Journal of Biosciences and Medicines, 2025, 13(1), 272-283
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Importance of Molecular Method for Detection of Microorganisms in the Exploration of the Infectious Etiology of Male Infertility in Burkina Faso

Michel Kiréopori Gomgnimbou[1,2,3*], Louis Robert W. Belem[1,2], Armel Moumouni Sanou[4], Claudel Fonessoubo Gbadjolbe[1], Arnaud Quetin Sanou[1], Kobo Gnada[1], Azouman Da[1], Etienne Bilgo[1,5]

[1]Equipe Biologie Moléculaire et Biotechnologies, Laboratoire de Recherche, Centre MURAZ, Institut National de Santé Publique, Bobo-Dioulasso, Burkina Faso
[2]Centre d'Excellence Africain en Innovations Biotechnologiques pour l'Elimination des Maladies à Transmission Vectorielle (CEA/ITECH-MTV), Université Nazi BONI, Bobo-Dioulasso, Burkina Faso
[3]Institut Supérieur des Sciences de la Santé (IN.S.SA), Université Nazi BONI, Bobo-Dioulasso, Burkina Faso
[4]Laboratoire de Recherche sur les Maladies Infectieuses et Parasitaire (LR-MIP), Institut de Recherche en Science de la Santé, Bobo-Dioulasso, Burkina Faso
[5]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-Dioulasso, Burkina Faso
Email: *gomikir@yahoo.fr

How to cite this paper: Gomgnimbou, M.K., Belem, L.R.W., Sanou, A.M., Gbadjolbe, C.F., Sanou, A.Q., Gnada, K., Da, A. and Bilgo, E. (2025) Importance of Molecular Method for Detection of Microorganisms in the Exploration of the Infectious Etiology of Male Infertility in Burkina Faso. *Journal of Biosciences and Medicines*, **13**, 272-283.
https://doi.org/10.4236/jbm.2025.131023

Received: December 14, 2024
Accepted: January 20, 2025
Published: January 23, 2025

Abstract

In Burkina Faso, as in other African countries, infertility has become a social burden for the population and a public health problem. Male infertility accounts for 30% to 40% of all infertility cases. The diagnosis of male infertility or hypofertility is often made by a simple laboratory analysis of sperm to explore sperm parameters. In most African countries, such as Burkina Faso, microbiological analysis in the context of sperm analysis is still not developed, and is carried out solely based on microscopy and traditional culture, which does not allow the growth of fragile and demanding bacteria. Our study investigated the microorganisms of sperm that may be involved in male infertility, using conventional bacteriology techniques and real-time PCR. However, it did not intend to perform a multivariate statistical association analysis to estimate the association of microorganisms with abnormal semen parameters. This prospective cross-sectional pilot study was carried out on patients who visited the bacteriology laboratory of Centre MURAZ, a research Institute in Burkina Faso, for male infertility diagnosis between 2 August and 31 August 2021. Bacteria were isolated and identified using standard bacteriology techniques. In parallel, common pathogenic microorganisms known to be asso-

ciated with male infertility were targeted and detected in the sperm using a multiplex real-time PCR assay. A total of 38 sperm samples were analyzed by bacteriological culture and bacteria isolated were *Staphylococcus aureus* (*S. aureus*) 5.55%, *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterococcus faecalis* (*E. faecalis*), *Streptococcus agalactiae* (*S. agalactiae*) and *Staphylococcus hoemalyticus* (*S. hoemalyticus*) respectively 2.70%. Real-time PCR targeted and detected *Chlamydia trachomatis* (*C. trachomatis*) at 7.89%, *Ureaplasma urealyticum* (*U. urealyticum*) at 21.05%, *Ureaplasma parvum* (*U. parvum*) at 18.42%, *Mycoplasma hominis* (*M. hominis*) at 15.79%, *Mycoplasma genitalium* (*M. genitalium*) at 10.53% and *Trichomonas vaginalis* (*T. vaginalis*) at 2.63%. *Neisseria gonorrhoeae* (*N. gonorrhoeae*) was targeted by the real-time PCR assay and was not detected (0%) in the tested semen samples. Our study highlights critical limitations of culture performance (low sensitivity), particularly in Burkina Faso, which has a total inability to detect microorganisms (fragile and demanding microorganisms) detected by PCR-based assays. There is therefore an urgent need to at least optimize culture, procedures and algorithms for detection of microorganisms associated with male infertility in clinical laboratories of Burkina Faso. The most effective solution is the routine implementation of molecular diagnostic methods.

Keywords

Male Infertility, Sperm, Microorganisms, Culture, Molecular Diagnostic, Burkina Faso

1. Introduction

Infertility is a social burden for the population, a difficult ordeal for couples to overcome. Its treatment can be stressful, invasive and costly. In some cases, it is the cause of marital discord or divorce. For a long time, women were helplessly blamed for a couple's infertility. The World Health Organization (WHO) defines infertility as the inability of a couple to conceive a child after one year or more of regular, unprotected sexual intercourse at the right time [1].

Infertility is a global public health problem affecting 10% - 20% [2] of couples of childbearing ages worldwide. Male infertility is present in 30% to 50% of cases [3]. It can be attributed to the male if there is an alteration in one or more sperm parameters, *i.e.* sperm concentration and/or motility and/or morphology [4].

Uropathogenic infections can lead to infertility and bacteria are typically involved in infection and inflammation leading to bacteriospermia.

Excessive leukocyte infiltration in the urogenital tract in response to bacterial load results in oxidative stress (OS). By interacting directly with sperm or producing reactive oxygen species (ROS), bacteria can cause infertility by affecting sperm characteristics such as motility, volume, capacitation, and hyperactivation [5] [6]. Sperm analysis is essential in the search for the cause of male infertility, but it

cannot diagnose or fully determine the primary causes of infertility [7]. For example, in around 30% of cases, the etiology of abnormal semen analysis parameters is not found. Thus, investigations involving the semen microbiome may bridge this gap [8]. The microbiome of semen is a field of increasing scientific interest, in recent years scientific evidence-based data from several studies demonstrated the existence of semen microbiome comprising different taxa, genus, and species of microorganisms originating from the urogenital tract [8]; also, it is now known that in addition to the pathogenic microorganisms (some of which are sexually transmitted) that can cause male infertility, the sperm microbiota can influence male fertility [8] [9]. Even the hypothesis that microbiota may be involved in male infertility is short-explored because this microbial niche is currently understudied compared with other areas of microbiome research. With the development and accessibility of Next-Generation-Sequencing technologies, there are more and more studies in this avenue [8].

Most previous studies have focused on the infected organs of the male genital tract, and few studies address the direct effect of microorganisms and their mechanisms of action on sperm.

The pathogens can induce various forms of damage to spermatozoa, such as DNA fragmentation, cell membrane peroxidation, and alteration of the acrosome [10]. This damage may be mediated by microorganisms' toxins and metabolites, or by their direct binding to spermatozoa and subsequent activation of signaling pathways linked to oxidative stress, apoptosis, and inflammation [10].

These induced changes can alter sperm parameters and subsequently lead to infertility. It appears that the effect of microorganisms on sperm function depends on the species and their concentration [10].

In Burkina Faso, studies on this issue are still inadequate, and national statistics are poorly publicized. Furthermore, in Burkina Faso, clinical laboratories, and diagnostic algorithms, did not perform appropriate tests for male infertility etiology investigations. Clinical laboratories just run traditional culture methods for current bacteria detection and identification. Also, infertility is a taboo subject. Couples in this situation are often led to adopt isolation strategies, which only serve to distance them from the real solutions [11]. According to data from Burkina Faso 2010 demographic and health surveys (DHS), infertility affected 1.1% of the general population (DHS, 2010). This survey focused solely on the social, economic, and environmental factors of infertility. The present study is the first of its kind, and its overall aim is to explore specifically targeted pathogenic microorganisms in sperm that may be involved in male infertility, using conventional bacteriology techniques and real-time PCR.

2. Methods

2.1. Study Site and Population

This prospective cross-sectional pilot study was conducted at the Centre MURAZ in the Bacteriology and Molecular Biology laboratories from 02 August to 31

August 2021. The Centre MURAZ is a research Institute founded in 1939 in Bobo Dioulasso, the second largest city in Burkina Faso, and has been part of the National Institute of Public Health (INSP) since 2018. The study population consisted of men with suspected infertility who presented to the Centre Muraz Bacteriology Laboratory for semen analysis, patients who had observed 72 hours of sexual abstinence and sent the sample within 30 min of collection., provided an ejaculate with sufficient volume, and gave their consent.

2.2. Sample Collection and Bacteria Isolation by Culture

To avoid contamination and spillage, collection conditions and precautions were explained to patients. Sperm samples were then collected by the patient in sterile sample cups obtained by masturbation or coitus interruptus after an abstinence period of at least 3 days, under WHO recommendations. Samples were delivered to the laboratory within 30 minutes of collection. Then the sperm were examined to determine volume, viscosity, pH, white blood cell presence, sperm concentration, motility, and normal morphology. Sperm samples were inoculated onto only specific chocolate agar medium supplemented with poly Vitex (Columbia agar is mixed to equal volume with hemoglobin, 500 ml each, and then enriched with 10 ml poyvitex) using a sterile pipet, then incubated at 37˚C in an oven at 5% CO_2 for 18 to 24 hours. A culture was considered positive if it met the following criteria: the microbial flora isolated was monomorphic with a threshold greater than 10^2 CFU/mL in the case of enterobacteria and greater than 10^4 CFU/mL for other bacteria such as enterococci, staphylococci or streptococci. A Gram stain was performed to guide the choice of VITEK cartridges and bacteria were identified using the VITEK 2®_COMPACT® automated system.

2.3. DNA Extraction and Multiplex Real-Time PCR Detection of Targeted Pathogenic Microorganisms from Sperm

Deoxyribonucleic acid (DNA) was extracted from sperm samples using the Qiagen Cador kit (Qiagen, 40724 Hilden, Germany) according to the manufacturer's procedure. Extracted DNA was stored at −20˚C until the analysis for detection of the targeted microorganisms. DNA extracts were analyzed for multiplex detection of *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *U. urealyticum*, *U. parvum*, *M. hominis* and *T. vaginalis* using VIASURE® Real-Time PCR Detection Kits (CerTest BIOTEC, Zaragoza, Spain). Amplification was performed using the CFX96TM Real-Time PCR System (Bio-Rad, California, USA). The amplification program was polymerase activation at 95˚C for 2 min, followed by 45 cycles of denaturation at 95˚C for 10 seconds and hybridization/extension at 60˚C for 50 seconds.

3. Results

3.1. Socio-Demographic Characteristics

The minimum age of our study patients was 19 years, the average was 36.15 years and the maximum age was 55 years. The [30 - 40] age group was the most

represented, followed by the [20 - 30] age group. The age bracket < 20 years was the least represented (Figure 1).

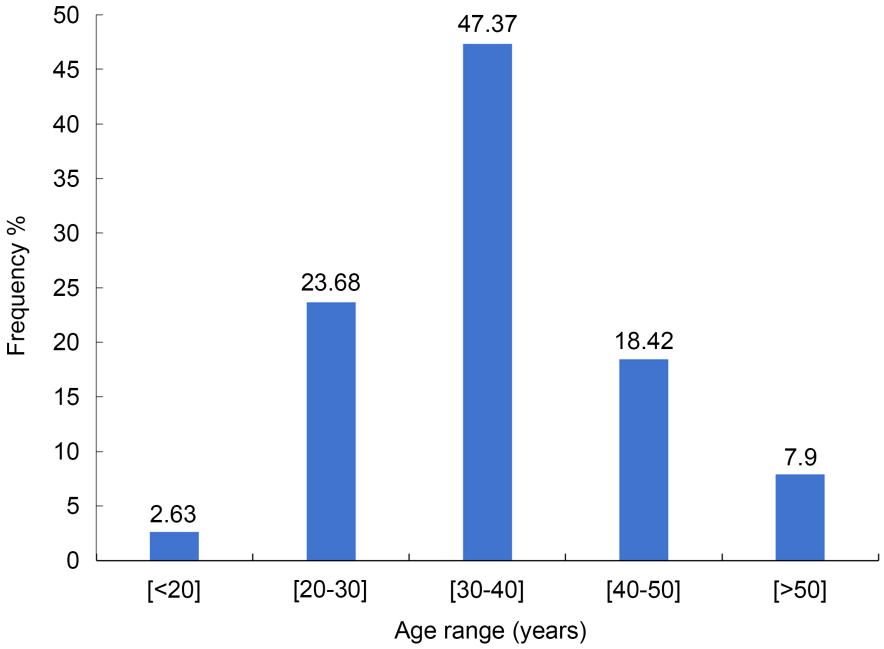

Figure 1. Patients (N = 38) distribution by age group.

By profession, shopkeepers were the most represented 26.31% (10/38), followed by civil servants 23.68% (9/38). Students were the least represented in this study, at 2.63% (1/38). Table 1 shows the distribution of patients by profession.

Table 1. Distribution of patients by profession.

Profession	Number	Frequency (%)
shopkeepers	10	26.31
Civil servant	9	23.68
Farmer	7	18.42
Worker	7	18.42
Unspecified	4	10.52
Student	1	2.63
Total	38	100.00

3.2. Bacteria Isolate by Culture

Of the thirty-eight (38) samples, twenty-one (21) strains were isolated in culture, with 6 bacterial species identified. *S. aureus* was the most frequently isolated, with a frequency of 5.55%, followed by *E. faecalis*, *S. agalactiae*, *S. hoemalyticus*, *K. pneumoniae* with a frequency of 2.70% each (Figure 2).

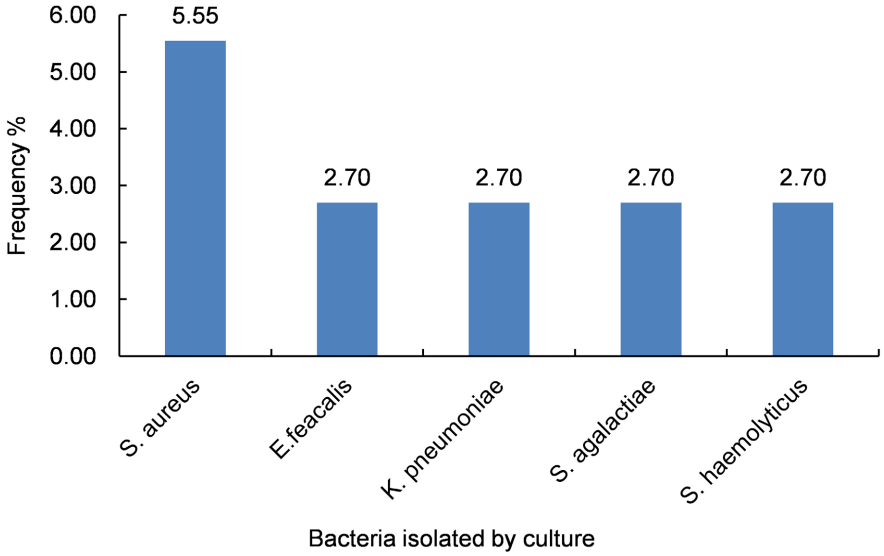

Figure 2. Frequency of distribution of bacteria isolated by culture.

3.3. Targeted Pathogenic Microorganisms Detected by Real-Time PCR

U. urealyticum was mostly detected by PCR with a frequency of 21.05%, followed by *U. parvum* with 18.42%, *M. hominis* at 15.79%, *M. genitalium* at 10.53% and *T. vaginalis* 2.63% (**Figure 3**). *N. gonorrhoeae* was not detected in this study.

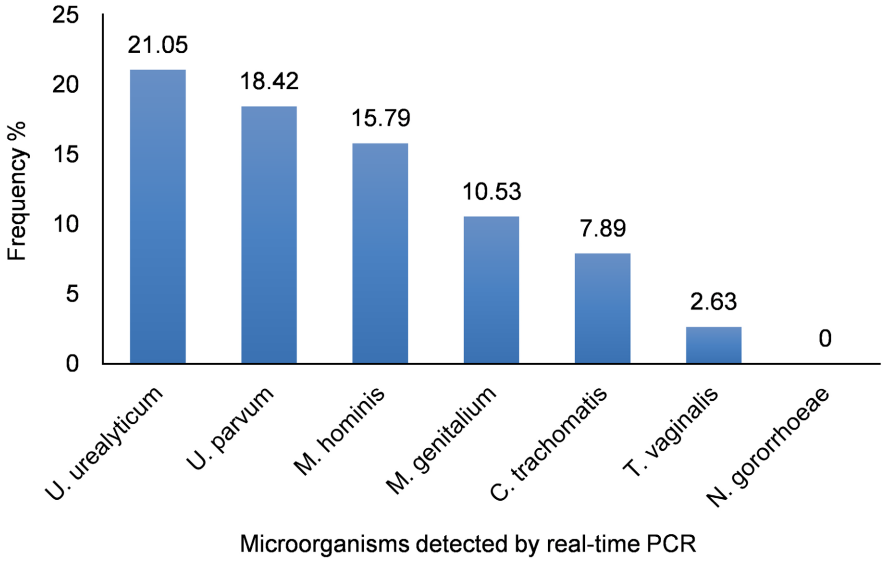

Figure 3. Frequency of pathogenic microorganisms detected by real-time PCR.

3.4. Microorganisms Potential Impact on Sperm Parameters

Table 2 shows the number and frequency of microorganisms detected in semen by the method. *U. urealyticum* was detected in sperm samples from patients (N = 38) with leukospermia in 4 individuals, asthenozoospermia in 2 individuals, and necrozoospermia in 2 individuals, but not oligozoospermia. *C. trachomatis*, *M.*

hominis, *M. genitalium,* and *S. aureus* (details in Table 3) were detected in some patients with leukospermia, asthenozoospermia, necrozoospermia, and oligospermia. On the other hand, bacteria detected by culture, *E. faecalis*, *S. agalactiae,* and *S. haemolyticus* were not associated with sperm parameter defects (Table 3).

Table 2. Frequencies (percentage) of microorganisms detected in patients' semen by method.

Microorganisms	Number (N = 38)	Percentage (%)
	qPCR method	
U. urealyticum	8	21.05
U. parvum	7	18.42
M. hominis	6	15.79
M. genitalium	4	10.53
C. trachomatis	3	7.89
T. vaginalis	1	2.63
N. gonorrhoeae	0	0
	Culture method	
S. aureus	2	5.26
S. agalactiae	1	2.63
S. haemolyticus	1	2.63
K. pneumoniae	1	2.63
E. faecalis	1	2.63

Table 3. Microorganisms detected distribution by patients' semen spermatic parameters.

Microorganisms	Sperm parameters of suspected infertility patients* (N = 38)				
	Leukospermia	Necrozoospermia	Asthenozoospermia	Oligospermia	Normal
			qPCR method		
U. urealyticum	4	2	2	0	0
U. parvum	3	0	0	0	4
M. hominis	2	2	2	1	0
M. genitalium	2	2	2	1	0
C. trachomatis	3	1	1	1	0
T. vaginalis	0	1	1	0	0
N. gonorrhoeae	0	0	0	0	0

Continued

			Culture method		
S. aureus	2	1	1	1	0
S. agalactiae	0	0	0	0	1
S. haemolyticus	0	0	0	0	1
K. pneumoniae	0	1	1	0	0
E. faecalis	0	0	0	0	1

*Some patients have multiple abnormalities in their semen.

4. Discussion

The mean age of our study population was 36.15 years. The younger age bracket of 30 to 40 years was in the majority. These results are similar to Mohammed Frikh *et al.*'s study which found an average age of 35.35 in Morocco in 2021. This result could be explained by the fact that this age bracket corresponds to the period when men were much more interested in reproduction in their married life. Another hypothesis could be that this age bracket corresponds to the period of late awareness on the part of the man to establish his share of responsibility for the couple's infertility. The distribution of patients according to profession showed that shopkeepers and civil servants were the majority in this study. These results show that infertility is not a problem of a single social stratum, but affects all social categories.

S. aureus was the most bacteria isolated by culture in this study with a rate of 5.55%. Our results are similar to those of Sanocka-Maciejewska *et al.* in Poland, who reported a rate of 7% [12]. Other species such as *E. faecalis, S. agalactiae, S. hoemalyticus, and K. pneumoniae* were isolated in low proportion in culture. Certain bacteria isolated by the culture-based method are part of the semen microbiome and their variation in quality or quantity are factors that influence male fertility. Some of the commensal opportunistic pathogenic bacteria can also influence male fertility. It is known that the culture of semen specimens from healthy individual men is positive in around 50% of cases. Recently, the use of high-throughput sequencing for semen microbiome studies has revealed the presence of bacteria such as *Corynebacterium*, *Lactobacillus*, *Pseudomonas*, *Prevotella*, *Streptococcus*, *Gardnerella*, *Enterococcus*, *Veillonella*, *Porphyromonas*, *Sneathia* and *Pelomonas* in semen samples from patients and healthy controls. Osadchiy *et al.* (2024) have found that bacteria in the *Pseudomonas* genus and *Lactobacillus iners* can play a role in male fertility [8].

These studies have also found potentially pathogenic bacteria such as *Finegoldia*, *Anaerococcus,* and others [8].

This study also revealed the lack of high sensitivity of culture-based, appropriate diagnostic algorithms in Burkina Faso for semen analysis in the exploration of male infertility.

Furthermore, this culture method with inappropriate culture media and diagnostic algorithm did not allow the isolation of fragile and demanding microorganisms such as *Mycoplasmes spp, Ureaplasma spp*, and *C. trichomonas*.

In contrast, qPCR showed in our study that *U. urealyticum* is the most common sexually transmitted pathogen in men, with a frequency of 21.05%. It was detected in the samples of some patients in common with certain sperm parameters such as leukospermia, asthenozoospermia, and necrozoospermia as reported by the study of Hannichi *et al.* in Tunisia in 2018 with a higher rate of 43.1% [13] [14]. Others have only reported a decrease in sperm count [15]. We also reported *U. parvum* infection at 18.42%. Our frequency results of these bacteria are corroborated by other studies, Gdoura *et al.* 2008 [11] [16] and Jalal *et al.* 2013 [17].

Regarding the possibility of microbial colonization alterations in the sperm of varicocele patients, research has indicated that the colonization of *U. urealyticum* in varicocele patients is substantially more than in healthy men. Although there isn't any concrete proof that *U. urealyticum* harms sperm, the oxidative stress that the parasite causes may hurt the quality of semen [18].

Other pathogenic microorganisms such as *C. trachomatis* (7.89%), *M. hominis* (15.79%), *M. genitalium* (10.53%), and *T. vaginalis* (2.63%) were reported in this study by molecular detection using qPCR.

Pathogenic microorganisms are responsible for silent infections in patients and are not systematically detected by conventional bacteriology [19]-[23].

Some studies have already demonstrated that this pathogen is responsible for male infertility. Roh *et al.* were able to show that *T. vaginalis* affects sperm fertilization capacity [24]. The germ is also responsible for sperm agglutination [25].

Male infertility may also arise from the loss of seminal ocilium caused by the coinfection of *C. trachomatis*, *U. urealyticum*, and *M. hominis* with varicocele inflammation [26]-[29].

These microorganisms, which cannot be isolated by culture in the absence of specific media, were detected by real-time PCR. As a result, real-time PCR can be used as a complement to culture for non-cultivable or difficult-to-cultivate microorganisms, making PCR the method of choice.

Infertile patients infected with pathogenic microorganisms such as pathogenic bacteria can regain their fertility if successfully treated with appropriate therapeutic management. There are some examples of infected infertile men by *M. genitalium* or *M. Hominis*, successfully treated with the improvement of the semen quality and wives of certain became pregnant 4 months after the treatment completion [19] [20] The aim of our study was not to perform a multivariate statistical association analysis to estimate the association of microorganisms with abnormal semen parameters but to demonstrate that in Burkina Faso there is a misdiagnosis of microorganisms involved in male infertility. There is therefore a need to improve the diagnosis of male infertility and the investigation of its causes, all of which would lead to good therapeutic management.

The results of this pilot study pave the way for a large and relevant study of

microorganisms that could be involved in infertility in Burkina Faso. This study using routine diagnostic samples has some limitations, such as the size of the samples, the lack of suitable culture media for the different micro-organisms that can be routinely isolated, and the lack of reagents to identify other viral pathogens involved in male infertility.

5. Conclusion

Sperm analysis is an essential tool in the search for the cause of male infertility. In this study, several pathogenic microorganisms were identified in sperm by culture or qPCR. Sexually transmitted pathogens identified by qPCR were more prevalent. Given the limitations of conventional bacterial culture, such as time, workload, and low sensitivity, PCR-based diagnostic methods, which offer rapid and sensitive detection of microbiota, should be used as a complement in clinical laboratories in Burkina Faso to strengthen the management of infertile men. In perspective, we will design a new study that will allow us to determine the clinical significance of microorganisms in male infertility and to explore other potential factors contributing to male infertility, such as genetic or lifestyle factors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] World Health Organization (2010) WHO Laboratory Manual for the Examination and Processing of Human Semen.

[2] Agarwal, A., Mulgund, A., Hamada, A. and Chyatte, M.R. (2015) A Unique View on Male Infertility around the Globe. *Reproductive Biology and Endocrinology*, **13**, Article No. 37. https://doi.org/10.1186/s12958-015-0032-1

[3] Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., *et al.* (2012) European Association of Urology Guidelines on Male Infertility: The 2012 Update. *European Urology*, **62**, 324-332. https://doi.org/10.1016/j.eururo.2012.04.048

[4] Sharlip, I.D., Jarow, J.P., Belker, A.M., Lipshultz, L.I., Sigman, M., Thomas, A.J., *et al.* (2002) Best Practice Policies for Male Infertility. *Fertility and Sterility*, **77**, 873-882. https://doi.org/10.1016/s0015-0282(02)03105-9

[5] Chen, J., Chen, J., Fang, Y., Shen, Q., Zhao, K., Liu, C., *et al.* (2023) Microbiology and Immune Mechanisms Associated with Male Infertility. *Frontiers in Immunology*, **14**, Article ID: 1139450. https://doi.org/10.3389/fimmu.2023.1139450

[6] Das, S., Roychoudhury, S., Dey, A., Jha, N.K., Kumar, D., Roychoudhury, S., *et al.* (2022) Bacteriospermia and Male Infertility: Role of Oxidative Stress. In: Kesari, K.K. and Roychoudhury, S., Eds., *Oxidative Stress and Toxicity in Reproductive Biology and Medicine*, Springer International Publishing, 141-163. https://doi.org/10.1007/978-3-030-89340-8_7

[7] Pandruvada, S., Royfman, R., Shah, T.A., Sindhwani, P., Dupree, J.M., Schon, S., *et al.* (2021) Lack of Trusted Diagnostic Tools for Undetermined Male Infertility. *Journal of Assisted Reproduction and Genetics*, **38**, 265-276. https://doi.org/10.1007/s10815-020-02037-5

[8] Osadchiy, V., Belarmino, A., Kianian, R., Sigalos, J.T., Ancira, J.S., Kanie, T., *et al.* (2024) Semen Microbiota Are Dramatically Altered in Men with Abnormal Sperm Parameters. *Scientific Reports*, **14**, Article No. 1068. https://doi.org/10.1038/s41598-024-51686-4

[9] Fraczek, M., Hryhorowicz, M., Gill, K., Zarzycka, M., Gaczarzewicz, D., Jedrzejczak, P., *et al.* (2016) The Effect of Bacteriospermia and Leukocytospermia on Conventional and Nonconventional Semen Parameters in Healthy Young Normozoospermic Males. *Journal of Reproductive Immunology*, **118**, 18-27. https://doi.org/10.1016/j.jri.2016.08.006

[10] Fraczek, M. and Kurpisz, M. (2015) Mechanisms of the Harmful Effects of Bacterial Semen Infection on Ejaculated Human Spermatozoa: Potential Inflammatory Markers in Semen. *Folia Histochemica et Cytobiologica*, **53**, 201-217. https://doi.org/10.5603/fhc.a2015.0019

[11] Sakande, J., Kabre, E., Ekue-Ligan, A., Ouedraogo, H. and Sawadogo, M. (2012) Relation entre les anomalies du spermogramme et les constituants biochimiques du liquide séminal de sujets consultant pour hypofertilité masculine à Ouagadougou. *International Journal of Biological and Chemical Sciences*, **6**, 1167-1178. https://doi.org/10.4314/ijbcs.v6i3.22

[12] Sanocka-Maciejewska, D., Ciupińska, M. and Kurpisz, M. (2005) Bacterial Infection and Semen Quality. *Journal of Reproductive Immunology*, **67**, 51-56. https://doi.org/10.1016/j.jri.2005.06.003

[13] Hannachi, H., Elloumi, H., Hamdoun, M., Kacem, K., Zhioua, A. and Bahri, O. (2018) La bactériospermie: Effets sur les paramètres spermatiques. *Gynécologie Obstétrique Fertilité & Sénologie* , **46**, 518-523. https://doi.org/10.1016/j.gofs.2018.03.014

[14] Lee, J.S., Kim, K.T., Lee, H.S., Yang, K.M., Seo, J.T. and Choe, J.H. (2013) Concordance of Ureaplasma Urealyticum and *Mycoplasma hominis* in Infertile Couples: Impact on Semen Parameters. *Urology*, **81**, 1219-1224. https://doi.org/10.1016/j.urology.2013.02.044

[15] Zhang, Q., Xiao, Y., Zhuang, W., Cheng, B., Zheng, L., Cai, Y., *et al.* (2014) Effects of Biovar I and Biovar II of Ureaplasma Urealyticum on Sperm Parameters, Lipid Peroxidation, and Deoxyribonucleic Acid Damage in Male Infertility. *Urology*, **84**, 87-92. https://doi.org/10.1016/j.urology.2014.04.014

[16] Gdoura, R., Kchaou, W., Ammar-Keskes, L., Chakroun, N., Sellemi, A., Znazen, A., *et al.* (2008) Assessment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, and *Mycoplasma genitalium* in Semen and First Void Urine Specimens of Asymptomatic Male Partners of Infertile Couples. *Journal of Andrology*, **29**, 198-206. https://doi.org/10.2164/jandrol.107.003566

[17] Jalal, H., Delaney, A., Bentley, N., Sonnex, C. and Carne, C.A. (2013) Molecular Epidemiology of Selected Sexually Transmitted Infections. *International Journal of Molecular Epidemiology and Genetics*, **4**, 167-174. https://www.ijmeg.org

[18] Peerayeh, S.N., Yazdi, R.S. and Zeighami, H. (2008) Association of Ureaplasma Urealyticum Infection with Varicocele-Related Infertility. *Journal of Infection in Developing Countries*, **2**, 116-119. https://doi.org/10.3855/t2.2.116

[19] Altmäe, S., Franasiak, J.M. and Mändar, R. (2020) The Seminal Microbiome in Health and Disease. *Nature Reviews. Urology*, **16**, 703-721.

[20] Winters, B.R. and Walsh, T.J. (2014) The Epidemiology of Male Infertility. *Urologic Clinics of North America*, **41**, 195-204. https://doi.org/10.1016/j.ucl.2013.08.006

[21] Yang, H., Zhang, J., Xue, Z., Zhao, C., Lei, L., Wen, Y., *et al.* (2020) Potential

Pathogenic Bacteria in Seminal Microbiota of Patients with Different Types of Dysspermatism. *Scientific Reports*, **10**, Article No. 6876. https://doi.org/10.1038/s41598-020-63787-x

[22] Ahmadi, M.H., Mirsalehian, A., Sadighi Gilani, M.A., Bahador, A. and Talebi, M. (2017) Asymptomatic Infection with *Mycoplasma hominis* Negatively Affects Semen Parameters and Leads to Male Infertility as Confirmed by Improved Semen Parameters after Antibiotic Treatment. *Urology*, **100**, 97-102. https://doi.org/10.1016/j.urology.2016.11.018

[23] Senior, K. (2012) Chlamydia: A Much Underestimated Sti. *The Lancet Infectious Diseases*, **12**, 517-518. https://doi.org/10.1016/s1473-3099(12)70161-5

[24] Ryu, J., Roh, J., Lim, Y., Seo, M. and Choi, Y. (2015) The Secretory Products of *Trichomonas vaginalis* Decrease Fertilizing Capacity of Mice Sperm *in Vitro*. *Asian Journal of Andrology*, **17**, 319-323. https://doi.org/10.4103/1008-682x.145070

[25] Benchimol, M., de Andrade Rosa, I., da Silva Fontes, R. and Burla Dias, Â.J. (2007) Trichomonas Adhere and Phagocytose Sperm Cells: Adhesion Seems to Be a Prominent Stage during Interaction. *Parasitology Research*, **102**, 597-604. https://doi.org/10.1007/s00436-007-0793-3

[26] Gonzalez-Jimenez, M.A. and Villanueva-Diaz, C.A. (2006) Epididymal Stereocilia in Semen of Infertile Men: Evidence of Chronic Epididymitis? *Andrologia*, **38**, 26-30. https://doi.org/10.1111/j.1439-0272.2006.00708.x

[27] Mehta, R., Sridhar, H., Kumar, B.V. and Kumar, T.A. (2002) High Incidence of Oligozoospermia and Teratozoospermia in Human Semen Infected with the Aerobic Bacterium Streptococcus Faecalis. *Reproductive BioMedicine Online*, **5**, 17-21. https://doi.org/10.1016/s1472-6483(10)61591-x

[28] Altmäe, S., Franasiak, J.M. and Mändar, R. (2019) The Seminal Microbiome in Health and Disease. *Nature Reviews Urology*, **16**, 703-721. https://doi.org/10.1038/s41585-019-0250-y

[29] Ahmadi, M.H., Mirsalehian, A., Gilani, M.A.S., Bahador, A. and Talebi, M. (2017) Improvement of Semen Parameters after Antibiotic Therapy in Asymptomatic Infertile Men Infected with *Mycoplasma genitalium*. *Infection*, **46**, 31-38. https://doi.org/10.1007/s15010-017-1075-3