

ORIGINAL ARTICLE

Identification of pathogens causing sexually transmitted infections in women attending a health center by multiplex RT-qPCR in Cochabamba, Bolivia

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Keywords:

sexually transmitted infections; STIs; risk factors; women; polymerase chain reaction; multiplex RT-qPCR (source: MeSH-NLM).

ABSTRACT

Objective. To identify pathogens causing sexually transmitted infections (STIs) using multiplex RT-qPCR and to determine associated risk factors in women attending the Research, Education and Services Center (CIES, by its Spanish acronym) in Cochabamba, Bolivia. **Methods.** A quantitative, observational, descriptive, cross-sectional study was conducted in 2024. The study population included 96 patients who met the inclusion criteria. Sociodemographic data, risk factors, and symptoms were collected using an interview-administered questionnaire. Pathogen detection was performed using multiplex RT-qPCR. Data were tabulated in Excel and analyzed in SPSS using descriptive statistics and chi-square or Fisher's exact tests, with $p < 0.05$. **Results.** Among all participants, 41.7 % were aged 18 to 27 years, 50 % were single, 63.5% had a higher educational level, and 71.9 % were from urban areas. Three and two microorganisms were identified in 30.2 % and 33.3 % of the analyzed samples, respectively. The most frequent pathogens were *Haemophilus ducreyi*, *Gardnerella vaginalis*, and *Ureaplasma parvum/urealyticum*. The risk factors associated with STIs were educational level ($p = 0.044$), unprotected sexual intercourse ($p = 0.037$), and vaginal discharge ($p = 0.017$). **Conclusions.** Multiplex RT-qPCR identified STI pathogens in women attending CIES in Cochabamba, with a high frequency of coinfections. *Haemophilus ducreyi*, *Gardnerella vaginalis*, and *Ureaplasma parvum/urealyticum* were the predominant pathogens. Unprotected sexual intercourse, educational level, and vaginal discharge were significantly associated with STIs.

Identificación de patógenos causantes de infecciones de transmisión sexual en mujeres de un centro de salud por RT-qPCR multiplex, Cochabamba, Bolivia

Palabras clave:

infecciones de transmisión sexual; ITS; factores de riesgo; mujeres; reacción en cadena de la polimerasa; RT-qPCR multiplex (fuente: DeCs-BIREME).

RESUMEN

Objetivo. Identificar patógenos causantes de infecciones de transmisión sexual (ITS) por RT-qPCR multiplex y factores de riesgo en mujeres del Centro de Investigación, Educación y Servicios – CIES, de Cochabamba, Bolivia. **Métodos.** Se desarrolló un estudio cuantitativo, observacional, descriptivo y de corte transversal durante la gestión 2024. La población incluyó a 96 pacientes que cumplieron los criterios de inclusión. Los datos sociodemográficos, factores de riesgo y síntomas fueron recolectados mediante un cuestionario aplicado por entrevista. La detección de patógenos se efectuó a través de RT-qPCR multiplex. Los datos fueron tabulados en Excel y analizados en SPSS mediante estadística descriptiva y pruebas χ^2 o exacta de Fisher, con $p < 0,05$. **Resultados.** De todas las participantes, el 41,7 % tenían entre 18 - 27 años, el 50 % estaban solteras, el 63,5 % presentaban un nivel de escolaridad superior y el 71,9 % provenían del área urbana. En el 30,2 % y 33,3 % de las muestras analizadas se identificaron tres y dos microorganismos, respectivamente. Los patógenos más frecuentes fueron el *Haemophilus ducreyi*, *Gardnerella vaginalis* y *Ureaplasma parvum/urealyticum*. Los factores de riesgo asociados con las ITS fueron: nivel de escolaridad ($p = 0,044$), relaciones sexuales sin preservativo ($p = 0,037$) y el flujo vaginal ($p = 0,017$). **Conclusiones.** La RT-qPCR multiplex identificó patógenos de ITS en mujeres atendidas en CIES Cochabamba, con alta frecuencia de coinfecciones. Predominaron *Haemophilus ducreyi*, *Gardnerella vaginalis* y *Ureaplasma parvum/urealyticum*. Las relaciones sexuales sin preservativo, el nivel de escolaridad y el flujo vaginal se asociaron significativamente con las ITS.

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INTRODUCTION

Currently, sexually transmitted infections (STIs) have reached an unusually high prevalence and have become a public health problem, particularly in developing regions such as Bolivia, where epidemiological surveillance and timely diagnosis remain limited ^(1,2). Very little information is available on the molecular characterization of sexually transmitted infections in the specific local context ^(1,3). Understanding the frequency and distribution of STI-causing pathogens, as well as their relationship with sociodemographic, behavioral, and clinical factors, is necessary to design effective prevention and control strategies ⁽⁴⁾.

Sexually transmitted infections are associated with several risk factors, such as early sexual debut, sexual intercourse without condom use, number of sexual partners, and alcohol consumption, all of which may increase the likelihood of acquiring one or more STIs. Clinical manifestations such as genital pain, vaginal discharge, or the presence of warts are not always present, making timely and accurate diagnosis more difficult ^(4,5). In this context, the use of molecular techniques for the detection of STI-causing pathogens, such as multiplex RT-qPCR, allows more accurate identification of the responsible etiological agents, even in cases of coinfection, thereby contributing to more appropriate and individualized clinical management ⁽⁶⁾.

At the international level, several studies have demonstrated the usefulness of molecular panels for STI diagnosis. Liu et al. ⁽⁷⁾, in China, reported frequent coinfections involving *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and *Mycoplasma genitalium* among women of reproductive age. Zhang et al. ⁽⁸⁾, also in China, reported prevalences of *Chlamydia trachomatis* and *Mycoplasma genitalium* of 7.1% and 3.8%, respectively, in the study population. Saavedra et al. ⁽⁹⁾, in Peru, evaluated coinfections and reported positivity rates of 14.38% for *Ureaplasma urealyticum*, 9.38% for *Chlamydia trachomatis*, and 1.25% for *Mycoplasma genitalium*.

At the local level, Cochabamba has cultural and social characteristics that may influence the prevalence and dynamics of STIs ⁽¹⁰⁾, particularly among women attending health centers. Despite this, few studies have incorporated a molecular approach into their analyses, limiting a comprehensive understanding of the problem and the development of evidence-based health policies ^(11,12). Sexual and reproductive health is an essential dimension of women's well-being; therefore, its assessment requires a comprehensive approach combining clinical, epidemiological, and molecular analyses ^(13,14).

Based on the above, this study was necessary to provide updated and context-specific evidence on the presence of STI pathogens among women in Cochabamba, as well as their association with risk factors and clinical manifestations, thereby contributing to the strengthening of prevention, diagnosis, and control strategies in sexual and reproductive health.

Accordingly, the objective of this study was to characterize sexually transmitted infections using multiplex RT-qPCR and to determine associated risk factors among women attending the Research, Education and Services Center (CIES, by its Spanish acronym) in Cochabamba, Bolivia.



METHODS

Study design and area

This was a quantitative, observational, descriptive, cross-sectional study conducted at the CIES Cochabamba, where participant recruitment, clinical evaluation, and sample collection were performed, as well as at the Molecular Biology Laboratory of the Faculty of Pharmaceutical and Biochemical Sciences, Universidad Mayor de San Simón, where molecular processing and analysis using multiplex RT-qPCR were carried out. The study was conducted in Cochabamba, Bolivia, during 2024.

Population and sample

The study population consisted of 121 women treated at the CIES Cochabamba during 2024. Of these, 96 (79.34%) met the inclusion and exclusion criteria and constituted the final analytical sample. The results also reflected a 95% confidence level and a maximum acceptable error of 4.57%. Women older than 18 years with a clinical diagnosis of an STI made by medical personnel, based mainly on direct clinical evaluation, and who provided signed informed consent were included. Women with incomplete data and those receiving pharmacological treatment were excluded.

Variables and data collection instruments

The independent variables were sociodemographic characteristics (age, educational level, marital status, and place of origin); risk factors (casual sexual partners, alcohol consumption, number of sexual partners, and sexual intercourse without condom use); and clinical symptoms (genital warts or scabs, genital pain or itching, vaginal discharge, and pain during urination). The dependent variable was the STI pathogen detected using the Bio-Speedy® multiplex RT-qPCR panel (15), LOT: 311091015STI, Ref: BS-STI-T-25.

An anonymous questionnaire was used to collect the independent variables, whereas an observation guide was used for the dependent variable.

Data collection techniques and procedures

An interview was used to collect sociodemographic characteristics, risk factors, and clinical symptoms. First, each participant was invited to take part in the study; the study was then explained in detail and all relevant information was provided. Women who agreed to participate subsequently signed an informed consent form.

For the detection of sexually transmitted infection pathogens, the Bio-Speedy® multiplex RT-qPCR panel ⁽¹⁵⁾ was used, which allows the simultaneous detection of 12 STI pathogens. Results were recorded using an observational technique.

Samples were collected by CIES medical personnel. Endocervical specimens were obtained using a genital swab from Qiagen Corporation ⁽¹⁶⁾, which was placed in its transport medium, sealed, coded, and transported in a sterile container at 2–8 °C to the Molecular Biology Laboratory. Nucleic acids were then extracted without prior treatment using the GeneAll Ribospin™ vRD commercial kit ⁽¹⁷⁾, following the manufacturer’s instructions.

Four PCR tubes were used for each sample, after which the PCR master mix was prepared. In the first tube, 10 µL of 2X Prime Script Mix + 5 µL of STI Oligonucleotide Mix 1 were added for the detection of herpes simplex virus type 1 (HSV-1), Mycoplasma genitalium, and herpes simplex virus type 2 (HSV-2). In the second tube, 10 µL of 2X Prime Script Mix + 5 µL of STI Oligonucleotide Mix 2 were added for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae. In the third tube, 10 µL of 2X Prime Script Mix + 5 µL of STI Oligonucleotide Mix 3 were added for the detection of Treponema pallidum, Trichomonas vaginalis, and Ureaplasma parvum/urealyticum. In the fourth tube, 10 µL of 2X Prime Script Mix + 5 µL of STI Oligonucleotide Mix 4 were added for the detection of Mycoplasma hominis, Haemophilus ducreyi, Streptococcus agalactiae, and Gardnerella vaginalis. Subsequently, 5 µL of nucleic acid from the sample was added to each tube, resulting in a total reaction volume of 20 µL. For the negative control, 5 µL of RNase-free water was used, whereas 5 µL of a mixture of pathogens and clones was used for the positive control. Amplification curves were analyzed using MIC and Bio-Rad software.

Data analysis

The results were tabulated and coded in a Microsoft Excel 2016 spreadsheet and then imported into SPSS

version 25. Descriptive analysis was performed by calculating absolute and relative frequencies for the variables studied. Associations between categorical variables were assessed using the chi-square test or Fisher’s exact test, as appropriate, with statistical significance set at $p < 0.05$ and a 95% confidence level.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki, as updated at the General Assembly of the World Medical Association held in Fortaleza, Brazil, in October 2013 ⁽¹⁸⁾. The study protocol was approved by the Bioethics Committee of the Faculty of Medicine, Universidad Mayor de San Simón, Cochabamba, Bolivia, under registration code CBE_UMSS_04_2025, issued on March 10, 2025, while ensuring respect for the rights of all research participants.

RESULTS

Among the participants, 41.7% were between 18 and 27 years of age. Regarding marital status, the most frequent group consisted of single women (50.0%). In terms of educational level, 63.5% had higher education. In addition, 71.9% were from urban areas (Table 1).

Table 1. Sociodemographic characteristics of women attending the Research, Education and Services Center (CIES, by its Spanish acronym)

Sociodemographic characteristics	n = 96	
	fi	%
Age group		
18-27 years	40	41.7
28-37 years	26	27.0
38-47 years	21	21.9
48-57 years	9	9.4
Marital status		
Single	48	50.0
Married	36	37.5
Cohabiting	10	10.4
Divorced	2	2.1
Educational level		
Primary	6	6.3
Secondary	29	30.2
Higher	61	63.5
Place of origin		
Rural	27	28.1
Urban	69	71.9

Table 2. Risk factors and clinical symptoms among women attending the Research, Education and Services Center (CIES, by its Spanish acronym)

Variables	n = 96	
	fi	%
Casual sexual partners		
Yes	22	22.9
No	74	77.1
Alcohol consumption		
Yes	48	50.0
No	48	50.0
Number of sexual partners		
1–3	80	83.3
4–6	16	16.7
Sexual intercourse without condom use		
Yes	17	17.7
No	79	82.3
Genital warts or scabs		
Yes	10	10.4
No	86	89.6
Genital itching or pain		
Yes	19	19.8
No	77	80.2
Pain during urination		
Yes	16	16.7
No	80	83.3
Vaginal discharge		
Yes	83	86.5
No	13	13.5

Regarding risk factors, 22.9% reported having casual sexual partners, 50.0% reported alcohol consumption, 83.3% had between one and three sexual partners, and 82.3% reported sexual intercourse without condom use. Regarding clinical symptoms, 10.4% presented genital warts or scabs, 16.7% reported pain during urination, 19.8% reported genital pain or itching, and 86.5% presented vaginal discharge (Table 2).

Regarding the pathogens detected using multiplex RT-qPCR, three and two microorganisms were identified in 30.2% and 33.3% of the samples analyzed, respectively (Figure 1).

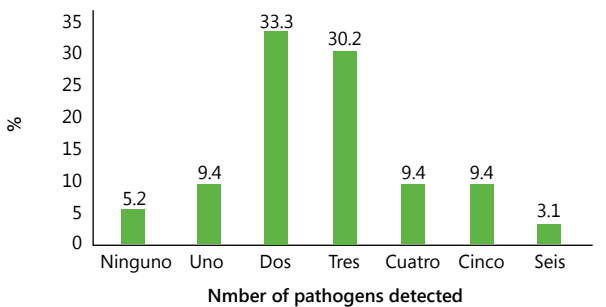


Figure 1. Percentage distribution according to the number of pathogens detected in participant samples.

The least frequently detected STI-associated pathogens were *Neisseria gonorrhoeae* (n = 1), *Treponema pallidum* (n = 1), *Streptococcus agalactiae* (n = 4), *Mycoplasma hominis* (n = 6), *Chlamydia trachomatis* (n = 9), *Mycoplasma genitalium* (n = 9), HSV-1 (n = 10), and HSV-2 (n = 12). In contrast, the most frequently identified pathogens were *Trichomonas vaginalis*, *Ureaplasma parvum/urealyticum*, *Gardnerella vaginalis*, and *Haemophilus ducreyi*, with 16, 59, 65, and 71 detections, respectively (Figure 2).

Among the sociodemographic characteristics, age, marital status, and place of origin were not associated

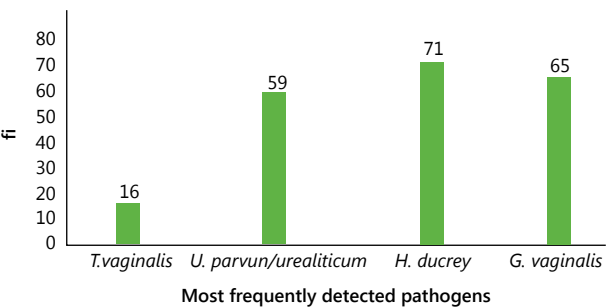


Figure 2. Most frequently detected pathogens in participant samples.

with the presence of STI pathogens ($p > 0.05$), whereas educational level showed a statistically significant association ($p = 0.044$). Regarding risk factors, no association with the presence of STI pathogens was observed for casual sexual partners, alcohol consumption, or number of sexual partners ($p > 0.05$). In contrast, sexual intercourse without condom use showed a significant association ($p = 0.037$). Among the clinical symptoms evaluated, only vaginal discharge was associated with the presence of STI pathogens ($p = 0.017$) (Table 3).

Table 3. Presence of STI pathogens according to sociodemographic characteristics, risk factors, and clinical symptoms

Variables	STI pathogens present		p-value
	Yes (n = 91)	No (n = 5)	
Age group			
18–27 years	37	3	0.795
28–37 years	25	1	
38–47 years	20	1	
48–57 years	9	0	
Marital status			
Single	45	3	0.774
Married	35	1	
Cohabiting	9	1	
Divorced	2	0	
Educational level			
Primary	6	0	0.044
Secondary	25	4	
Higher	60	1	
Place of origin			
Rural	25	2	0.618
Urban	66	3	
Casual sexual partners			
Yes	20	2	0.322
No	71	3	
Alcohol consumption			
Yes	46	2	0.646
No	45	3	
Number of sexual partners			
1–3	76	4	0.837
4–6	15	1	
Sexual intercourse without condom use			
Yes	77	2	0.037
No	14	3	
Genital warts or scabs			
Yes	10	0	0.434
No	81	5	
Genital itching or pain			
Yes	19	0	0.579
No	72	5	
Pain during urination			
Yes	16	0	0.586
No	75	5	
Vaginal discharge			
Yes	81	2	0.017
No	10	3	

Table 4. Sociodemographic characteristics, risk factors, and clinical symptoms associated with STI pathogens

Variables	Pathogens	p-value
Sociodemographic characteristics		
Age group	<i>Mycoplasma genitalium</i>	0.036
Educational level	<i>Mycoplasma hominis</i>	1.81E-5
Marital status	<i>Trichomonas vaginalis</i>	0.031
Place of origin	<i>Streptococcus agalactiae</i>	0.033
Risk factors		
Casual sexual partners	<i>Mycoplasma genitalium</i>	0.014
	<i>Trichomonas vaginalis</i>	0.004
Sexual intercourse without condom use	<i>Ureaplasma parvum/urealyticum</i>	0.005
Clinical symptoms		
Genital warts or scabs	<i>Chlamydia trachomatis</i>	0.018
	<i>Neisseria gonorrhoeae</i>	0.003
Pain during urination	<i>Mycoplasma hominis</i>	0.001
	<i>Haemophilus ducreyi</i>	0.009

Mycoplasma hominis infection was associated with educational level and the presence of genital warts or scabs; *Mycoplasma genitalium* infection was associated with age group and casual sexual partners; *Trichomonas vaginalis* infection was associated with marital status and casual sexual partners; *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections were associated with genital warts or scabs; place of origin was associated with *Streptococcus agalactiae* infection; and sexual intercourse without condom use was associated with *Ureaplasma parvum/urealyticum* infection (Table 4).

 **DISCUSSION**

In this study, STIs among women attending a health center in Cochabamba were molecularly characterized using multiplex RT-qPCR, allowing the simultaneous detection of multiple pathogens and the identification of coinfections. This molecular approach has demonstrated high sensitivity and specificity, as shown in several international studies, including those conducted by Lee et al. ⁽¹⁹⁾ in South Korea, Suntok et al. ⁽⁶⁾ in Uganda, and Liu et al. ⁽⁷⁾ in China, which highlight its usefulness for achieving accurate and timely diagnosis, particularly in infections with nonspecific symptoms or in cases involving multiple microorganisms.

The results showed a high frequency of coinfections, since three and two pathogens were identified in 30.2% and 33.3% of the analyzed samples, respectively. This is comparable to the findings reported by Lee et al. ⁽¹⁹⁾, in which 54.3% of the analyzed samples tested positive for at least one pathogen and coinfections were frequent among female patients. Similarly, studies conducted by Grad et al. ⁽²⁰⁾ in Romania and Pella-Saavedra et al. ⁽⁹⁾ in Peru reported the detection of coinfections, with positivity rates of approximately 30.0% among the analyzed samples and high frequencies for specific pairs of microorganisms. These findings support the relevance of multiplex RT-qPCR as a method for the diagnosis and identification of STI-causing pathogens.

The most frequently detected pathogens in the present study were *Haemophilus ducreyi*, *Gardnerella vaginalis*, and *Ureaplasma parvum/urealyticum*. Similar findings were reported in Austria, where *Mycoplasma hominis* was detected in 21.5% and *Ureaplasma* spp. in 7.5% of the analyzed samples, with both microorganisms showing a higher prevalence among women with symptoms of genital infection ⁽²¹⁾.

On the other hand, *Mycoplasma genitalium* and *Chlamydia trachomatis* were each detected in 9.4% of the samples in this study, findings similar to those reported by Kirkoyun et al. ⁽²²⁾ and Pella-Saavedra et al. ⁽⁹⁾, who detected *Mycoplasma genitalium* in 7.8% and *Chlamydia trachomatis* in 9.38% of samples. These findings reinforce the epidemiological consistency of these pathogens among women of reproductive age, despite regional differences.

Regarding *Neisseria gonorrhoeae* and *Treponema pallidum*, both were identified at a low frequency (1.0%) in the analyzed samples. This low prevalence is comparable to that reported by de Souza Mendes et al. ⁽²³⁾ and could be explained by more timely clinical care for acute symptoms or by limitations in sampling sensitivity. In Bolivia, the presence of herpes simplex virus (HSV) and human papillomavirus (HPV) has previously been documented among rural women, with high prevalence rates ^(1,24), indicating that viral STIs also represent an important public health problem in the region.

Regarding risk factors, sexual intercourse without condom use was significantly associated with the presence of STIs ($p = 0.037$), a finding consistent with the studies by Grad et al. ⁽²⁰⁾ and Kirkoyun et al. ⁽²²⁾, in which this behavior was strongly associated with infections caused by *Ureaplasma* spp., *C. trachomatis*, and *M. genitalium*. In contrast, variables such as alcohol consumption were not significantly associated with

STIs, possibly because of underreporting or the social perception of these behaviors, a common limitation in studies based on self-reported questionnaires ⁽²⁵⁾.

In the analysis of clinical symptoms, vaginal discharge was the only symptom significantly associated with the presence of STIs ($p = 0.017$), which is consistent with the literature recognizing this sign as one of the most sensitive, although nonspecific, manifestations of genital infections ^(26,27). However, the low frequency of other symptoms, such as genital pain or warts, highlights the need for sensitive diagnostic methods such as RT-qPCR to identify STIs in asymptomatic patients or those with mild symptoms.

The relationship between specific pathogens and individual variables also yielded relevant findings: *Mycoplasma genitalium* was associated with casual sexual partners ⁽²⁸⁾, *Trichomonas vaginalis* with marital status ⁽²⁹⁾, and *Ureaplasma parvum/urealyticum* with sexual intercourse without condom use ^(30,31), indicating that the epidemiological patterns of STIs may vary according to the infectious agent and sociodemographic context. Similar associations have also been identified in studies conducted by Yamani et al. ⁽³²⁾ and Jacques-Aviñó et al. ⁽³³⁾.

Regarding *Mycoplasma* species, *Mycoplasma genitalium* showed a statistically significant association with age group ($p = 0.036$) and casual sexual partners ($p = 0.014$), a finding consistent with that reported by Sethi et al. ⁽³⁴⁾ and Miranda et al. ⁽³⁵⁾, who described a higher frequency of this infection among women with more than one sexual partner and in groups with greater sexual exposure. In turn, *Mycoplasma hominis* was significantly associated with educational level ($p = 1.81 \times 10^{-5}$), a result similar to that reported by Wang et al. ⁽³⁶⁾, in which a lower educational level was associated with higher detection rates of this microorganism. This association could be explained by conditions of social vulnerability and unequal access to sexual health information and services.

One of the main strengths of the present study was the use of the multiplex RT-qPCR panel, which enabled the simultaneous detection of 12 pathogens, the identification of coinfections, and the assessment of clinical and epidemiological associations. In addition, the study provides valuable and updated data on the epidemiology of STIs in an understudied local setting such as Cochabamba, Bolivia.

The limitations include the cross-sectional design, which precludes establishing causal relationships, as well as the lack of subsequent clinical follow-up, which

limits assessment of clinical progression and response to treatment. Likewise, the relatively small sample size limits the extrapolation of the findings to a broader population, although the results provide a solid basis for future studies using longitudinal designs and more representative samples.

The incorporation of multiplex RT-qPCR into routine STI screening at local health centers is recommended, together with sexual health education focused on consistent condom use, in order to reduce coinfections, improve local detection, and guide specific treatment.

Conclusions

Sexually transmitted infections among women in Cochabamba showed a high frequency of coinfections, with *Haemophilus ducreyi*, *Gardnerella vaginalis*, and *Ureaplasma parvum/urealyticum* being the most frequently detected pathogens. Educational level, sexual intercourse without condom use, and the presence of vaginal discharge were significantly associated with the presence of STIs. In addition, the use of multiplex RT-qPCR enabled the simultaneous detection of 12 pathogens, making it a useful tool for timely diagnosis and epidemiological surveillance.



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Authorship contribution

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MEA: Conceptualization, methodology, and final review of the manuscript.

SVF: Investigation, data curation, and final review of the manuscript.

JPEA: Resources, writing – original draft, and writing – review and editing.

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Conflict of interest statement

The authors declare no conflicts of interest.