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ORIGINAL ARTICLE

Clinical Application of RNA Simultaneous Amplification and Testing for Pathogens Associated with Urogenital Infections

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SUMMARY

Background: **RNA simultaneous amplification and testing (RNA-SAT) is a sensitive, specific, and rapid test method for the detection of pathogens in clinical samples. Herein, we used RNA-SAT to understand the detection status and distribution of common pathogens causing infections in patients with clinical urethritis to provide early clinical diagnosis and treatment.**
Methods: **Urine or swab samples were collected from 1,594 patients with suspected urinary tract infections between April 2022 and August 2023. RNA-based detection of common urogenital pathogens, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium*, was performed. Infection rates in different genders and ages were analyzed. Results of pathogen cultures were recorded during the same period.**
Results: **Majority of the patients included in the study were outpatients (99.25%, 1,582/1,594). Out of the 1,594 patients, 828 (51.94%) had a urogenital pathogen infection. *U. urealyticum* had the highest detection rate (37.50%, 555/1,480), followed by *C. trachomatis* (17.13%, 223/1,302), *N. gonorrhoeae* (10.45%, 127/1,215), and *M. genitalium* (9.98%, 127/1,273). Coinfections of *U. urealyticum*, *C. trachomatis*, and/or *N. gonorrhoeae* were observed in 13.56% (174/1,283) of tested samples. Total RNA detection rate did not significantly differ between sexes; however, the detection rate of *U. urealyticum* and *N. gonorrhoeae* in females was significantly higher and lower than in males, respectively. Further, detection rate showed a decreasing trend with age. Finally, the RNA-SAT approach showed a higher detection rate for *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* than the conventional culture approach did.**
Conclusions: ***U. urealyticum*** **and *C. trachomatis* are the main pathogens responsible for urogenital tract inflammation. RNA-SAT can identify major pathogen infections at the early stages of the disease, effectively compensate for the deficiency of culture-based detection, and aid in early clinical diagnosis, and hence intervention.**
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KEYWORDS

genitourinary tract infection, RNA-SAT, urogenital pathogen, pathogen detection rate

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INTRODUCTION

Urethral genital inflammation is the inflammation of the urethra and/or genitalia, mainly caused by gonococcus (*Neisseria gonorrhoeae*) or non-gonococcus (such as *Chlamydia trachomatis*, *Mycoplasma genitalium*, or *Trichomonas vaginalis*) pathogens. The most common signs and symptoms of urogenital pathogen infections include itching, burning pain, difficulty urinating or frequent urination, mucopurulent urethral discharge, urethral erythema, and other discomfort symptoms in the urethra or vagina; however, a few patients can be asymptomatic. The diagnostic criteria include the typical signs and symptoms, exposure history, and detection of the relevant pathogens [1-3]. The rapid and accurate identification of pathogens in urogenital infections plays a crucial role in their diagnosis and treatment in clinical settings and helps provide targeted treatment for patients, thereby minimizing the risk of transmission and long-term complications [4–6].

Real-time constant temperature fluorescence RNA amplification detection technology (RNA simultaneous amplification and testing [RNA-SAT]) is a sensitive, specific, and rapid test method for detecting pathogens in clinical samples [6-8]. It combines nucleic acid extraction, amplification at constant temperature, and fluorescence-labeled hybrid probe detection technology, and has been used for the detection of pathogens in tuberculosis, respiratory infections, and urethritis [9–11]. In this study, we performed RNA-SAT to understand the detection status and distribution of common urogenital pathogens responsible for infections in patients with clinical urethritis, such as *C. trachomatis*, *Ureaplasma urealyticum*, *N. gonorrhoeae*, and *M. genitalium*, to aid early clinical diagnosis.

MATERIALS AND METHODS

Study subjects

A total of 1,594 patients with urinary and reproductive tract infections who first visited the First Affiliated Hospital of Zhejiang University School of Medicine were recruited from April 2022 through August 2023. Urine and female cervical or vaginal swabs and male urethral swabs were collected for RNA-SAT analysis. Female cervical swabs, vaginal secretions, and male urethral secretions were used for the culture methods. The study protocol adhered to the principles of the Declaration of Helsinki (2013) and was approved by the Ethics Review Committee of the First Affiliated Hospital School of Medicine, Zhejiang University (the ethics committee number is 2021-092, and the approval date is February 4, 2021). All participants understood the procedures and consented to participate in the study.

Sample collection

For the collection of urethral swabs, male participants were instructed to not urinate 1 - 2 hours before sampling. A sterile swab was inserted into the urethra (3 - 4 cm), rotated, retained for 20 - 30 seconds, removed, and then stored in a sterile collection tube containing RNA preservation solution. For the collection of cervical or vaginal swabs, mucus and pus were wiped using a cotton swab. Subsequently, a sterile skim cotton swab was inserted for 20 - 30 seconds into the cervical tube through the scale epithelial junction or vaginal rotation. The swab was then removed and placed in the aforementioned RNA sample preservation tube.

For urine samples, the first urination in the morning or that after a long period of non-urination (at least 1 hour) was collected into a sterile tube. The urethral opening was not cleaned prior to collection. Two milliliters of the collected urine were transferred into an RNA sample storage tube, mixed, and then examined. All specimens were sent for testing within 24 hours. Specimens that could not be submitted on time for testing were stored at 4 - 8°C or -20°C.

Pathogen testing

The *C. trachomatis*, *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* pathogen RNA fluorescent probe kit (Shanghai Rendu Biotechnology Co., Ltd., Shanghai, China) was thawed at 25°C and shaken thoroughly. This experiment involved the use of the automatic loading mode of the AutoSAT fully automatic all-in-one machine provided by Shanghai Rendu Biotechnology Co. The instrument scans the sample barcode, reads the information in the laboratory information system Autos AT, and collects basic information, such as sample position, type, and test items. After confirming the accuracy of these settings, the experiment was conducted. The detection results were judged based on the real-time fluorescence signal appearance time and intensity, combined with the positive and negative controls. The experimental results were automatically transmitted to the laboratory information system and reported as positive or negative.

Statistical analysis

GraphPad Prism (GraphPad Software Inc., Boston, MA, USA) was used for statistical analysis. Data are presented as numbers and percentages (%). The chi-squared test was used to compare differences between groups. $p < 0.05$ was considered a statistically significant difference.

RESULTS

Clinical outcomes

Out of the 1,594 patients included in this study, 1,376 were male, aged 15 - 78 years (median: 32 years), and 218 were female, aged 18 - 71 years (median: 31 years). The age range of the patients was 21 - 40 years, and majority were male (Figure 1A). Majority of the patients (1,582/1,594, 99.25%) were outpatients from urology, dermatology, and gynecology departments (Figure 1B).

Table 1. Positive cases and percentage of patients from different genders and sample sources [n (%)].

	Gender		χ^2	p	Sample type		χ^2	p
	male	female			urine	swab		
Total	710 (51.60%)	118 (54.13%)	0.48	0.49	765 (51.07%)	63 (65.62%)	7.66	0.006
C. trachomatis	201 (17.68%)	22 (13.33%)	1.92	0.17	210 (17.31%)	13 (14.61%)	0.43	0.51
U. urealyticum	446 (34.92%)	109 (53.69%)	26.33	< 0.0001	498 (35.83%)	56 (62.22%)	25.14	< 0.0001
N. gonorrhoeae	118 (11.17%)	9 (5.66%)	4.49	0.03	120 (10.61%)	8 (9.52%)	0.10	0.75
M. genitalium	115 (10.33%)	12 (7.50%)	1.25	0.26	118 (9.95%)	9 (10.34%)	0.01	0.91

Table 2. Positive and negative cases of patients in different age groups.

	Age (years)											
	15 - 20		21 - 30		31 - 40		41 - 50		51 - 60		61 - 78	
	+	-	+	-	+	-	+	-	+	-	+	-
Total	24	16	363	293	297	289	67	127	33	61	5	19
C. trachomatis	14	22	100	228	77	393	26	134	4	73	2	20
U. urealyticum	15	21	237	366	205	340	67	115	27	63	3	21
N. gonorrhoeae	7	22	58	457	41	396	19	135	2	60	1	17
M. genitalium	7	21	66	459	34	431	13	149	7	64	0	22

+, positive; -, negative.

Table 3. Comparison of pathogen detection results between RNA-SAT and culture methods (n = 55).

RNA-SAT	Culture method		Total
	positive	negative	
Positive	7	44	51
Negative	0	4	4
Total	7	48	55

In most cases, general medical examination (physical examination) and urethritis or urinary tract infection examination were conducted (Figure 1C). Urine specimens comprised 93.98% (1,498/1,594) of all samples. Prevalence of *C. trachomatis*/*U. urealyticum*/*N. gonorrhoeae*/*M. genitalium* in the study population

Out of the 1,594 urine or swab samples, 828 (51.94%) were positive for urogenital pathogen RNA. The most common infection was *U. urealyticum*, with a positivity rate of 37.50% (555/1,480), followed by *C. trachomatis* (17.13%, 223/1,302), *N. gonorrhoeae* (10.45%, 127/1,215), and *M. genitalium* (9.98%, 127/1,273).

Additionally, 1,283 samples were tested for coinfections. A total of 174 samples (13.56%) were positive for coinfections, mainly with *U. urealyticum*, *C. trachomatis*, or *N. gonorrhoeae*. The overall positivity and detection rates of the different pathogens are shown in Figure 2.

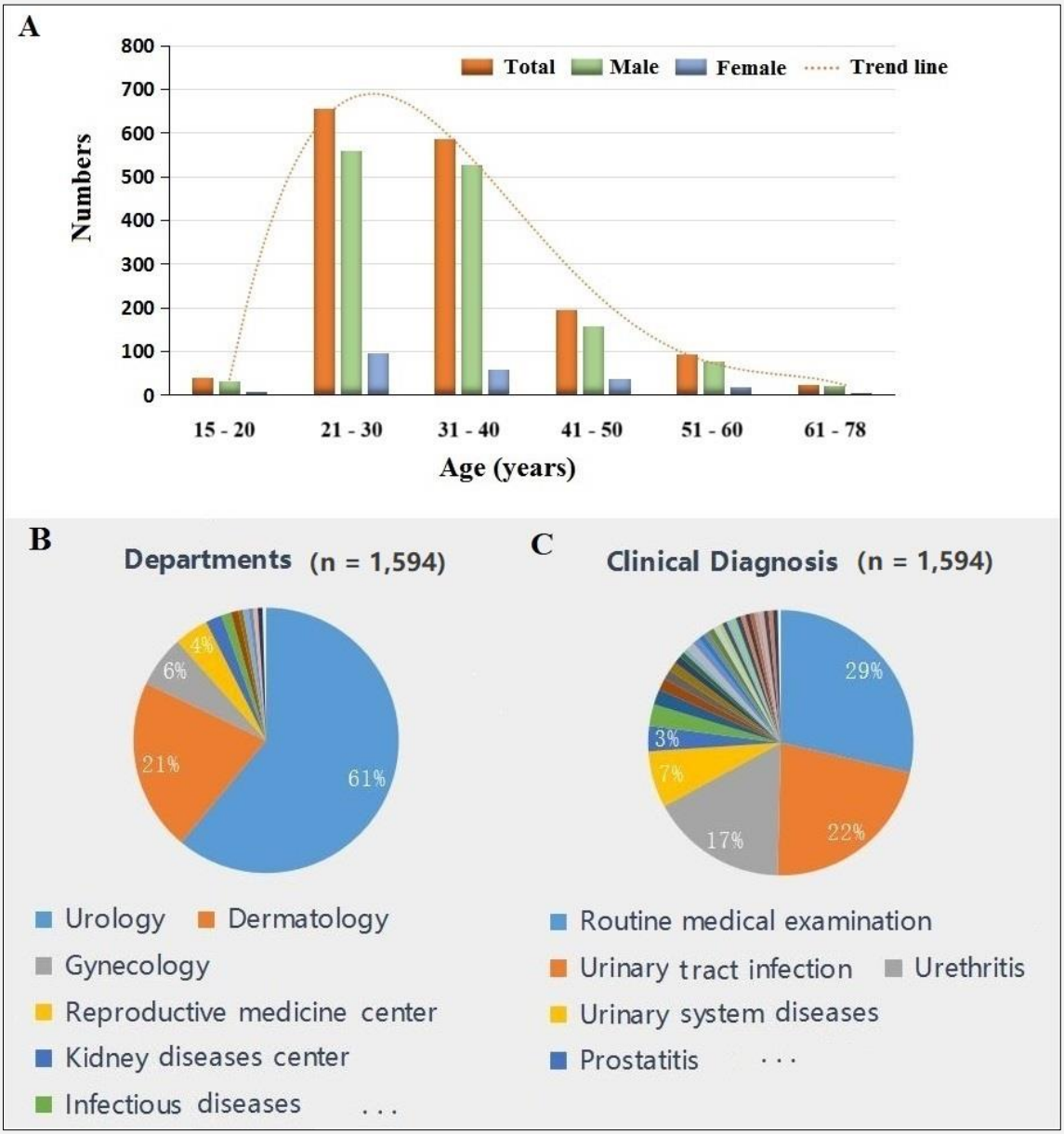

Figure 1. Clinical data of 1,594 patients with suspected urinary tract infections.

A - Age and gender distribution; most patients were between 21 and 40 years old and were predominantly male. B - Distribution of patients visiting different departments. C - Clinical diagnosis.

Pathogen distribution in patients of different gender, age, and sample sources

Out of the 1,376 male patients, 710 (51.60%) tested positive for urogenital pathogens. Among the 218 female patients, 118 (54.13%) tested positive for urogenital pathogens. No significant difference was observed in the overall detection rate between male and female patients. However, the detection rate of *U. urealyticum* was significantly higher in female patients than it was in male patients (53.69% vs. 34.92%, $p < 0.0001$), whereas the *N. gonorrhoeae* detection rate was significantly lower in female patients than it was in male patients (5.66% vs. 11.17%, $p = 0.03$) (Table 1). The overall pathogen and *U. urealyticum* detection rates in urine

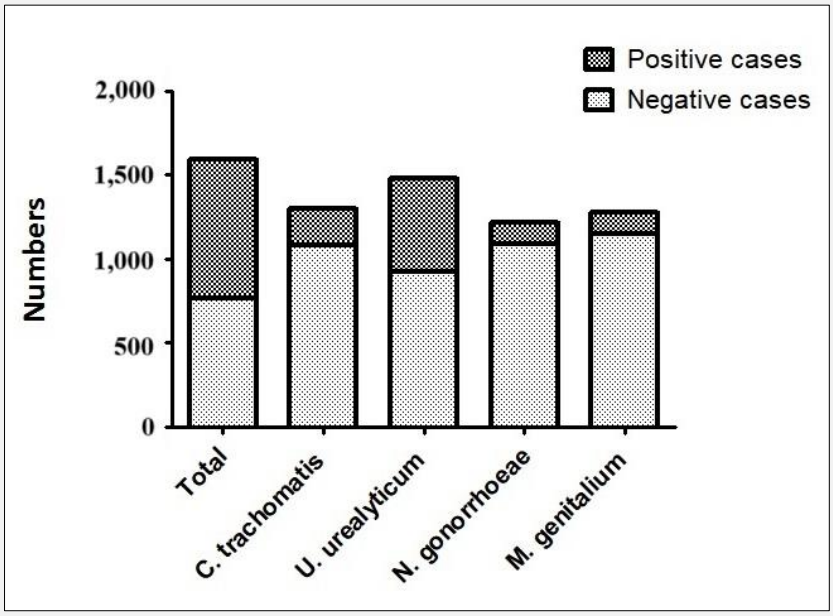

Figure 2. Distribution of overall and individual pathogen detection rates of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* RNA in 1,594 specimens.

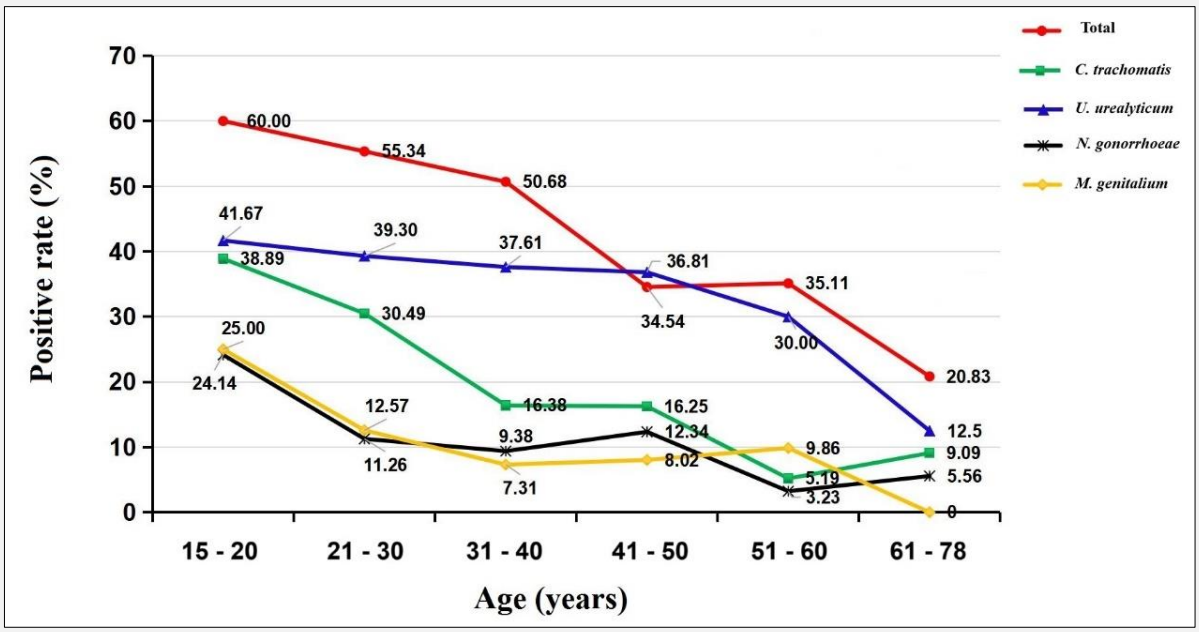

Figure 3. Overall pathogen and *C. trachomatis*, *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* detection rates of patients of different age groups.

and swab samples were also considerably different (Table 1).

To assess pathogen distribution based on age, the patients were divided into six age groups (15 - 20, 21 - 30, 31 - 40, 41 - 50, 51 - 60, and 61 - 78 years). The total number of pathogens detected in each group is shown in Table 2. Majority of the patients were aged 21 - 40 years, and pathogen detection rate gradually decreased with age. Among all age groups, *U. urealyticum* had the highest detection rate (Figure 3).

Comparative analysis between RNA detection and culture methods

Fifty-five samples were simultaneously analyzed for the detection of *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* using RNA-SAT and culture methods. The positive detection rate of RNA-SAT and culture method were 92.73% (51/55) and 12.73% (7/55), respectively, and the difference between them was statistically significant ($\chi^2 = 70.61$, $p < 0.0001$). As shown in Table 3, the total compliance rate for the two methods was 20.00% (11/55); the positive compliance rate was 100% (7/7), and the negative compliance rate was 8.33% (4/48). Overall, the RNA-SAT method exhibited a higher detection rate than culture methods.

DISCUSSION

C. trachomatis, *U. urealyticum*, *N. gonorrhoeae*, and *M. genitalium* are common sexually transmitted pathogens that cause urogenital infections [11,12]. Urinary tract infections caused by *C. trachomatis* and *M. genitalium* can lead to urethritis, cervicitis, prostatitis, infertility, and other diseases and affect important physiological functions, leading to low birth weight, neonatal pneumonia, premature birth, and other serious complications [11,13]. The diagnosis of urinary tract genital inflammation is mainly based on epidemiological history, clinical manifestations, and comprehensive analysis of laboratory tests. Pathogen detection is the most direct basis for the diagnosis of infection [3,4]. In recent years, clinicians have paid increasing attention to the detection of urogenital tract infections. A clear diagnosis at the acute stage of infection can prevent excessive use of antibiotics, which has important clinical significance for the prevention and treatment of various diseases, including urogenital tract infections.

Our results showed that the RNA-SAT approach had a detection rate of 51.94% (828/1,594), wherein *U. urealyticum* (37.50%, 555/1,480) was the most commonly detected urogenital pathogen. Furthermore, 1,283 specimens were analyzed for coinfections, and 174 specimens (13.56%) were found to be coinfected with two or more pathogens, mainly *U. urealyticum*, *C. trachomatis*, and/or *N. gonorrhoeae*, suggesting that coinfections are common in genitourinary tract infections.

Comparing the pathogenic examination results of patients of different genders, it was found that there was no significant difference in the overall detection rate between males and females, but the detection rate of UU was the highest among all patients, and the UU detection rate of female patients was significantly higher than that of male patients, similar to the report by Xu et al. [14]. *U. urealyticum* is a common mycoplasma pathogen of the human urogenital tract that can cause diseases under specific conditions (such as changes in the internal environment or decreased resistance) [6,11]. Further, the patients were mainly 21 – 40 years old, and the detection rate gradually decreased with age.

Currently, the main pathogen detection methods for urogenital tract infections include direct microscopy, culture, and immunological and molecular biology-based detection. Each method has its advantages and disadvantages. For example, while direct microscopy is a fast, simple, intuitive, and specific method, it relies on the expertise of the examiner, is easily affected by external environmental factors, and has low sensitivity. Similarly, while drug susceptibility and detection can be examined using the conventional culture method, this method has a long experimental cycle and low detection sensitivity [3], necessitating newer approaches to circumvent these limitations, which is facilitated by the development of molecular biology technology. RNA-SAT is a newer generation nucleic acid detection method that has high sensitivity and good specificity for urogenital and sexually transmitted pathogens. It can detect pathogenic nucleic acids in urine and swabs simultaneously, which has played an important role in the diagnosis of genitourinary tract infections and sexually transmitted diseases, and it has been increasingly used for the detection of pathogens in clinical samples [7,15]. Simultaneous amplification and testing technology is used to detect pathogenic RNA to detect live bacteria and aid early diagnosis; because RNA is decomposed in the environment within hours, it can accurately and sensitively reflect acute infection and can also be used to assess response of the pathogen to treatment [4,15]. In the present study, the positive coincidence rate of infection caused by *M. genitalium* and *N. gonorrhoeae* detected by SAT technology was 100%, and the negative coincidence rate was only 8.33%. These results indicate that the RNA-SAT method had a higher detection rate compared with the conventional culture method. In addition, RNA-SAT can be used to analyze urogenital tract secretions and urine samples. As a noninvasive sampling specimen, urine samples replace swabs, with advantages of convenient sampling, shorter collection and processing time, low biohazard risk, and increased follow-up compliance in clinical settings, which facilitates patients and reduces the work burden for clinicians.

In conclusion, RNA-SAT provides a new sampling and detection method for the diagnosis of urogenital tract infection pathogens and is a test method that needs to be promoted and adopted in clinical settings.

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Declaration of Interest:
The authors have no competing interests to declare.

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