



Prevalence, Sensitivity to Antibiotics and Serotypes of Streptococcus b Carriage in Pregnant Women in Algiers

S Mahrane^{1,2*}, H Riad¹ and Z Ghechi^{1,2}

¹Central Biology Laboratory, CHU Hussein Dey, Algiers, Algeria

²Faculty of Pharmacy, Department of Pharmacy, Microbiology Laboratory, Algeria

*Corresponding Author: S Mahrane, Central Biology Laboratory, CHU Hussein Dey, Algiers, Algeria.

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Abstract

Objective: The objective of this work is to determine the carriage rate of Streptococcus b in pregnant women in the last trimester, to specify the serotypes, to assess the antibiotic sensitivity of the carried strains and to determine the support of the resistance to macrolides and aminoglycosides.

Material and Methods: This was a prospective study from March 1, 2015 to September 30, 2015, which included 389 of patients consulting at the Gynecology and Obstetrics Department, Nafissa Hamoud university hospital Hussein Dey Algiers, Algeria, it has been carried out according to the recommendations of the CDC.

The harvested strains benefited from a study of antibiotic sensitivity, a search of resistance gene to macrolide (ermA, erm B and Mef A) and aminoglycosides aad6 and Aph3 and serotyping were detected by multiplex PCR.

Results: The carriage rate found was 22.6%. All strains were sensitive to betalactamines, 50% were resistant to macrolides and 25% to aminoglycosides. A predominance of the ermb gene and the aph3 gene was found; with predominance of the serotype Ib.

Conclusion: The carriage rate of streptococcus b is highly significative represented mainly by serotype Ib, strains are sensitive to betalactamines but 50% proved resistant to macrolides, prophylactic measures must be instituted to prevent transmission.

Keywords: *Streptococcus agalactiae*; Gynecology; Serotypes

Introduction

Streptococcus b or *Streptococcus agalactiae* is a Gram-positive cocci classified by Rebecca Lancefield in group B of the family Streptococcaceae; at present, about ten serotypes are described [1]. *S. agalactiae* was originally described as responsible for mastitis in cattle [2], its involvement in human pathology was established only in the 1960s [3].

S. agalactiae is a commensal germ of the gastrointestinal and genitourinary tract; in pregnant women, in the world the rate of maternal colonization varies from 1.6% to 36% [4,5]. The vaginal colonization of *S. agalactiae* is intermittent and transient: only

30 to 40% of women colonized in early pregnancy are still at childbirth [6]. The frequency of this asymptomatic colonization seems to vary according to the geographical and ethnic origin of the patients [7]. Maternal colonization by *S. agalactiae* is responsible for colonization and neonatal infection [8]. In terms of sensitivity to antibiotics: betalactamines and aminoglycosides remain the molecules of choice for the treatment, but over the last years it has been noted an emergence of resistance towards these molecules and especially to macrolides which have an interest in antibioprophyllaxis in women with streptococcus b, allergic to betalactamines. The aim of this work is to determine the carriage rate of streptococcus b in pregnant women in the last trimester, to

specify the serotypes, to evaluate the antibiotic sensitivity of the strains of carriage and to determine the support of the macrolide resistance. and aminoglycosides.

Material and Methods

This was a prospective study from March 1, 2015 to September 30, 2015, which included 389 patients consulting at the Gynecology and Obstetrics Department of the Nafissa Hamoud university Hospital Hussein Dey Algiers, Algeria.

The calculation of the required sample size with a risk $\alpha = 5\%$ and a precision level equal to $1/5^{\text{th}}$ of P (theoretical percentage) was based on a prevalence of streptococcal carriage in the general population of 22% in North Africa [9]. The gestational age of the patients was between 34 and 37 weeks, which constituted an inclusion criteria. All women who were on antibiotics or who received antibiotic therapy, within the 15 days prior to the collection regardless of type and duration, were excluded. For each patient we performed a vaginal swab and an anal sample according to the recommendations of the CDC. The two swabs were immersed in a Todd Hewitt Enrichment Broth supplemented with antibiotics (nalidixic acid and colistin) and incubated for 18H at 35°C. Isolation is carried out from each broth on fresh blood agar incubated for 18 to 24 hours under CO₂ at 35°C. The identification of Streptococcus B strains was performed by the Api strepto (biométrieux) gallery; for each isolated strain an antibiogram was performed according to the Clinical and Laboratory Standards Institute criteria (CLSI, 2014); the list of antibiotics tested is as follows: -Penicillin G, ampicillin, erythromycin, clindamycin, pristinamycin, rifampicin, chloramphenicol, levofloxacin, tetracycline, gentamicin, vancomycin, ofloxacin for the kanamycin critical diameters were those of CA-SFM. After extraction of the DNA by the phenol/chloroform technique, we proceeded to search for the macrolide resistance genes *ermA*, *erm B* and *Mef A* by multiplex PCR according to the protocol described by Hraoui [10] and aminoglycoside resistance genes *aad6* and *Aph3* according to the Ioprado [11], this research concerned strains that showed resistance in the antibiogram. Serotyping was performed by multiplex PCR according to the protocol described by Monica Impéri [12]. PCR products were resolved by electrophoresis on 1% agarose gels.

Statistical analysis. Pearson’s x2 test was used to assess inter-group significance. Statistical significance was set at P-value ≤ 0.05 .

Results

The number of patients was 389 parturients. The average age of gestants is 31.7 years: IC [31.18-32.22] with extremes ranging from 25 to 45 years. Parturient recruitment according to gestational age was homogeneous (Figure 1). The rate of carriage by cumulating the two sites (anal and vaginal) is 22.6% (Figure 2), The rate of carriage according to the sampling site is 17% for vaginal site and 15.4% for anal site. We noted that the rate of colonization increased from 34 weeks to 36 weeks of gestation, to be stabilized between 36 and 37 weeks at around 28%; there are no difference between the two sites (P = 0.883).

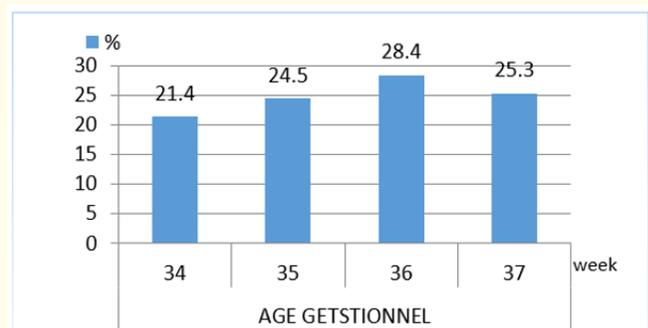


Figure 1: Distribution of parturients by gestational age.

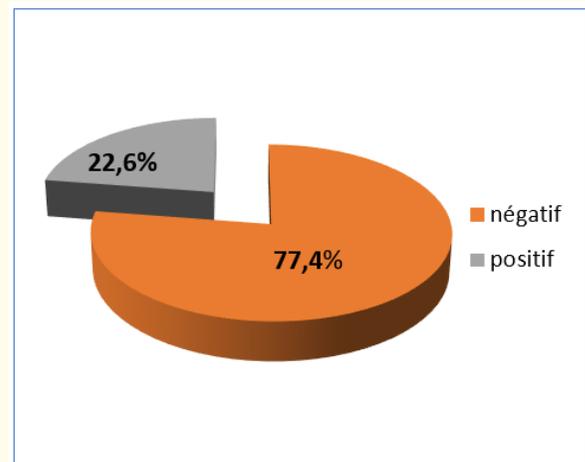


Figure 2: Carriage rate of Streptococcus agalactiae in pregnant women.

The number of isolates strains of streptococcus b was 88, all of them were sensitive to penicilline, ampicilline, fluoroquinolones and vancomycine but they presented a high percentage of resistance to other antibiotics: tetracycline (81.1%), erythromycin (52.8%), clindamycin (47.2%), kanamycin (32.7%) and 9.4% of the isolates had a resistance to gentamicin (Figure 3).

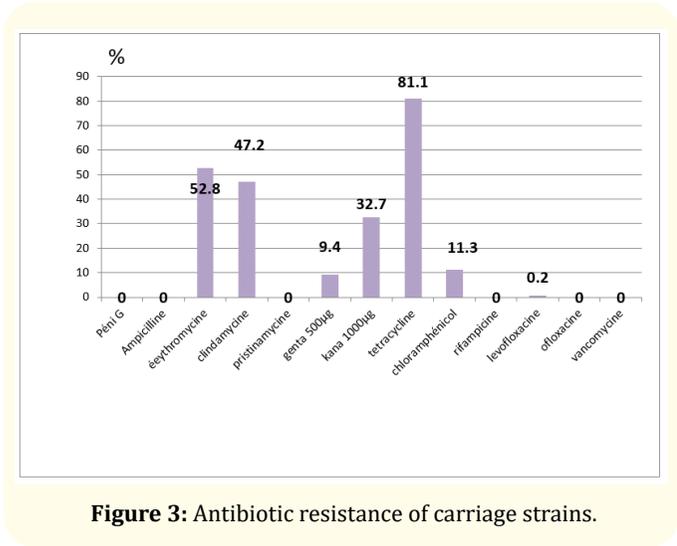


Figure 3: Antibiotic resistance of carriage strains.

The search for mef A, ermA, ermB macrolide resistance genes involved 31 strains. Half of the strains possessed the ermB gene (53.3%), the ermA + ermB gene association was present in 20% of the strains. and in 26.7% the association ermB + mef A was found. There was no correlation between the phenotype observed and the genotype found. the search for the genes Aph3 and aad6 concerned the strains having expressed resistance to one of the two molecules namely: gentamicin or kanamycin; the number of strains tested was 20. We found a predominance of the Aph3 gene alone, which was present in 78.9% of strains tested against only 20% of strains harboring the aad6 + aph3 gene.

The results of serotyping showed a predominance of serotype Ib at 40.4% followed by serotype III and serotype Ia at 23.1% and 21.1%, respectively. Other serotypes were found but poorly represented, such as serotype II, VI, V and VI. The results are shown in figure 4.

Discussion

The rate of colonization that we found is 22.6%, this rate corresponds to that advanced by STOLL and al in 1998 which has

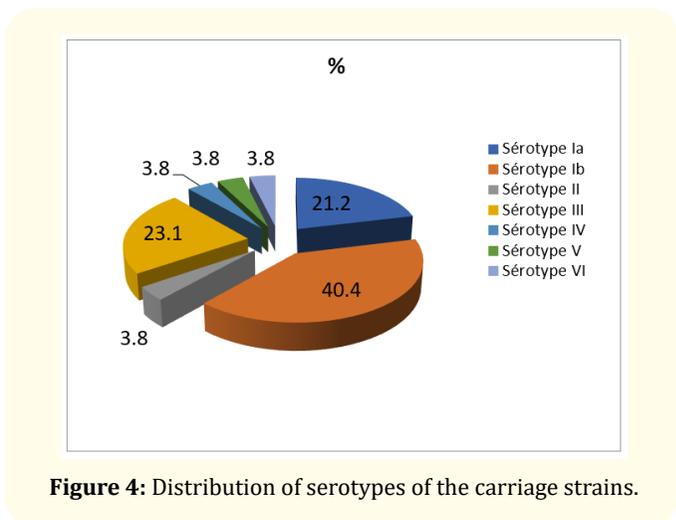


Figure 4: Distribution of serotypes of the carriage strains.

noted a portage rate in North Africa of 22% [9]. The result is similar to the one reported in 2003 by Ait in Constantine and two of its suburbs (east of Algeria) is 26%, this difference can be explained by the fact that the Constantine study is a multicenter study unlike our which is mono-centric [13]. In the Maghreb, similar results are reported by Moroccan teams: 23.3% in the Fez region [14] 20.2% in the Marrakech region [15]. In Tunisia the rate reported by Jerbi is much lower (12.94%) [16]. The worldwide rate of colonization by Streptococcus agalactiae published by Kwatra is 17.9%, he specifies that disparity exists between different regions of the globe: with Africa leading the way with a colonization rate of 22.4% followed by the American continent with a colonization rate of 19.7% and not far behind Europe with a colonization rate of 19% and lastly the South East Asian regions with a low rate of around 11.1% [17]; this disparity in the rate recorded worldwide can be explained by the choice of the sampling site: according to the recommendations of the CDC 2010 it is necessary to carry out screening by targeting the vaginal and anal site while some authors are interested only in the vaginal site, the study published by Almessegad [18] that targeted the vaginal site for the study of carriage would explain the low rate found in Ethiopia which is 13.7% far from the rate proposed for Africa by Kwatra [17].

The prevalence of carriage may be influenced by the technique used: Morita demonstrated that the chromogenic medium gives better results because it detected non-hemolytic colonies, he found a positivity rate of 21% with the GSF against 22.3% for the ChromoID of Biomérieux [19]. The same conclusions are made

by Xie which demonstrates the superiority of the chromogenic medium compared to the fresh-blooded Columbia with antibiotics, he found a carriage rate of 6% on GSF against 10% with the medium Granada [20]. Tazi reaches the same conclusions: she notes the ease and the facility provided by chromogenic media for the detection of streptococcus b colonies, and notes the excellent performance of molecular biology techniques in terms of sensitivity and specificity [21]. Bigdani found rate 27.7% using the culture and it reaches 43.8% with PCR at the vaginal site, the same result is obtained at the anal site with a colonization rate of 30.7% for culture and 41.6% with PCR [22].

The results of the studies on the relationship between carrying and gestational age vary from an author to another. We did not find any relationship between gestational age and colonization, similar results were found by Alemseged., *et al.* [18] however, Khan and collaborator [23] note the increase in colonization rate of gestants in parallel with gestational age; this same finding is made by the team of Joachim and collaborator [24].

All isolated strains of carriage have been found to be sensitive to betalactamines, similar results are reported by different authors who find 100% sensitivity to betalactamines [10,25,26]. However, other authors have reported resistance rates, on the order of 2 to 4% against betalactamines [20,27]. According to KIMURA the support of this resistance is linked to a modification of PLP (149) [28]. We report the isolation of one strain resistant to levofloxacin, resistance to fluoroquinolones remains low or exceptional in the world [18,29].

The aminoglycoside resistance remains low according to the authors [10,11], however 9.4% of our strains have a resistance to gentamicin, the resistance to kanamycin is much higher (32%), this is due to the frequent use of amikacin in hospital practice in our country. Hraoui in Tunisia found among these strains 1.3% resistance to gentamicin and 3.1% to streptomycin [10].

The macrolide resistance found is 50%, similar rates are reported by different authors [29,30] but other found rates much higher as in the study of Suhaimi and Wang who detected a resistance rate in the order of 80% [31,32] while others noted a low rate of resistance as Del Pilar who advances a rate of 10% [25,33]. for tetracyclines, all the authors agree on a very high resistance rate of about 80% [10,31,33]. The resistance to chloramphenicol

is variable: Hraoui found only 3.1% resistant strains, Suhaimi does not describe any strain resistant to this molecule [10,31]. In our series the rate is much higher (27%), this would be due to use this antibiotic as an additive by veterinarians.

The distribution of genetic supports differs from one study to another but the majority found that *ermB* is predominant: Similar results are found in South Africa where the *ermB* gene is predominant [10,26,34-36].

No correlation between the observed phenotype of macrolide resistance and the gene harbored was found in our series: thus, both MLSBc and MLSBi strains had the *ermB* gene as a resistance gene; this finding was made by different authors [37,38].

The study of genetic support for aminoglycoside resistance revealed the predominance of the *Aph3* gene (78.9%) and the *Aad6 + Aph3* association at 21.1%. Resistance to aminoglycosides remains rare, very few authors have examined the question, in Argentina it represents only 1.7% and the genetic support found is *Aad6-Aph2*, [11] Poyart report a rate resistance in the order of 9.7% and the genetic support is essentially *Aph3 + Aad6et* in some *Aph3* strains [21].

The results obtained for the distribution of carriage serotypes demonstrate the predominance of three serotypes: serotype Ia (40.4%), serotype III (23.1%) and serotype Ib (21.2%), the other serotypes described are serotype II, IV, V and VI. Bergal regains a predominance of serotype V (44.6%), followed by serotype II and III (19%) and Ia (8%), so there is variability between regions as our study was conducted on the Algiers and Bergal in the east of Algeria [26], however, in other countries, other serotypes different from those found in Algeria are reported by other authors from different geographical regions [36,39,40].

Conclusion

The carry rate of streptococcus b in pregnant women is 22.6% in Algeria, strains of *Streptococcus agalactiae* isolated in a carriage situation are mainly serotype Ib, all of which are sensitive to betalactamines, however Macrolide used in case of allergy to betalactamines have a high resistance rate.

Acknowledgements

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