruitment of neutrophils to the site of infection through bacterial vaginosis (BV) condition in presence of *E. coli*, *E. faecalis*, *Staph. aureus*, and *C. albicans* [13]. Additionally to, a pro-inflammatory cytokines (IL-6) that involving in B-cell differentiation, and (INF-α) which contributing in stimulate the leukocytes infiltration and remodeling the tissues [14]. Reduction of *Lactobacillus spp*. Halts vaginal pH from being preserved in the normal range (3.8–4.5), and when some pathogens have the capacity to form biofilms [15], they can impulse a cascade of negative conditions in the host, such as incessant infections, inflammation, and symptoms related with genitourinary syndrome caused by a combination of pathogens that are difficult to treat [16]. The objective of this study is understanding the alternations in the vaginal milieu through menopause transition which is significant not only for recognizing the pathophysiology implicit in menopausal symptoms but also for marking targeted therapeutic interferences.

2. Methodology

The present study is conducted between September 2024 and December 2024, and the samples were obtained from the Maternity and Pediatric Hospital and private gynecology clinic in Al-Muthanna province. The included 100 participants women, divided in to four groups according to last menstrual cycle, group1: participants women with continuous menstrual cycle (25 pre-menopause), group 2: participants women with less than 1 years last menstrual cycle (25 peri-menopause), group 3: participants women with 1 to less than 2 years last menstrual cycle (25 menopause). In addition to, 25 participants women with more than 2 years last menstrual cycle (25 post-menopause). Those who had undergone hysterectomy or oophorectomy, and pregnancy women were excluded.

Vaginal swab sample and mid-stream urine samples were obtained and stored into Amies transport media at 2-8°C, inside a safety box until reach to the lab.

DeMan Rosga Sharpe broth (MRS) for *Lactobacillus spp*., sabouraud dextrose broth was employed for activate *Candida spp*., Although, brain heart infusion broth (BHI) utilized in activates other bacterial types. Selective media utilized to diagnosis a different bacterial species. HiCrome *Lactobacillus* Selective agar use for isolation and differentiation between different species of *Lactobacillus*, HiCrome UTI agar media facilitates and accelerate the identification of some gram-positive bacteria and some gram-negative bacteria based on the difference in the shape and color of the colonies. HiCrome Candida Differential agar media is recommendation for ease identification and isolation of *Candida spp*. In addition to, several biochemical test including IMVIC test, catalase, coagulase, and oxidase tests [17].

3. Ethical approval

The principles summarized in the Declaration of Helsinki which served as the basis for conduction of this study. Verbal consent was acquired and the questionnaire form was registered from the participants prior to obtained the samples. the study protocol examined and approved by a local ethics committee.

4. Statistical analysis

Statistical analysis is conducted using SPSS(Social Statistical Package Science) version 24. The chi-square test is applied with a significance level of $P \leq 0.01$ to asses rates [18].

5. Results

A total of 100 samples were collected from Al-Muthanna province, divided into pre-menopausal , peri-menopausal , menopausal and postmenopausal women. [figure 1] shows The proportion of participants with homogenous and diverse bacterial growth rates 84.0% in peri-menopausal , 76.0% in menopausal , and 92.0% in post-menopausal participants respectively in compared with only (12.0%) of pre-menopausal participants was had a diverse growth (P = 0.001).

Microbial profiles of the vaginal microbiota characterized by low *L. acidophilus* in peri-menopausal (12.0%), menopausal (16.0%), and post-menopausal (24.0%) participants in compared with pre-menopausal (52.0%) participants (P = 0.025).

The most isolated bacterial pathogen was *E. faecalis* [Figure 2], rates were 64.0%, 56.0%, and 60.0% for peri-menopausal, menopausal and post-menopausal participants respectively in compared with pre-menopausal (12.0%) participants (P = 0.027). Followed by *E. coli* [Figure 3].

With rates 3 (12.0%) in premenopause, 17 (68.0%) in peri-menopause, 13 (52.0%) in menopause, and the rate in post-menopause was 11 (44.0%), (P=0.024). Also theirs a prevalence in *Staph. aureus* species and show a significant differences in the percentage between the study groups, 2 (8.0%) in peri- menopause, 8 (32.0%) in menopause, 7 (28.0%) in post-menopause, and no growth in pre- menopause, (p = 0.036). In addition to, *C. albicans* was showed an apparent growth in all study group, but with no significant difference, 3 (12.0%) in pre-menopause, 6 (24.0%) in peri- menopause, 1 (4.0%) in menopause, and 7 (28.0%) in post-menopause , (p = 0.148). [Table 1] show non-significant association between residency and Bacterial growth type in vaginal swab culture in all study groups.

The association between urogenital symptoms that are related with Genitourinary Syndrome (GSM) and bacterial growth type in vaginal swab culture has been carried out and the results were demonstrated in [Table 2]**.** In pre-menopause the present results show non-significant association between urogenital symptoms and bacterial growth, (P < 0.05). But in peri-menopause, menopause, and post-menopause groups, there was significant association between urogenital symptoms and bacterial growth (P> 0.05).

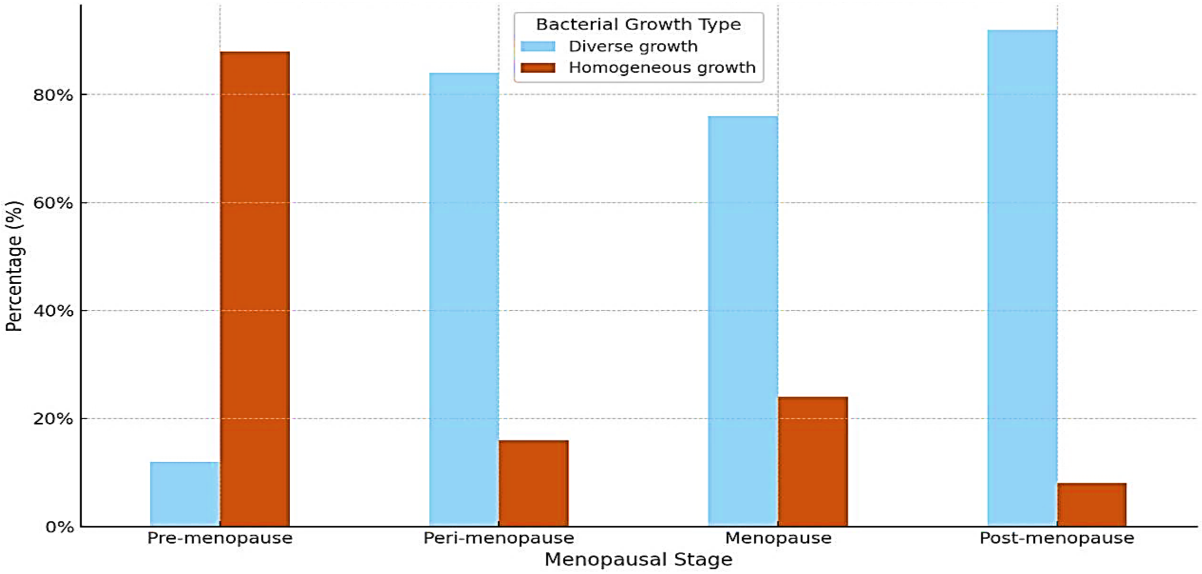

Figure (1): Bacterial growth in pre-menopausal, peri-menopausal, menopausal and postmenopausal women of vaginal swab culture.

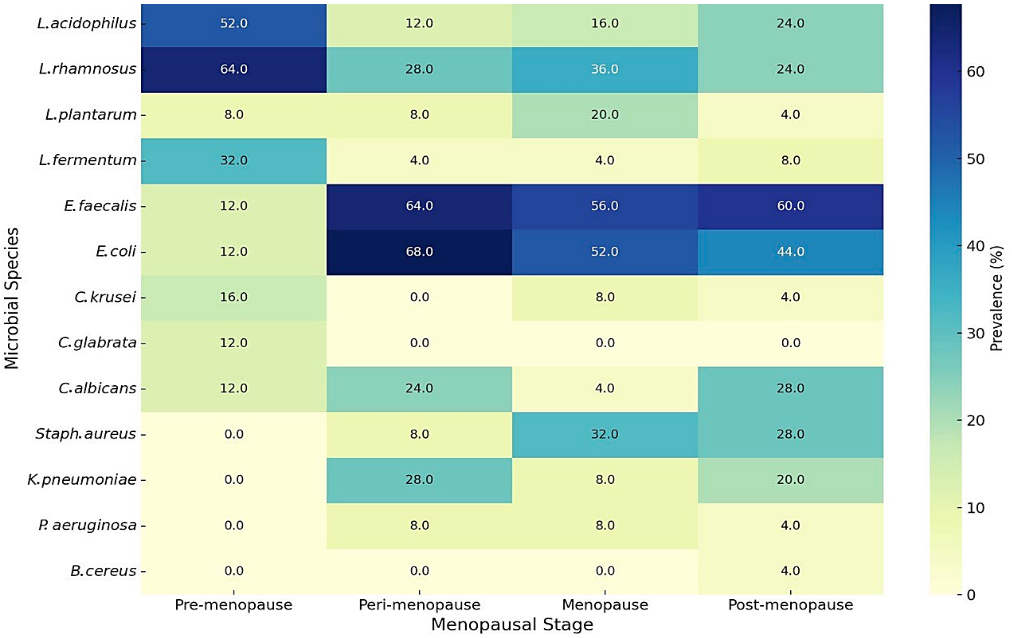

Figure (2): Heatmap of microbial species prevalence across menopausal stages.

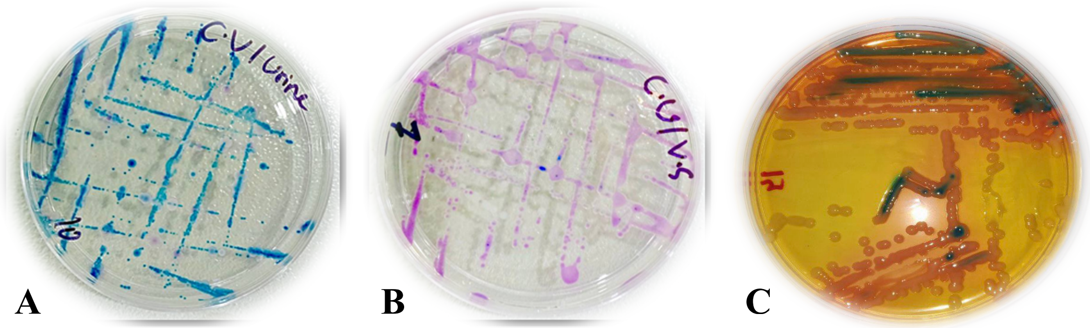

Figure 3: Show the bacterial isolates on HiCrome UTI agar, A- *E. faecalis*, B- *E. coli*, C- *Lactobacillus spp*. on HiCrome Lactobacillus agar.

Table 1: Association between residency and Bacterial growth in vaginal swab culture.

Groups		**Residency**	
		Urban	**Rural**
Peri-menopause	Diverse	12 (57.1%)	9 (42.9%)
	Homogenous	1 (25.0%)	3 (75.0%)
	P value	0.238	
Menopause	Diverse	10 (52.6%)	9 (47.4%)

	Homogenous	3 (50.0%)	3 (50.0%)
	p-value	0.910	
Post-menopause	Diverse	13 (56.5%)	10 (43.5%)
	Homogenous	0	2 (100.0%)
	p-value	0.125	

Table 2: Association between Bacterial growth type and some Urogenital symptoms related with GSM.

Groups		Frequent urination	Burning	Itching	Genital irritation	Vaginal discharges	Vaginal dryness
Pre-menopause	Diverse	3 (37.5%)	3 (37.5%)	2 (33.3%)	3 (75.0%)	3 (23.1%)	3 (75.0%)
	Homogenous	5 (62.5%)	5 (62.5%)	4 (66.7%)	1 (25.0%)	10 (76.9%)	1 (25.0%)
	P value	0.480	0.480	0.414	0.137	0.052	0.137
Peri-menopause	Diverse	18 (94.7%)	14 (87.5%)	11 (100%)	12(100%)	14 (87.5%)	10 (90.9%)
	Homogenous	1 (5.3%)	2 (12.5%)	0	0	2 (12.5%)	1 (9.1%)
	P value	0.001**	0.003**	0.001**	0.001**	0.003**	0.001**
Menopause	Diverse	13 (86.7%)	14 (100%)	11 (91.7%)	12(100%)	7 (87.5%)	16 (88.9%)
	Homogenous	2 (13.3%)	0	1 (8.3%)	0	1 (1.5%)	2 (11.1%)
	P value	0.005**	0.001**	0.004**	0.001**	0.034**	0.001*
Post-menopause	Diverse	19 (95.0%)	16 (94.1%)	12 (100%)	11 (84.6%)	4 (80.0%)	20 (90.9%)
	Homogenous	1 (5.0%)	1 (5.9%)	0	2 (15.4%)	1 (20.0%)	2 (9.1%)
	p-value	0.001*	0.001**	0.001**	0.013**	0.108	0.001**

6. Discussion

This study findings is compatible with a previous study conducted by several researchers [19, 20], which explained that a high levels in (E2) hormone at pre-menopause women stimulate vaginal epithelium thickening, which contribute in the accumulation of glycogen that metabolizing by *Lactobacillus spp*., that maintain a healthy vaginal pH (<4.5) and produce antimicrobial substances such as (Lactic acid, hydrogen peroxide, and bacteriocin), which serves against pathogenic or opportunistic bacteria. While at peri-menopause, menopause, and post-menopause, dysbiosis take place through the drop in Estradiol-2 level, vaginal epithelium thinning, low glycogen storage, reduction in vaginal microbiota (*Lactobacillus*), and rising in the vaginal pH (5.0-7.0). Thu, these factors contributing in the promote the pathogenic, anaerobic, and opportunistic bacterial growth [21].

The high prevalence of *E. faecalis* from vaginal isolated samples could be due to the significant virulence factors of this bacterial species including biofilm formation and a capacity to adherence to the urogenital tracts , a wide ability to survive in severe environments [22]. Additionally to, many of *E. faecalis* strains considered as multi-drug resistance including the Cephalosporins [23]. Due to the menopausal physiological changes, the Vaginal tract microenvironment was represented as a reservoir to the uropathogenic *E. coli* colonization (UPEC), which contributing in vaginal infections and recurrent UTIs [24]. An opportunistic infections by *Staph. aureus* and *C.albicans* in menopausal women was also presented due to hormonal changes, reduction in immune response, and mucosal alternations which leading to microbial over-growth and imbalance. Resulting in an aerobic vaginitis (AV) and vulvovaginal candidiasis (VVC) [25].

The non-significant results which included the association between residency and bacterial growth type in the study group perhaps due to the small number of samples included in this study. In contrast to a previous study [26], proved there is a significant difference in microbial diversity between urban and rural females, which a rural women was had a higher prevalence of opportunistic and pathogenic microbes, appeared as a result of influence the vaginal milieu by external environmental factors, hygiene, and life style. While urban women exhibited a high prevalence of *Lactobacillus* microbiota, that offering a protection agents the bacterial infections.

After menopause, reduction in serum level of Estradiol-2 (E2), decline in progesterone level, and increase in serum level of follicle stimulating hormone (FSH), leading to thinning of the urogenital tracts epithelium, reduce in vaginal lubrication, and decrease in the blood flow and elasticity [27]. Such alternations create a favorable microenvironment for dysbiosis. Additionally to, reduction in Lactobcillus spp., and this will rise the vaginal pH, resulting in growth of opportunistic microbes and uropathogens such as (*E. faecalis*, *E. coli*, *Staph. aureus*, *C. albicans*, etc.) [28]. That have role in existence of several urogenital symptoms includes vaginal dryness, burning, itching, irritation, and discharges [29]. These are most common manifestations in post-menopausal women, and these chronic conditions expressed as genitourinary syndrome of menopause (GSM), which is another term of the vaginal atrophy [30].

7. Conclusion

A significant shift in the composition of vaginal microbiota across menopausal stages, a considerable reduction in *L. acidophilus* was detected with advancing the menopausal status. The diverse bacterial growth was distinctly more predominant in peri-menopause, and post-menopause participants compared to pre-menopause women, indicating that a hormonal fluctuations may contribute in a vaginal microbial diversity. *E. faecalis* featured as most frequently pathogen that isolated from menopausal group, followed by *E.coli*. Remarkably, a significant correlation between a bacterial growth was found in peri-menopausal, menopausal, and post-menopausal groups and the emerging urogenital symptoms, supporting an association between the altered vaginal microbiota and Genitourinary Syndrome of Menopause (GSM). While, this correlation was not significant in participants at peri-menopause stage. These results are highlight on the importance of monitoring an aging women vaginal microbiota, for an early identification and management of complication related-GSM.

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