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Molecular Diagnosis of Sexually Transmitted Infection Reveals a High Frequency of *Neisseria gonorrhoeae* Infection among Male Urethritis Patients and Highlights the Usefulness of Molecular Testing for Clinical Diagnosis in Burkina Faso

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Abstract

Sexually transmitted infections (STIs) represent a public health problem due to their high prevalence worldwide and the emergence of multidrug resistance of responsible microorganisms. Medical laboratory diagnosis of sexually transmitted genital infections by traditional methods as culture remains extremely delicate, difficult or impossible (to find extremely fragile organisms that can be cultured). Thus, molecular techniques constitute an alternative to improve accurate diagnostic, personalized patient treatment, and public health. A total of 83 clinical samples including urethral discharge and urine samples from individual patients with symptoms of urethritis received were analyzed using traditional methods and a commercial real-time PCR (qPCR)

method. Out of 83 urethritis patients, n = 55 (66.26%) were positive for at least one of the STI pathogens detected by qPCR. qPCR assay was more sensitive (50/83, positive cases) compared to culture (15/83, positive cases) and light microscopy (28/83, positive cases). The most prevalent NTD pathogen in the suspected patients was *N. gonorrhoeae* with 60.24% (50/83) based on real-time PCR diagnosis. Among the positive cases of STI pathogens, *Neisseria gonorrhoeae* had the highest frequency 49/55 (89.01%) followed by low frequencies of *Trichomonas vaginalis* 4/55 (7.27%) and *Chlamydia trachomatis* 1/55 (1.82%). This highlights the high prevalence of *N. gonorrhoeae* infection in male urethritis patients and a very important misdiagnosis using traditional routine methods in Burkina Faso by medical laboratories. Thus, this situation may negatively impact patients' personalized treatment and care and public health with the possible rapid emergence of multidrug-resistant strains. This study also highlights the urgent need to optimize culture for the diagnosis of NTD pathogens in Burkina Faso and the usefulness and the need for the introduction of molecular diagnostic methods in routine diagnosis for the detection of NTD pathogens in the medical laboratories in Burkina Faso.

Keywords

Molecular Diagnostic Methods, Pathogens, Sexually Transmitted Infection, Urethritis, Burkina Faso

1. Introduction

Urethritis or inflammation of the urethra, is the most common syndrome in symptomatic male urethritis, accompanied by dysuria, pruritus, and urethral discharge. Most cases of infectious urethritis are caused by *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and/or *Chlamydia trachomatis* (*C. trachomatis*) [1]. Sexually Transmitted Infections (STI) are the leading cause of infectious urethritis and pose a serious public health concern [2]. The number of sexual partners, prior sexual education, inconsistent condom usage, and prior STI are the most significant factors that have been found to affect the appearance of an STI [3].

Among the pathogens responsible for urethritis, *N. gonorrhoeae* is a sexually transmitted pathogen of very deep concern and has been classified by the World Health Organization (WHO) as a priority pathogen due to the worldwide emergence of multidrug-resistant isolates and the rapid increase in antimicrobial resistance rates. To make matters worse, extensively drug-resistant (XDR) strains of *N. gonorrhoeae*, which is resistant to ceftriaxone and highly resistant to azithromycin have emerged in several countries in recent years. For example, several European countries have been affected by XDR isolates of *N. gonorrhoeae* since 2022, with 4 cases reported in Austria, France, and the United Kingdom, and 2 more cases in France in 2023 [4]-[6]. Caméléna F 2024, Michaela Day, Clara Maubaret Cambodia also reported 3 cases of XDR isolates of *N. gonorrhoeae* between

2021 and 2022 [7]. All these strains were genetically related.

In addition to gonococcal urethritis, there is urethritis caused by other pathogens and in almost half of these cases of non-gonococcal urethritis, no specific agent is identified, despite extensive microbiological testing [8]. According to the WHO, surveillance data on gonococcal and chlamydial infections are also very limited in low- and middle-income countries. Most of the information available on STIs in these countries comes either from research studies or population surveys [9].

Medical laboratory diagnosis of genital infections remains extremely delicate, as it is often difficult or even impossible to find extremely fragile germs that can be cultured, hence the use of molecular techniques [10]. It is estimated that one million people a day contract an STI, of which around 498.91 million STIs are considered curable, namely those due to *Treponema pallidum* (syphilis), *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis*, occur worldwide each year, with the highest rates recorded in sub-Saharan Africa and Latin America and the Caribbean, followed by the Western Pacific Region [9] (ONUSIDA 2012). Since the onset of HIV/AIDS infection, there has been renewed interest in STIs, because on the one hand, they are cofactors of the infection and, on the other, their proper management can reduce the acquisition of HIV/AIDS infection.

In many low- and middle-income countries, there is a problem with accurate diagnosis of STIs. This is due to the lack of appropriate techniques. Clinical laboratories do not perform high-performance tests, the traditional culture method lacks sensitivity (lack of appropriate culture media), and molecular assays are absent from routine testing. This situation leads to inadequate management of STI patients and possible expansion of STI outbreaks as well as outbreaks of XDR strains.

Our study aimed to estimate the prevalence and frequency of the most common STI pathogens involved in male urethritis by an effective diagnostic method, real-time polymerase chain reaction (real-time PCR) or qPCR, and to evaluate the diagnostic performance of the culture method.

2. Material and Methods

2.1. Study Population

This study included male patients over 10 years of age with clinical symptoms suggestive of urethritis (see supplemental data in the Excel file for details) who consulted clinical services and were received at the Clinical Biology Laboratory of the Centre MURAZ, Bobo-Dioulasso, Burkina Faso. Thus, the clinical specimens from the patients used were from routine diagnosis, urethral swabs, and first urine. There was no specific recruitment and enrolment of patients for this study.

2.2. Sample Collection and Processing

This cross-sectional study was conducted from 2020 to 2021. From a patient

presenting with a urethral discharge, the skin was collected using a swab soaked in PCR grade water and then inserted into a 1.5 ml tube and 0.2 ml of the transport medium (Sacace amplification kit transport medium) was added and then shaken vigorously for 15 to 20 sec and stored at −20˚C until DNA extraction. Urine sampling consisted of collecting 10 to 20 ml of the morning's first stream of urine or 2 hours after the last micturition in a sterile jar. Urine samples were stored at 2˚C - 8˚C for no more than 24 hours, or frozen at −20˚C/80˚C. Urine was centrifuged for 30 minutes at 3000 rpm, then the supernatant was discarded and approximately 200 µl of the pellet was stored at −20˚C before DNA extraction. This study was approved by the ethical committees of the Health Science Research, Burkina Faso No 2024-042/MSHP/MESRI/CERS.

2.3. Microscopic Analysis and Microorganism Isolation by Culture

Microscopic examination in the fresh state of the specimen (urines or urethral swabs) was carried out, making it easier to visualize the presence of *T. vaginalis* due to its movements (**Figure 1(A)**). Gram stain and examination in microscopy were performed to visualize intra- and extracellular coffee-bean Gram-negative diplococci, often rare on the smear, suggestive of *N. gonorrhoeae* (**Figure 1(B)**). Then, all samples were inoculated onto chocolate agar medium supplemented with poly vitex, and incubated at 37˚C in an oven at 5% CO_2 for 18 to 24 hours and bacteria were identified with Neisseria-Hemophylus (NH) card using the VITEK 2®_COMPACT® automated system (Biomérieux, Marcy l'Etoile, France).

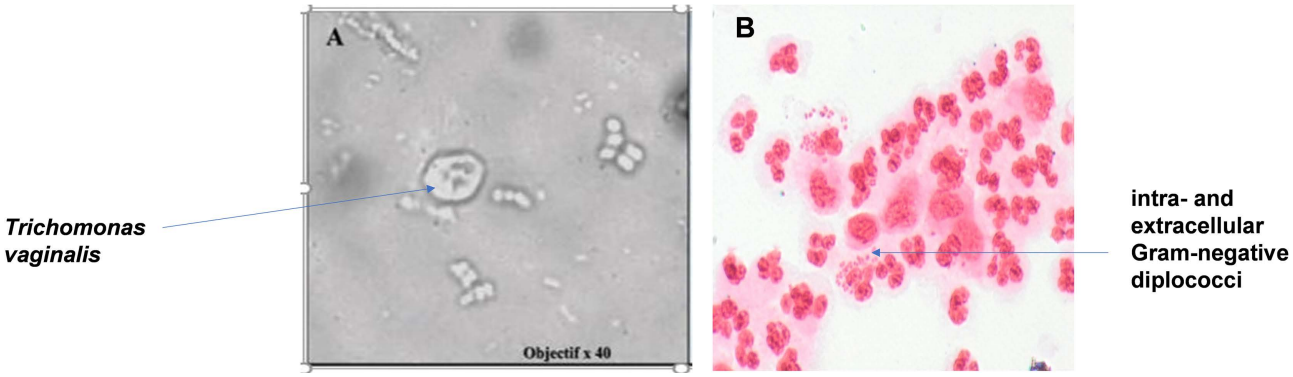

Figure 1. Microscopic examination. A: examination of fresh specimen visualizing *Trichomonas vaginalis*, B: observation of Gram-negative diplococci from urethral specimen (Laboratory of Bacteriology, Centre MURAZ, Bobo-Dioulasso, Burkina Faso).

2.4. Multiplex Molecular Detection of Sexually Transmitted Pathogens by a Multiplex Real-Time PCR Assay

DNA was extracted essentially from urine and urethral swab clinical samples using DNA-Sorb-A nucleic acid extraction kit (Sacace BIOTECHNOLOGIES®, 22100 Como, Italy) following the protocol provided by the manufacturer. The extracted DNA was stored in a freezer at −20˚C until used for the molecular analysis.

DNA extracts were screened by Sacace BIOTECHNOLOGIES® (22100 Como, Italy) Real-Time PCR kit for multiplex detection (*Trichomonas vaginalis*/*N.*

gonorrhoeae/*C. trachomatis* Real-TM, CE) of the 3 STD urethritis-causing pathogens, *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis*. Briefly, the reaction mixture was prepared in a final volume of 25.5 µL including 10 µL of DNA extract, 10 µL of PCR-mix-1-FRT, 5 µL of PCR-mix-2-FRT, and 0.5 µL of TaqF DNA polymerase. The amplification cycles program was 1 cycle at 95˚C for 10 min followed by 5 cycles at 95˚C for 5 s; 60˚C for 20 s; 72˚C for 15 s and finally 40 cycles of 95˚C for 5 s; 60˚C for 30 s and 72˚C for 15 s. The quality control of the reaction was monitored by internal controls, negative controls, and positive controls used in the assay.

2.5. Data analysis

Data were entered into Microsoft Excel 2016 and statistical analysis was performed using the software R version 4.3.3 to determine the prevalence of sexually transmitted diseases (STD) pathogens responsible for urethritis in men (*N. gonorrhoeae*, *T. vaginalis*, and *C. trachomatis*) directly targeted by the trioplex real-time PCR assay. Socio-demographic characteristics of patients to pathogens or diseases were also analyzed. In addition, we compared the performance obtained by R analysis (sensitivities and specificities) of microscopy and culture, 2 traditional methods used in routine diagnosis of these pathogens to the real-time PCR technique.

3. Results

3.1. Socia-Demographic Characteristics of Urethritis Patients and Pathogens Detected

Sociodemographic characteristics and clinical symptoms of some patients were missed (Supplementary Data, in Excel file) because of the poor quality of the biological test orders and the clinical and sociodemographic information provided by the physicians, who did not fully follow the recommendations of the Health Care Quality Management System. Our collected data on sociodemographic characteristics showed that patients' ages ranged from 16 to 60 years, with the most exposed age group being 16 - 30 years with 44.58%, followed by 30 - 40 years with 21.68%. The youngest patient included in the study was 16 years old. Single patients are most represented in this study population (45.78%), and the various occupational categories were grouped into shopkeepers, students, civil servants, workers, drivers, and farmers (Table 1).

3.2. Prevalence and Frequency of STI—Causing Pathogens in Male Urethritis Patients Based on Molecular Testing

In the present study, 66.26% (55/83) of the specimens (patients) were positive for urethritis-causing pathogens detected by triplex qPCR and 33.74% (28/83) were negative (Table 2). *N. gonorrhoeae* was the most common pathogen in the population of suspected study patients with 60.24% (50/83), followed by rare cases of *T. vaginalis* with 4.82% (4/83) and *C. trachomatis* with 2.41% (2/83) (Table 2).

Table 1. Pathogen frequencies by patients' socio-demographic characteristics.

Characteristics	Frequencies of pathogens detected by qPCR method				
Profession	*C. trachomatis*	*N. gonorrhoeae*	*T. vaginalis*	No pathogen detected	p-value
Biologist	0 (0.00%)	2 (4.00%)	0 (0.00%)	0 (0.00%)	
Breeder	0 (0.00%)	1 (2.00%)	0 (0.00%)	0 (0.00%)	
Driver	0 (0.00%)	1 (2.00%)	0 (0.00%)	0 (0.00%)	
Electrician	0 (0.00%)	1 (2.00%)	0 (0.00%)	0 (0.00%)	
Farmer	0 (0.00%)	1 (2.00%)	0 (0.00%)	0 (0.00%)	
Follow-up Evaluator	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (3.57%)	
Glazier	0 (0.00%)	1 (2.00%)	0 (0.00%)	0 (0.00%)	0.70
Primary pupil	0 (0.00%)	4 (8.00%)	0 (0.00%)	2 (7.14%)	
Shopkeeper	0 (0.00%)	2 (4.00%)	2 (50.00%)	5 (17.86%)	
Soldier	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (3.57%)	
Student	0 (0.00%)	5 (10.00%)	0 (0.00%)	3 (10.71%)	
Teacher	0 (0.00%)	3 (6.00%)	1 (25.00%)	2 (7.14%)	
Not Respond	2 (100.00%)	29(58.00%)	1 (25.00%)	14(50.00%)	
		Age			
]16, 30]	0	27 (55.10%)	0 (0.00%)	12 (42.85%)	
]30, 40]	0	9 (18.37%)	3 (75.00%)	6 (21.43%)	
]40, 50]	0	4 (8.16%)	1 (25.00%)	2 (7.14%)	0.08771
]50, 60]	0	0 (0.00%)	0 (0.00%)	1 (3.57%)	
Not Respond	1	9 (18.37%)	0 (0.00%)	7 (25.00%)	
		Marital status			
Married	1 (100.00%)	13 (26.53%)	2 (50.00%)	10 (35.71%)	
Single	0 (0.00%)	23 (46.94%)	2 (50.00%)	13 (46.43%)	0.5829
Not Respond	0 (0.00%)	13 (26.53%)	0 (0.00%)	5 (17.85%)	

Table 2. Prevalence of the 3 pathogens targeted by the qPCR assay.

Pathogens		Number (N = 83)	Prevalence (%)
N. gonorrhoeae	Positive	50	**60.24**
	Negative	33	39.76
C. trachomatis	Positive	2	**2.41**
	Negative	81	97.59

Continued

T. vaginalis	**Positive**	**4**	**4.82**
	Negative	79	95.18
All pathogens**	Positive*	56	**67.46**
	Negative	27	32.53

**(*N. gonorrhoeae*, *T. vaginalis* and *C. trachomatis*) *(one co-infection with *C. trachomatis* and *N. gonorrhoeae* that is considered as a single positive specimen (or patient).

Of the 55 urethritis cases caused by sexually transmitted pathogens, the most frequent pathogen causing urethritis was *N. gonorrhoeae* with 89.01%. Infections by *T. vaginalis*, *C. trachomatis*, or co-infections were rare, respectively 7.27%, 1.82%, and 1.82% *N. gonorrhoeae*/*C. trachomatis* (Figure 2).

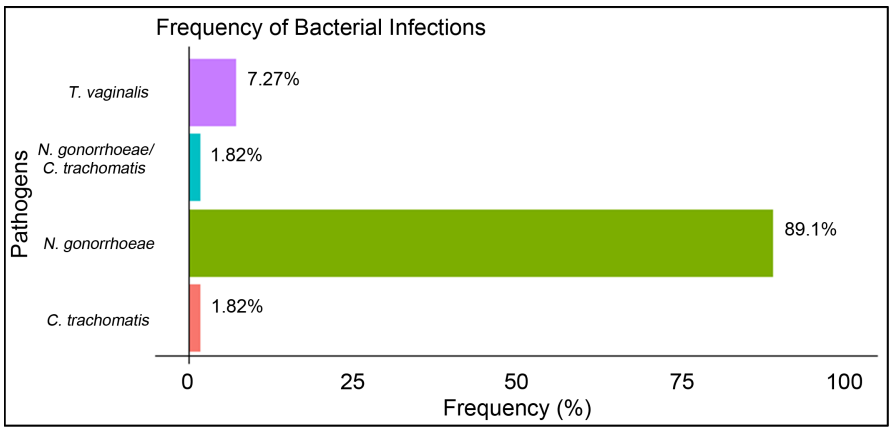

Figure 2. Frequencies of pathogens detected by the qPCR method (N = 55 of positive samples).

3.3. Diagnostic Performance of Microscopy and Culture Compared to Real-Time PCR (qPCR) for the Detection of Pathogens

The detection and identification of STI pathogens was performed using light microscopy, the culture method, and a real-time PCR (qPCR) assay. Light microscopy of fresh specimens or after Gram staining may allow observation of microorganisms and guide subsequent examination in the case of positive specimens. The culture method, which uses appropriate culture media, can allow the isolation and identification of any bacterial pathogen. In this study, the qPCR assay used targeted 3 of the most common sexually transmitted pathogens causing male urethritis, *i.e.*, *N. gonorrhoeae*, *T. vaginalis*, and *C. trachomatis*.

The comparison of light microscopy and culture results with qPCR for the detection of all pathogens shows low sensitivities of light microscopy and culture, respectively 28/55 (50.91%) and 15/55 (27.27%). Table 3 shows the details of the comparison of light microscopy and culture results with qPCR.

The diagnostic performance of the culture method (allow for detection and identification at the species level) compared to the qPCR for the detection of the

3 pathogens targeted by the qPCR or all the pathogens showed a low sensitivity of the culture for *N. gonorrhoeae* (30%). In particular, these results showed a total inefficiency of the culture in the context of Burkina Faso for the detection of *T. vaginalis* and *C. trachomatis* (Table 4).

Table 3. Comparison of light microscopy and culture results with qPCR.

		qPCR		
		Positive	Negative	p-value
Light microscopy	Positive	28 (100.00%)	0 (0.00%)	3.517e−06*
	Negative	27 (49.09%)	28 (50.91%)	
Culture	Positive	15 (100.00%)	0 (0.00%)	0.002266*
	Negative	40 (58.82%)	28 (41.18%)	

Table 4. Diagnostic performance of the culture method compared to the qPCR assay for pathogens detection.

Pathogens	Culture diagnostic performance	
	Sensitivity (%)	Specificity (%)
N. gonorrhoeae	30.00	100.00
T. vaginalis	0.00	100.00
C. trachomatis	0.00	100.00
All pathogens	27.27	100.00

4. Discussion

Our study aimed to estimate the prevalence and frequencies of common NTD pathogens involved in urethritis in male patients in Burkina Faso using a commercial qPCR assay and to compare the performance of traditional diagnostic methods with qPCR.

Of 83 urethritis patients, n = 55 (66.26%) were positive for at least one of the STI pathogens detected by qPCR. qPCR assay was more sensitive (50/83, positive cases) compared to culture (15/83, positive cases).

The most exposed age group in our study population was the group aged 16 - 30 years (44.58%), representing the youngest patients. Sexual activity must be highest in this age group, which did not frequently use methods of protection against sexual contact.

The most common NTD pathogen detected was *N. gonorrhoeae* with a high prevalence of 60.24% (50/83) and frequency (within only positive cases of an NTD pathogen) 49/55 (89.01%). In addition to *N. gonorrhoeae* 4 and 2 positive cases were detected respectively for *T. vaginalis* and *C. trachomatis*. One case of co-infection with *N. gonorrhoeae* and *C. trachomatis* was found in this study. *N. gonorrhoeae* with its multidrug resistance and extensive drug resistance emergence is a worldwide concern pathogen and many countries have run *N. gonorrhoeae* surveillance programmes [4]. For example, Burkina Faso has included *N. gonorrhoeae* resistance in its antimicrobial resistance (AMR) surveillance of bacterial pathogens of concern [11] [12]. Contrary to the results of our study showing a high prevalence of *N. gonorrhoeae* in cases of urethritis, the 2022 and 2023 reports of AMR surveillance in Burkina Faso involving 22 sentinel laboratories reported no cases of *N. gonorrhoeae* isolation in Burkina Faso [11] [12]. This is an alarming situation that calls into question the technical capacity of Burkina Faso's clinical laboratories, the performance of the public health surveillance programme, and the relevance of the algorithm for the management of *N. gonorrhoeae* infections in Burkina Faso.

Our results provide evidence that clinical laboratories in Burkina Faso are not able to isolate *N. gonorrhoeae* from clinical specimens, and certainly not able to isolate *C. trachomatis* and *T. vaginalis* found in our study. An additional concern is the multidrug and extensively drug-resistant strains of *N. gonorrhoeae*, which are on the rise worldwide and for which phenotypic drug susceptibility testing cannot be performed in the absence of the isolate.

The reasons for the total failure of clinical laboratories in Burkina Faso to isolate *N. gonorrhoeae* and other STI bacterial pathogens include the use of inappropriate culture media, technical problems in culture with insufficient saturation with 5% CO_2 in the incubator, the time taken from collection to isolation on the appropriate medium and the quality of the enriched medium used. Overall, the low diagnostic performance of STI pathogens based on traditional routine methods in Burkina Faso by clinical laboratories may lead to a very important misdiagnosis of STI. The negative impact on personalized treatment and care for patients, and public health with the possible rapid emergence of multidrug-resistant strains, is considerable.

This study also highlights the urgent need to optimize culture for the diagnosis of STI pathogens in Burkina Faso. As the diagnostic performance of culture is also low compared to qPCR, this study highlights the usefulness and the need for the introduction of molecular diagnostic methods in routine diagnosis for the detection of STI pathogens in the medical laboratories in Burkina Faso. Our study showed that the diagnostic performance of culture even using appropriate culture media was still low, 30% for the detection of *N. gonorrhoeae*. In our study, we did not use appropriate culture media to isolate *C. trachomatis* and *T. vaginalis*, which explains the lack of detection of these pathogens by the culture method.

However, based on qPCR, *T. vaginalis* was the second STI pathogen isolated in

this study and *C. trachomatis* was the third, each with low prevalence. Dwari *et al.* (2018) in India found *N. gonorrhoeae* (61.42%) and *C. trachomatis* (45.9%) [13]. Rietmeijer *et al*., 2018 in Zimbabwe, conducted similar studies giving *N. gonorrhoeae* (73.5%) and *C. trachomatis* (22.5%) [14]. These literature research data corroborate the high prevalence of *N. gonorrhoeae* worldwide but show a high prevalence of *T. vaginalis* and *C. trachomatis* compared to our results.

These sexually transmitted infections have few symptoms in men, with the most common symptom being acute anterior urethritis, while gonococcal infections in women have no specific symptoms [15] [16] but have important consequences if not detected early and treated successfully (*N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*). If undetected and untreated, they can lead to chronic pelvic pain, infertility, and ectopic pregnancy, as well as facilitating HIV acquisition and transmission [17].

Untreated chlamydia can lead to trachoma, a major cause of blindness, and lymphogranuloma venereum, which can cause chronic proctitis, rectal stenosis, and genital lymphoedema in both men and women after anal intercourse [14]. STIs are, therefore, a serious public health problem that affects both men and women. In addition to early diagnosis which is crucial for prompt treatment [15] [17], public health measures to control these diseases can include a variety of means. These include encouraging men to use condoms correctly and consistently and educating them about the importance of protecting themselves during sexual activity. Accessibility of condoms is an important part of this prevention strategy. It is also important to promote frank and open dialogue to create an atmosphere conducive to informed decision-making and to reduce the stigma associated with STIs [18]. Because STIs can have an impact on mental health and quality of life. Finally, improving the sexual and reproductive health of men and their partners is an important component of all these packages of measures [15] [19] [20]. One strategy is to create an STI control program, similar to Burkina Faso's existing tuberculosis, malaria, and HIV control programs. A program would allow the country to mobilize internal and external funds and strengthen control methods. This study had limitations including the lack of appropriate media to isolate *C. trachomatis*, *T. vaginalis* and other STI pathogens. The qPCR assay kit does not take into account all the pathogens involved in urethral discharge. The antibiogram was not performed due to the lack of an appropriate E-test and the lack of certain information on the patient's report card.

Despite these limitations, this study provides evidence-based results on the importance of *N. gonorrhoeae* infection, the inadequate quality (low sensitivity) of results from clinical laboratories isolating STI pathogens in Burkina Faso using the culture method, and the usefulness of molecular methods.

This study also highlights the urgent need to optimize culture for the diagnosis of STI pathogens in Burkina Faso and the need for the introduction of molecular diagnostic methods in routine diagnosis for the detection of STI pathogens in the clinical laboratories in Burkina Faso.

5. Conclusion

This study shows the high prevalence of *N. gonorrhoeae* infection in male urethritis in Burkina Faso, but unfortunately poor patient care and public health prevention on all fronts due to the low diagnostic performance of the routine culture method and the lack of antibiotic susceptibility testing. This study also highlights the urgent need to optimize culture for the diagnosis of STI pathogens, the need to introduce molecular diagnostic methods into routine diagnosis for the detection of STI pathogens in clinical laboratories, and the need to strengthen the STI surveillance system in Burkina Faso. These measures are very important and urgent to prevent STI outbreaks (especially *N. gonorrhoeae* infection outbreaks) in many foci in Burkina Faso, as the vulnerable exposed population has increased dramatically in recent years. Due to the security crisis, there were approximately 2 million internally displaced persons (IDPs) in March 2024, including many sexually active and vulnerable adolescents. Urgent action, including effective screening methods and sexual and reproductive health interventions, is therefore warranted for this specific population.

Author Contributions

M.K.G: Writing—original draft, Methodology, Conceptualization; A.D: Conceptualization, Writing—original draft, Methodology; L. R. W. Belem: Writing—original draft; K. G: Methodology; K.R: Statistical analyse; A.Q: Methodology; A. M. S: Review & Editing; S. Sa: Conceptualization, Validation, Writing—original draft; D.K: Conceptualization, Writing—original draft.

Conflicts of Interest

The authors declare no competing interests.

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