ORIGINAL ARTICLE

The first Prenatal Group B Strep (GBS) Screening in Late Pregnancy in Algerian population (Northeast Algeria)

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ABSTRACT

Background: Group B *Streptococcus* (GBS) can cause severe pneumonia, sepsis and meningitis in neonates and causes one of the most prevalent causes of invasive neonatal infections. Prenatal screening and prenatal antibiotic prophylaxis can prevent maternal transmission of *S.agalactiae* during delivery.
Aim: To determine the maternal risk of maternal carriage of group B streptococcus over time, to offer reliable epidemiological data to the health staff working at maternity Meriem Bouatoura, Batna (Northeast Algeria) or Even all maternity hospitals in Algeria.
Methods: In this prospective study, vaginal specimens (lower third) were collected from 150 pregnant women. The samples were cultured on 5% sheep blood and Chromagar Orientation. The method of confirming the identification of GBS was he agglutination test using the PASTOREX® Strep Kit. The antibiotic susceptibility testing was performed using the Kirby Bauer method.
Results: A total of 150 patients received in 34 weeks of amenorrhea. A total of 15 patients were GBS positive, a percentage rate of 10%. Regular vaginal culture in pregnant third trimester screening was done, with evaluation of risk factors and treatment of risk of infection in infants is considered.
Conclusion: There are a fairly large proportion of positive cases to alarm health personnel about the need to put in place a surveillance plan on the emergence of this dangerous strain.
Keywords: Streptococcus agalactiae, Pregnant Women, Batna, Northeast Algeria.

INTRODUCTION

Streptococcus agalactiae (Group B *streptococcus*, GBS) is a Gram-positive species, commensal with the human gastrointestinal and genitourinary flora; it is responsible for serious diseases in newborns and more rarely in pregnant women[1]. It is considered the main agent involved in maternal- fetal infections, sepsis and meningitis of the newborn term. Because of the importance of colonization in women and the pathogenicity of this bacterium, screening, prevention and treatment strategies have been developed[2].

The only truly effective way to prevent neonatal infections is to screen any woman with GBS at the time of delivery for effective antibiotic treatment. Knowing that no screening has been initiated before at the specialized hospital mother and child "Meriem Bouatoura " Batna and that according to research no similar studies at the national level have been published. The objective is to determine the rate of maternal carriage of GBS in the long term and to offer reliable epidemiological data to the health staff working at the level of maternity Meriem Bouatoura Batna or all the Algerian maternity hospitals as well as to inform them of the real risk of carriage of GBS correlating with the literature.

This makes this work original, given the data collected and its important impact on public health, and this is due to the fact that through this kind of work it is possible to establish a clear screening strategy to prevent any neonatal meningitis or other infections caused by this species.

The genus *steptococcus* is one of the most diverse bacterial genera. It comprises more than 50 species and the classification has been a source of considerable confusion[3]**.** Until recently, the classification was entirely based on the external characteristics of the organisms. Certain characteristics of the initial classification scheme remain useful to the clinician, such as hemolytic profiles and serotype. Molecular methods have refined and clarified the relationships between the various species of streptococci[4] (Kawamura Y et al, 1995)[5].

It should be noted that a variety of cocci Gram-positive catalase negative belonging to other genres is often incorrectly referred to simply as "strep". The biochemical differentiation and clinical characteristics of these genera are discussed elsewhere. The first subdivision of the genus *streptococcus* was based on the ability to lyse the red blood cells of sheep[6].

METHODS

Study framework: We carried out a prospective study in the specialized mother and child hospital "Meriem Bouatoura" Batna (Northeast Algeria) between April 15, 2019 and July 1, 2019. The study was carried out in the bacteriological laboratory of the Meriem Bouatoura Batna maternity. The patients are pregnant women in the last trimester of pregnancy, who have not taken any antibiotics in the last three days. Patient clinical information is collected using a Group B *Steptococcus* fact sheet.
Clinical data: Patient clinical information is collected using a GBS screening fact sheet.
Cytobacteriological urine test: It is a microbiological examination which allows both to diagnose a urinary tract infection by identifying the germ responsible and to help choose the best treatment. It is the most requested exam in medical practice and its interpretation is relatively easy, in

theory. A lack of rigor in the stages of its realization can nevertheless lead to results of fairly average quality and, consequently, unreliableas well aslook for asymptomatic bacteriuria by GBS. Generally this test begins with the microscopic examination and continues with the macroscopic examination. The first urine or urine that has remained in the bladder for 4 hours was collected and reception was before two hours in the laboratory. Morning urine has been processed by two methods:

Microscopic examination: Cytobacteriological examination of the urine is a microbiological examination which allows both to diagnose a urinary tract infection by examining the shape and mobility of the germs and therefore to identify them precisely (bacteriological examination) and also to determine the presence of leukocytes, d erythrocytes, crystals and yeasts (cytological examination). The direct examination is carried out by homogenization using the vortex of the urine sample then depositing a drop using a Pasteur pipette on a very clean Nageotte cells, covered by a cover glass. The counting is done using the x40 magnification Nageotte cell: The different elements that can exist in the urine are red cells, leukocytes, yeasts, sometimes accompanied by the presence of glucose in the urine of epithelial cells, crystals, cylinders and germ.

Macroscopic examination after culture: This examination helps to determine the shape, relief, consistency and appearance of colonies on a Petri dish with the naked eye. In this examination, the culture medium is Chromagar Orientation agar and blood agar, it was used to search for fine greenish blue colonies, and the seeding is done using a 10 µl calibrated loop by tight streaks. Agar, then the seeded dishes is incubated at 37 °C (5% CO2) for 24 to 48 hours. Reading is done by observing the plates with the naked eye.

Vaginal sampling: Perform a vaginal swab (lower third) The placement of the speculum is unnecessary and is not recommended for vaginal sampling. The reception was before half an hour at the laboratory and preferably done at the laboratory level. Two examinations were carried out:

Direct microscopy of unstained wet mounts and Gram stained specimens was performed.

Gram staining: Gram positive bacteria retain the purple coloration of gentian (or crystal violet) and have a blue tint under the microscope. Gram negative bacteria can be discolored, removing the purple coloration of Gentian Violet with an alcohol - acetone solution. The bacteria are then stained red with safranine (or basic fuchsin).

Materials and reagents used

Binocular microscope with objectives 10 and 100 Immersion oil Gram dye box containing: Gentian violet or violet crystal (Reagent 1) Lugol's solution (Reagent 2) Alcohol-acetone bleach solution (Reagent 3) Basic fuchsin. Using a clean blade, identify the blade with a pencil using the identification number (ID), the initial of the participant and the date. Make a thin smear on the slide. And dry the slide in the open air

Culture under atmosphere at 5% CO 2 for 24-48 hours: Fresh blood agar to look for beta-hemolytic colonies and on chromagar orientation agar (BioMérieux) to look for fine greenish blue colonies.

GBS strain identification test: The method of confirming GBS identification is the agglutination using the PASTOREX® Strep Kit
(Bio-Radn France).

RESULTS

Maternal Age: The median age of mothers before childbirth was 27 years (**Table 1).**

Table 1: Maternal age

Patient	Age (years)
P1	27
P2	27
P3	25
P4	28
P5	20
P6	22
P7	23
P8	33
P9	34
P10	29
P11	24
P12	23
P13	31
P14	28
P15	33

Gestational age at the time of vaginal sampling: Gestational ages were expressed in completed weeks of amenorrhea after the date of onset of pregnancy indicated in the medical record. The results obtained show that the average gestational age at which the sample was taken was 34 weeks. The High Health Authority recommends a vaginal sample between 34 and 38 weeks of amenorrhea, all examinations have been carried out in this interval. Compliance with official recommendations is therefore 100% for this criterion.

Sampling: A total of 150 patients underwent a sample in 34 weeks of amenorrhea. A total of 15 patients were positive for GBS, a percentage rate of 10%.

Results of microscopic morning urine exams

Direct examination- Culture: The microscopic observation in the fresh state was carried out using a Nageotte cell at 40 × magnification. In a culture in an atmosphere at 5% CO2 for 24 to 48 hours, on blood agar, then the inspection for the presence of fine greenish blue colonies.

Results of microscopic examinations vaginal sample

Direct review: The microscopic examination was carried out without coloring the sample by direct observation between slide and cover slip (fresh technique), it made it possible to show the presence of different cellular elements, the table below shows the different elements that are present in each patient (Table 2)

Table 2: The different cellular elements those are present in each patient

Patient	leukocytes (>10^4/ml)	red blood cells (>10^4 /ml)	epithelial cell (>10^4 /ml)
P1	-	-	-
P2	+	-	-
P3	-	-	-
P4	+	-	-
P5	-	-	-
P6	-	-	-

P7	-	+	-
P8	-	-	-
P9	-	-	+
P10	--	-	-
P11	+	+	-
P12	-	-	+
P13	-	-	-
P14	-	-	-
P15	+	-	+

• The positivity threshold for inflammatory reactions in adult patients is 10^4 leukocytes per ml
• The normal rate of red blood cells is less than 10^4 red blood cells per ml.
• The evaluation of the epithelial cell level is correlated with a probable contamination when the threshold of 10^4 cells per ml is exceeded. It was found that only one woman (patient n°11) had a positive inflammatory reaction in the urine culture , the small number of patients we cannot judge a relationship between a positive inflammatory reaction and the carriage of GBS in pregnant women **(Figure 1)**

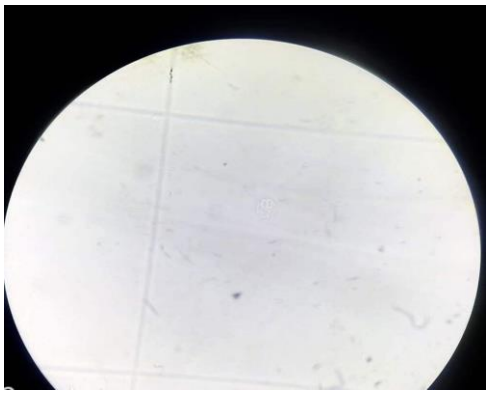

Figure 1. Microscopic observation at 40x magnification of cytobacteriological urine test of a patient with GBS positive.

Appearance of colonies

From different samples, the isolation of the strains on the Chromagar orientation agar medium was carried out for the search for fine greenish blue colonies **(Figure 2**), it made it possible to examine the morphology of the colonies and on fresh blood agar to search for beta colonies, hemolytic, with a generally mucous appearance their diameter is between 1 and 1.5 mm **(Figure 3).** A total of 15 patients were GBS positive, patients had a positive culture for GBS in the vaginal culture. The GBS was positive by Blood agar and on an orientation chromagar medium (BioMérieux).

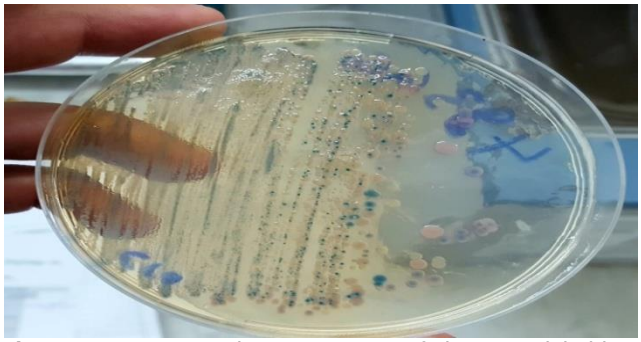

Figure 2. Macroscopic appearance of the greenish blue colonies of the GBS on an orientation chromagar medium (BioMérieux)

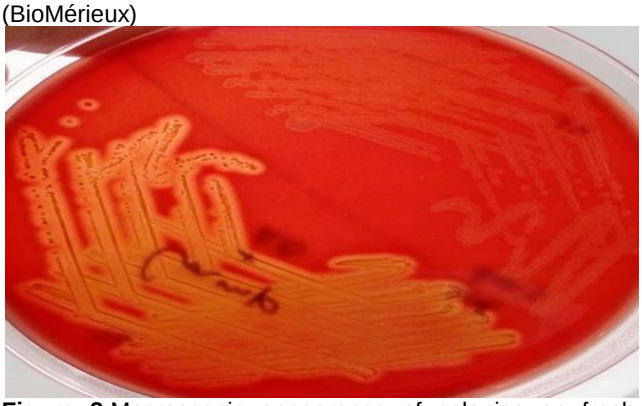

Figure 3. Macroscopic appearance of colonies on fresh blood agar.

Gram stain examination

The observation of the strains studied after Gram staining with "G x 100", revealed that the latter are Gram positive which have a chain-shaped cocci. They can be isolated, in pairs or grouped in clusters (**Figure 4**).

Results of tests to identify the GBS strain was able to confirm the presence of GBS by observing an agglutination **(Figure 5)**

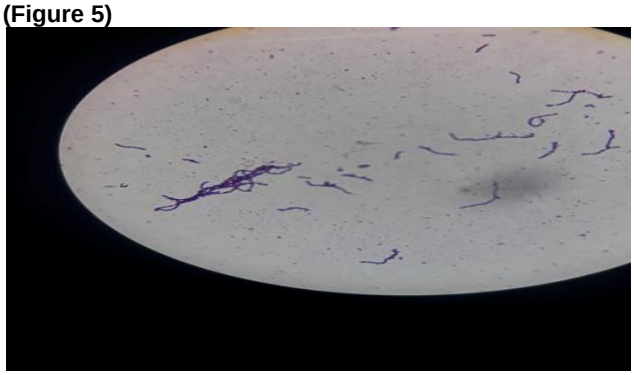

Figure 4. Microscopic observation after Gram staining.

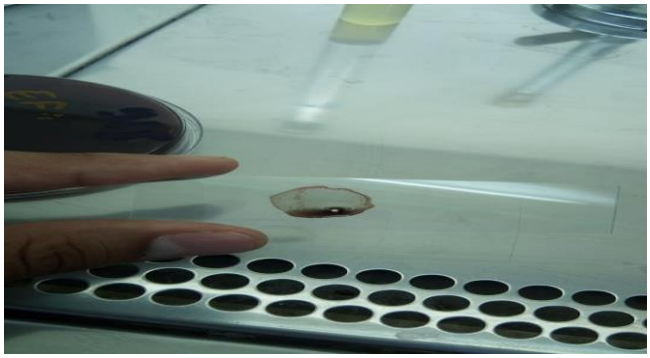

Figure 5. The agglutination results using the PASTOREX® Strep Kit.

Antibiotic susceptibility testing was performed on all GBS isolates using the Kirby-Bauer method **(Table 3)**

Table 3: Resistance (%) of 15 strains of Streptococcus agalactiae (GBS) isolated from screening samples

Drugs	Sensitivity (%)	Resistance (%)
Penicillin	64%	36%
Tetracycline	28%	72%
Levofloxacin	17%	83%
Spiramycin	54%	46%
Chloramphenicol	0%	100%
Erythromycin	29%	71%
Clindamycin	45%	55%
Norfloxacine	76%	24%

DISCUSSION

This result is close to that found in the study by[2] who found a percentage of 20.2% in the region of Marrakech, Morocco and the one in the data of French literature where 10% of pregnant women are positive. In Canada, between 11% and 19.5% of pregnant women carry GBS. In Tunisia, a study of 294 patients demonstrated a carriage rate of 12.98%[2]**.**

Recommendations for GBS testing are numerous. The American Academy of Pediatrics (AAP), the National College of Gynecologists and Obstetricians French ANAES recommend GBS screening in asymptomatic patients without a history of infections and maternal-fœtales associated with antibiotic per-partum when screening Positive[2]. The American College of Obstetrics and Gynecology associated with the PAA and the Center for Disease Control and Prevention (CDC) proposed in 1996 two options, considered equivalent, that of systematic screening and that based on the search for risk factors justifying the start of personal antibiotherapy[7]. In our study, asymptomatic carriage of group B streptococcus in pregnant women during the last trimester of pregnancy was 10%, which correlates with the literature results. In France, the number of pregnant women with GBS has been estimated at 10%, which corresponds to 75,000 pregnant women per year [8] In Canada, maternal colonization ranges from 11% to 19.5%[9]. In the United States, asymptomatic vaginal carriage of GBS has been estimated at 20 to 30% of pregnant women in late pregnancy[10]. The prevalence of genital and anal carriage of group B streptococcus in developing countries is estimated at 9% in India, 8% in Asia Pacific, 18% in sub-Saharan Africa, 17% in North Africa and East, 12% in Central or South America [8]**.** In Tunisia the prevalence was estimated at 12.92% in a population of 294 patients screened[11]. In a prospective study conducted in Morocco at the level of Marrakech, the carrying rate was 20.2%[2]. The rate of carriage also varies according to the sampling sites. In our study, the samples were taken only at the genital level; the midwives of the hospital mother and children " Meriem Bouatoura" Batna were not able to take rectovaginal samples for a shortage of staff and a high number of pregnant women. In a study conducted in Fes, anal carriage alone was estimated at 11.7%, while vaginal carriage alone was estimated at 3.3%, and vaginal and anal carriage at 23.3%[12]**.** Similarly in a Brazilian series and 35 positive samples out of 207 women screened, 15 were only on anal sampling, 43%[13]. Studies conducted in North America involve the combination of a systematic rectal specimen explaining a carrier regularly greater than 18%[14]. On the other hand, in France, this levy is deemed unnecessary in the absence of demonstrated interest in terms of maternal- fetal infections[2]. The risk of infection is proportional to the density of the carrier. It has been shown that the risk of transmission is related to the inoculum of the GBS present. The factors studied during pregnancy at the time of screening (age, parity, gynecological obstetric history, threat of preterm delivery, cesarean delivery) did not show any real interest as factors significantly associated with carriage. From GBS, although the literature on GBS screening is plentiful, few studies have focused specifically on the risk factors for maternal carriage of this germ. The prophylactic antibiotics during labor should be brief, intense, loading dose and intravenously with molecules narrow spectrum[15].

A study from Trinidad Orrett FA et al showed a significant trend of increasing prevalence with increasing age[16]. However Javanmanesh F et al found no significant association with age and education[17].

ANAES[2] and the American Academy of Pediatrics recommend early initiation of antibiotic therapy during labor because its effectiveness is optimal from the second injection. It would take at least 4 hours to obtain an effective ampicillin level in the amniotic fluid and thus effectively reduce transmission. Local preventive antibiotic therapy during pregnancy does not decrease the rate of childbirth.

GBS is resistant to penicillin in 36% of cases, Tetracycline in 72% of cases, Levofloxacin in 83% of cases, Spiramycin in 46%, Chloramphenicol in 100% of cases, Erythromycin in 71% of cases, to Clindamycin in 55% of cases and to Norfloxacin in 24% of cases.

This multi-antibiotic-resistant strain poses serious treatment problems, limits the choice of antibiotic therapy and sometimes leads to a therapeutic impasse.

CONCLUSION

From day to day the GBS in its pathogenic form shows us how dangerous it is especially to newborns. It is necessary and even legitimate to pay more attention to this transmissible bacterium from mother to child, especially in Algeria and according to our research no publication has been found.

According to our modest study, there are a fairly large proportion of positive cases to alarm health personnel about the need to put in place a surveillance plan on the emergence of this dangerous strain. We will try to develop our screening techniques either during sampling or at the laboratory level using molecular biology techniques especially that, according to the biography, it is always better to use molecular biology methods (mainly PCR). To confirm the results obtained**.**

Conflict of interest: The authors have no conflicts of interest to declare for this study.

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