

Research Article

Sex-Based and Age-Specific Trends in *Gardnerella vaginalis* Infection: An Epidemiological Study in Republic of Korea, 2018-2022

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Abstract: Sexually Transmitted Infections (STIs) are an important global public health issue caused by various pathogens. STI symptoms can manifest at different times, depending on the sex, age, and infection route. Here, we aimed to assess the occurrence of STIs in the Republic of Korea, explore associated global trends, and identify pathogens with high positivity rates to inform future health policies. A total of 177,599 samples were collected by U2Bio (Jangwon Medical Foundation, Seoul, Republic of Korea), a testing agency that conducts STI tests for general practitioners and semi-hospitals. Among these, 49,395 specimens were positive for *Gardnerella vaginalis* (GV) and were analyzed retrospectively based on sex, age, co-infection, and sample collection method. Among the pathogens isolated in the positive group, GV showed the highest prevalence at 27.8% (n = 49,395/177,599). Males were more frequently infected than females, with a higher positivity rate in younger males (20-29 years; 42.6%) than in age-matched younger females (14.3%). The highest prevalence was detected in younger males (42.6%) and older females (> 90 years; 21.4%) among the different male and female groups. GV showed co-infection with up to nine other STIs, with the highest co-infection rate with *Ureaplasma* spp. In summary, GV infections vary by sex, age, and specimen type, with higher rates in males despite its association with bacterial vaginosis in females. These findings suggest the need for targeted diagnostic strategies, improved STI screening programs, and tailored public health interventions to mitigate infection risks based on sex and age demographics.

Keywords: Coinfection, *Gardnerella vaginalis*, Sexually transmitted infections, *Ureaplasma parvum*, *Ureaplasma urealyticum*

Introduction

The incidence of Sexually Transmitted Infections (STIs) is a major global public health concern, with various pathogens affecting populations differently based on sex, age, transmission routes, and other factors. Studies on sex-specific positivity rates have shown that although males provide more samples for STI testing, a higher proportion of samples from females test positive (Dias *et al.*, 2022; Kreisel *et al.*, 2021). This observation aligns with the findings that the vaginal microbiota, including species such as *Gardnerella* spp., contributes to Bacterial Vaginosis (BV) and facilitates other STIs. These elevated positivity rates in females emphasize the need for targeted

STI prevention and treatment in males to mitigate urethritis and related complications (Du *et al.*, 2022; Fu *et al.*, 2022; Kenyon *et al.*, 2013).

Age-specific studies have indicated that infection rates tend to vary with age, with the highest rates observed among females of reproductive age and a surge in specific age brackets (Minichiello *et al.*, 2012; Choe *et al.*, 2011). For instance, previous studies in India demonstrated that males aged 30-39 years exhibit the highest positivity rates, which aligns with the findings of our previous study (Vora *et al.*, 2011). In terms of sample collection methods, studies in the Republic of Korea reported the highest positivity rates in samples obtained via swabs, which is

consistent with our previous findings (Park *et al.*, 2020). However, other studies have highlighted high positivity rates obtained with urine samples for asymptomatic infections in males, suggesting a need to balance diagnostic accuracy with patient convenience when selecting collection methods (Kılıç *et al.*, 2022)

Research on temporal trends has demonstrated that increased testing volumes often result in varied incidence rates, particularly among younger age groups, with patterns differing by age and region. These findings highlight the importance of conducting regional and country-specific research to inform public health strategies (Kenyon *et al.*, 2013; Makuza *et al.*, 2022). Additionally, previous studies have documented co-infections of BV with other STIs, such as those caused by *Chlamydia* spp., *Neisseria* spp., and *Ureaplasma* spp. These findings align with our earlier findings (Sarier, 2019) and emphasize the need to understand inter-STI relationships to improve co-infection prevention and management strategies (Sarier, 2019).

As STI rates continue to rise, research using diverse methodologies to identify trends and contributing factors remains active. While previous studies have primarily explored STI prevalence in broader contexts, this study focuses on *Gardnerella Vaginalis* (GV) infections, analyzing sex-based, age-specific, and specimen-type-related trends over a five-year period in the Republic of Korea. By evaluating GV positivity rates in different demographic groups and specimen types, we aim to provide insights that could inform targeted STI prevention and treatment strategies.

Materials and Methods

Sample Collection

This retrospective analysis was conducted using 177,599 samples collected between September 2018 and December 2022 by U2Bio (Jangwon Medical Foundation, Seoul, Republic of Korea). Samples were collected using four different methods: swabs, urine, semen, and other fluids (blood and vaginal fluid).

DNA Extraction

DNA extraction was performed using the Bioneer's DNA Extraction Kit (Seoul, Republic of Korea), following the manufacturer's protocol.

Real-Time PCR

Sample analysis was conducted using real-time PCR with a Bioneer STI Kit (Bioneer, Seoul, Republic of Korea). To detect *Gardnerella vaginalis* (GV), the target gene 16S rRNA was used (Table 1), and PCR amplification was performed according to the manufacturer's protocol under the following cycling

conditions: 45 cycles of 95°C for 5 min, 95°C for 5 s, and 55°C for 5 s.

Table 1: The target gene and product size (base pair) for the detection of each STI

Detected pathogen	Target gene	Product size (bp)
<i>Candida albicans</i>	5S rRNA	101
<i>Chlamydia trachomatis</i>	ompA	88
<i>Gardnerella vaginalis</i>	16S rRNA	130
<i>Herpes simplex virus type I</i>	us4	111
<i>Herpes simplex virus type II</i>	Gg	86
<i>Mycoplasma genitalium</i>	MaPa	131
<i>Mycoplasma hominis</i>	Gap	88
<i>Neisseria gonorrhoeae</i>	16S rRNA	90
<i>Treponema pallidum</i>	polA	136
<i>Trichomonas vaginalis</i>	Beta tubulin	111
<i>Ureaplasma parvum</i>	ureC	138
<i>Ureaplasma urealyticum</i>	uREc	125

The PCR kit components were provided in a ready-to-use, lyophilized, single-tube format and included primers, dual-labeled fluorogenic (TaqMan) probes, DNA polymerase, dNTPs, and stabilizers. The kit was thawed at room temperature (18-22°C) for 10 min before use. The kit components included STI8A-Plex Premix, STI8B-Plex Premix, STI8A, B Positive Control (PC) DNA, Internal Positive Control (IPC) DNA, DEPC-treated distilled water (no-template control, NTC), DEPC DW, and optical sealing film.

The samples used for analysis included urine and vaginal swabs, which were stored at 4-8°C for up to six days. For extended storage, samples were aliquoted and stored at -80 to -20°C. Nucleic acids were extracted from the collected samples using the ExiPrep™ 16Dx system (Bioneer, Seoul, Republic of Korea).

Prior to testing, PCR premix tubes were prepared, including four tubes (two NTC and two PC tubes). All reagents, controls, and samples were vortexed for at least 10 s and then spun down to obtain accurate results. To compensate for the potential sample loss, the volume of each tube was maintained at a slightly higher value for each sample.

Forty-five microliters of each mixture were dispensed into PCR premix tubes, and 5 µL of NTC was added to the NTC wells. The wells were then sealed using an optical sealing film. The tubes were transferred to separate

spaces to avoid contamination, and 5 µL of PC was added to each PC well. To avoid confusion, each well was labeled according to the respective sample.

The optical sealing film was trimmed using scissors or a blade to fit each tube, and the film was firmly applied using an applicator. To ensure complete mixing of the PCR premix and DNA, the tubes were centrifuged using ExiSpin™ (Bioneer, Seoul, Republic of Korea) at 2500 rpm with 1 s spin-down/hard vortexing for 20 s, which was repeated for 20 cycles. Upon completion, the tubes were loaded onto an Exicycler™ 96 real-time thermal block (Bioneer, Seoul, Republic of Korea) for analysis.

Following amplification, the fluorescence amplification curves were reviewed, and the validity of the experiment was assessed by confirming the accuracy of the NTC, PC, and IPC, ensuring that all procedures were properly conducted.

Statistical Analysis

In this study, statistical analysis was performed to analyze the yearly trend of 12 STD infections, differences between age groups, positive rates according to gender, and differences according to sample collection methods. The chi-square (χ^2) test was applied to evaluate the statistical significance of differences within subgroups. STD-positive and negative expected values were calculated by multiplying the total number of people in each subgroup by the total number of STD-positive and negative rates, and then dividing by the total number of study subjects. If the p-value was less than 0.05, it was considered significant. All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA).

Results

Sex-Based GV-Positivity Rate

Of the 177,599 STI samples collected, 49,395 were positive for GV (Table 2). The majority of the samples were from males accounting for 77.2% (137,027/177,599) of the samples, whereas females accounted for 22.8% (40,572/177,599). The overall GV-positivity rate across all samples was 27.8%, with a higher positivity rate in females (49.3%) than that in males (21.5%; Table 1).

Age-Specific GV Infection

GV positivity was the highest among individuals in their 20s, with males exhibiting a 28.3% higher positivity rate than females in this age group. In the 20-39-year age range, males had a higher GV positivity rate than females, and this trend continued in individuals aged 40-59 years. However, among

individuals aged 90-99 years, females had a 15.4% higher GV positivity rate than males (Fig. 1).

Similarly, the overall STI positivity rates were lower in 20-69-year-old females than those in age-matched males. Nevertheless, the overall STI positivity showed an increasing trend in females older than 70 years (Fig. 2).

Table 2: Analysis according to sex, age, sample reception period, and specimen type

		N	%
Sex	Male	137,027	77.2
	Female	40,572	22.8
	Sex difference	96,455	55.4
	≥9	28	0.0
Age (years)	10-19	2,773	1.6
	20-29	43,877	24.7
	30-39	43,793	24.7
	40-49	32,072	18.1
	50-59	26,705	15.0
	60-69	18,674	10.5
	70-79	7,528	4.2
	80-89	2,032	1.1
	90-99	117	0.1
	2018	5,912	3.3
Sample reception period	2019	25,394	14.3
	2020	33,885	19.1
	2021	42,764	24.1
	2022	69,644	39.2
	Swab	21,879	12.3
Specimen type	Urine	143,043	80.5
	Semen	372	0.2
	Other	12,305	6.9
	Total	49,395	27.8
GV positivity	Male	29,394	21.5
	Female	20,001	49.3
GV Only Infection	Total	17,313	35.0
	Male	11,061	63.9
	Female	6,252	36.1

Rate of GV Positivity Based on Sample Collection Methods

Of all samples collected from 2018 to 2022, urine samples were the most frequently collected specimen type (143,043 samples), followed by swabs (21,879 samples). Urine samples had the highest collection rate among males in terms of the ratio by the sample collection method, whereas swabs were more commonly used for females.

Considering the positivity rate of the collection method, swabs had a higher positivity rate than urine, particularly in females than in males. In contrast, the positive detection rate of the collection method was higher for urine samples than for swab samples. A difference in positivity rate was observed based on sex, suggesting differences according to the sample type (Table 3).

Among the GV-positive samples in males, urine showed the highest ratio (91.0%), and in females, swabs showed the highest ratio (61.4%). These findings suggest a sex-based preference for sample collection methods and differences in GV detection rates (Fig. 3).

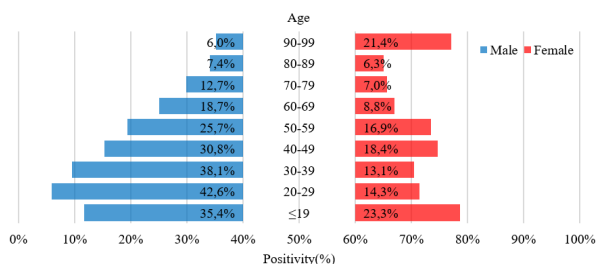


Fig. 1: Gardnerella vaginalis positivity assessed and analyzed across different age groups in both male and female groups

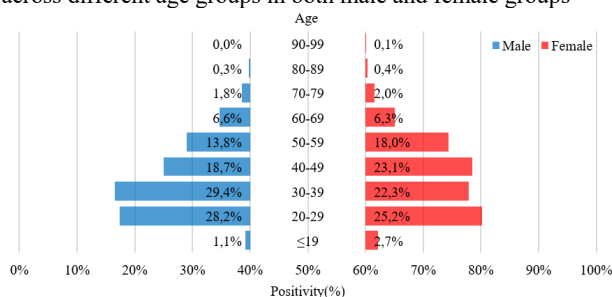


Fig. 2: STI positivity rates across different age groups in both males and females

Table 3: Positivity rate of each sample collection method according to sex

	Sample collection method	Positivity		Negativity	
		N	%	N	%
Total	Swab	15,561	71	6,318	29
	Urine	62,284	44	80,759	56
	Semen	114	31	258	69
	Other	5,194	42	7,111	58
Male	Swabs	441	61	285	39
	Urine	53,056	42	72,614	58
	Semen	114	31	258	69
	Other	4,128	40	6,131	60
Female	Swabs	15,120	71	6,033	29
	Urine	9,228	47	8,145	53
	Semen	0	0	0	0
	Other	1,066	52	980	48

Annual Changes and Positivity Rate Change

The number of samples collected increased each year compared to that in their preceding years, with the largest increase observed in 2019 (429.5% increase compared to 2018) (Table 4).

The overall STI positivity rate by year showed a downward trend from 2018 to 2022, with the highest rate observed in 2018 (56.0%) and the lowest in 2022 (41.3%). Stratification of the positive samples for males and females showed an increasing trend, with the highest

number of positive cases observed in 2022 for both males and females. Despite the increased test volume, the positivity rates in males and females showed no trend. The highest positivity rate for males was observed in 2019 (71.8%), while it was highest in 2021 (35.3%) for females. Nevertheless, the positivity rate tended to decrease in males and increase in females, indicating sex-related differences. GV positivity rates followed a decreasing trend, dropping from 38.8% in 2018 to 22.1% in 2022. However, the highest GV positivity rate was observed in 2019 for males (45.3%) and 2021 for females (27.7%) (Table 5).

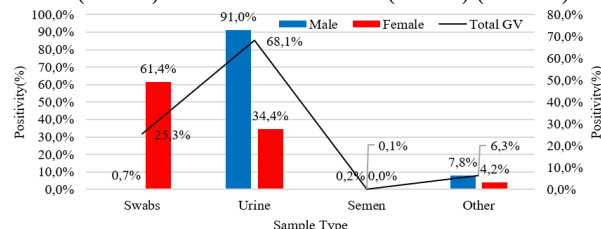


Fig. 3: GV positivity rates based on sample collection methods for different sexes. The right axis shows GV positivity (%) presented by bars, and the line graph shows the total GV positivity

Table 4: Sample collection, collection rate, and annual increase in sample collection. Sample reception ratio represents the ratio of all received samples to the corresponding year

Period	Number of samples	Sample reception ratio (%)	Increase ratio (%)
2018	5,912	3.3	-
2019	25,394	14.3	429.5
2020	33,885	19.1	133.4
2021	42,764	24.1	126.2
2022	69,644	39.2	162.9

Co-Infection of GV and STIs

Of the 49,395 GV-positive samples, 35.0% (17,313/49,395) showed GV-only infection. Among these, males accounted for 63.9% (11,061/17,313), and females accounted for 36.1% (6,252/17,313; Table 2).

Co-infections with 2-9 pathogens were observed in the samples, with GV co-infected with up to nine pathogens. Among the two-pathogen co-infections, GV co-infection accounted for 32.5% (27,066/83,153) of the total positive samples, of which co-infection with *Ureaplasma Parvum* (UP) was the most prevalent at 44.2% (11,963/27,066).

For three-pathogen co-infections, 9.9% (8,262/83,153) of the total positives were observed. Among these, 26.3% (2,171/8,262) showed co-infection of GV with *U. Urealyticum* (UU) and *Mycoplasma Hominis* (MH), and 21.6% (1,784/8,262) were co-infected with GV with UP and MH.

Four-pathogen co-infections were observed in 2.0% (1,716/83,153) of the samples, with GV co-infections with UU, MH, and UP in 27.0% (463/1,716) of these samples.

Five pathogen co-infections were detected in 0.3% (304/83,153) of the total positive samples. GV co-infection with UU, MH, UP, and Chlamydia Trachomatis (CT) comprised 18.1% (55/304), and GV co-infection with CT, UU, MH, UP, and Herpes Simplex Virus 2

(HSV2) comprised 11.5% (35/304) of the total samples with five-pathogen co-infection. Furthermore, 11.2% (34/304) of the total samples with five-pathogen co-infection revealed co-infection with GV, UU, MH, UP, and HSV2.

Table 5: Sample positivity rate based on sex and annual change in *Gardnerella vaginalis* infection

	2018	2019	2020	2021	2022
Reported STI-positive sample	3,312	13,850	18,204	18,967	28,820
%	56.0	54.5	53.7	44.3	41.3
Reported STI-negative sample	2,600	11,544	15,681	23,797	40,824
%	44.0	45.5	46.3	55.6	58.6
Reported positive sample in males	2,379	10,612	12,368	12,273	20,107
%	71.8	76.6	67.9	64.7	69.8
Reported positive samples in females	933	3,238	5,836	6,694	8,713
%	28.2	23.4	32.1	35.3	30.2
Reported GV-positive samples	2,291	8,998	11,458	11,275	15,373
%	38.8	35.4	33.8	26.4	22.1
Reported GV-positive sample in males	1,457	6,269	6,818	6,016	8,834
%	44.0	45.3	37.5	31.7	30.7
Reported GV-positive sample in females	834	2,729	4,640	5,259	6,539
%	25.2	19.7	25.5	27.7	22.7

Six-pathogen co-infections accounted for 58 of the 83,153 total positive samples. Co-infection with GV, CT, UU, *Mycoplasma Genitalium* (MG), MH, and UP was observed in 19.0% (11/58), and that with GV, CT, UU, MH, CA, and UP was observed in 17.2% (10/58) of the total samples with six-pathogen co-infection.

Seven-pathogen co-infections were observed in 5 of 83,153 positive samples, comprising GV co-infection with CT, UU, MH, CA, UP, and HSV2 in 40.0% (2/5); GV co-infection with CT, UU, MG, MH, UP, and HSV2 in 20.0% (1/5); GV co-infection with CT, UU, MH, CA, UP, and HSV1 in 20.0% (1/5); and GV co-infection with *Neisseria Gonorrhoeae* (NG), CT, UU, MG, MH, and UP in 20.0% (1/5) samples.

Nine-pathogen co-infection was observed in only one sample, which was coinfecting with NG, CT, UU, MH, *Trichomonas Vaginalis* (TV), CA, UP, and HSV2.

UP was present in all co-infections, followed by UU and MH, which also had high co-infection rates.

Among males, GV infection alone accounted for 11,061 positive samples, with co-infections observed in 13,588 (two pathogens), 4,184 (three pathogens), 526 (four pathogens), 59 (five pathogens), and six (six pathogens) samples.

In females, GV infection alone accounted for 6,252 positive samples, with co-infections observed in 8,791 (two pathogens), 3,516 (three pathogens), 1,140 (four pathogens), 244 (five pathogens), 52 (six pathogens), and 5 (seven pathogens) samples. Additionally, one sample showed a nine-pathogen co-infection.

Male samples exhibited a maximum of six co-infecting pathogens, whereas female samples showed co-infections with up to nine pathogens (Fig. 4).

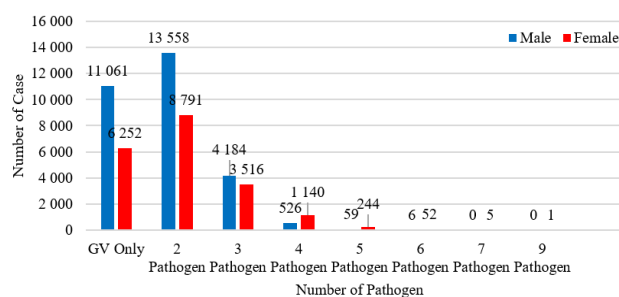


Fig. 4: Sex-based comparison of GV-only infections and STIs caused by co-infection. The y-axis shows the number of infected people, and the x-axis shows the number of pathogens involved in the infection

Discussion

Sex-Based Positivity Rate

This study found that a higher number of samples were collected from males than from females, suggesting greater testing rates among males. However, the positivity rate was higher in females, indicating a possible sex-based difference in the GV infection prevalence.

In females, *Gardnerella* spp. plays a significant role in maintaining and disrupting the vaginal microbiota, contributing to BV. GV also promotes infection with other STIs, necessitating the prevention and treatment of females with high infection rates (Rosca *et al.*, 2020; Chacra *et al.*, 2023). In addition, although the positivity rate was lower in males than in females, the number of samples received was higher for males than for females. Urethritis and other complications may occur in males, indicating the need to prevent GV infection in males (Boyanova *et al.*, 2021).

GV is a common infection in females and is typically limited to the urological system in males. However, GV-related lung complications have been identified in males with alcohol addiction, and cerebrospinal fluid infections have been detected in some young males (Lu *et al.*, 2022). These findings highlight the need for infection prevention and appropriate treatment strategies that extend beyond the positivity rates in both males and females. Furthermore, GV has been associated with various complications, including infections outside the urological system, underscoring the importance of further research on its impact in other areas.

Age-Related GV Infections

The average age of patients with GV infection observed in this study was 26-33 years. In addition, the GV infection rate tended to decrease in individuals under the age of 89 years and increase again in those older than 90 years. GV showed positivity across all age groups, with an average positivity rate of 30.8% for groups younger than 59 years. GV showed the highest positivity rate in the older age group, followed by UP.

According to a Greek study, *Ureaplasma* spp. infection is common in pre-adolescent underage females, and GV is a common infection in pregnant adult females (Sianou *et al.*, 2017). In addition, *Ureaplasma* spp. infections prevail in pregnant and postmenopausal adult females (Sianou *et al.*, 2017). The infection rates in this previous study were similar to those observed in females at the average pregnancy age in this study.

In a study of males with suspected STI-associated urogenic symptoms, GV had the highest positivity rate (15.9%), with an average age of 37 years. This was highly consistent with the findings of a previous study, which

showed the highest positivity rate of 29.4% in males under the age of 30-39 years (Kılıç *et al.*, 2022). Furthermore, an Australian study highlighted an increase in STIs, such as CT, NG, and TP, among older people (Louise *et al.*, 2020).

While our findings are not entirely consistent with those of previous studies, they emphasize the importance of further research on STI infections in younger individuals with high positivity rates and in older adults to inform future prevention and treatment strategies.

Positivity Rate Based on Sample Collection Method

In this study, differences were observed in the proportion of samples collected using different methods, depending on sex. However, both females and males had the highest positivity rates among the swab samples. These findings contrast with previous findings, which showed higher positivity rates in urine samples than in swab samples from males with asymptomatic urological infections (Kılıç *et al.*, 2022). In addition, higher microbial loads have been detected in urine and swab samples from BV-positive females, indicating that both sample types are suitable for GV detection (Naicker *et al.*, 2020).

In studies using alternative methods, such as droplet digital PCR, both urine and swab samples showed high amplification coefficients, confirming their effectiveness for GV detection. Although urine collection is more inconvenient than swab collection, it may be a viable alternative (Naicker *et al.*, 2020b).

This study showed that while swab samples had the highest positivity rate, the number of urine samples was greater than that of swab samples, and urine samples showed a higher overall positivity rate than swab samples. These findings indicate that both urine and swab samples are effective for GV detection. However, urine samples are often preferred owing to the discomfort associated with swab collection. Therefore, improvements in the swab collection method are recommended to enhance diagnostic accuracy and patient convenience.

Year-by-Year Change and Positivity Rate Change

The number of STI tests increased over the study period, with the incidence of some STIs rising while others remained unchanged. The highest incidence was observed in younger age groups (Naicker *et al.*, 2020a). The prevalence of STIs did not vary significantly by month or region; however, differences in prevalence were observed according to age (Naicker *et al.*, 2020b).

A study conducted in the United States reported a 39.0% increase in the number of emergency room visits related to STIs nationwide, with CT being the most common cause of STIs. Half of the cases involved young females, highlighting a public health crisis (Wee *et al.*,

2018). Similarly, a study in Uganda analyzed trends in infection rates among young females and reported a 14.3% increase in positivity rates from 2006 to 2011, accompanied by a 143-sample increase. However, by 2016, the positivity rate had decreased by 13.2% despite a 9,832-sample increase (Chacra *et al.*, 2021). Aligning with these findings, the present study showed that the number of samples has increased over the past five years. Nevertheless, we observed changes in the positivity rates, with the prevalence of some pathogens increasing and that of others decreasing. The differing prevalence patterns by pathogen underscores the need for rapid pathogen-specific diagnostics. Additionally, the varying trends in the incidence of STIs across regions and countries emphasize the need for research on national and regional trends in STI prevalence to guide public health strategies.

Co-infection of GV and STIs

We confirmed that up to nine pathogen co-infections could be transmitted, with seven co-infections involving GV. Among these, UP had the highest co-infection rate, and the relationship between the two pathogens needs to be confirmed. In females with infertility, *Ureaplasma* spp. tended to co-occur with GV in the cervix (Wee *et al.*, 2018). In addition, when the balance of the normal vaginal microbiota is disrupted, the loss of lactobacilli allows for the proliferation of other STIs, such as those caused by UU and MH (Chacra *et al.*, 2021).

There may also be a correlation between BV and STIs, suggesting that the prevalence of both single and multiple infections related to STI-induced microorganisms is high in females with BV (Chacra *et al.*, 2021; Chacra *et al.*, 2023). However, additional research is required to understand the association between BV and STIs (Chacra *et al.*, 2023).

BV is associated with fertility issues, including infertility, which in turn increases susceptibility to STIs (Shvartsman *et al.*, 2023; Morrill *et al.*, 2020). BV, which is characterized by a high prevalence of GV, is closely associated with infertility (Kyono *et al.*, 2018). This association is due to inflammation, sperm antigen immunization, the presence of bacterial toxins, and an increased risk of STIs (Ravel *et al.*, 2021).

Furthermore, the reduced presence of GV and *Lactobacillus* spp. in the vagina is associated with infertility in females. The specific enzymes produced by GV, including sialidases, may facilitate the entry of HPV and other STI-causing bacteria into the vaginal environment, thereby promoting pathogen growth (Gholizadeh *et al.*, 2023).

The co-infection rate of GV and *Ureaplasma* spp. is high, and further research is needed to explore how *Ureaplasma* spp., with their elevated co-infection rates,

may increase the risk of viral infections, such as HPV and HIV, as suggested in previous studies (Shvartsman *et al.*, 2023; Morrill *et al.*, 2020; Kyono *et al.*, 2018; Ravel *et al.*, 2021; Gholizadeh *et al.*, 2023).

Conclusion

This study confirmed the differences in the positivity rate of GV based on sex and specimen type, emphasizing the importance of selecting appropriate specimens for accurate diagnosis and identifying specimens with the highest positivity rates for each pathogen.

Although the overall positivity rate from 56.0% to 41.3% decreased each year, the total number of positive samples increased from 3,312 to 28,820, suggesting an increased prevalence of infection. Furthermore, the positivity rate decreased in males and increased in females, although more samples were obtained from males. As these findings are based on data from a single institution, according to institution variations may exist because data from other institutions were not included. Therefore, further research comparing positivity rates across different institutions is necessary to assess whether differences exist based on the sample collection method.

Same the other study GV exhibited the highest positivity rate 49.3%, particularly as it caused BV in the females. however GV only infection shows a higher rate in males 63.9% than in females 36.1%. The results of this study can contribute to more effective STD prevention by developing customized strategies for each pathogen in consideration of sex, age, and sample collection methods when establishing future health policies by using them as basic data that are expected to show high positivity rates in certain sex.

However, because this study has limitations in collecting and analyzing data from a single institution, it is necessary to increase the reliability of the study by collecting and comparing data from several institutions, and there is also a need for further research.

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Author's Contributions

Hyeok-Jin Kwon, Sun-Gyu Kim, and Jae Kyung Kim: Conceptualization, methodology, formal analysis,

writing, original draft, Writing review and editing, and final approval.

Junmin Lee and Dongin Seok: Conceptualization, Data curation, writing - original draft, writing review and editing, and final approval.

Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the Clinical Research Review Committee of Dankook University (Institutional Review Board DKU, Certificate No. DKU 2021-04-002).

References

- Chacra, A. L., & Fenollar, F. (2021). Exploring the global vaginal microbiome and its impact on human health. *Microbial Pathogenesis*, 160, 105172. <https://doi.org/10.1016/j.micpath.2021.105172>
- Chacra, A. L., Ly, C., Hammoud, A., Iwaza, R., Mediannikov, O., Bretelle, F., & Fenollar, F. (2023). Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission? *Microorganisms*, 11(10), 2470. <https://doi.org/10.3390/microorganisms11102470>
- Boyanova, L., Marteva-Proevska, Y., Gergova, R., & Markovska, R. (2021). Gardnerella vaginalis in urinary tract infections, are men spared? *Anaerobe*, 72, 102438. <https://doi.org/10.1016/j.anaerobe.2021.102438>
- Choe, H.-S., Lee, S.-J., Kim, C. S., & Cho, Y.-H. (2011). Prevalence of sexually transmitted infections and the sexual behavior of elderly people presenting to health examination centers in Korea. *Journal of Infection and Chemotherapy*, 17(4), 456-461. <https://doi.org/10.1007/s10156-010-0191-0>
- Dias, S. P., Brouwer, M. C., & van de Beek, D. (2022). Sex and Gender Differences in Bacterial Infections. *Infection and Immunity*, 90(10), e00283. <https://doi.org/10.1128/iai.00283-22>
- Du, M., Yan, W., Jing, W., Qin, C., Liu, Q., Liu, M., & Liu, J. (2022). Increasing incidence rates of sexually transmitted infections from 2010 to 2019: an analysis of temporal trends by geographical regions and age groups from the 2019 Global Burden of Disease Study. *BMC Infectious Diseases*, 22(1), 574. <https://doi.org/10.1186/s12879-022-07544-7>
- Fu, L., Sun, Y., Han, M., Wang, B., Xiao, F., Zhou, Y., Gao, Y., Fitzpatrick, T., Yuan, T., Li, P., Zhan, Y., Lu, Y., Luo, G., Duan, J., Hong, Z., Fairley, C. K., Zhang, T., Zhao, J., & Zou, H. (2022). Incidence Trends of Five Common Sexually Transmitted Infections Excluding HIV From 1990 to 2019 at the Global, Regional, and National Levels: Results From the Global Burden of Disease Study 2019. *Frontiers in Medicine*, 9, 851635. <https://doi.org/10.3389/fmed.2022.851635>
- Gholizadeh, O., Yekanipour, Z., Kivi, S., Farahbod, F., Aqayinejad, M., Tasoujizade, Y., Yasamineh, S., Amini, P., Akbarzadeh, S., Hajiesmaeili, M., & Poortahmasebi, V. (2023). Review of the evidence of the effects of human papillomavirus infection and Gardnerella vaginalis, and their co-infection on infertility. *Microbial Pathogenesis*, 175, 105980. <https://doi.org/10.1016/j.micpath.2023.105980>
- Kenyon, C., Colebunders, R., & Crucitti, T. (2013). The global epidemiology of bacterial vaginosis: a systematic review. *American Journal of Obstetrics and Gynecology*, 209(6), 505-523. <https://doi.org/10.1016/j.ajog.2013.05.006>
- Kılıç, M., Besli, Y., Köseoglu, E., Palaoglu, E. K., & Esen, T. (2022). Gardnerella vaginalis: Is it an Underestimated Cause of Urinary Symptoms in Males? *Infectious Diseases and Clinical Microbiology*, 4(3), 172-177. <https://doi.org/10.36519/idcm.2022.172>
- Kreisel, K. M., Spicknall, I. H., Gargano, J. W., Lewis, F. M. T., Lewis, R. M., Markowitz, L. E., Roberts, H., Johnson, A. S., Song, R., St. Cyr, S. B., Weston, E. J., Torrone, E. A., & Weinstock, H. S. (2021). Sexually Transmitted Infections Among US Women and Men: Prevalence and Incidence Estimates, 2018. *Sexually Transmitted Diseases*, 48(4), 208-214. <https://doi.org/10.1097/olq.0000000000001355>
- Kyono, K., Hashimoto, T., Nagai, Y., & Sakuraba, Y. (2018). Analysis of endometrial microbiota by 16S ribosomal RNA gene sequencing among infertile patients: a single-center pilot study. *Reproductive Medicine and Biology*, 17(3), 297-306. <https://doi.org/10.1002/rmb2.12105>
- Lu, H., Du, Y., Pan, T., Lou, Z., Li, H., Liao, Y., & Wang, L. (2022). Gardnerella vaginalis purulent meningitis in an adolescent male: a case report. *BMC Neurology*, 22(1), 217. <https://doi.org/10.1186/s12883-022-02733-y>
- Makuza, J. D., Soe, P., Jeong, Dahn., Nisingizwe, M. P., Dushimiyimana, D., Umutesi, J., Nshimiyimana, L., Maliza, C., Serumondo, J., Remera, E., Rwibasira, G. N., Tuyishime, A., & Riedel, D. J. (2022). Epidemiology of sexually transmitted infections: trends among patients screened for sexually transmitted infections in rwandan health facilities 2014-2020. In *BMC Infectious Diseases* (Vol. 22, p. 701). <https://doi.org/10.1186/s12879-022-07685-9>
- Minichiello, V., Rahman, S., Hawkes, G., & Pitts, M. (2012). STI epidemiology in the global older population: emerging challenges. *Perspectives in Public Health*, 132(4), 178-181. <https://doi.org/10.1177/1757913912445688>

- Morrill, S., Gilbert, N. M., & Lewis, A. L. (2020). Gardnerella vaginalis as a Cause of Bacterial Vaginosis: Appraisal of the Evidence From in vivo Models. *Frontiers in Cellular and Infection Microbiology*, 10, 168. <https://doi.org/10.3389/fcimb.2020.00168>
- Naicker, D., Ramsuran, V., Naicker, M., Dessai, F., & Abbai, N. (2020). Quantitative detection of bacteria associated with BV in urine versus swab samples using droplet digital PCR. *International Journal of Infectious Diseases*, 101, 446. <https://doi.org/10.1016/j.ijid.2020.09.1169>
- Park, S.-H., Hwang, K.-A., Ahn, J. H., & Nam, J.-H. (2020). Evaluation of Multiplex Polymerase Chain Reaction Assay for the Simultaneous Detection of Sexually Transmitted Infections Using Swab Specimen. *Journal of Bacteriology and Virology*, 50(1), 44-54. <https://doi.org/10.4167/jbv.2020.50.1.044>
- Ravel, J., Moreno, I., & Simón, C. (2021). Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. *American Journal of Obstetrics and Gynecology*, 224(3), 251-257. <https://doi.org/10.1016/j.ajog.2020.10.019>
- Rosca, A. S., Castro, J., Sousa, L. G. V., & Cerca, N. (2020). Gardnerella and vaginal health: the truth is out there. *FEMS Microbiology Reviews*, 44(1), 73-105. <https://doi.org/10.1093/femsre/fuz027>
- Sarier, M. (2019). Prevalence of Polymicrobial Infection in Urethritis. *Journal of Urological Surgery*, 6(3), 180-183. <https://doi.org/10.4274/jus.galenos.2019.2405>
- Shvartsman, E., Hill, J. E., Sandstrom, P., & MacDonald, K. S. (2023). Gardnerella Revisited: Species Heterogeneity, Virulence Factors, Mucosal Immune Responses, and Contributions to Bacterial Vaginosis. *Infection and Immunity*, 91(5), e00390-22. <https://doi.org/10.1128/iai.00390-22>
- Sianou, A., Galyfos, G., Moragianni, D., & Baka, S. (2017). Prevalence of vaginitis in different age groups among females in Greece. *Journal of Obstetrics and Gynaecology*, 37(6), 790-794. <https://doi.org/10.1080/01443615.2017.1308322>
- Vora, R., Anjaneyan, G., Doctor, C., & Gupta, R. (2011). Clinico-epidemiological study of sexually transmitted infections in males at a rural-based tertiary care center. *Indian Journal of Sexually Transmitted Diseases and AIDS*, 32(2), 86-89. <https://doi.org/10.4103/0253-7184.85410>
- Wee, B. A., Thomas, M., Sweeney, E. L., Frentiu, F. D., Samios, M., Ravel, J., Gajer, P., Myers, G., Timms, P., Allan, J. A., & Huston, W. M. (2018). A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, 58(3), 341-348. <https://doi.org/10.1111/ajo.12754>