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Epidemiological Trends of Sexually Transmitted Infections Among Women in Cheonan, South Korea, 2006–2012

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A lack of investigation in specific regions has impeded the understanding of epidemiological trends in the prevalence of sexually transmitted infections (STIs) in South Korea. To help fill this research gap, this study used multiplex polymerase chain reaction (mPCR) to determine the prevalence of STIs detected in clinical specimens collected from women in Cheonan, South Korea between August 2006 and November 2012, and analyzed the prevalence of STIs according to age, bacterial pathogen, and time period. Of the 1,618 specimens collected from 1,523 patients, 536 (35.2%) tested positive for at least 1 pathogen, with 407 (25.2%) testing positive for 1 pathogen, 103 (6.4%) for 2 pathogens, 20 (1.2%) for 3 pathogens, and 6 (0.4%) for 4 pathogens (n = 697 pathogens total). The median ages of all patients and of STI-positive patients were 37.8 and 33.3 years, respectively, and both decreased annually over the study period. *Mycoplasma hominis* (MH) was detected in 62.1% of the positive specimens, *Ureaplasma urealyticum* (UU) in 28.4%, *Chlamydia trachomatis* (CT) in 23.1%, *Trichomonas vaginalis* (TV) in 7.8%, *Mycoplasma genitalium* (MG) in 6.5%, and *Neisseria gonorrhoeae* (NG) in 2.1%. Whereas the prevalence of MH, MG, and TV infection did not vary greatly over the study period, that of UU decreased by one-fifth and that of both CT and NG increased 4-fold. The results indicate great variability in the rates of infection with each pathogen and a decreasing trend in overall STI prevalence, age of patients seeking STI testing, and age of STI-positive patients.

Keywords: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, sexually transmitted infection, *Trichomonas vaginalis*, *Ureaplasma urealyticum*

Introduction

Defined as a broad but relatively well-defined group of infections, sexually transmitted infections (STIs) are typically caused by an acute presentation that can progress to a chronic clinical condition [24]. Reduction of complication rates and disease spread [18] requires the use of rapid and reliable laboratory techniques that can identify the causative pathogen or normal flora, the most common of which have been found to include *Trichomonas vaginalis* (TV), *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), and *Neisseria gonorrhoeae* (NG) [2, 10, 15, 27, 28].

However, major challenges exist with the use of current diagnostic methods in STI detection. For example, in the clinical diagnosis of bacterial vaginosis, the uses of current criteria, such as Amsel's criteria, or techniques, such as determination of Nugent's score [5, 21, 29], lead to inconclusive results in 30% of symptomatic women [22], and have a sensitivity and specificity of 92% and 77%, respectively [7]. Moreover, these methods not only require that testing personnel have a certain level of experience but have also been reported to have questionable diagnostic accuracy due to the ambiguous terms used [1, 19]. Fortunately, polymerase chain reaction (PCR)-based methods have been developed to detect vaginosis pathogens that appear to overcome the disadvantages of conventional criteria and techniques, having been found to have a high level of specificity (95–100%) and sensitivity (95–100%) in investigations of their functionality [19, 20, 22].

Recent reports that an STI often involves not one but rather a group of highly variable pathogens [16, 26] have

prompted a search for methods capable of simultaneously identifying multiple pathogens in a single clinical sample [9, 13, 26]. Two such methods are the dual priming oligonucleotide (DPO) PCR system (Seegene, Seoul, Korea), a recently developed nucleic acid amplification technique [9, 23], and multiplex PCR (mPCR), a PCR technique that allows for simultaneous amplification of more than one target nucleic acid, including those of six the bacterial pathogens, TV, MH, UU, CT, MG and NG, in one test tube [29].

According to the Korea Centers for Disease Control and Prevention (KCDC), at least 7,000 individuals are diagnosed each year with STI in South Korea [6]. Several studies, most of which were conducted under the auspices of large organizations, such as the KCDC, have described the distribution and types of STIs identified in the South Korean population. However, these studies primarily aimed at collecting national data using surveys and reporting the results of statistical analysis of the data rather than conducting direct testing of one region over a specified period to identify the types of STIs and the extent of multiple infections. The lack of investigation into specific areas has impeded understanding of the epidemiological trends of STI prevalence in South Korea. To assist in collecting the foundational data necessary for establishing laboratory diagnostic systems and preventative measures, this study performed multiplex PCR (mPCR) to determine the prevalence of STIs in clinical specimens collected from a large sample of women in Cheonan and analyzed the distribution of STI prevalence according to age, bacterial pathogen, and time period.

Materials and Methods

Materials

From August 2006 to November 2012, 1,618 endocervical swab specimens were consecutively obtained from 1,523 female patients attending STD clinics in the Dankook University Hospital for STI screening. Each collected specimen was tested in a week by mPCR for STI screening, and the epidemiological data were acquired by retrospective clinical analysis.

Methods

DNA extraction. The collected clinical specimens were stored at −70°C until isolation of DNA for the mPCR assay. The swab specimen was suspended in PBS and collected by centrifugation at 13,000 ×*g* for 10 min. The supernatant was discarded and the pellet was resolved in PBS. DNA for the mPCR assay was extracted using a QIAamp DNA Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions. Briefly, approximately 200 µl of each specimen was used as starting material for the DNA isolation. Concentrations of the extracted DNA samples were measured using a Nanodrop 1000 (Thermo Fisher Scientific, USA)

Multiplex PCR. The mPCRs for the STIs were conducted with the Seeplex STD Detection Kit (Seegene, Korea) according to the manufacturer's instructions, using a PTC 200 PCR system (MJ Research, USA). Briefly, 5 µl of extracted DNA was added in the PCR tube of the Seeplex STD Detection Kit that contains the 5 µl of primer probe mixture and 10 µl of mPCR premix for six types of STI pathogens, including TV, MH, UU, CT, MG, and NG. Detection of the six types of STI pathogens was performed simultaneously in a single mPCR using a combination of primers for each pathogen. The target genes and their size of the six types of STIs are listed in Table 1. The initial PCR step was performed at 94°C for 15 min, then by 40 cycles of the following conditions; 94°C for 30 sec, 63°C for 90 sec, and 72°C for 90 sec. The final cycle was followed by an extension step at 72°C for 10 min to complete any partial polymerizations. Positive and negative PCR controls containing standardized viral RNA extracts and nuclease-free water, respectively, were included in each run. The kit includes amplification of the *Arabidopsis* cellulose synthase (*CesA*3) gene as an internal control (IC), which is designed to detect the presence of PCR inhibitors. An IC was included in the PCR mix to detect the presence of PCR inhibitors. PCR products were separated by ethidium-bromide-stained 2% agarose gel electrophoresis and visualized under UV light.

Table 1. Detection targets of the mPCR assays in various pathogens involved in STIs.

	Target	Gene	Size (bp)
Arabidopsis (internal control)	*CesA3*		719
Trichomonas vaginalis	*Actin*	*L05468*	580
Mycoplasma hominis	*Gap*	*Aj243692*	502
Ureaplasma urealyticum	*Urease*	*AF085729*	435
Chlamydia trachomatis	*Cryptic plasmid*	*M19487*	348
Mycoplasma genitalium	*gyrA*	*L43967*	253
Neisseria gonorrhoeae	*Por A pseudogene*	*AJ223447*	214

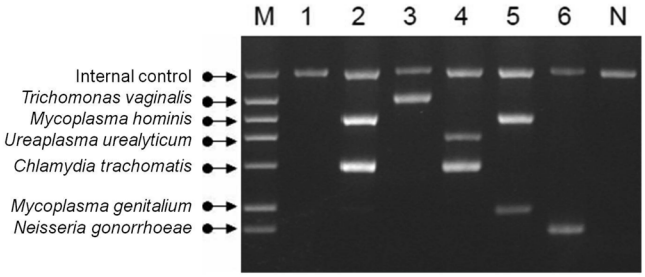

Fig. 1. Performance of the mPCR assay using 5 samples containing no pathogens (Lane 1), 3 pathogens (Lane 2), 1 pathogen (Lane 3), 2 pathogens (Lane 4), 2 pathogens (Lane 5), 1 pathogen (Lane 6), and a negative control (Lane N).
Lane M, molecular markers. Internal control (800 bp), *Trichomonas vaginalis* (580 bp), *Mycoplasma hominis* (502 bp), *Ureaplasma urealyticum* (435 bp), *Chlamydia trachomatis* (348 bp), *Mycoplasma genitalium* (253 bp), and *Neisseria gonorrhoeae* (214 bp).

Results

Performance of mPCR assay amplified and differentiated the six pathogens, which could be observed individually or as components of mixed infections in the clinical samples (Fig. 1). Of the 1,618 specimens collected from 1,523 patients, 536 specimens (35.2%) tested positive for pathogens. A total of 697 pathogens were detected, indicating a rate of single and co-infection of 75.9% (407/697) and 24.1% (129/697), respectively (Table 2). The median age of all patients was 37.8 years (range 10.7–81.0 years); the median age of patients with STI-positive samples was 33.3 years; and the median age of patients with single, dual, triple, and quadruple infections was 28.9, 38.7, 48.4, and 17.0 years, respectively (Fig. 2). When the study population was divided by age group by 10-year intervals, the age 40–49 year group contained the highest number of STI pathogen-positive patients (457), whereas the 10–19 year group had the highest STI-positive rate (47.4%; 55/116; Table 3).

Table 2. General STI results and ratios of isolated pathogens.

	Number	(%)
Patients	1,523	100.0
Positive patients	509	33.4
Specimens	1,618	100.0
Positive specimens	536	33.1
Mycoplasma hominis	333	62.1
Ureaplasma urealyticum	152	28.4
Chlamydia trachomatis	124	23.1
Trichomonas vaginalis	42	7.8
Mycoplasma genitalium	35	6.5
Neisseria gonorrhoeae	11	2.1

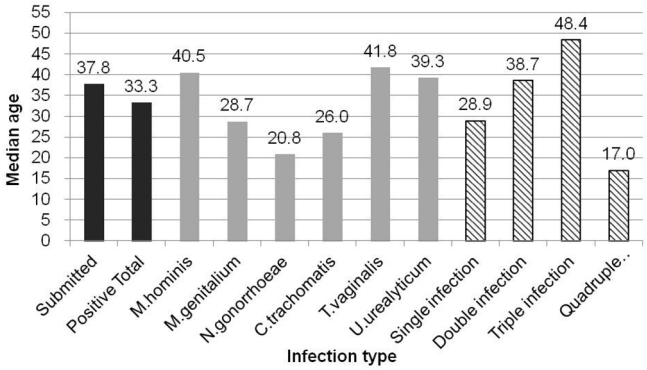

Fig. 2. Median age of STI-positive patients tested during 2006–2012 in Cheonan, Korea.
The Y-axis represents the median age, whereas the X-axis represents the infection type. The gray bar indicates the type of infection pathogen, and the diagonal bar represents the number of pathogens detected.

Table 3. Distribution of patients according to age group and detected pathogen.

Age group	No. of total patients (%)		No. of pathogen detected patients (%)		Positive rate (%)	No. of MH	No. of UU	No. of CT	No. of TV	No. of MG	No. of NG	No. of detected pathogen
10 ~ 19	116	(7.6)	55	(10.8)	(47.4)	34	8	28	3	6	5	84
20 ~ 29	322	(21.1)	122	(24.0)	(37.9)	53	32	52	6	14	3	160
30 ~ 39	426	(28.0)	117	(23.0)	(27.5)	74	39	33	9	5	1	161
40 ~ 49	457	(30.0)	152	(29.9)	(33.3)	122	52	8	19	7	1	209
50 ~ 59	145	(9.5)	50	(9.8)	(34.5)	40	15	3	5	2	1	66
60 ~ 69	44	(2.9)	11	(2.2)	(25.0)	8	6	0	0	1	0	15
70 ~ 79	12	(0.8)	2	(0.4)	(16.7)	2	0	0	0	0	0	2
80 ~ 99	1	(0.1)	0	(0.0)	(0.0)	0	0	0	0	0	0	0
Total	1,523	(100.0)	509	(100.0)	(33.4)	333	152	124	42	35	11	697

Table 4. Distribution of specimens according to year and detected pathogen.

Year (yr)	No. of total specimens	No. of pathogen detected specimens	Positive rate (%)	No. of MH		No. of UU		No. of CT		No. of TV		No. of MG		No. of NG		No. of detected pathogen
				No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
2006[a]	75	41	(54.7)	30	(54.5)	15	(27.3)	4	(7.3)	3	(5.5)	3	(5.5)	0	(0.0)	55
2007	261	110	(42.1)	68	(45.3)	47	(31.3)	20	(13.3)	4	(2.7)	11	(7.3)	0	(0.0)	150
2008	220	81	(36.8)	49	(49.5)	28	(28.3)	13	(13.1)	6	(6.1)	2	(2.0)	1	(1.0)	99
2009	239	76	(31.8)	50	(47.2)	26	(24.5)	14	(13.2)	9	(8.5)	6	(5.7)	1	(0.9)	106
2010	187	55	(29.4)	32	(45.7)	13	(18.6)	16	(22.9)	5	(7.1)	3	(4.3)	1	(1.4)	70
2011	286	87	(30.4)	51	(46.4)	16	(14.5)	27	(24.5)	8	(7.3)	5	(4.5)	3	(2.7)	110
2012[b]	350	86	(24.6)	53	(49.5)	7	(6.5)	30	(28.0)	7	(6.5)	5	(4.7)	5	(4.7)	107
Total	1618	536	(33.1)	333	(47.8)	152	(21.8)	124	(17.8)	42	(6.0)	35	(5.0)	11	(1.6)	697

[a]Aug. ~ Dec. 5 months in 2006 year.

[b]Jan. ~ Nov. 11 months in 2012 year.

Regarding the causative pathogen, MH was detected in 62.1% (333/536) of the 536 positive specimens, UU in 28.4% (152/536), and CT in 23.1% (124/536), whereas TV, MG, and NG were each detected in less than 10% (7.8%, 6.5%, and 2.1%, respectively; Table 2). Regarding the age of patients by causative pathogen, the median age of patients with a TV-positive sample was 41.8 years, that of patients with a MH-positive sample was 40.5 years, that of patients with a UU-positive sample was 39.3 years, and that of patients with a NG-sample was 20.8 years (Fig. 2). Classification of the 697 pathogens according to age group revealed that the largest number of pathogens (209) was detected in the 40–49 year group, followed by the 30–39 year, 20–29 year, and 10–19 year groups (Table 3).

Table 5. Monthly STI-positive rates.

Period	No. of total patients	No. of pathogen detected specimens	Positive rate (%)
Jan.	132	55	(41.7)
Feb.	102	36	(35.3)
Mar.	137	47	(34.3)
Apr.	151	40	(26.5)
May	134	39	(29.1)
Jun.	132	46	(34.8)
Jul.	133	59	(44.4)
Aug.	150	52	(34.7)
Sep.	139	30	(21.6)
Oct.	148	43	(29.1)
Nov.	142	46	(32.4)
Dec.	118	43	(36.4)

Analysis of annual STI incidence revealed that the highest PCR-positive rate had been recorded in 2006 (54.7%; 41/75), followed by 2007 (42.1%; 110/261) and 2008 (36.8%; 81/220; Table 4). Analysis of the monthly PCR-positive rate revealed that the highest had been recorded in July (44.4%; 59/133), followed by January (41.7%; 55/132) and December (36.4%; 43/118; Table 5). Analysis of changes in incidence over time revealed that the median age of STI-positive patients had increased between 2006 and 2008, from 38.0 years in 2006 to 39.9 years in 2007 to 40.2 years in 2008, but had then decreased to 29.4 years by 2012 (Fig. 3).

Regarding the detection rate by pathogen, the mean annual detection rates for MH and MG remained at approximately 47.8% and 5.0%, respectively, from 2006 to 2012. In contrast, the mean annual detection rate for CT increased from 7.3% to 28.0% and that of NG from 0% to 4.7%, whereas that of UU decreased from 27.3% to 6.5%, from 2006 to 2012 (Table 4).

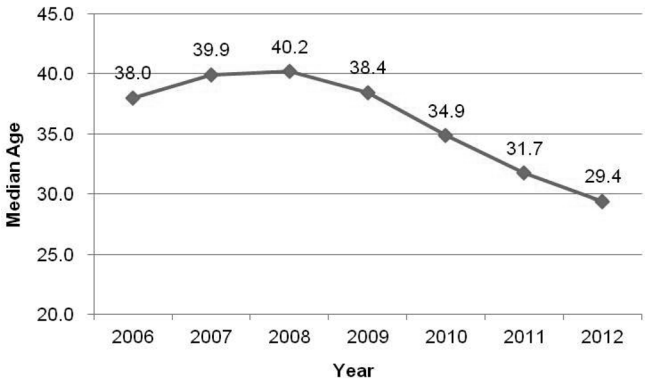

Fig. 3. Median age of STI patients during 2006–2012.

Table 6. Frequency of single-, double-, and multiple-positive infections based on mPCR detection assays.

Single positive microorganism	No.	(%)	Double positive microorganism		No.	(%)	Multiple positive microorganism				No.	(%)
MH	234	(43.7)	MH	UU	45	(8.4)	MH	TV	UU		8	(1.5)
CT	76	(14.2)	MH	CT	18	(3.4)	MH	CT	UU		6	(1.1)
UU	68	(12.7)	MH	TV	9	(1.7)	MH	CT	TV	UU	3	(0.6)
MG	15	(2.8)	TV	UU	8	(1.5)	MH	MG	UU		2	(0.4)
TV	9	(1.7)	MG	CT	6	(1.1)	NG	TV	UU		2	(0.4)
NG	5	(0.9)	MH	MG	5	(0.9)	MH	MG	CT		1	(0.2)
			CT	UU	5	(0.9)	MG	CT	UU		1	(0.2)
			NG	CT	3	(0.6)	MH	MG	CT	TV	1	(0.2)
			MG	UU	2	(0.4)	MH	MG	CT	UU	1	(0.2)
			CT	TV	2	(0.4)	MH	MG	NG	CT	1	(0.2)
Total	407	(75.9)			103	(19.2)					26	(4.9)

Of the 407 patients with a single infection, 234 tested positive for MH (43.7%), 76 for CT (14.2%), 68 for UU (12.7%), 9 (1.7%) for TV, and 5 (0.9%) for NG. Co-infection with MH and UU was most frequently observed (43.7%, 45/103) in the 103 patients with double infection and co-infection with MH, TV, and UU (40.0%, 8/20) in the 20 patients with triple infections. Of the 6 patients with quadruple infections, 3 (50%) tested positive for MH, CT, TV, and UU (Table 6).

Discussion

Few studies have simultaneously investigated the relative frequency of detection of CT, NG, MG, MH, and UU in cervical samples and, to our knowledge, none before this study has examined the frequency of their detection in Cheonan, South Korea. According to the KCDC, the total reported number of STI cases increased from 27,915 in 2001 to 32,872 in 2002 before beginning to decrease in 2003 such that it had decreased to 12,486 cases in 2007 [16]. The findings of the present study confirm this decreasing trend, as the number of STI-positive patients in the sample was found to have decreased from 110 in 2007 to 81 in 2008 to 76 in 2009.

Regarding the causative pathogen detected in the specimens, the prevalence of MH, UU, and CT was found to be 62.1%, 28.4%, and 23.1%, respectively. These rates are in accord with those of Yu and Lee [29], who reported rates of 53.7% (116/216), 39.8% (86/216), 28.7% (62/216), 11.1% (24/216), 10.6% (23/216), and 6.0% (13/216) for MH, UU, CT, MG TV, and NG detection, respectively, over the same period in Seoul, Korea. They are also in accord with those of Kim *et al.*'s [14] study of male patients, performed at Seoul in 2011, which reported rates of 26.0% (112/430), 9.1% (39/430), 6.7% (29/430), 0.5% (2/430) 0.2% (1/430), and 0.2% (1/430), for UU, MH, CT, NG, TV, and MG detection, respectively. In contrast, other studies have reported a total prevalence of MH and UU infection of 44.4% and 18.9%, respectively, in Seoul in 2006 [17]. The prevalence of CT was 6.5% [4] in Estonian, 9% in Swedish [11], and 11% in Slovenian studies [12], both performed also on patients attending STD clinics. In our study, the trend of CT prevalence of 23.1% is coincidence of the study of Yu and Lee [29] in the Seoul area but higher compared with areas of Europe [4, 11, 12].

Regarding age, the median age of all patients examined in this study was 37.8 years, and the median age of STI-positive patients was 33.3 years. Interestingly, the median age of all patients decreased annually, from 40.2 years in 2008 to 38.4 years in 2009 to 34.9 years in 2010, as did the median age of STI-positive patients, falling from 40.2 years in 2008 to 29.4 years in 2012. Differences in prevalence according to race and socioeconomic status have been reported [8], and differences by sex have also been suggested.

The results of the present study indicate that the prevalence of infection with several of the pathogens examined varies annually. The prevalence of MH, MG, and TV infection did not vary greatly over the study, with that of MH ranging from 45.3% to 54.5%, of MG from 2.0% to 7.3%, and of TV from 2.7% to 8.5%, between 2006 and 2012. In contrast, the prevalence of UU decreased by approximately one-fifth between 2007 and 2012, from 31.3% in 2007 to 6.5% in 2012, while that of both CT and NG increased more

than 4-fold, from 7.3% and 0.0%, respectively, in 2006 to 28.0% and 4.7%, respectively, in 2012.

STIs have become a major public health issue in the United States as well as in South Korea. The prevalence of chlamydia, which showed a large increase in prevalence, is the highest of all STIs in the US population and increasing, with the total number of cases reported having increased from 970,000 in 2005 to 1,030,000 in 2006 and having increased since, especially among sexually active individuals aged between 15 and 24 years [3, 16]. The findings regarding chlamydia are in accord with those of the 2006 surveillance report of the US Centers for Disease Control and Prevention [7], which reported that 19 million new STI cases are recorded every year, approximately half of which are in individuals aged between 15 and 24 years. In contrast, Kim *et al*. [14] and Shipitsyna *et al*. [25] found the mean age of the patients in their studies to be 45.4 ± 8.1 years and 36 (range 15–61) years, respectively. Although differences with respect to the period and region investigated in these studies must be acknowledged, the findings all indicate that the average age of STI-positive patients appears to be decreasing.

The coexistence of various sexually transmitted microorganisms, a very common phenomenon, has been attributed to several factors, including a common route of transmission, the sexual behavior of the host, and the presence of resident flora [13]. In the present study, 75.9% (407/536) of all STI-positive cases were infected with one pathogen and 24.1% (129/536) with multiple pathogens. Among those infected with more than one pathogen, 19.2% (103/536) were infected with 2 pathogens, 3.7% (20/536) with 3 pathogens, and 1.1% (6/536) with 4 pathogens. These findings are in accord with those of Samra *et al*. [24], who detected a single pathogen in 51 (45.1%) and multiple pathogens in 24 (21.2%) of the specimens examined in their study.

The aim of this study was to investigate the prevalence of STIs caused by CT, NG, MG, UU, MH, and TV infections in Cheonan, South Korea. Although slight variations in STI distribution according to time period, region, and social environment were observed, the results indicated a decreasing trend regarding both the total number of STI-positive patients and the STI incidence in the Cheonan region. Although the incidence of infection with CT and/or UU is increasing, the age of those seeking STI testing and the age of STI-positive patients are decreasing, indicating a correlation between the increase in the positive result rate for CT and a decrease in the mean age of STI-positive patents. Further in-depth studies should explore the nature of this correlation and the indications of the other findings of this study and other studies of STI prevalence and characteristics. We hope that these results will be used as baseline data for future clinical studies.

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