Rev. Inst. Med. Trop. Sao Paulo
2016;58:83
http://dx.doi.org/10.1590/S1678-9946201658083

ORIGINAL ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY OF *Streptococcus agalactiae* ISOLATED FROM PREGNANT WOMEN

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SUMMARY

Introduction: Group B streptococcus (GBS) or *Streptococcus agalactiae* can colonize the gastrointestinal and genitourinary tracts and has been considered one of the most important risk factors for the development of neonatal disease. The present study evaluated the antimicrobial susceptibility of GBS isolates from pregnant women who were attended at a public health service in Northern *Paraná*, Brazil. **Methods**: A descriptive analytical cross-sectional study was performed with 544 pregnant women, at ≥ 35 weeks of gestation. One hundred and thirty-six GBS isolates from pregnant women were tested for antimicrobial susceptibility. **Results**: All of the GBS isolates showed susceptibility to the drug that is most frequently used for intrapartum prophylaxis: penicillin. Resistance to clindamycin and erythromycin was detected, thus decreasing the options of prophylaxis in women who are allergic to penicillin. **Conclusions:** Additional studies should be conducted to increase the knowledge of GBS sensitivity profile to antimicrobials in other health centers.

KEYWORDS: *Streptococcus agalactiae*; Antimicrobial susceptibility; Penicillin; Clindamycin; Erythromycin.

INTRODUCTION

Group B streptococcus (GBS) or *Streptococcus agalactiae* can colonize the gastrointestinal and genitourinary tracts and has been considered one of the most important risk for the development of neonatal disease. GBS is often associated with medical intercurrences during pregnancy and the postpartum period and be associated with life-threatening disease in newborns due to sepsis, pneumonia, and meningitis[1].

GBS colonization rates in pregnant women vary according to socioeconomic, cultural, and demographic conditions as well as the methods used for detection. Prenatal GBS screening is recommended by the Centers for Disease Control and Prevention (CDC) by means of specimens harvested from the vaginal introitus and perianal region from all the pregnant women between 35 and 37 weeks of gestation[2].

Studies conducted in the United States found that 10-36% of pregnant women were GBS carriers, with 50-65% of vertical transmission rates. Health surveys in India showed a low rate of colonization (1.6-1.76%), although the rate of vertical transmission is consistent with the ones reported in other countries (53-56%)[3].

Penicillin G administered intravenously is the drug of choice for intrapartum prophylaxis, but ampicillin is an acceptable alternative[2]. In pregnant women allergic to penicillin, cefazolin is recommended when the risk of anaphylaxis is low. Clindamycin and erythromycin are indicated in cases in which there is a high risk of anaphylaxis. Vancomycin should be used in pregnant women allergic to penicillin when there is resistance to clindamycin and erythromycin or when susceptibility to these drugs is unknown[4]. However, the CDC[2] claims that the efficacy of the above alternatives to betalactamics, including cefazolin, clindamycin, erythromycin, and vancomycin has not been measured in controlled trials. In addition, the ability of these last three drugs to reach bactericidal levels in the fetal circulation and in the amniotic fluid is very limited[2].

Some studies have performed the screening of circulating GBS isolates in pregnant women using vaginal and rectal samples and they have reported a reduction of susceptibility to penicillin, > 50% of resistance to macrolide and clindamycin[5], 23.0% of resistance to erythromycin, and 1.3% of resistance to levofloxacin[4].

There is a need to increase the GBS screening in public and private clinics during the recommended gestational period so as to provide adequate prophylaxis in colonized pregnant women, whenever necessary. In Brazil, there are some data on maternal GBS colonization showing

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different rates[6-8], but there is no official recommendation by the Brazilian Health Ministry regarding the GBS screening in all the pregnant women in order to perform prophylaxis. To achieve the adequate prophylaxis, the knowledge of GBS susceptibility to the primarily used drugs in a given population is needed.

Based on the recommendation of the CDC[2], the present study evaluated the antimicrobial susceptibility of *Streptococcus agalactiae* isolates from pregnant women who were attended at a public health service in Northern *Paraná*, Brazil.

METHODS

A descriptive analytical cross-sectional study was performed with 544 pregnant women from September 2011 to March 2014. Vaginal specimens and anorectal specimens were collected from pregnant women, at ≥ 35 weeks of gestation, by separate sterile swabs. Patients came from 21 municipalities of Northern *Paraná*, Brazil. One vaginal and one anorectal swabs were immediately plated on 5% defibrinated sheep blood agar-SBA (Himedia, *Curitiba*, *Paraná*, Brazil) and incubated at 35-37 °C for 18-24 h. Other two swabs obtained from both sites were submerged in 2 mL of HPTH medium[8], with sterile defibrinated sheep blood (Laborclin, *Pinhais*, *Paraná*, Brazil). After incubation for 18-24 h at 35-37 °C, samples were subcultured in SBA and incubated at 35-37 °C for 24-48 h. Two others, one vaginal and one anorectal swabs were cultured in Todd-Hewitt medium (Himedia, *Curitiba, Paraná*, Brazil) supplemented with 8 µg/mL gentamicin (Inlab, *São Paulo*, Brazil) and 15 µg/mL of nalidixic acid (Inlab, *São Paulo*, Brazil) according to the manufacturer's instructions and incubated at 35-37 °C for 18-24 h.

One hundred and thirty-six colonies suspected of being GBS were identified by a latex agglutination test using the Streptococcal Grouping Kit (Oxoid, Hampshire, UK) according to the manufacturer's instructions after the presumptive identification by the following tests: Gram stain, catalase, bacitracin (0.04 U; *Diagnósticos Microbiológicos Especializados*, *Araçatuba, SP*, Brazil), trimethoprim-sulfamethoxazole susceptibility (1.25 µg; Newprov, *Curitiba, PR*, Brazil), hippurate hydrolysis and bile-esculin.

All of the analyses were performed in the Laboratory of Medical Bacteriology, Department of Clinical Analysis and Biomedicine, State University of *Maringá, Paraná*, Brazil. The study was approved by the Ethics and Human Research Committee, State University of *Maringá* (process n° 236/2011).

All of the GBS isolates were tested for antimicrobial susceptibility using the disk-diffusion test (Kirby-Bauer) on Mueller-Hinton agar supplemented with 5% sheep blood using a standardized GBS suspension to 0.5 MacFarland standards, prepared from fresh bacterial cultures according to the Clinical and Laboratory Standards Institute (CLSI)[9]. The following antibiotics were tested: cefotaxime, clindamycin, chloramphenicol, erythromycin, levofloxacin, penicillin, tetracycline, and vancomycin (*Diagnósticos Microbiológicos Especializados, Araçatuba, SP*, Brazil). Additionally, the detection of inducible resistance was performed using the D-zone test, and the results were interpreted according to the CLSI[9].

RESULTS

Of the 544 pregnant women who participated in the study, 136 (25%) were positive for GBS based on the combination of the three culture media in the two clinical specimens.

All of the GBS isolates were susceptible to penicillin, vancomycin, and cefotaxime. We found that 11 (8.1%) of the GBS isolates were resistant to erythromycin; three (2.2%) of these had a constitutive resistance to clindamycin (cMLSB, macrolide, lincosamide, and streptogramin B). The eight (5.9%) erythromycin-resistant GBS isolates, which showed to be susceptible to clindamycin or intermediately resistant were submitted to the D-zone test. These erythromycin-resistant GBS isolates showed to be positive in the D-zone test, indicating that these isolates were clindamycin-resistant (inducible MLSB phenotype). None of the isolates showed the M phenotype (resistance to erythromycin only). Six isolates (4.4%) were intermediately resistant to chloramphenicol. In the present study, a high rate of GBS resistance (82.3%) to tetracycline was detected (Table 1).

Table 1
Antimicrobial susceptibility profile of *Streptococcus agalactiae* isolated from pregnant women in Northern *Paraná,* Brazil

Drugs	Susceptibility (%)	Intermediate (%)	Resistant (%)
Penicillin	100 (136/136)	-	-
Vancomycin	100 (136/136)	-	-
Levofloxacin	99.3 (135/136)	0.7 (1/136)	-
Tetracycline	14 (19/136)	3.7 (5/136)	82.3 (112/136)
Chloramphenicol	95.6 (130/136)	4.4 (6/136)	-
Cefotaxime	100 (136/136)	-	-
Clindamycin	91.9 (125/136)	2.2 (3/136)*	5.9 (8/136)**
Erythromycin	91.9 (125/136)	-	8.1 (11/136)

* Detected as inducible clindamycin resistant after the D -zone test. ** Three isolates with constitutive clindamycin resistance and five with inducible clindamycin resistance after the D-zone test.

DISCUSSION

Penicillin is the first-line agent recommended for prophylaxis and treatment of GBS diseases. Our study confirms the uniform susceptibility to penicillin, vancomycin and cefotaxime similar to some previous reports[10-12]. In our study, to perform the susceptibility testing to penicillin, the CLSI[9] recommendation was carried out. However, according to Kimura *et al.* (2009)[13] the employed methodology has a limitation to detect isolates with eventual reduced susceptibility to penicillin. Kimura *et al.* (2009)[13] referred to a new test that offers a promising, easy, and reliable way to detect isolates with this reduced susceptibility to penicillin. In the future, the new method should be performed and compared in order to improve the susceptibility testing in this situation, and thus promoting a better case analysis for the treatment of GBS-related diseases.

Although the CDC[2] recommends the regular use of penicillin as the drug of choice for the antimicrobial prophylaxis in pregnant women who are colonized with GBS, physicians should be alert to the possibility that pregnant women may be allergic to this drug. According to some authors, 8% to 10% of pregnant women, reported allergy to this drug[14,15].

The observed rates of resistance or even higher rates of resistance to erythromycin, clindamycin and chloramphenicol observed in our study corroborates data reported by Lambiase *et al*. (2012)[10], Konikkara *et al*. (2013)[11] and Emaneini *et al*. (2014)[16] that found rates as high as 45.0%. The rates of GBS resistance to erythromycin and clindamycin indicate that caution should be taken when using these antimicrobials for GBS prophylaxis. Susceptibility tests need to be performed to guide the choice of antimicrobial drugs used for prophylaxis in pregnant women and to determine the resistance profile of GBS to the most used drugs.

Regarding *Streptococcus* spp., resistance to macrolide can emerge by two mechanisms. One of these is the methylation of ribosomes, which prevents the erythromycin from binding with the erythromycin ribosomal methylase, encoded by *erm* genes. The methylated ribosomes confer resistance not only to macrolides but also to lincosamides, as clindamycin. Some *erm* genes have been described and in some strains the *erm*-type resistance is expressed constitutively (cMLSB) therefore inducing the bacterial resistance to clindamycin[17]. The constitutive resistance to clindamycin is a phenomenon that was detected in three isolates, corroborating the data reported by Dutra *et al*. (2014)[18]. However, high levels of cMLSB in GBS have been reported[16].

Regarding tetracycline, which was widely used in the 1970s because of its broad spectrum of action, low toxicity, and low cost, a high rate of GBS resistance (82.3%) was detected in our study. High resistance to this drug was reported in other countries, including Tunisia (97.3%)[19] and Iran (96%)[16]. Currently, its use is restricted because the emergence of resistance appears to be related to the high use of antibiotics[19].

CONCLUSION

In conclusion, the present study confirmed the high antimicrobial susceptibility of GBS to the drug that is most frequently recommended for intrapartum prophylaxis, penicillin. A considerable number of GBS isolates were resistant to clindamycin and erythromycin, thus decreasing the options for prophylaxis in pregnant women who are allergic to penicillin. Additional studies should be conducted to increase the knowledge of GBS susceptibility profile to antimicrobials in other health centers.

ACKNOWLEDGEMENTS

We thank all the nurses of the Units Health and their teams for their support that were essential for the accomplishment of this work and Mariana Digieri Cavalheiro for her help in data collection.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: SCCSM, RFC, SMP. Performed the collection of biological material: SCCSM, FTRS, ABC. Performed the analysis and compilation of data: SCCSM, RFC, MDBC, SMP. Performed the sensitivity tests: MO, NCSS, MBBR, RAFP. Wrote the manuscript: SCCSM, RFC, SMP. All the authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors have stated explicitly that there are no conflicts of interest regarding this article.

FUNDING

This work was partially supported by the *Araucaria* Foundation for the Support of Scientific and Technological Development of the state of *Paraná*, Brazil.

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Received: 11 January 2016
Accepted: 13 June 2016