American Journal of Biochemistry and Biotechnology

Original Research Paper

The Underestimated Prevalence of *Ureaplasma* Infections: A Comprehensive Epidemiological Study in South Korea (2018-2020)

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Article history
Received: 12-06-2023
Revised: 13-09-2023
Accepted: 13-10-2023

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Abstract: *Ureaplasma* infections are frequently underestimated due to potential inaccuracies in data collection from symptomatic patients. This study aimed to accurately assess the prevalence of *Ureaplasma Urealyticum* (UU) and *Ureaplasma Parvum* (UP) infections among outpatients aged 0-70 years in South Korea between 2018 and 2020. A total of 59,381 clinical samples, including swabs and urine specimens, were collected and analyzed using multiplex real-time polymerase chain reaction, for identifying UU and UP infections. The study revealed a significant prevalence of UU and UP infections, with 8,486 (14.2%) and 10,660 (17.9%) cases, respectively. The highest positive rates were observed among individuals aged 20-29 years. Notably, in women, infection rates with UP were substantially higher than with UU. Co-infections with both UU and UP were detected in 291 men and 612 women who tested positive for *Ureaplasma* infections. These findings underscore the elevated risk of *Ureaplasma* transmission in both adult and infant populations, highlighting the importance of understanding and addressing this often underestimated public health concern. This study contributes valuable data on *Ureaplasma* infections in the South Korean population, shedding light on their prevalence and distribution, which can further inform public health interventions.

Keywords: Multiplex Real-Time Polymerase Chain Reaction, Sexually Transmitted Disease, *Ureaplasma Urealyticum*, *Ureaplasma Parvum*

Introduction

Sexually Transmitted Infections (STIs) are a global health concern, having a significant impact on both developing and developed countries (Caruso *et al*., 2021; Hanna *et al*., 2020). These infections are caused by various pathogens including viruses, bacteria, and parasites, and manifest with a diverse range of clinical symptoms and signs (Hanna *et al*., 2020). Effective antibiotic treatments are available for most STIs, but when left untreated, they can lead to severe short-and long-term complications and untreated individuals can serve as sources of further infection, contributing to the escalation of the STI epidemic (Eisinger *et al*., 2020).

The high prevalence of asymptomatic cases complicates the management of STIs, underscoring the substantial burden they place on public health (Caruso *et al*., 2021). Among the wide array of STIs, infections by *Ureaplasma Urealyticum* (UU) stand out as one of the most common, affecting sexually active women in particular, with a prevalence ranging from 40-80% among asymptomatic individuals (Caruso *et al*., 2021). UU is an opportunistic pathogen primarily found in the reproductive organs of women of childbearing age (Krause, 2008; Gdoura *et al*., 2007).

UU infections have been linked to various clinical conditions, including non-sterile urethritis, male infertility, bacterial vaginitis, chronic endometriosis, pelvic inflammation, and abortion (Caspi *et al*., 1971; Braun and Besdine, 1973; Weidner *et al*., 1978; Biernat-Sudolska *et al*., 2011). Moreover, UU and *Ureaplasma Parvum* (UP) infections have the potential

to rapidly spread within populations and are associated with cervical epithelial infections, human papillomavirus infections, and neonatal deaths (Quinn *et al*., 1985). In the context of male infertility, UP appears to be more pathogenic than UU, affecting progressive sperm motility and the secretory functions of the epididymis (Zhou *et al*., 2018). However, the precise mechanisms underlying these effects remain unclear (Zhou *et al*., 2018).

While culture-based methods are commonly used to detect *Ureaplasma* spp., these methods lack specificity, with results reporting infections as UU without distinguishing between UU and UP, leading to misdiagnosis (Horner *et al*., 2018). In addition, multiple Real-Time Polymerase Chain Reaction (m-RT PCR) tests are frequently employed without differentiating between these *Ureaplasma* species (Cao *et al*., 2007).

Despite the significance of *Ureaplasma* infections, studies focusing on the epidemiological trends of UU and UP are limited in scope, with few investigations examining specific patient groups, such as males or females, or considering the different age brackets of patients. This study seeks to bridge this knowledge gap by analyzing the epidemiological patterns of UU and UP infections in Korea between September 2018 and December 2020. The insights gained from this research aim to inform the development of innovative diagnostics and treatments, ultimately contributing to the reduction in the incidence and transmission of STIs. By doing so, this study addresses a critical public health issue and offers a basis for significant advancements in the field of sexually transmitted infections.

Materials and Methods

Sample Collection

Clinical samples (n = 59,381) were gathered from male and female patients aged 0-70 years who visited primary and secondary medical facilities in South Korea between September 2018 and December 2020. The samples were classified as swabs, urine, and other materials (such as catheters or pus). Molecular analysis of these biological specimens was conducted at the U2 Bio Laboratory in Seoul, Korea. Urine specimens were obtained from a single urination and stored in containers without any additives. To summarize the procedure, 10 mL of urine was centrifuged at 1500× g for 20 min. After discarding the supernatant, the cellular pellets were collected for nucleic acid extraction. Swab samples collected from the vagina, cervix, and urethral duct were subjected to centrifugation at 13,000× g for 5 min. Nucleic acids were extracted from the cellular pellets following the removal of the supernatant. These clinical specimens were preserved at 70°C until further analysis. This study was conducted as a qualitative test for sexually transmitted pathogens without measuring the concentration of extracted DNA. However, to ensure the sensitivity of the test, the test was performed within the confirmed range using internal control substances for purposes of quality control.

This study was approved by the Dankook University Institutional Review Board (IRB file No. 2021-03-056). This study was conducted in accordance with the tenets of the Declaration of Helsinki. This was a retrospective study; tests were performed by a medical institution for diagnosis. Patient consent was not required because we did not use personal information.

Nucleic Acid Extraction

DNA for the Multiple Real-Time Polymerase Chain Reaction (M-RT PCR) assay was extracted using an ExiPrep™ Dx bacteria genomic DNA Kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The concentration of the extracted DNA was measured using the AccuPower® STI8A-plex real-time PCR Kit (Bioneer) and AccuPower® STI4C-plex real-time PCR Kit (Bioneer). This study was conducted at a testing agency in Korea using a commercial multiplex PCR kit (Bioneer, Daejeon, Korea) for the detection of various sexually transmitted pathogens. The kit used in the study was approved by the Korean ministry of food and drug safety and passed the certification review by the Korean society of laboratory medicine.

Multiple Real-Time Polymerase Chain Reaction Analysis

M-RT PCR analysis was performed using the AccuPower® STI8A-Plex and AccuPower® STI4C-plex real-time PCR Kits using the Exicycler™ 96 real-time quantitative thermal block system (Bioneer) according to the manufacturer's protocol. The amplification protocol comprised one cycle at 95°C for 5 min and 45 cycles at 95°C for 5 s and 55°C for 5 s. The threshold cycle was determined according to the manufacturer's instructions. Eight pathogens, namely, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, UU, *Mycoplasma genitalium*, *Candida albicans*, *Gardnerella vaginalis*, UP, and *Treponema pallidum*, were evaluated. In this study, we focused on UU and UP.

Statistical Analysis

Statistical analyses for this study were carried out using SAS version 9.4, developed by SAS Institute Inc. in Cary, North Carolina, USA. These analyses included descriptive statistical assessments and frequency analyses. The data pertaining to the detection of UU and UP DNA using real-time PCR were assessed based on the patient's gender, age, and specimen type. Findings with a significance level of $p<0.05$ were regarded as statistically significant.

Results

Ureaplasma Urealyticum and Ureaplasma Parvum Infections Based on Age

The number of patients with UU infections was 8,486 in total (14.2%), with 6,752 (14.7%) male patients and 1,734 (12.8%) female patients (Table 1).

The highest positivity rate was found in individuals in the age group 20-29 years. The positivity rate decreased with age in both sexes. The average age of the total population, men and women, who tested positive, was 37.4, 36.9, and 39.4 years, respectively (Table 1).

The number of patients with UP infection was 10,660 in total (17.9%), with 5,670 (12.3%) male patients and 4,990 (36.8%) female patients. Men had a higher positivity rate than women. The highest positivity rate in the male population was observed in the age groups of 30-39 and 20-29 years. The positivity rate increased with age and then decreased after the age of 40 years. The highest positivity rate in women was observed in the age groups of 20-29 and 40-49 years. A similar positivity rate was observed in individuals in their 20-40 s. Thereafter, the positivity rate decreased with age. The average age of the population that tested positive was 38.9 years for the total population, 38.7 years for men, and 39.2 years for women (Table 1). Both UU and UP positivity rates were not significantly associated with age ($p = 0.066$ and 0.063, respectively; Table 1). However, a distinct infection pattern was observed according to the age group. However, this pattern did not reach statistical significance.

Co-Infection of Ureaplasma Urealyticum and Ureaplasma Parvum

Among the 19,146 patients who tested positive for UU or UP, the number of co-infections was 703 in total: 291 in male patients and 612 in female patients. For both sexes, the population in the 20-29-year age group was associated with the highest positivity rate, with 85 (29.2%) male and 199 (32.5%) female individuals, respectively (Fig. 1). The positivity rate of co-infection decreased with age. However, this relationship did not reach statistical significance.

Comparison of Positivity Rates of Ureaplasma Urealyticum and Ureaplasma Partum Based on Sex

In male patients, the positivity rate of UU was higher than that of UP and it increased with a decrease in age. In men, the rate of UP infection was almost identical to that of UU infection. In women, the highest positivity rate (29.0%) of UU was observed in the 20-29-year age group (Fig. 2). The positivity rate decreased with increasing age. Among the female patients with UP, the positivity rate was high in individuals in their 20 and 40 s. However, this relationship did not reach statistical significance.

Comparison of Positivity Rates Based on Sample Type

The positivity rates of UU and UP were compared based on the type of clinical specimen tested. Swab, urine, or other (catheter or pus) samples were used. The swab specimen was associated with the highest positivity rate. The detection rate of UP in women (44.3%) was the highest in the swab specimen. Among the swabs, the detection rate of UU in men (16.8%) was the highest, followed by UU in women (15.6%) and UP in men (9.5%) (Fig. 3). The second-highest positivity rate was associated with urine samples; here, the detection rate of UP (30.2%) in women was the highest, followed by UU (15.2%) in men, UP (12.4%) in men and UU (10.3%) in women (Fig. 3). In the other samples, the detection rate for UP (25.5%) was the highest in women, followed by UP (12.9%) in men, UU (11.5%) in men and UU (8.4%) in women (Fig. 3). However, this relationship did not reach statistical significance.

Table 1: Distribution of UP and UU positivity rates based on sex and age

	Positivity rate for UU							Positivity rate for UP					
	Total (n = 59,381)	(%)	Male (n = 45,833)	(%)	Female (n = 13,548)	(%)		Total (n = 59,381)	(%)	Male (n = 45,833)	(%)	Female (n = 13,548)	(%)
Positive, n (%)	8,486	(14.2)	6,752	(14.7)	1,734	(12.8)	Positive, n (%)	10,660	(17.9)	5,670	(12.3)	4,990	(36.8)
Negative, n (%)	50,895	(85.8)	39,081	(85.3)	11,814	(87.2)	Negative, n (%)	48,721	(82.1)	40,163	(87.7)	8,558	(63.2)
Age, years							Age, years						
≤19	193	(2.3)	122	(1.8)	71	(4.1)	≤19	231	(2.2)	79	(1.4)	152	(3.0)
20-29	2,784	(32.8)	2,281	(33.8)	503	(29.0)	20-29	2,895	(27.2)	1,605	(28.3)	1,290	(25.9)
30-39	2,350	(27.7)	2,002	(29.6)	348	(20.1)	30-39	2,764	(25.9)	1,642	(28.9)	1,122	(22.5)
40-49	1,494	(17.6)	1,133	(16.8)	361	(20.8)	40-49	2,383	(22.3)	1,092	(19.3)	1,291	(25.9)
50-59	1,059	(12.5)	744	(11.5)	285	(16.5)	50-59	1,666	(15.6)	808	(14.3)	858	(17.2)
60-69	471	(5.5)	344	(5.1)	127	(7.3)	60-69	564	(5.3)	353	(6.2)	211	(4.2)
≥70	135	(1.6)	96	(1.4)	39	(2.2)	≥70	157	(1.5)	91	(1.6)	66	(1.3)
Average age, years	37.4		36.9		39.4		Average age, years	38.9		38.7		39.2	
P	0.066						P	0.063					

UP: *Ureaplasma Parvum*; UU: *Ureaplasma Urealyticum*

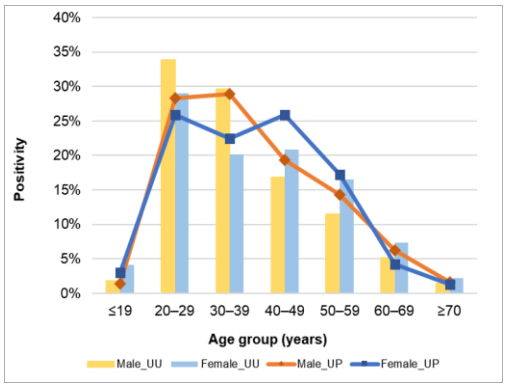

Fig. 1: Co-infection with UU and UP. The orange bar shows the classification of UU and UP co-infection in women by age and the gray bar shows the classification of UU and UP co-infection in men by age. A trend line is included for each color. UU: *Ureaplasma Urealyticum*; UP: *Ureaplasma Parvum*

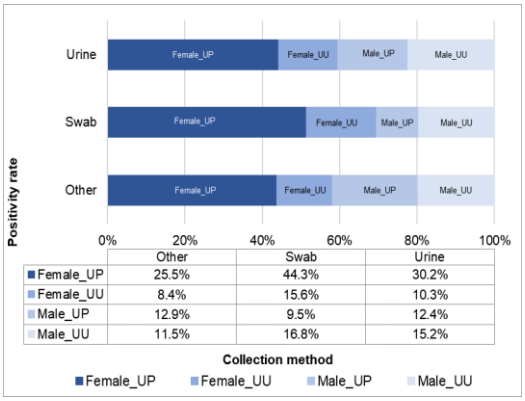

Fig. 2: Comparison of UU and UP positivity rates based on age and sex. The line represents UP and the bar represents UU. Orange represents male patients and blue represents female patients. UU: *Ureaplasma Urealyticum*; UP: *Ureaplasma Parvum*

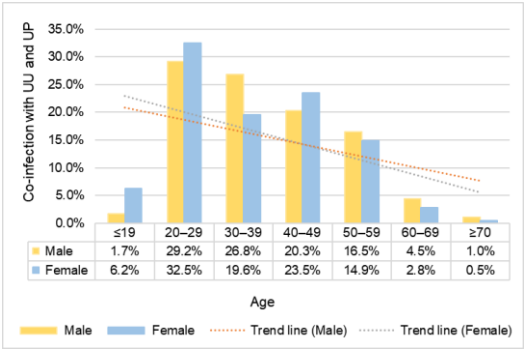

Fig. 3: Comparison of positivity rates based on sample type. The green bar represents the UP positivity rate in women and the brown bar indicates the UU positivity rate in women. The orange bar represents the UU positivity rate in men and the yellow bar represents the UP positivity rate in men. Different rates are indicated by stacked bars. UU: *Ureaplasma Urealyticum*; UP: *Ureaplasma Parvum*

Discussion

In this study, we conducted a comprehensive analysis of UU and UP infections in Korea during the period from September 2018 to December 2020. Our findings reveal distinct patterns in the prevalence of UU and UP infections based on age, gender, and sample type. Notably, the highest incidence of UU and UP infections was observed among individuals aged 20-29 years. However, it is essential to delve deeper into the implications and context of these findings. Comparing our results with previous studies, we find some discrepancies in UU and UP positivity rates across different age groups and regions. For instance, studies in the United States and China reported the highest UU positivity rates in the 30-39-year age group (Wang *et al*., 2016; Zhu *et al*., 2016). In contrast, our study identified the highest UU positivity rate among individuals aged 20-29 years. These variations may reflect differences in sample populations, geographical locations, or healthcare-seeking behaviors, emphasizing the need for context-specific research to better understand these dynamics.

Furthermore, the observed decrease in UU positivity rates after the age of 40 in both men and women contrasts with previous findings that UU is often isolated in 40-80% of cervical samples in women of childbearing age (Cassell *et al*., 1993; Waites *et al*., 2005). Our results suggest that UU may not follow the same prevalence trends in Korean populations as in populations from other regions. The reasons for these variations warrant further investigation. This gender-based difference in UP prevalence raises questions about potential biological or behavioral factors contributing to these disparities. Further research is needed to elucidate the underlying causes of these differences. Co-infections of UU and UP were more prevalent in women than in men, in line with the findings of Del Prete *et al*. (2017). This is consistent with our study. This observation underscores the importance of considering co-infections in clinical practice, as they can have unique clinical implications and may necessitate tailored treatment approaches.

While our study provides valuable insights into the changing positivity rates of UU and UP infections, it is crucial to acknowledge the limitations of the study. Anonymized data limited our ability to assess patients' symptoms and basic conditions, which could have provided valuable context. Additionally, the absence of studies on *Ureaplasma* co-infections with other STI-causing pathogens calls for further research in this area. Finally, the relatively short duration of the study may limit the generalizability of our findings over a more extended period. Notwithstanding these limitations, our study contributes to understanding the epidemiological trends of UU and UP infections in Korea. We have identified variations in positivity rates across age and gender groups and provided

a comprehensive analysis of over 50,000 samples, utilizing a consistent testing method within a single institution. The implications of our findings extend to the development of strategies aimed at safeguarding public health and reducing the incidence and transmission of UU and UP. Furthermore, our study offers a foundational dataset for future clinical investigations into UU and UP infections. Long-term studies are essential to elucidate regional disparities and evaluating co-infections with other STI-causing agents should be a priority in patients presenting with clinical symptoms. In conclusion, this research sheds light on the changing landscape of UU and UP infections in Korea, emphasizing the need for ongoing surveillance, context-specific interventions, and a deeper exploration of co-infections and their clinical implications.

Conclusion

STIs could lead to several diseases and their prevalence varies according to sex and age. UU and UP are important bacterial pathogens that cause chronic and asymptomatic genital infections. In this study, we examined the epidemiological trends of UU and UP infections in Korea between September 2018 and December 2020. In this study, the incidence of UU and UP infections was the highest in the 20-29-year age group. The incidence of UP infection tended to decrease in men in their 40 s and older and in women in their 50 s and older.

Additionally, it is worth noting that limited statistics existed prior to the use of molecular biological testing for UU and UP, highlighting the need for comprehensive data to inform healthcare practices effectively. Future studies should delve into the clinical characteristics of UU and UP infections, providing a more holistic understanding of these conditions.

In conclusion, our research contributes valuable insights into the changing landscape of UU and UP infections in Korea. These findings underscore the importance of ongoing surveillance and the necessity for detailed studies on co-infections, resistance patterns, and clinical characteristics. By addressing these gaps in knowledge, we can develop more effective strategies to combat UU and UP infections and ultimately improve public health outcomes. Furthermore, follow-up studies on bacterial STIs, such as UU and UP, are essential to advance our understanding of these infections and their impact on individuals and communities.

Acknowledgment

We extend our sincere gratitude to the publisher for their invaluable support in bringing this research article to a wider audience. Their resources and platform have played a pivotal role in sharing our findings and contributing to the field of research. Additionally, we acknowledge the collective efforts of the dedicated editorial team for their meticulous review and editing of our work, which has greatly enhanced its quality. Furthermore, we wish to express our appreciation to all individuals and institutions who may have been involved in various capacities, including data collection and analysis, contributing to the successful completion of this study. Your contributions have been integral to the research process. Thank you for the collaborative efforts that have made this publication possible.

Funding Information

The authors have not received any financial support or funding to report.

Author's Contributions

Jae Eun Choi and Bo Kyeung Jung: Made substantial contributions to the conception and design of the study. These authors contributed equally to this study.

Eun Ju Oh and Jae Kyung Kim: Made substantial contributions and acquisition and analysis of the data.

Ethics

This investigation was carried out in line with the declaration of Helsinki's fundamental principles and was given approval by Dankook University's institutional review board (Republic of Korea) (No. 2021-04-002).

Competing Interests

The researchers state that they have no opposing interests.

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