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Comparative Study of Microbiological and Molecular Diagnosis (Bac Multi-Screen-Real-™kit) of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus spp.* Strains from Urinary and Vaginal Secretions in Ouagadougou (Burkina Faso)

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Abstract

Introduction: The last few decades have seen considerable progress in the diagnosis and medical management of infectious diseases. Among these diseases, urogenital infections caused by *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus spp.* remain a major cause of morbidity and mortality in Ouagadougou (Burkina Faso). The aim of this study was to evaluate the performance of the Bac Multi-Screen-Real-™kit compared with the culture technique in the diagnosis of *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus spp.* strains from vaginal and urinary secretions in Ouagadougou. **Materials and Methods:** This was a descriptive study covering a period of 6 months. Molecular analyses were performed on urine and vaginal swabs from patients admitted to Hôpital Saint Camille de Ouagadougou (HOSCO) who had previously been diagnosed using the culture technique in the laboratory.

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Results: Ninety-two (92) patients participated in this study and *Streptococcus* germs were detected in 14 of them (15.22%) by real-time PCR versus 1 of them (1.09%) by culture technique. Among the four *streptococcus* look for, *Streptococcus spp.* (*S. spp.*) and *Streptococcus agalactiae* (*S. agalactiae*) were only found in our study population. Compared to culture technique, PCR technique has shown a specificity, a sensitivity, a Positive predictive value and a Negative predictive value of 100,100,100 and 85% respectively. **Conclusion:** Real-time PCR is an excellent method due to its short duration of performing and the sensitivity in the diagnosis of germs involved in urinary and vaginal infections. Another merit of this kit is the detection of *Streptococcus agalactiae*, not by culture technique.

Subject Areas

Molecular Biology/Microbiology

Keywords

Real-Time PCR, Bac Multi-Screen-Real-^{TM}kit, *Streptococcus*

1. Introduction

Urogenital infections are a real problem in the health sector and occupy an important place among the infectious pathologies affecting the urinary and reproductive tracts [1]. Urinary tract infections are the second most common infectious disease in the world after upper respiratory tract infections, affecting more than 150 million people [2]. The overall prevalence of urinary tract infections in sub-Saharan Africa is 32.12%. The highest prevalence (67.6%) was recorded in South Africa, followed by Nigeria (43.65%) and Zambia (38.25%) [3]. They can affect the lower and/or upper parts of the urinary tract. In Burkina Faso, the prevalence of urinary tract infections is relatively low (0.7%) in children aged 0 - 15 years [4]. In adults, according to a study based on 330 samples taken at the maternal and child health department of Saint Camille Hospital, the prevalence of urinary tract infections was 18.5% [5]. In another study carried out in 2018, the prevalence of urinary tract infections was 27.5% [6]. These studies show the extent of urinary tract infections in Burkina Faso. Most urinary tract and vaginal infections are caused by bacteria such as streptococci. Streptococci are a heterogeneous group of bacteria comprising several species that colonise and/or infect humans and animals [7] [8]. In human medicine, streptococcal infection is a public health problem. In terms of mortality and morbidity, four species of streptococci are of major importance in human disease: Group A *Streptococcus* (*Streptococcus pyogenes*), Group B *Streptococcus* (*Streptococcus agalactiae*), Pneumococcus (*Streptococcus pneumoniae*) and *Streptococcus spp.* [7]. The species implicated in urogenital infections are generally identified by two main techniques: latex agglutination and bacterial culture [9]. In Burkina Faso, diagnosis of these infections is generally based on culture. Recently, molecular tech-

niques based on genome amplification have been developed. Wherever they have been used, genomic amplification techniques such as polymerase chain reaction (PCR) have improved the diagnostic performance of the bacteria responsible for these infections [10]. Thanks to their high sensitivity and specificity, these new techniques can detect bacterial DNA even in very small quantities in biological fluids [11]. Additionally, the PCR essay is faster than the culture method which has a slow turnaround time, about 36 to 72 h before results can be issued. Another feature of the PCR essay like Bac Multi-Screen-Real-™kit is the simplicity of the kit due to the concentration of the different items used into the microtubes ready to use. This allows us to guarantee the reliability of the results that will be obtained. The aim of this study was to detect four species of *Streptococcus* using PCR and standard microbiological cultures in patients who came to the health facility laboratory for cyto bacteriological examination.

2. Materials and Methods

2.1. Type, Period and Context of Study

This was a descriptive study over a 6-month period from February 2023 to July 2023. The study was conducted in Burkina Faso (Ouagadougou) in the biomedical analysis laboratory of the Hospital Saint Camille de Ouagadougou (HOSCO) and in the molecular biology laboratory of the Pietro AnnigoniBiomolecular Research Centre (CERBA). The samples were collected at the Hospital Saint Camille de Ouagadougou (HOSCO) and the molecular analyses were carried out at the CERBA.

2.2. Study Population, Sampling and Samples

The study concerned all patients who came to the HOSCO for a cyto bacteriological examination including culture technique. The patients included are those given their consent to participate in the study. We conducted face-to-face interviews with patients to gain their consent and then ask information thus as name, age and sexe. Then, samples (urine and vaginal swabs) of these patients were looked for among all samples after laboratory analysis. The urine samples were taken in sterile cryotubes and the vaginal samples on two different swabs. A standard culture was performed on these samples at the HOSCO biomedical analysis laboratory on the sampling day. For molecular analysis the samples were stored in the fridge for 48 hours at most until testing which was performed at CERBA.

2.3. Bacteriological Analysis

Streptococci were identified using conventional methods. After sampling, the samples were subjected to macroscopic analysis to determine their appearance. The samples were then inoculated onto ordinary agar (e.g. BCP or Uriselect) and incubated at 37˚C for 24 hours. From colonies obtained on ordinary culture media, young isolated cultures were replicated on fresh blood agar and re-incubated at 37˚C for 24 hours. Blood agar made it possible to read the haemolytic charac-

ter of the fastidious bacteria. Biochemical tests were carried out to correctly identify the associated bacterial species. These included a catalysis test and tests for free coagulase, oxidase and DNase, which enabled the different streptococcal species to be differentiated.

2.4. Molecular Analysis

2.4.1. Extraction of Bacterial DNA

Genomic DNA extraction was performed with DNA-Sorb-B kit from Sacace Biotechnologies following the manufacturer's protocol instructions. That included cell lysis, DNA precipitation, washing and elution of DNA. The DNA extract was quantified to evaluate its concentration and purity with the spectrophotometry method (Nanodrop). The extract which had a good concentration and purity were stored at −20˚C for PCR testing.

2.4.2. PCR Amplification

Detection of the four *Streptococcus* germs (*Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus spp*) was performedby multiplex real-time PCR using Bac Multi-Screen-Real-^{TM}kit and Sacycler-96 thermalcycler (both of Sacace Biotechnologies, Italy). The Bac Multi-Screen-Real-^{TM}kit used, at most the four *Streptococcus* germs, allows the detection of 21 other enterobacteria germs. This kit is ready for use due to all PCR items (nucleotides, primers, probes, buffer and MgCl2) are concentrated in microtubes and we need to add only 10 µL of Taq polymerase and 5 µL DNA extract. In order to make sure of the results of PCR we used for each run we used three controls which are Negative Control of Extraction (NCE), Positive Control of Amplification (C+) and Negative Control of Amplification (NCA). Finally, the PCR microtubes containing 35 µL of the reaction mix were transferred to the SaCycler-96 Real-Time PCR v.7.3 plate thermal cycler (Sacace Biotechnology, Italy). The amplification programme consisted of a first step of a single cycle of 80˚C for 1 min and 94˚C for 1 min 30 s then a second step of 5 cycles consisting of 94˚C for 30 seconds and 64˚C for 15 seconds and finally a third step of 45 cycles consisting of 94˚C for 10 seconds and 64˚C for 15 seconds.

2.4.3. Interpretation of Multiplex Real-Time PCR Results

The results were interpreted using Real Time_PCR v7.9 software on the computer associated with the thermo cycler by the crossing or not crossing of the threshold line by the fluorescence curve. The different germs were then identified following the manufacturer's protocol described in Table 1.

2.5. Data analysis

PCR and culture results were entered and analyzed using Excel 2016 software. We then calculated the sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of PCR, using bacterial culture as the reference for strain diagnosis.

Table 1. Detection of targets in FAM, HEX, ROX channels and Cy5 fluorophores.

PCR Reaction mix	Tube N°	Family	Hexagon	Rox	Cy5	Colour
PCR-1 reaction mix	1	Tunneler*	IC	Marker	-	Blue
	2	*Streptocoque pyogènes*	IC	-	-	uncolored
	3	*Citrobacterfreundii*	IC	-	*Citrobacterkoseri*	
	4	*Burkholderia spp.*	IC	-	-	
	5	*Streptocoque pneumoniae*	IC	-	*Streptocoquespp*	
	6	*Staphylocoque aureus*	IC	-	*Staphylocoque spp.*	
	7	*Klebsiella pneumoniae/ Klebsiellaocytoca*	IC	-	*Klebsiella pneumoniae*	
	8	*Acinetobacter spp.*	IC	-	-	
PCR-1 reaction mix	9	*Enterobacter Cloaques*	IC	-	*Serratia marcescens*	uncolored
	10	*Stenotrophomonas maltophilie*	IC	Marker	*Haemophilus spp.*	Blue
	11	*Haemophilus grippe*	IC	-	-	uncolored
	12	*Morganellamorganii*	IC	-	*Entérobactéries*	
	13	*Enterococcus spp.*	IC	-	*console HMC*	
	14	*Escherichia coli*	IC	-	*Pseudomonas aerugineux*	
	15	*Streptocoque agalacties*	IC	-	*Proteus spp.*	
	16	*Achromobacter ruhlandii*	-	-	*Achromobacter xylosoxidans*	

IC: Internal control.

Sensitivity (Se): Ability of a test to detect as ill those subjects who have the disease in a given population; also measures the ability of a test to eliminate false negatives.

Specificity (Sp): Ability to correctly identify the non-diseased among those who do not have the disease in a given population; thus measures the ability of a test to eliminate false positives.

Positive predictive value (PPV): The positive predictive value is the probability that the disease is present when the test is positive. A diagnostic strategy with a PPV of 100% means that all positive results correspond to patients.

Negative predictive value (NPV): The negative predictive value is the probability that the disease is not present when the test is negative. A diagnostic strat-

egy with an NPV of 100% means that all negative results correspond to people who are not ill.

$$Se = Tp100/Tp + Fn$$

$$Sp = Tn100/Tn + Fp$$

$$VPP = Tp/Tp + Fp$$

$$VPN = Tn/Tn + Fn$$

Tp (true positives) represents the number of sick individuals with a positive test;

Fp (false positives) represents the number of non-sick individuals with a positive test;

Fn (false negatives) represents the number of sick individuals with a negative test;

Tn (true negatives) represents the number of non-diseased individuals with a negative test.

3. Results

3.1. Socio-Demographic Characteristics

A total of 92 patient samples were analyzed, among which there were 37 (40.22%) men and 55 (59.78%) women (sex ratio of 0.67). The mean age in the study population was 36.17 ± 18.97 years, with extremes of 0.5 and 77 years. The majority of our patients were in the [19 - 50] age group, with a proportion of 57.61% (Table 2). There was a female predominance (9/14; 64.28%) than male (5/14; 35.72) in the population study. That gives a sex ratio of 0.70 in favor of women.

3.2. Prevalence of Streptococcal Infection in Our Study

The overall prevalence of streptococcal infection was determined by bacterial culture and real-time PCR in our study. Real-time PCR showed a higher frequency (15.38%, 14/92) than bacterial culture technique (1.09%, 1/92). According to the two types of samples in our study, the prevalence of streptococcal was 13.51% (10/74) from urine and 22.22% (4/18) from vaginal samples (Figure 1) by PCR method. By the culture method we have found one streptococcal case only in the vaginal (Figure 1).

Table 2. Study population distribution by age group and sex..

Age (years)	Sex		Total
	Men	Women	
[0, 5 - 18]	2	5	7
[19 - 50]	22	31	53
[51 - 77]	13	19	32
Total	37	55	92

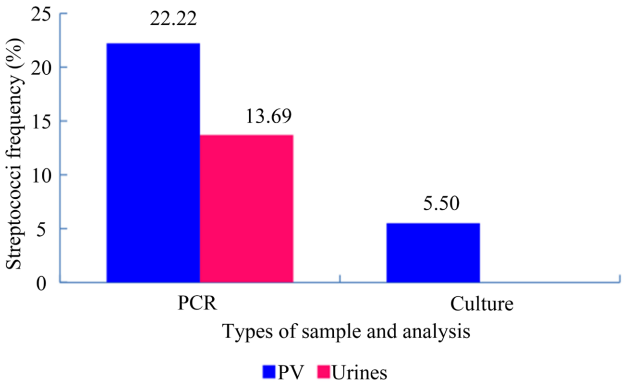

Figure 1. Breakdown by pathological product (%).

3.3. Species Frequencies of Streptococci Identified

The strain most detected in this study was *Streptococcus spp*. By culture method, the one streptococci detected was *Streptococcus spp.* and among the fourteen detected by PCR method, thirteen was also *Streptococcus spp.* strain. The *Streptococcus agalactiae* was the second strain of streptococci identified by the PCR technique only (not detected by culture technique). Most of germs detected were in single infections, onlyone case of co-infection (*Streptococcus agalactiae* and *Streptococcus spp)* was found (Table 3).

3.4. Performance of the Bac Multi-Screen-™Kit and Bacterial Culture with Culture as the Reference Method for Screening for *Streptococcus* Strains

The performance of the Bac-Multi-Screen-Real-™kit was determined by calculating its sensitivity, specificity, positive predictive value and negative predictive value (Table 4).

Table 3. Frequency of germs identified.

	Streptococcus pyogenes	*Streptococcus agalactiae*	*Streptococcus pneumoniae*	*Streptococcus spp.*
Culture Bacterial	0	0	0	1
Multiplex real-time PCR	0	1	0	13

Table 4. Evaluation table for PCR and bacterial culture with culture as the reference method for the detection of *Streptococcus* strains.

Multiplex real-time PCR	Bacterial culture «Gold standard»		Total
	Positive	Negative	
Positive	1	13	14
Negative	0	78	78
Total	1	91	92

Sensitivity (Se): 100%; Specificity (Sp): 100%; Positive predictive value (PPV): 100%; Negative predictive value (NPV): 85%.

4. Discussion

Analyses of vaginal secretions and urine samples from 92 patients at Saint Camille Hospital in Ouagadougou revealed that 14 samples (15.22%) showed streptococcal bacterial infection. The study also revealed that *Streptococcus* infects patients of both sexes, with a strong female predominance of 64.28% (9/14), giving a sex ratio of 0.70. This result is similar to that of Ouedraogo *et al.* in 2022 in Burkina Faso, who found a proportion of 56.15% of women suffering from urinary incontinence compared with 43.85% of men [12]. Benhiba *et al*., in 2015, during their study on the epidemiology and antibiotic resistance of urinary tract infections in Marrakech, Morocco, also observed a sex ratio of 1.12 [13]. It is estimated that 20% of the world's adult female population will develop at least one urinary tract infection, compared with less than 0.1% of men. The strong female predominance of this infection could be explained by the proximity of the terminal digestive tract and the urogenital system, associated with a urethra in women [14]. In addition, the vagina has commensal flora that could be pathogenic for the urinary tract. Other factors, such as pregnancy, the use of contraceptives and the use of tampons during menstruation, could also increase the risk of urinary tract infection.

The age range of our patients was 0.5 to 77 years, with a mean age of 36.17 ± 18.97 years. This could be explained by the fact that streptococcal bacterial infections occur at all ages. More than half (57.14%) of our strains came from patients aged between 19 and 50, which could be explained by the fact that the patients recruited at the HOSCO are mainly adults. Our results differ from those of Mlugu *et al*., who found that the prevalence of urinary tract infections was 41% (141/344) and that elderly people (≥60 years) were five times more likely to have a urinary tract infection than adolescents ($p < 0.001$) in the Tanzanian population [2].

The strains isolated in our study came mainly from vaginal samples (22.224%) and urinary samples (13.51%). These results clearly illustrate the pathogenic role of streptococci in various infectious pathologies. Genital infections in women could be explained by a number of factors, including intensive sexual activity, frequent vaginal irrigation and poor hygiene.

In this study, out of four (4) germs tested, we detected only 1.09% *Streptococcus agalactiae* and 15.22% *Streptococcus spp*. Over 80% of urinary tract infections are caused by *Escherichia coli* and other Gram-negative bacteria, including *Pseudomonas spp*., *Klebsiella spp*. and *Acinetobacter spp*. However, Gram-positive bacteria, including enterococci, staphylococci and streptococci, are also important uropathogens [15]. Urinary tract infections caused by *Streptococcus agalactiae* are also common in pregnant, diabetic and immunocompromised individuals, as well as in those with pre-existing urological abnormalities, and account for approximately 1 to 2% of all monomicrobial urinary tract infections [16] [17].

Analysis of the results showed that 92.86% of S. *spp.* were detected by PCR and that these germs were not detected by culture. Culture is the gold standard

in traditional bacteriology. It is no longer sufficient to guarantee a reliable diagnosis within a timeframe that allows rapid treatment of the patient. In fact, it has a number of limitations, including antibiotic therapy prior to biological diagnosis in the laboratory, difficulty in differentiating a precise strain of *streptococcus* and loss of viability of bacteria during transportation of samples to the laboratory. All things considered, culture has a number of advantages. It allows an antibiogram to be produced, which is known for its role in monitoring bacterial resistance and optimising treatment, and it also allows replicable strains to be preserved for further investigations on the living germ.

The aim of our study was to compare the culture technique with real-time PCR. PCR detects significantly more bacterial germs than culture. A sensitivity and specificity of 100% were determined by the PCR test using bacterial culture as the reference for strain diagnosis. In this study, real-time PCR proved to be a better diagnostic tool, in terms of both sensitivity and specificity, than the culture-based diagnostic technique. It reduces operating time and considerably speeds up the delivery of results, while limiting the risk of contamination; it can detect several germs at the same time with Bac Multi-Screen Real-^{TM}kit.

5. Conclusion

The aim of our study was to compare real time PCR with the standard method which is the culture in the diagnosis of strains of *streptococcus pyogenes*, *streptococcus agalactiae*, *streptococcus pneumoniae* and *streptococcus spp.* In this, we found that these four strains of *streptococcus* can be detected rapidly and reliably by a PCR assay than culture essay using vaginal and urines samples. This study also showed that the rate of incidence of *streptococcus* is underestimated by culture essays. A comparison of the results of the Bac Multi-Screen-Real-^{TM}kit with those of culture shows us that the Bac Multi-Screen-Real-^{TM}kit can be used alongside culture to increase the diagnostic capacity of bacterial infections, given the role of culture in antibiotic susceptibility testing. Despite the relatively higher cost of PCR than that of the culture it will be more useful for routine regarding short duration, high specificity and high sensitivity of PCR test.

Conflicts of Interest

The authors declare no conflicts of interest.

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