

RESEARCH ARTICLE

Coinfections with multiple sexually transmitted pathogens in Republic of Korea, 2018–2020

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Abstract

Background: Sexually transmitted infections (STIs) can have serious consequences, and the global STI incidence remains high. However, there is little information on the frequency of STIs with multiple pathogens according to age. Accordingly, we conducted a study to determine the trends of coinfection with sexually transmitted pathogens according to age in the Republic of Korea from 2018 to 2020.

Methods: From January 2018 to December 2020, 65,191 samples of swab, urine, and other types submitted for STI screening were obtained from U2Bio Co. Ltd. (Seoul, Republic of Korea). Multiplex polymerase chain reaction, a sensitive and rapid method for simultaneous detection of STIs caused by multiple different pathogens, was performed using an AccuPower STI4C-Plex Real-Time PCR kit, AccuPower STI8A-Plex Real-Time PCR kit, and AccuPower STI8B-Plex Real-Time PCR kit with an Exicycler 96 Real-Time Quantitative Thermal Block.

Results: Of the 65,191 samples tested, 35,366 (54.3%) tested positive for one or more sexually transmitted pathogens. The prevalence of coinfections with two or more sexually transmitted pathogens was inversely proportional to age. Furthermore, the rates of coinfection with sexually transmitted pathogens and age distribution differed according to sex and the sexually transmitted pathogen type.

Conclusion: This study confirmed that a significant proportion of patients with STIs are coinfecting with multiple pathogens. Public health managers could use these results to recognize and prevent STIs according to age.

KEYWORDS

coinfections, community health, multiplex polymerase chain reaction, Republic of Korea, sexually transmitted infections

1 | INTRODUCTION

Sexually transmitted infections (STIs) are caused by pathogens commonly transmitted through sexual contact, particularly vaginal, anal, or oral sex.^{1,2} STIs continue to be a serious problem. The global

incidence remains high, but most STIs are asymptomatic.³ It is estimated that more than 500 million people between the ages of 15 and 49 years have genital infections caused by the herpes simplex virus (HSV).³ Pathogens that cause STIs include bacteria, parasites, and viruses.⁴ Bacterial STIs include chlamydia, gonorrhoea, and syphilis;

Sun Jung Lee and Tae Su Jang contributed equally to this project and should be considered co-first authors.

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parasitic STIs include trichomoniasis; and viral STIs include human immunodeficiency virus, HSV, and hepatitis B virus infection.⁵ They cause symptomatic or asymptomatic infections of genital organs, which may lead to infertility, pregnancy complications, cancer, and even death.⁵ If left untreated, STIs can have serious consequences, including neurological manifestations, infertility, mother-to-child transmission, and birth defects.⁶

Various methods are used to diagnose STIs, including culture or serological tests for each specific pathogen, but these methods are generally time-consuming and lack sensitivity for identifying STIs in asymptomatic patients.^{7,8} Considering the frequency of coinfection with STIs, the high number of asymptomatic cases, and the difficulties associated with culture methods, nucleic acid amplification testing is an appropriate method for diagnosing STIs.⁴ Multiplex polymerase chain reaction (mPCR) is a sensitive and rapid method for simultaneous detection of STIs caused by multiple pathogens in clinical laboratories and has recently attracted attention as a method for detecting sexually transmitted pathogens using molecular diagnostics.^{4,8}

A significant proportion of patients with STIs are coinfecting with multiple pathogens.⁷ In this study, patients were tested for coinfections with 12 different sexually transmitted pathogens: *Candida albicans*, *Chlamydia trachomatis*, *Gardnerella vaginalis*, HSV1, HSV2, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Ureaplasma parvum*, and *Ureaplasma urealyticum*. A symbiotic relationship between *T. vaginalis* and *M. hominis* has been reported in a Chinese study.⁹ A novel *Mycoplasma* strain, *Candidatus Mycoplasma girerdii*, has been identified in vaginal secretions of women, and it has been shown that this strain may be associated with trichomoniasis.⁹ In Madrid, Spain, *T. vaginalis* was shown to play a key role in the risk of acquiring infections with other genitourinary pathogens, such as *N. gonorrhoeae* and *C. trachomatis*.¹⁰ *M. genitalium*, which is considered an independent risk factor for cervicitis in women, was found only in *C. trachomatis*-positive samples.¹¹ A study conducted in Croatia also showed a significant association between *M. genitalium* and *C. trachomatis* infections.¹¹ *M. genitalium* and *M. hominis* infections have also been reported to be significantly associated.¹¹

The number of STIs continues to increase worldwide.¹² Although there are multiple reports on STIs caused by multiple pathogens, there is little information on the frequency of STIs with multiple pathogens according to age. Therefore, this study aimed to investigate the trend of coinfection by age in patients in the Republic of Korea screened for STIs using mPCR from 2018 to 2020.

2 | MATERIALS AND METHODS

2.1 | Materials

From January 2018 to December 2020, 65,191 samples of swab, urine, and other types (catheter, pus, and tissue) from patients who were tested for STIs, excluding those aged 0 and over 100 years old, were obtained from U2Bio Co. Ltd. (Seoul, Republic of Korea) for STI screening.

2.2 | Methods

2.2.1 | DNA extraction

For mPCR, the clinical specimens were stored at -70°C until DNA isolation. DNA for the mPCR assay was extracted using the ExiPrep Dx Bacteria Genomic DNA Kit (Exicycler 96, Bioneer, Daejeon, Republic of Korea) according to the manufacturer's instructions.

2.2.2 | Multiplex polymerase chain reaction

PCR analysis was performed using AccuPower STI4C-Plex Real-Time PCR kit (Exicycler 96, Bioneer, Daejeon, Republic of Korea), AccuPower STI8A-Plex Real-Time PCR kit (Exicycler 96, Bioneer, Daejeon, Republic of Korea), and AccuPower STI8B-Plex Real-Time PCR kit (Exicycler 96, Bioneer, Daejeon, Republic of Korea) with an Exicycler 96 Real-Time Quantitative Thermal Block (Exicycler 96, Bioneer, Daejeon, Republic of Korea) according to the manufacturer's protocol. The amplification protocol consisted of one cycle of 5 min at 95°C , 5 s at 95°C , and 5 s at 55°C .

After amplification for 96 cycles, 12 different sexually transmitted pathogens (*C. albicans*, *C. trachomatis*, *G. vaginalis*, HSV1, HSV2, *M. genitalium*, *M. hominis*, *N. gonorrhoeae*, *T. pallidum*, *T. vaginalis*, *U. parvum*, and *U. urealyticum*) were detected. In this study, the data of patients who tested positive for more than one of the 12 pathogens were analyzed.

2.3 | Statistical analysis

Statistical analysis was performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics such as frequencies, percentages, and means were computed.

3 | RESULTS

From January 2018 to December 2020, a total of 65,191 samples were received for STI testing, of which 35,366 (54.3%) tested positive for one or more sexually transmitted pathogens (Tables 1 and 2).

Analysis of the rate of coinfection with sexually transmitted pathogens according to age revealed that the number of pathogen types identified decreased with increasing age. The mean age of patients coinfecting with two types of pathogens was 36 years, and the age of the individual coinfecting with nine types of pathogens was 19 years (Table 3).

The rates of coinfection with sexually transmitted pathogens and age distribution differed according to sex. Coinfections in male patients were more frequent in the 30- to 39-year age group, whereas the age of female patients with coinfections varied from 19 to 40 years (Figure 1).

The prevalence of coinfection differed according to the sexually transmitted pathogen type. Among them, *N. gonorrhoeae* and *C. trachomatis* showed a high rate of single infection but a low rate of coinfection, and *N. gonorrhoeae* showed the lowest rate of coinfection with two or more other sexually transmitted pathogens. Conversely, *M. hominis* showed a low rate of single infection and a high rate of coinfection. Among patients with *M. hominis* or *T. vaginalis* infections, the majority had coinfections with more than two types of pathogens (Figure 2).

4 | DISCUSSION

This study showed that the rate of coinfection with multiple pathogens differed according to the age group and that it was higher in younger individuals. A previous study from China reported a high prevalence of STIs in adults between the ages of 20 and 39 years.² This is because young adults have a lower level of awareness of the

risks associated with sexual activity and may engage in sexual activity with multiple sexual partners and unprotected sex.^{2,12} In addition, according to a study conducted in New Mexico, the USA, the

TABLE 3 Mean age of patients with coinfections with multiple types of sexually transmitted pathogens according to the number of different pathogens detected

Number of different pathogens detected	Number of positive samples	Mean age (years)
1 type	18,037	37.6
2 types	12,065	36.0
3 types	4140	35.4
4 types	881	32.7
5 types	196	30.7
6 types	43	26.1
7 types	3	22.9
9 types	1	19

TABLE 1 Number of specimens that tested positive for each type of sexually transmitted pathogen (N = 35,366)

Pathogen	Number of positive specimens
<i>Candida albicans</i>	2068
<i>Chlamydia trachomatis</i>	4674
<i>Gardnerella vaginalis</i>	22,747
Herpes simplex virus 1	194
Herpes simplex virus 2	1358
<i>Mycoplasma genitalium</i>	2118
<i>Mycoplasma hominis</i>	3527
<i>Neisseria gonorrhoeae</i>	1638
<i>Treponema pallidum</i>	22
<i>Trichomonas vaginalis</i>	182
<i>Ureaplasma parvum</i>	11,521
<i>Ureaplasma urealyticum</i>	9330

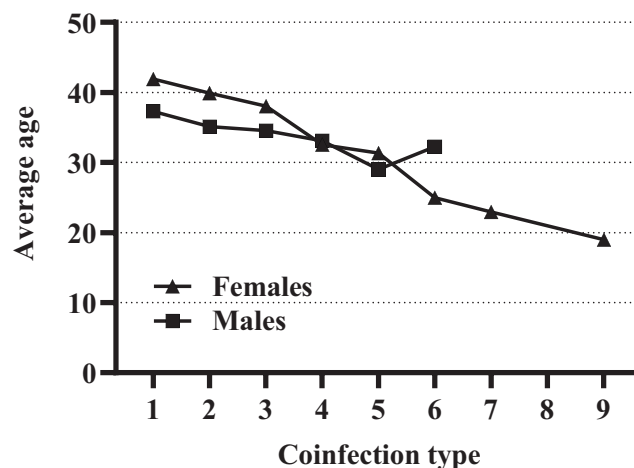


FIGURE 1 Mean age according to sex and the number of coinfections. Triangles and squares represent the mean age of female and male patients, respectively

TABLE 2 Rates of coinfection with each sexually transmitted pathogen

	NG	CT	UU	MG	MH	TV	GV	Candida	UP	TP	HSV 1	HSV 2
Single	32.01	32.15	39.16	33.32	39.85	45.55 ^a	40.54	43.55	40.11	30.29	32.66	42.37
2 types	29.48	29.64	37.35	31.20	39.73	46.53	39.31	39.14	39.61	29.00	32.92	38.57
3 types	29.33	28.86	37.14	31.09	38.75	40.76	37.63	37.58	37.87	35.00	32.91	38.39
4 types	28.39	28.76	35.24	29.23	35.61	40.30	34.60	33.93	35.04	27.75	26.38	36.95
5 types	25.63	26.06	31.30	25.81	30.91	45.47	30.51	29.87	31.41	24.50	N/D	36.57
6 types	23.50	24.50	25.97	26.82	24.51	25.80	25.81	25.32	25.88	N/D	28.00	30.53
7 types	N/D	23.00	23.00	22.00	23.00	N/D	23.00	23.50	23.00	N/D	23.00	23.00
8 types	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
9 types	19	19	19	N/D	19	19	19	19	19	N/D	N/D	19

Abbreviations: *Candida*, *Candida albicans*; CT, *Chlamydia trachomatis*; GV, *Gardnerella vaginalis*; HSV1, Herpes simplex virus 1; HSV2, Herpes simplex virus 2; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; N/D, Not Detected; NG, *Neisseria gonorrhoeae*; TP, *Treponema pallidum*; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

^aHighest percentage.

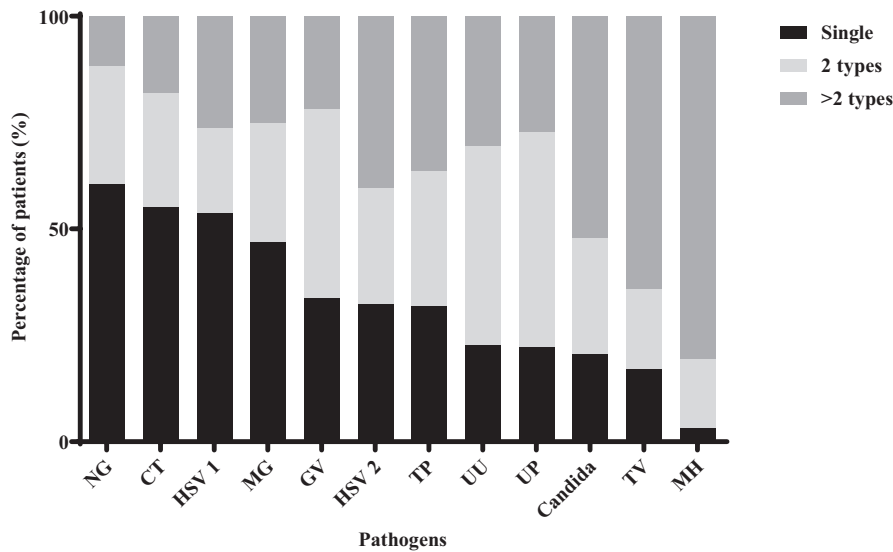


FIGURE 2 Differences in sexually transmitted infections according to the pathogen. Black, light gray, and dark gray represent single, two types, and more than two types of infection, respectively. *Candida*, *Candida albicans*; CT, *Chlamydia trachomatis*; GV, *Gardnerella vaginalis*; HSV1, Herpes simplex virus 1; HSV2, Herpes simplex virus 2; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; NG, *Neisseria gonorrhoeae*; TP, *Treponema pallidum*; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*

highest rate of coinfection with *M. genitalium*, *C. trachomatis*, and *T. vaginalis* occurred in women aged 20–29 years and decreased with increasing age.¹³

In this study, the rate of coinfections differed according to sex. In males, the rate of coinfection was the highest in the 30- and 39-year age group, but in females, the age trend was less marked. According to a study on women of childbearing age conducted in South Africa, coinfections (*T. vaginalis* and *C. trachomatis*, or *T. vaginalis* and *N. gonorrhoeae*) were mainly observed in women under the age of 30 years.¹⁴ Moreover, according to another study conducted in South Africa, the prevalence of coinfection was 56.2% among women with bacterial vaginosis, and 53.4% among women with nonulcerative STIs, with an average age of 26 years.¹⁵

This study showed that the rate of coinfection varied according to the pathogen type. Patients with *N. gonorrhoeae* and *C. trachomatis* infection had a low rate of coinfection, whereas those infected with *M. hominis* and *T. vaginalis* had a high rate of coinfection with other sexually transmitted pathogens. A study conducted in an STI clinic in Birmingham, Alabama, USA, found that coinfection with *M. genitalium* in women with *C. trachomatis* was uncommon, and that it was present in only 7.3% of the coinfection patients.¹⁶ A study of pregnant women who visited a hospital in Ghana showed that *Candida* (53%) coinfection was common in women with *T. vaginalis* infection.¹⁷ In addition, in another study from Iran in which coinfection with sexually transmitted pathogens was confirmed using mPCR, 10/300 patients (3.3%) tested had coinfections confirmed, including 2 cases of *C. trachomatis*/*N. gonorrhoeae*, 3 cases of *C. trachomatis*/*T. vaginalis*, and 5 cases of *N. gonorrhoeae*/*T. vaginalis* coinfections.⁷ According to a study conducted in Beijing, China, among the patients with coinfections, 60.6% of men and 71.4% of women were coinfecting with *U. urealyticum* and *C. trachomatis*.²

This study has several limitations. First, it is not possible to determine the characteristics of the sexual partner (same-sex or opposite-sex, occupation, or education level). Second, as this was a retrospective study that used laboratory records, we did not have data on the clinical characteristics of the patients.

In this study, data on coinfection with STIs were collected and analyzed by age and bacterial species. These results could help public health managers recognize and prevent STIs in various age groups. Therefore, the study results can be used for educational purposes and to develop public health policies related to STIs by recognizing the differences in the STI profile according to age group.

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

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