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The relation between urinary and vaginal microbiomes in urge urinary incontinence

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Abstract

Background: Urge incontinence is considered when a patient suddenly have an urge to void urine, in this situation there is an unnecessary contraction of the urinary bladder that leads to leakage of urine despite the closed bladder by holding sphincter muscles.
Objective: This study aimed to assess the association between urinary and vaginal microbiomes using 16S rRNA gene sequencing in those with urge urinary incontinence.
Patients and methods: A case-control study in which one hundred women who attended outpatient clinic of Baghdad Teaching Hospital during period from January 2021 to December 2021. In this study vaginal swabs and catheterized urine samples were collected from fifty women who proved to have urge urinary incontinence and another fifty samples were taken from controls. Cusco speculum high vaginal swab taken from all wall of vagina and after that under aseptic technique, folly's catheter inserted and midstream urine collected. The collected specimens transport to laboratory for genetic study, where these specimens, examined by two genomic DNA isolation and real Time PCR for qualitative detection of urinary and vaginal bacteria.
Results: There was no significant association regarding age and body mass index in both groups (p=0.883 for age and p= 0.9 for body mass index). *Enterococci* contributed 49% of an individual's urinary and 45% of their vaginal samples with moderate positive correlation (r=0.564) and significant association (p=0.005). *Gardnerella* contributed 31% of an individual's urinary and 24% of their vaginal samples with moderate positive correlation (r=0.463) and significant association (p=0.001). *E coli* were 14% of an individual's urinary and 12% of their vaginal samples with strong positive correlation (r=0.822) and significant association (p=0.001). Other bacteria that had weak correlation and non-significant association were *Urealyticum* 26% urinary and 15% vaginal, (r=0.184) (p=0.214). *S aureus* 3% urinary and 0% vaginal, (r=0.172) (p=0.246). Finally, *M hominis* was 10% urinary and 4% vaginal, (r=0.241) (p=0.101).
Conclusion: Positive associations between vaginal and urinary genera in urge urinary incontinence. *E coli* had high correlation between urine and vagina while other microbiomes as *Gardnerella* and *Enterococci* have moderate correlation and other microbiomes (*Urealyticum*, *S aureus* and *M hominis*) had weak correlation between urine and vagina in urge urinary incontinence.

Keywords: Urge urinary incontinence, urinary tract infection, vaginal micro biomes

Introduction

Urge urinary incontinence

Urinary incontinence occurs when patients have a sudden urge to urinate. In urge incontinence, the bladder contracts unnecessarily, forcing urine to flow through the sphincter that keeps the bladder closed. Other names for this mode overactive bladder (OAB)

1. Bladder spasm
2. Spasmodic bladder
3. Irritated bladder
4. The instable detrusor [1].

Urge incontinence can be result due to infection, bladder CA, inflammation of the bladder, blockage in the form of bladder stones, brain or nerve problems such as multiple sclerosis or stroke, or damage to nerves such as the spinal cord [2].
Some factors can increase the risk of urinary incontinence, including:
Natural pregnancy and childbirth
Adiposity and obesity
Family history of urinary incontinence

Aging, although urinary incontinence is not an inevitable part of aging [3].

Urge incontinence is the second most common cause of urinary incontinence; almost 3 out of 10 cases of incontinence are related to urge incontinence. It can occur at any age, but usually begins in younger age. Women suffer more than men. The prevalence of urge incontinence in adult women varies widely (from 5% to 72%), with a concordance between different studies of approximately 30% [4].

Immediate evaluation for urge incontinence should always include a thorough history, physical examination, and investigations. The history should include the nature of the patient's incontinence, the onset and duration of incontinence symptoms, whether the patient is bothered by the symptoms, and any concomitant factors that may influence incontinence, such as the number and type of daily incontinence for discussion. Enuresis fluid intake and bladder stimulants (caffeine, soda, artificial sweeteners, alcohol, etc.), frequent or intermittent urination, comorbidities (eg, diabetes,urinarytract infections). [5].

Therefore, a thorough physical examination should include a pelvic and genitourinary examination, as well as a neurological examination, assessment of gait, mental status, and body mass index (BMI). Examination of the abdomen may reveal tenderness at the angle of the vertebrae, palpable formations in the pelvis and bladder. A positive Valsalva test for more than 6 seconds may indicate pelvic organ prolapse. A digital pelvic floor exam provides information about the tone and strength of your pelvic floor. In addition, speculum examination may reveal vaginal atrophy, dermatitis associated with urinary incontinence, and extraurethral discharge/fistula [6].

Urge incontinence patients should undergo a urinalysis, and some patients may require a post-void residual urine volume (PVR), cystoscopy, and urodynamic studies. Video dynamic studies are standard in the evaluation of patients with urinary incontinence, but are commonly used to evaluate complex cases of incontinence [7].

The usual approach to treating stress urinary incontinence is a gradual treatment program that begins with noninvasive behavioral changes, followed by devices and medication, and finally surgery for those whose symptoms do not respond to initial treatment. First-line treatment includes patient education about bladder health and habits, lifestyle changes, changes in bladder emptying routine, dietary changes, and behavioral therapies such as avoidance of bladder stimulants (eg, caffeine, smoking), muscle training pelvic floor muscles (PFMT) and biological feedback [8].

Biofeedback is a technique designed to strengthen the rectal and urethral sphincters and pelvic floor muscles and help women control their bladder. Biofeedback can help women learn which muscles to use, when to use them, and how hard to contract to prevent leakage. There are different types of biofeedback, but one of them is the insertion of a probe into the vagina. When you contract the muscles, the pressure of the tube appears on the computer screen, as if trying to block the passage of water. A physical therapist or nurse teaches women how and when to contract the muscles to maintain effective bladder control. They will learn to use the screen as a guide First, the page is hidden from prying eyes so they can trust it. Women through time it is necessary Improve coordination and control of the sphincter and pelvic floor muscles. If you train them during a biofeedback program, the strength of these muscles will also improve. Another form of biology is the use of ultrasound in real time. The probe is placed in the perineum and an image of the pelvic organs is displayed on the screen. When a woman contracts her pelvic floor muscles, she can see what is happening around her bladder and bowels. Movement of the therapist Or A professional nurse can adjust the tension of your muscles by watching them on the screen. Unfortunately, this type of biofeedback is currently available only in specialized centers [9].

If behavioral changes, such as bladder training, do not relieve immediate symptoms of incontinence, we choose to try various medical, interventional, and surgical treatments. Medical treatments include: oxybutynin (Ditropan), solifenacin (bladder), and Botox injections. Interventional treatments include electrical stimulation such as sacral nerve stimulation and transcutaneous spinal nerve stimulation. Surgical procedures for incontinence include increasing bladder capacity, limiting nerve impulses to control muscles, and changing the direction of urine flow [10].

Urinary tract infections

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting approximately 150 million women worldwide each year. Urinary tract infections require treatment with age and account for 50-60% of adult women, mainly due to antibiotic resistance [11].

These infections are caused by a variety of pathogens, but recent research suggests that the vaginal, intestinal, and urinary microbiota play an important role in regulating disease activity [12].

Microorganisms establish close contact with a person during development and at the junction of the external part of the body with the endoseptum, various body environments, for example, the skin, nose, mouth, intestines, vagina and genitourinary tract, which, in turn, form the target microorganism [13].

Epidemiology

Urinary tract infections are a very common bacterial infection among women. They usually occur between the ages of 16 and 35, with 10% of women affected each year and 40-60% at least once in their lifetime. Relapses are common, with about half occurring within a year. Urinary tract infections are at least four times more common in women than in men [14].

Aim of the study

The aim of the study is to assess the relation between urinary and vaginal microbiomes using 16S rRNA gene sequencing in those with urge urinary incontinence.

Patients and methods

This is a case-control study in which one hundred women who attended outpatient clinic of Baghdad Teaching Hospital during period from January 2021 to December 2021. In this study vaginal swabs and catheterized urine samples were collected from 50 women who proved to have urge urinary incontinence and another 50 samples were taken from controls.

Inclusion criteria

- Healthy mentally & physically women.
- Women diagnosed with urge urinary incontinence.

Exclusion criteria

- Women who had any type of STD or other symptomatic vaginal infections.
- Diabetes mellitus.
- Immune compromise Women.
- Women were using diaphragms, douches, or spermicides.
- Pregnant women.
- Those that received diuretics, antibiotics and vaginal

suppositories.
- Those with symptomatic UTI and urinary tract abnormality
- Those with mental disease
- Those with neurological disease as (Alzheimer disease, multiple sclerosis, spinal cord injury, stroke).
- Women with stress and mixed urinary incontinence
- Those with prolapse
- Those with TAH
- Those with urinary stones

Data collection
According to forma that include
- Age
- Parity
- Mode of delivery (whether vaginal or caesarean deliveries).
- Menopause.
- Urinary diary
- Past medical history (DM, immune disorders or neurological problems).
- Drug history (diuretics, antibiotics or vaginal suppositories).
- Past gynecological history (recurrent infections, vaginal lump, heaviness, prolapse, menstrual disturbances or fibroids).
- Past surgical history: (TAH, repair).
- Past obstetrical history: (instrumental delivery, large baby).
- Sexual history: (sexual partner infection).
- GIT history: (bowel symptoms).

Examinations
1. General examination
Vital signs (BP, PR, TEMP), weight, height, BMI, mental status and mobility.

2. Abdominal examination
Palpate the kidneys, or if there is bladder distension, abdominal mass.

3. Speculum examination
For vaginal infection (discharge)

4. Pelvic examination
- Appearance of local tissue, excoriation of vulval itching.
- Presence of atrophy
- Urethral abnormality
- Pelvic organ prolapses
- Bimanual examination of uterus

5. Vaginal examination
Anterior vaginal wall prolapse (cystocele) or posterior vaginal wall prolaps (rectocele).
Bonneys test -ve

Investigations
- GUE
- U/S
- Urodynamic study

Specimen collection
At outpatient clinic of Baghdad teaching hospital and after taking consent from patients in lithotomy position, examination done with Cusco speculum high vaginal swab taken from all wall of vagina and after that under aseptic technique, folly's catheter inserted (2 ways folys catheter used with size14) and midstream urine collected.

Collected specimens (urine samples, vaginal swabs) kept on refrigerator at temperature (4-8 C°) then transport to laboratory for genetic study.

Laboratory methods
The collected specimens (vaginal swabs and urine samples) were examined into two main steps:

Genomic DNA isolation:
Total DNA (Genomic, mitochondrial, Bacterial and viral DNA) isolated from the vaginal and urine samples for molecular studies was applied using genomic DNA purification kits of Qiagen (QIAamp DNA Mini Kit /Germany).

Real Time PCR for qualitative detection of urinary and vaginal bacteria:
The PCR-based methods have been used successfully for the detection of pathogen
Program Rotor-Gene 2000/3000 consists of the following:
Reaction Volume (μl): 13
Hold: 95º - 15 min
Cycling: 45 cycles

Statistical analysis
- Data were analyzed SPSS IBM program
- Data are presented as means ± variance compared using an unpaired *t*-test.
- Chi-square or Fisher s exact' tests compared means and percentages when appropriate.
- Probability values < 0.05 were considered statistically significant in all analyses.

Results
A total of 50 women with urge urinary incontinence (UUI) and comparable 50 without UUI (control group) were involved in this study with age ranged from (25-55) years.

Table (3-1) showed that the main age group were between (45-55) years with mean age of (42.4±8.8) years in case group while (41.5±10) years in control group. There was non-significant difference between both groups regarding the age group (p=0.883). In addition, the mean BMI were (27.4±5) in case group and (27.1±5.4) in control group, no significant difference was observed between two groups concerning BMI (p= 0.9). Regarding menopause, no significant difference was observed between the two groups (p=0.564) whilst significant differences were shown in regard with parity (p=0.043) and mode of delivery (p=0.043).

Table 3-1: Demographic criteria of both case and control groups

	UUI (n=50) No. (%)	Control (n=50) No. (%)	P value
Age			
25-34	8 (16%)	10 (20%)	0.883*
35-44	17 (34%)	16 (32%)	
45-55	25 (50%)	24 (48%)	
Mean ± SD	42.4±8.8	41.5±10	
BMI			
Normal	17 (34%)	15 (30%)	0.9*
Overweight	20 (40%)	23 (46%)	
Obese	13 (26%)	12 (24%)	
Mean ± SD	27.4±5	27.1±5.4	
Menopause			
Premenopausal	42 (84%)	44 (88%)	0.564**
Postmenopausal	8 (16%)	6 (12%)	
Parity			
Nulliparous	9 (18%)	18 (36%)	0.043**
Multiparous	41 (82%)	32 (64%)	
Mode of delivery			
Vaginal delivery	34 (68%)	24 (48%)	0.043**
Caesarean section	16 (32%)	26 (52%)	

* Unpaired *t*-test

** Chi-square test

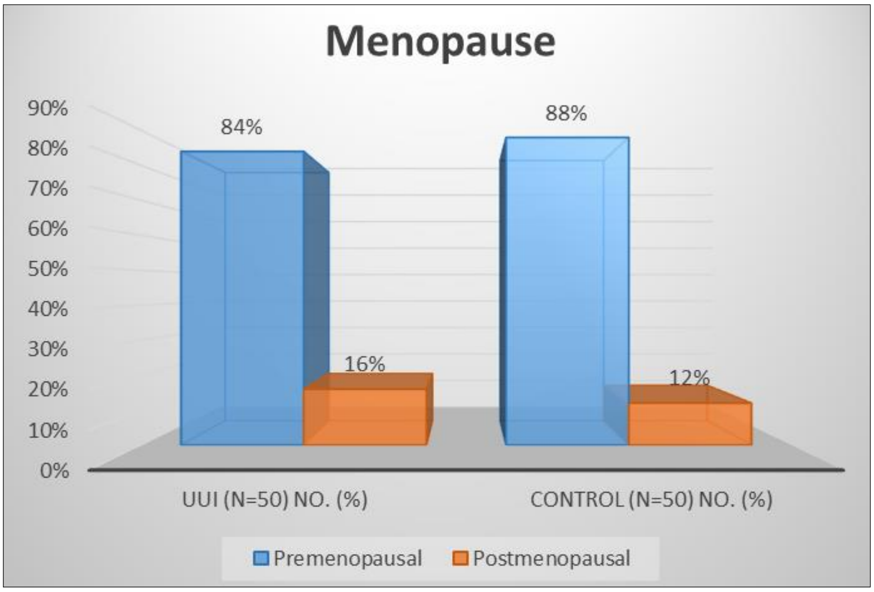

Fig 3-1: Menopause in the study groups

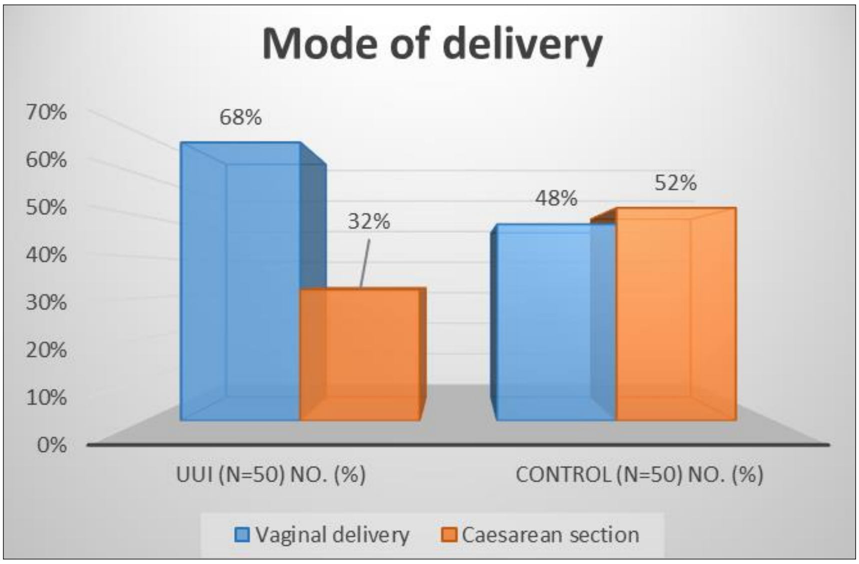

Fig 3-2: Mode of delivery of the study groups

Table (3-2) explained contribution of bacterial genera in urinary and vaginal samples in both UUI and control groups. *Enterococci, Gardnerella, E coli, Urealyticum, S auerus* and *M hominis* were extant together in vagina and urine. *Enterococci* was the most abundant pathogen in cooperation urine and vagina. Followed by *Gardnerella*, *Urealyticum*, *E coli*, *M hominis* and *S aureus*.

Table 3-2: Contribution of bacterial genera to individual's urine and vaginal samples in both groups

	UUI (n=50)		**Control (n=50)**	
	Urine	**Vagina**	**Urine**	**Vagina**
Enterococci	31	29	18	16
Gardnerella	19	16	12	8
E coli	8	8	6	4
Urealyticum	15	7	11	8
S aureus	1	1	2	0
M hominis	5	0	5	4

Table (3-3) explained contribution of bacterial genera to individual's urine and vaginal samples in UUI group. *Enterococci* were found alone in 9 cases of urine sample and 22 cases found together with other microbiomes. In vaginal samples, *Enterococci* were detected in 16 cases alone while in 13 cases with other bacteria. *Gardnerella vaginalis* were found alone in 3 cases of urine sample and 16 cases found together with other microbiomes. In vaginal samples, *Gardnerella* were detected in 9 cases alone while in 7 cases with other bacteria. *E coli* were found alone in 5 cases of urine sample and 3 cases found together with other microbiomes. In vaginal samples, E.coli were detected in 8 cases alone while in no cases with other bacteria. Other bacteria were less commonly existing in urine and vagina.

Table 3-3: Contribution of bacterial genera to individual's urine and vaginal samples in UUI group

Genera	Urine	Vagina
Enterococci	9	16
Gardnerella vaginalis	3	9
E coli	5	8
Urealyticum	0	1
S aureus	1	1
M hominis	5	0
Enterococci + *Gardnerella*	10	7
Enterococci + *Urealyticum*	6	6
Gardnerella + *Urealyticum*	3	0
Enterococci + *Gardnerella* + *Urealyticum*	3	0
Enterococci + *E coli* + *Urealyticum*	3	0

Table (3-4) explained contribution of bacterial genera to individual's urine and vaginal samples in control group. *Enterococci* were found alone in 7 cases of urine sample and 11 cases found together with other microbiomes. In vaginal samples, *Enterococci* were detected in 4 cases alone while in 12 cases with other bacteria. *Gardnerella vaginalis* were found alone in 2 cases of urine sample and 10 cases found together with other microbiomes. In vaginal samples, *Gardnerella* were detected in 2 cases alone while in 6 cases with other bacteria. *E coli* were not found alone in urine sample but 6 cases found together with other microbiomes. In vaginal samples, no *E coli* were detected alone while in 4 cases with other bacteria. Other bacteria were less commonly existing in urine and vagina.

Table 3-4: Contribution of bacterial genera to individual's urine and vaginal samples in control group

Genera	**Urine**	**Vagina**
Enterococci	7	4
Gardnerella vaginalis	2	2
Urealyticum	2	2
Enterococci + *Gardnerella*	4	4
Enterococci + *E coli*	2	2
Gardnerella + *E coli*	2	0
Enterococci + *Urealyticum*	0	2
Urealyticum + *M hominis*	2	0
E coli + *Urealyticum*	2	2
Gardnerella + *Urealyticum*	2	0
Enterococci + *Gardnerella* + *M hominis*	0	2
Enterococci + *Gardnerella* + *S aureus*	2	0
Enterococci + *Urealyticum* + *M hominis*	3	2

Table (3-5) observed the Percent and correlation of bacterial genera to individual's urine and vaginal samples in UUI group. *Enterococci* contributed 31% of an individual's urinary and 29% of their vaginal samples with moderate positive correlation (r=0.675). *Gardnerella* contributed 19% of an individual's urinary and 16% of their vaginal samples with moderate positive correlation (r=0.672). *E coli* were 8% of an individual's urinary and 8% of their vaginal samples with strong positive correlation (r=0.928).

Other bacteria that had weak correlation were *Urealyticum* 15% urinary and 7% vaginal, (r=0.182). Finally, *M hominis* was 5% urinary and 0% vaginal, (r=0.154). *S aureus* 1% urinary and 0% vaginal, (r=0.320).

Table 3-5: Percent and correlation of bacterial genera to individual's urine and vaginal samples in UUI group

Bacteria	**Contribution in urine samples**	**contribution in vaginal samples**	**Correlation (r)***
Enterococci	31%	29%	0.675
Gardnerella	19%	16%	0.672
E coli	8%	8%	0.928
Urealyticum	15%	7%	0.182
S. aureus	1%	0%	0.320
M. hominis	5%	0%	0.154

*Pearson's correlation

Table (3-6) observed the Percent and correlation of bacterial genera to individual's urine and vaginal samples. *Enterococci* contributed 18% of an individual's urinary and 16% of their vaginal samples with moderate positive correlation (r=0.65).

Gardnerella contributed 12% of an individual's urinary and 8% of their vaginal samples with moderate positive correlation (r=0.44). *E coli* were 6% of an individual's urinary and 4% of their vaginal samples with moderate positive correlation (r=0.53).

Urealyticum 11% urinary and 8% vaginal, strong correlation (r=0.63). *S aureus* 2% urinary and 0% vaginal, weak correlation (r=0.12). Finally, *M hominis* was 5% urinary and 4% vaginal, strong correlation (r=0.88).

Table 3-6: Percent and correlation of bacterial genera to individual's urine and vaginal samples in control group

Bacteria	Contribution in urine samples	Contribution in vaginal samples	Correlation (r)*
Enterococci	18%	16%	0.65
Gardnerella	12%	8%	0.44
E coli	6%	4%	0.53
Urealyticum	11%	8%	0.63
S. aureus	2%	0%	0.12
M. hominis	5%	4%	0.88

*Pearson's correlation

Discussion

This study involved 50 ladies with urge urinary incontinence (UUI) and comparable 50 without UUI (control group) with age ranged from (25-55) years.

The results of this study demonstrated a strong correlation between urinary and vaginal microbes regarding ***E coli*** followed by *Enterococci* and *Gardnerella*. Weak correlations were seen with other microbiomes.

The intestine, vagina, and bladder are three anatomical sites commonly involved in the pathogenesis of UTIs. The resident microbiota acts as a potential reservoir of uropathogenic bacteria or protects us from the possibility of developing a UTI [15].

Urinary tract infections are mainly caused by uropathogenic Escherichia coli, which is responsible for more than 80% of community-acquired infections, while healthcare-associated infections are associated with Staphylococcus, Klebsiella, Enterobacter, Proteus, and Enterococcus. Moreover, an increase in the number of E. coli in the intestine is associated with E. coli bacteriuria and the development of E. coli urinary tract infections [16].

Karstensand colleagues presented evidence supporting the presence of bacterial communities in the bladder of women with and without lower urinary tract symptoms. Mainly, they found, that increased symptoms and episodes of urge incontinence are associated with features of the urinary tract microbial community that may be clinically relevant [17].

Brannon and colleagues highlighted the ability of E.coli to persist in the vagina after a UTI, demonstrating that various urinary E.coli isolates not only adhere to vaginal cells in mouse models of acute and chronic infection, but they also invade vaginal cells. In contrast, vaginal colonization model showed that vaginal colonization can subsequently infect the bladder with pathogens and cause rUTIs. These results demonstrated the ability of E. coli to create an intracellular vagal reservoir where the pathogen is protected from extracellular stressor before causing ascending infection, as supported by our study [18].

Klein *et al.*, in terms of bacterial species, the samples contained comparable numbers of Enterobacteriaceae, a few non-fermenting gram-negative bacteria, but significantly more gram-positive cocci and gram-positive bacilli. In particular, Alloscardivia omnicolens, *Gardnerella vaginalis, Actinomyces* spp. and *Actinotignum schaalii* were significantly more abundant in samples incubated and processed by standard laboratory automation, a study we are not compatible with due to the large sample size (35,564), compared to our study sample size [19]

According to Pearce *et al*. Urinary urge incontinence is an underdiagnosed urinary disorder characterized by symptoms similar to a urinary tract infection, including urinary frequency and urge to urinate. The recent discovery of the urinary tract microbiome calls for clarification of whether bacteria play a role in causing UUI. We have shown that the urinary tract microbiomes of UUI and non-UUI differ between groups in both sequences and cultures evidence. Compared to the non-UUI microbiome, sequencing experiments showed that the UUI microbiome contained an increase in *Gardnerella* and a decrease in Lactobacillus. Nine genera (*Actinobaculum*, *Actinomyces*, *Aerococcus*, *Arthrobacter*, *Corynebacterium*, *Gardnerella*, *Oligella*, Staphylococci and Streptococci) grew more than the INN group. In addition, this study does not agree with our study because of the large sample size and longer duration, so we need more samples to identify these microbiomes. [20]

Our study, disagreed the results Yuko M. Komso *et al.*, they pointed an important relationship between the vaginal and urinary microbiome, and that Lactobacillus predominates in both urine and vagina (samples No. = 212). Several other bacteria are also highly correlated between vagina and urine, including *Gardnerella* (r = 0.70), Prevotella (r = 0.64), and Ureaplasma (r = 0.50). This connection has implications for studying the manipulation of the genitourinary microbiome to prevent urinary incontinence and treat conditions such as urinary tract infections [21].

Eric T. Leung Used traditional bioinformatics and they characterized the vaginal and urinary tract microbiomes and analyzed the utility of concurrent microbial networks for understanding urinary tract infections in women. Common microbial networks can be used to understand lower urinary tract symptoms and their relationship to the urinary tract and vaginal microbiome. Lactobacillus-related bacteria in the urinary tract microbiome of women with UMN are uropathogenic for urinary tract infections. They also found that lactobacillus-related bacteria are uropathogen associated with urinary tract infections, on the other hand, *Aerococcus* found to be important pathogens in the microbial community of the vaginal microbiome of women with UUI, these findings inconsistent with ours [22].

Tomislav Meštrovi'c and his co-workers supported our study , they showed that vaginal dysbiosis can lead to E. coli colonization and rapid recurrence of UTIs, while changes in the urinary microbiome can precede UTIs and other pathologies in the urinary tract [23].

Another study consistent with our findings involved three roles of vaginal bacterial populations in UTIs and urinary tract health. First, the vagina can be a reservoir for E.Coli, the most common cause of urinary tract infections, and other known uropathogens. Second, several species of vaginal bacteria are often detected in urine cultures but are not considered urinary pathogens, and other species of vaginal bacteria are poorly characterized because of their variable nature. Third, some vaginal bacteria that are not widely considered uropathogens can enter the urinary tract, cause damage or immunomodulation, and alter the balance of host-pathogen interactions and affect the outcome of uropathogenesity [24].

As in our study, Seifu *et al*. reported that urinary tract infections were prevalent in the study area, with uropathogens were Escherichia coli being the most isolated, followed by staphylococci [25].

Conclusion

This study illustrates positive associations between vaginal and urinary genera in urge urinary incontinence. *Enterococci* was the most abundant in both urine and vagina but *E coli* had high correlation between urine and vagina while Other microbiomes as *Gardnerella* had moderate correlation and others as (*Urealyticum*, *S aureus* and *M hominis*) had weak correlation between urine and vagina in urge incontinence.

Also we conclude that microbiomes as *Enterococci* and *Gardnerella* can cause more infection in combination with other microbes than alone while E.coli can cause infection alone rather than in combination with other bacteria

Recommendation

Good vaginal hygiene, treatment of vaginal infection and urinary tract infection can decrease risk of urge urinary incontinence.

Further study needed to find correlation between gut E.coli which is the most predominant intestinal bacteria with both vaginal and urinary E.coli.

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