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Prevalence of Group B Streptococcus among Pregnant Women in Bobo-Dioulasso (Burkina Faso)

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Abstract

Background: Group B Streptococcus (GBS) or *Streptococcus agalactiae*, which asymptomatically colonizes the female genital tract, is one of the leading causes of septicemia, meningitis and pneumonia in neonates. This study was conducted in Bobo Dioulasso, Burkina Faso to determine the prevalence of GBS colonization among pregnant women. **Methods:** Six hundred and eleven (611) pregnant women were screened for GBS colonization between July and December 2016. Vaginal swab samples were aseptically collected from the subjects after oral informed consent. Standard microbiological methods were used to isolate and identify GBS isolates. The antibiotic susceptibility profile of GBS isolates was assessed using the Kirby-Bauer disk diffusion method. **Results:** Colonization prevalence was 6.05%. No risk factors associated with the carriage rate was statistically identified. All isolates were susceptible to Amoxicillin, Ampicillin, Cefotaxime, Levofloxacin, Vancomycin and Nitrofurantoin. Resistance to antibiotics was found for erythromycin (35.14%), lincomycin (16.22%) and penicillin G (10.81%). **Conclusion:** Although a low carriage (6.05%) rate and isolates were susceptible to many antibiotics found in this study, a policy of systematic screening of pregnant women at least in the third trimester must be promoted.

Keywords

Group B Streptococcus, Prevalence, GBS Carriage, Burkina Faso

1. Background

Streptococcus agalactiae, commonly called group B streptococcus (GBS) is a commensal bacterium of the digestive tract. Then, intermittent colonization of genital tract can occur. These bacteria can become pathogenic for pregnant women in certain circumstances and lead to premature birth, puerperal fever… In fact, an ascending infection can cause maternal, fetal and early-onset neonatal disease (days 0 - 6), leading to maternal death, stillbirth and/or neonatal death or morbidity [1] [2].

The bacterial epidemiology of neonatal meningitis places GBS in the lead. GBS is the main cause of neonatal bacterial meningitis. According to a study at maternity hospitals in Abidjan, Ivory Coast, GBS infections represented 61.5% of neonatal bacterial meningitis with a mortality rate of 44.06% [3]. GBS is also responsible for 15% - 25% of puerperal fevers with or without bacteremia [4].

Despite its pathogenicity, GBS remains one of the microorganisms for which no vaccine is available [5]. Screening is needed to evaluate vaginal colonization by GBS and to study the pattern of antibiotic susceptibility in order to have an appropriate intervention against these infections. According to studies conducted in several countries, the prevalence of this vaginal colonization by GBS in women is varied: United States of America (14%) [6], India/Pakistan (12%) [6], Belgium (12%) [7] and Togo (2.5%) [8].

Unfortunately, we are unaware of a study in which the prevalence of vaginal colonization by GBS was assessed in Burkina Faso and there is no national recommendation on systematic vaginal sampling after antenatal examinations. Yet the risk of maternal-fetal transmission is markedly high during childbirth. Antibiotic prophylaxis is based solely on risk factors. A multi-state study in United States of America indicated that effectiveness of screening-based approach was greater (above 50%) than the one of antibiotic-based approach during the prevention of perinatal GBS infections [9]. Also antibiotic-based approach strategy increases the resistance of bacteria to antibiotics. To evaluate the harm, this first study in Burkina Faso was conducted to determine the prevalence, the risk factors of maternal colonization with GBS and the antibiotics susceptibility of GBS isolates.

2. Methods

2.1. Study Area

A cross-sectional observational study led over 6 months (from July to December 2016) involving pregnant women who attended the antenatal clinics of five ur-

ban health centers of Bobo-Dioulasso below:

1) **Sikasso Cira** health and social promotion centre is a first level of health system. It is located at the center of Bobo-Dioulasso. The average number of annual consultations was 6422 with 451 antenatal consultations.

2) **Farakan** health and social promotion centre is also a first level of health system, located at the East of Bobo-Dioulasso city. The average number of annual consultations was 23,713 with 1310 prenatal consultations.

3) **Colsama** health and social promotion centre is the first level of health system at the south of Bobo-Dioulasso city. The average number of annual consultations was 38,818 with 1278 prenatal consultations.

4) **Accart-ville** health and social promotion centre is a first level of health system: it is located at the west of Bobo-Dioulasso city. The average number of annual consultations was 40,568 with 1996 prenatal consultations.

5) **Sourô Sanou University Teaching Hospital** (SS-UTH) is the major healthcare and the reference center for the southern and western regions of Burkina Faso. It has 521 beds distributed through different departments (medicine, surgery, gynaecology obstetrics and paediatrics). The annual number of hospitalizations ranges from 15,000 to 20,000 patients among 19.825 consultations. The number of means of prenatal consultation is 132.

2.2. Study Population

From July to December 2016, 611 consecutive pregnant women were recruited at the five health centers (SS-UTH: n = 16, Colsama: n = 28, Accart-Ville: n = 167, Farakan: n = 99, Sikasso Cira: n = 48). Pregnant women receiving antibiotic treatment within the last two weeks prior to recruitment were excluded.

2.3. Specimen Collection

Samples were collected in a prenatal consultation room. One-swab sample was taken per participant without setting up a speculum by swabbing the vaginal cavity. Sterile cotton swab should clean vaginal cavity and imperatively wall of the lower half of the vagina, the vulvar opening, and the vulva to load up vaginal secretions. They were immediately seeded on Fresh Blood Agar (FBA) plus Colistin and Nalidixic Acid (ACN) and incubated in a jar containing a carbon dioxide generator pending shipment to the laboratory.

2.4. Specimen Culture Isolation and Identification, Microscopy and Antigen Detection

The Petri dishes were incubated at 37˚C for 24 to 48 hours in an atmosphere enriched with 5% CO_2. Then, Gram staining was performed in order to observe the isolates under microscope. Catalase test, latex agglutination test with anti-B antiserum (PASTOREXTM STREP, BIO-RAD, France) and CAMP test were also realized to identify isolates. Strain *Staphylococcus aureus* ATCC 25928 was used as reference strain.

2.5. Data Analysis

We typed data using Epi Info TM version 7.2.0.1. The results were analyzed using the SPSS software (Version 20) and evaluated statistically by Chi square test. Level of significance was taken to be p-values < 0.05. The figures were represented using Excel 2010 software. A pregnant woman was colonized by GBS when culture, antigen detection and CAMP-test were positives.

2.6. Antibiotic Susceptibility Testing

We used the Kirby-Bauer method for antibiotics Disk susceptibility testing. Agar plate has been inoculated with a suspension of GBS' strain, and specific antibiotic disks have been placed on the agar surface. Then, agar plate was incubated between 35˚C and 37˚C in an atmosphere containing 5% CO_2 during 16 to 18 h. Once the incubation completed, the diameter of inhibition zone was measured. The interpretation of the antibiotic susceptibility test has been performed according to The French Society of Microbiology guidelines [10]. The GBS's trains were tested on different antibiotic disks which included Penicillin G (1 µg), Amoxicillin (30 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Erythromycin (15 µg), Lincomycin (15 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Nitrofurantoin (300 µg), Tetracycline (30 µg) and Sulfamethoxazol-Trimethoprime (23.75 µg + 1.75 µg).

3. Results

During the study, 611 pregnant women were screened, at a minimum, maximum and average age of 14, 42 and 26 years respectively. Most of the women were married (93.62%). With reference to the level of schooling, the percentage was 43.21%, 29.46%, 25.04% and 2.29% corresponding to never went to school, went to school up to primary, secondary, and university level respectively (**Table 1**). Four hundred and twenty-nine of the women (70.2%) were pauci- or multipara. Seventy-two (11.78%) of the women had at least four children and one hundred and ninety-three (31.59%) did not have any child. The lowest parity was one and the highest one was eight with an average of 1.17. Almost the half of women (49.76%) coming for consultation, were in the third trimester of pregnancy while only 19.80% and 30.44% were in the first and second trimester respectively (**Table 2**).

Upon bacteriological analysis, thirty-seven (37) strains of GBS have been isolated from 37 pregnant women, representing a bacterial carriage rate of 6.05% (37/611).

The sociodemographic characteristics of GBS carriers (women) are shown on (**Table 3**). Any risk factor associated with the carriage was identified. Among the subjects that were colonized with GBS, GBS colonization was higher among women with 35 years old and more (10.87%) according to 3.64% in younger (26 - 29) women. The prevalence of GBS colonization was higher among women schooled up to primary level than those who did not school or reached second-

ary or higher level. Regarding the marital status, the prevalence of GBS carriage was not significantly different with 5.13% among unmarried women and 7.12% among women living in couples.

The highest colonization of the SGB was found in the group of female employees and craftswomen (7.06%) and the low colonization among resellers (4.83%). The lowest rate of carriage was observed among those who didn't go to school (4.55%) (Table 3).

None of the sociodemographic characteristics analysed could explain GBS portage and there was no statistically significance between our groups studied.

The prevalence was higher among women in the first trimester of pregnant women (8.26%) followed by those in the third trimester (6.58%) and in the second trimester (3.76%). The difference was not statistically significant ($p = 0.23 > 0.05$) (Table 4).

The parity was from zero to five children with an average of 1.54. The rate of colonization by GBS in women who gave birth at least four times (8.33%) was higher than the one in those who delivered between two and four times (4.37%). However, this difference was not statistically significant ($p = 0.4 > 0.05$) (Table 4).

The gravidity was between one and six with an average of 2.76. There was no significant difference between the colonization rate in women who got pregnant once (7.14%) and those who got pregnant at several times (5.59%) ($p = 0.46 > 0.05$).

Antibiomicrobial susceptibility pattern of the 37 GBS isolates from pregnant women against 12 antimicrobial agents is presented in Figure 1. All strains were susceptible (100%) to Ampicillin, Amoxicillin, cefotaxime Vancomycin, levofloxacin and Nitrofurantoin. Resistance was observed for penicillin (10.81%), erythromycin (35.14%), lincomycine (16.22%) ciprofloxacin (13.51%), tetracycline (83.78%) and trimethoprim-sulfamethoxazole (100%).

Table 1. Distribution of women according to marital status and schooling level.

Factor	Number	Percentage (%)
Marital status		
Single	39	6.38
Married	572	93.62
Totals	611	100
Schooling level		
None	264	43.21
Primary	180	29.46
Secondary	153	25.04
University	14	2.29
Totals	611	100

Table 2. Distribution of patients by gestational, parity and gestational age.

Factor	Number	Percentage (%)
Gravidity		
1	182	29.8
>1	429	70.2
Totals	611	100
Parity		
Nulliparous (0)	193	31.59
Primiparous	163	26.68
Pauciparous	183	29.95
Multiparous (+4)	72	11.78
Totals	611	100
Gestational age (trimester)		
1st	121	19.80
2nd	186	30.44
3rd	304	49.76
Totals	611	100

Table 3. Sociodemographic characteristics of GBS carriers (women).

Variable	Number	Number of GBS isolates	Percentage (%)	p-value
Age group (years)				
<20	107	10	9.34	
20 - 24	215	12	5.58	
25 - 29	110	4	3.64	**0.23**
30 - 34	133	6	4.51	
≥35	46	5	10.87	
Totals	611	37		
Marital status				
Single	39	2	5.13	**0.25**
Married	572	35	6.12	
Totals	611	37		
Main occupation				
Housewife	314	20	6.37	
Retailer	145	7	4.83	
Employees and craftswomen	85	6	7.06	**0.58**
Student and pupils	67	4	5.97	
Totals	611	37		
Schooling level				
None	264	12	4.55	
Primary	180	14	7.78	**0.55**
Secondary	153	10	6.54	
University	14	1	7.14	
Totals	611	37		

Table 4. Obstetric history and GBS carriers (women).

Variables	Numbers	Number of GBS isolates	Percentage (%)	p-value
		Gravidity		
Primigravida	182	13	7.14	**0.46**
Multigravida	429	24	5.59	
Totals	611	37		
		Parity		
Nullipare (0)	193	15	7.77	
Primiparous (1)	163	8	4.9	**0.4**
Paucipara (2 - 3)	183	8	4.37	
Multipare (+4)	72	6	8.33	
Totals	611	37		
		Gestational age (trimester)		
1st	121	10	8.26	
2nd	186	7	3.76	**0.23**
3rd	304	20	6.58	
Totals	611	37		

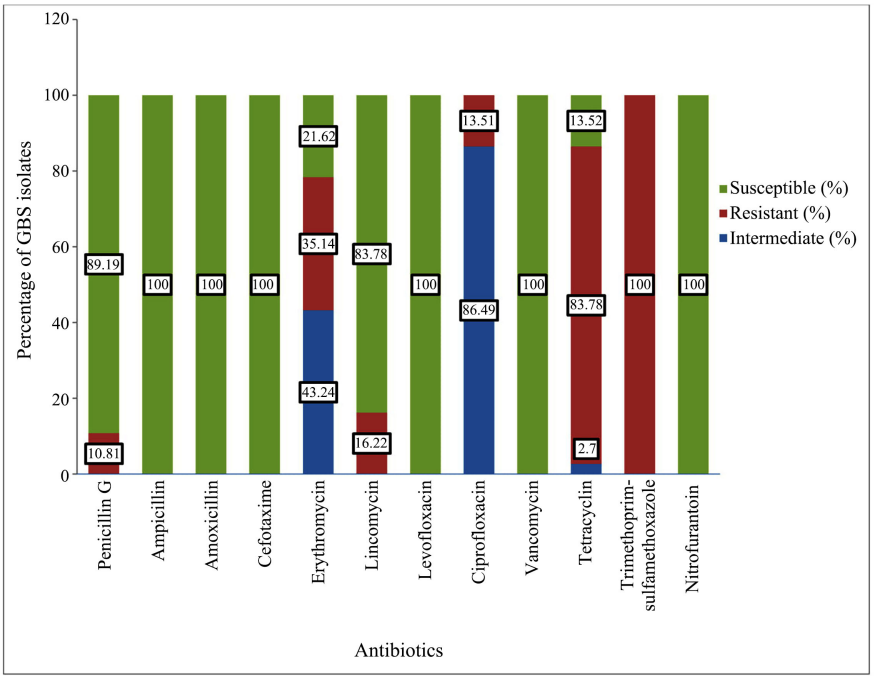

Figure 1. *In vitro* activity of 12 antibiotics on the 37 isolated GBS strains (n = 611).

4. Discussion

We carried out a prospective cross-sectional study. Inclusions were made with pregnant women who presented for prenatal consultation. Some patients were on antibiotic therapy and others refused to enter the study. These aspects did not

allow them to be included, thus limiting the possibilities for inclusion. On the other hand, we did not have the HIV status that would have better broadened the scope of our results. Despite these limitations, the results of our study remain scientifically valid.

In this study, the prevalence of Group B *Streptococcus* (GBS) colonization among pregnant women in Bobo-Dioulasso was found to be 6.05%. However, the prevalence varied according to the health centers: 4.16% at the Sikasso Cira health and social promotion center 10.10% at the Farakan Health and Social Promotion Center. Loulergue *et al.* in France, reported different observations; out of 23 study centers where the same isolation technique and different collection techniques were used, the prevalence ranged from 5.1% to 22.5% [11].

The fact that we used the same sampling technique and isolation method might explain this slight variation of GBS colonization prevalence in our study. It should be mentioned, that the overall GBS carriage rate varies widely, depending on the country, the sampling sites, the period of screening and the study population.

However, in our study, the prevalence of GBS vaginal carriage is comparable to the 6.7% rate found by Foumane *et al.* in Cameroon [12] where the study was conducted under the same conditions. Despite its low rate, GBS carriage was greater than the prevalence (2.5%) found by Salou *et al.* in Togo [8]. This difference with the Togolese study may be due to the cultural practice of the study population. Behaviors such as intimate toilets incorrectly performed in our context may explain the high rate.

Indeed, when the women realize the toilets from bottom of their genital area to the top it will favour the contamination of the genital mucosa by the bacteria from intestinal flora, particularly the vaginal bacteria at high risk of infection (VBHRI). In addition, these toilets cause moisture that promotes bacterial overgrowth. Among these bacteria, we can mention: GBS and other β-hemolytic *streptococci*, *Escherichia coli* and other *Enterobacteriaceae*, *Haemophilus influenzae* and other Gram-negative bacilli. This helps to increase the rate of vaginal contamination by GBS in these women.

However, the prevalence found in our study remains low compared to those reported by Onipede *et al.* in Nigeria (11.3%) [13], Musa *et al.* in Ethiopia (20.9%) [14], and Alham *et al.* in Morocco (20.2%) [15]. These differences could be explained by the fact that only women in the third trimester of pregnancy were included in these studies. Hormonal imbalance during the 3rd trimester, which favours the fixation of bacteria other than lactobacilli, would justify this high prevalence of GBS. In studies carried out in some European countries, higher rate were obtained, such as France 16.7% [16] and Belgium 22% [7].

This may be justified by the fact that in Africa the context of the high prevalence of genital infection where other germs such as *Candida* spp, *Gardnerella vaginalis* and *Mobiluncus* spp have the same ecology as GBS. The latter being a fragile germ, the metabolites of other bacteria are harmful to its growth. As results, the other germ can take over.

In our study, we collected only vaginal samples. In the study carried by Bennouna *et al.* in Morocco, the prevalence of GBS anal carriage was estimated at 11.7% whereas the one for GBS vaginal carriage was 3.3%. The global prevalence of GBS carriage in this study was found to be 23.3% [17]. Regarding the study conducted by Agricola *et al.* in Tanzania, vaginal carriage alone was evaluated at 12.2% compared to 5% for anal with a global GBS carriage of 23% at the two sites [18]. The various prevalences reported in these studies would explain the fact that the rate of GBS carriage also varies according to the sites of sampling. The higher vaginal rate found in Tanzania could be justified by sexual contaminations.

In our study, screening was performed regardless of the period of pregnancy. Thus, the carriage was estimated at 8.6% in the first quarter, 3.76% in the second quarter, and 6.58% in the third quarter. This disparity could be explained by the reasons for consulting women in the first trimester of our series such as repeated abortion, multiple cesarean sections, and pregnancy-related pathology. We could assume that may be this is a more high risk population and there is selection bias in collecting the samples.

In the present study, no statistically significant association was observed for GBS colonization in the study subjects based on the sociodemographic characteristics. Although the literature on GBS screening is plentiful, few studies have really focused on risk factors for maternal carriage. Similar findings have been reported by Bennouna *et al.* as well as by Alham *et al.* both in Morocco [15] [17]. However, the extensive study carried out by Stapleton *et al.* in the United States of America, which involved 40.459 colonized women compared to 84.268 controls [19], the high level of population study, was significantly associated with the carriage of GBS.

Our results could be justified by the level of study of our population, which is predominantly dominated (72.67%) by uneducated people and those at primary school. Women of high level of study realize an excessive genital hygiene and sometimes introduce cosmetic products into their vagina. These practices cause the imbalance of the vulvovaginal flora by scraping *Döderlein bacilli* useful for protection against the invasion of other germs.

Regarding the susceptibility of strains to antibiotics, GBS strains isolated in our study were all (100%) susceptible to amoxicillin, ampicillin, cefotaxime, levofloxacin, vancomycin and nitrofurantoin. Similar findings have been reported in studies conducted by Ferjani *et al.* in Tunisia [19] and Loulergue *et al.* in France [11]. In addition to this good susceptibility to the antibiotics mentioned, resistance to other antibiotic has been found.

We reported in our series that 10.81% of GBS isolates were resistant to penicillin G. Although some studies have reported a lack of resistance to penicillin G [8] [20], the prevalence found in our study is close to 9.4% found by of Agricola *et al.* in Tanzania. In their study, out of sixty-nine (69) strains isolated, six (6) strains were resistant to penicillin G [17]. This resistance of our series could be

justified by the frequent use of this molecule in the past at the level of our sanitary system and/or the phenomenon of self-medication. Indeed, penicillin G was a broad-spectrum and easily accessible antibiotic. This penicillin was used during the empirical treatment of several infections such as purulent meningitis, sexually transmitted infections (syphilis and gonorrhea) and infections caused by *Staphylococcus aureus*.

Macrolides are given in cases of allergy, as an alternative to beta-lactams for peri-partum antibiotic prophylaxis to GBS infection. The study of the susceptibility of these molecules reported that 35.14% of the strains were resistant to erythromycin, and 16.22% to lincomycin.

The resistance of GBS to erythromycin has been studied by several authors who reported resistance rates that ranged from 6.9% to 51.3% [11] [13] [14] [19]. The high rate of erythromycin resistance found in our study is explained by its wide prescription in the first line. Indeed, erythromycin is not used only in cases of allergy to beta-lactams but especially when beta-lactams are finished.

In addition, the side effect related to the digestive intolerance of this molecule generates poor compliance during treatment that could lead to macrolide resistance. This large-scale use promotes awareness of GBS that will develop resistance mechanisms. Failure to follow treatment will create antibiotic-induced stress that increases the rate of genetic exchange including that of genes conferring resistance.

In the study of Ferjani *et al.* in Tunisia resistance to lincomycin, a molecule that can serve as an alternative to erythromycin, has been estimated at 46.2% [19]. This low resistance rate observed during our study is due to the moderate prescription of this molecule related to its availability in our context.

In our study, 13.51% of strains were resistant to ciprofloxacin. Musa *et al.* reported similar result of 13.8% during their work on the prevalence of GBS in Ethiopia [14]. The easy access, the broad spectrum and the exclusively oral route of administration could explain this resistance observed in our study. Thus ciprofloxacin is used for empirical treatment of frequent infections in our health system such as urinary tract infections and typhoid fever.

In our study, 83.78% of strains were resistant to tetracycline. This rate is close to 81.7% of resistant strains found by Loulergue *et al.* during their work on the antibiotic susceptibility of strains of vaginal GBS isolated in France [11].

This high proportion is justified by the existence of the acquired resistance to tetracycline that is common, mainly by acquisition of a ribosomal protection protein encoded by the Tet (M) gene.

5. Conclusions

In this first study of vaginal colonization by GBS in Burkina Faso, we found a carriage rate of 6.05% among pregnant women in Bobo-Dioulasso. This prevalence was low compared to the ones obtained by other authors. Nevertheless, it allows the first time to show that the vaginal carriage of the SGB is a reality in our coun-

try. No risk factors associated with the carriage of GBS were found in our study.

All strains isolated were susceptible to amoxicillin, ampicillin, cefotaxime, levofloxacin, vancomycin and nitrofurantoin. Resistance was detected to penicillin G (10.81%), erythromycin (43.24%) and lincomycin (16.22%). Neonatal infection due by early onset GBS is a therapeutic emergency, and the severe outcome resulting in neonatal meningitis justifies the need to screen the GBS vaginal carriage at the last stage of pregnancy and a preventive intrapartum antibiotics in case of vaginal colonization by the bacteria.

Abbreviations

GBS: Group B Streptococcus; SS-UTH: Souro Sanou University Teaching Hospital; FBA: Fresh Blood Agar; ACN: Colistin and Nalidixic; CAMP: Christie-Atkins-Munch-Pertersen; ATCC: American Type Culture Collection; SPSS: Statistical Package for the Social Sciences; VBHRI: Vaginal Bacteria at High Risk of Infection.

Ethics Approval and Consent to Participate

The patients' oral consents were sought and gained by explaining to them the objectives of the study and its benefits. Then, questionnaire on demographic data and obstetric history was filled for each participant. The study was approved by the regional health direction.

See **Additional File** (2016/0027/MS/RHBS/DRS).

Availability of Data and Materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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Sourô Sanou University Hospital supported this research. In fact Sourô Sanou University Hospital brought the reagents and consumables for the collection of samples, the bacteriological identification and antibiogram of GBS strains.

Authors' Contributions

ASO suggested the initial hypothesis, designed the study, acquired and analysed all data presented. The first drafting of the manuscript was undertaken by ASO, YS, DAS, AV, SS, SO, FM, AP, MB, LS and SG were equally involved in helping to develop the hypothesis and the critical appraisal/re-writing of the manuscript.

All authors have read and approved the final manuscript.

ASO takes overall responsibility for the integrity of the work.

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Conflicts of Interest

The authors declare that they have no competing interests.

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Additional File

- Authorization of the Regional Health Direction
- This document made it possible to carry out the collection in all the sites without any problems and facilitated the participation of the participants.

MINISTERE DE LA SANTE
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REGION DES HAUTS-BASSINS
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DIRECTION REGIONALE DE LA SANTE

BURKINA-FASO
Unité-Progrès-Justice

Bobo-Dioulasso, le 29 JUIN 2016

2016-E-0027 /MS/RHBS/DRS

Le Directeur Régional

Rèf :

A

Monsieur SAWADOGO Yacouba,
Etudiant en MASTER
TBM, Matricule 220 753 W

-=-Bobo-Dioulasso-=-

Objet : Autorisation de collecte de prélèvements

Suite à votre demande du 21 juillet 2016, relative à l'autorisation de collecte de prélèvement pour la réalisation de l'étude dont l'intitulé est ***«Etude de la prévalence et du profil de sensibilité aux antibiotiques de stréptococcus du groupe B chez les femmes enceintes à Bobo Dioulasso»*** dans le cadre de son mémoire de fin d'étude.

Par la présente, j'ai le plaisir de vous informer que je marque mon accord pour la réalisation de la dite étude.

Toutefois, vous prendrez attache avec l'équipe cadre du district sanitaire de Dô, et la Direction régionale de la santé des Hauts-Bassins pour les aspects pratiques. Aux termes de l'étude, une copie du rapport sera déposée dans le District Sanitaire de Dô, et à la DRS/HBS.

Veuillez recevoir, Monsieur, l'expression de mes salutations distinguées.

Ampliations :
- DS-Dô
- Archives/chrono

Ministère de la Santé ★ Direction Régionale de la Santé ★ Hauts-Bassins (DRS)
Le Directeur

Docteur Ziemlé Clément MEDA
Médecin, PhD en santé publique

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