Streptococcus group B serotype distribution in anovaginal isolates of women in term pregnancy

Małgorzata Romanik [1,2], Krzysztof Nowosielski [2], Ryszard Poręba [2], Urszula Sioma-Markowska [3], Gayane Martiroisian [1], Jan Groborz [1]

1 Department of Medical Microbiology, Medical University of Silesia, Katowice, Poland
2 Department of Gynecology and Obstetrics, Specialist Teaching Hospital in Tychy, Poland
3 Department of Women's Health, Faculty of Health Sciences Medical University of Silesia, Katowice, Poland

Correspondence to: Krzysztof Nowosielski, MD., PhD.
Department of Gynecology and Obstetrics, Specialist Teaching Hospital in Tychy, Edukacji 102, 43-100 Tychy, Poland.
e-mail: krzysnowosilcow@yahoo.com

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Abstract

BACKGROUND: To evaluate Streptococcus group B (GBS) serotype distribution in anovaginal isolates of women in term pregnancy and to assess the correlation of the distribution with socio-epidemiological variables and neonatal outcomes.
DESIGN: An observational study.
SETTINGS: Department of Gynecology and Obstetrics, Specialist Teaching Hospital in Tychy, Poland.
POPULATION: 80 women between 37 and 40 gestation weeks with preserved fetal membranes and who had not been treated with antibiotics for at least two weeks before the study.
MATERIAL AND METHODS: The specimens from the vagina and the rectum of pregnant women were collected. GBS colonization tests were conducted in compliance with Centers for Disease Control and Prevention recommendations. Serotyping of the isolates was performed using the Essum GBS Serotyping Kit (Umea, Sweden) according to manufacturer's instruction. Mein outcome measures. GBS serotype distribution in the population of Polish women in term pregnancy.
RESULTS: In the studied group of 80 pregnant women GBS colonization rate was 28.7%. Four GBS serotypes were observed (Ia, V, III and II). Serotype Ia was the most predominant – 43.47%. For GBS Ia, V and III serotypes, no significant difference in the prevalence of diabetes mellitus and neonatal outcomes was observed. Only in one case early-onset sepsis was diagnosed in the neonate and serotype Ia was determined.
CONCLUSIONS: 1) From among four identified GBS serotypes in the population of Polish pregnant women, serotype Ia was the most dominant. 2) For GBS serotypes, no significant difference in the prevalence of diabetes mellitus and neonatal outcomes was observed. 3) Active immunization aimed for preventing GBS colonization in mothers should include not only serotypes V, II and III but also Ia in order to be an effective and safe in preventing life threatening neonatal infections.

ORIGINAL ARTICLE

INTRODUCTION

Streptococci group B (GBS), colonizing the anogenital region of pregnant women, are frequently involved in the neonatal (51% early-onset sepsis – EOS and 49% late-onset invasive disease – LOD) and maternal (postpartum sepsis, chorioamnionitis, endometritis, urinary tract infections) infections worldwide (Gray *et al.* 2007; Lin *et al.* 2011; Stoll *et al.* 2011; Romanik *et al.* 2011; Kaambwa *et al.* 2010; Häkansson & Källén 2006; Lin *et al.* 1998). The intrapartum maternal chemoprophylaxis of colonized gravidas with β-lactams is currently recommended by Centers for Disease Control and Prevention (CDC) (MMWR 2010). However, common use of antibiotics in prophylaxis in pregnant women may lead to the following complications:

- development of GBS strains resistant to antibiotics administered in intrapartum maternal prophylaxis,
- growth of other bacterial species resistant to antibiotics,
- increase the contribution rate of other bacteria species in neonatal infections,
- development of the new microorganisms that are pathogenic for the newborns and neonates (Moore *et al.* 2003).

The intrapartum antibiotic therapy of GBS recommended by CDC may not prevent the late complications of GBS infections in neonates or may be the cause of allergic reactions in mothers; for that reasons in might not be the proper method of prophylaxis. The use of active immunization for preventing the GBS colonization of gravidas may be, in the nearest future, effective and safe way of life-threatening neonatal infection prophylaxis (Shen *et al.* 2000; Melin 2011). However, the knowledge of GBS serotypes distribution in pregnant women is crucial to prepare such vaccines.

The aim of this study was to evaluate the GBS serotype distribution and to assess the correlation of that distribution with socio-epidemiological variables and neonatal outcomes.

MATERIAL AND METHODS

One hundred and sixty specimens from the vagina and the rectum of 80 pregnant women between 37 and 40 gestation weeks hospitalized in the Maternity Ward of the Department of Gynecology and Obstetrics, Medical University of Silesia in Tychy, Poland, were examined. All study samples (two from each patient) were collected by physicians and transported to the laboratory within one hour. All tested gravidas had preserved fetal membranes and had not been treated with antibiotics for at least two weeks before the test (the complete characterization of studied group was presented in our previous study) (Romanik *et al.* 2011).

GBS colonization tests were conducted in compliance with CDC recommendations, using liquid Todd Hewitt Broth (bioMérieux, France) and then subcultured onto sheep blood agar plates. Additionally, solid selective differentiation Granada medium (bioMérieux, France) was used, onto which specimens collected from vaginal vestibule and rectum were inoculated directly, as previously described (Romanik *et al.* 2011).

Isolated strains were identified as GBS based on the following criteria: their morphological features (colony characteristics including a narrow zone of β-hemolysis and Gram positive staining), serological (Slidex Strepto-Kit bioMérieux, France) and biochemical (rapid ID 32 Strep bioMérieux, France) tests, and the growth and orange pigment formation on Granada agar (bioMérieux, France).

Serotyping of the isolates was performed using the Essum GBS Serotyping Kit (Umea, Sweden) according to manufacturer's instruction. The kit recognizes the nine polysaccharide antigens: Ia, Ib, II, III, IV, V, VI, VII, VIII.

Statistical analysis was performed with the Statistica 8,0 computer software (StatSoft, Krakow, Poland). Fisher's exact test was used for qualitative variables and Kruscal-Wallis test – for quantitative variables. The p-level ≤ 0.05 were accepted as statistically significant.

RESULTS

In the investigated group of 80 pregnant women GBS colonization rate was 28.7%. Four GBS serotypes, specified by the polysaccharide capsule, were observed (Ia, V, III and II). Serotype Ia was the most predominant – 43.47%, followed by V (7 from 23) and III (5 from 23). We compared the occurrence of GBS serotypes isolation from anovaginal region with the selected socio-demographic characteristics and medical history (Table 1). Serotype II was not included in the analysis as only one gravida was colonized with GBS serotype II. For GBS Ia, V and III serotypes, no significant difference in the prevalence of diabetes mellitus and neonatal outcomes was observed. Only in one case early-onset sepsis was diagnosed in the neonate and serotype Ia was determined.

DISCUSSION

The data on the rate of GBS colonization in Polish gravidas vary from 3.3% up to 30% (Romanik *et al.* 2011; Brzychczy-Włoch *et al.* 2010; Brzychczy-Włoch *et al.* 2011). Additionally, the presence of GBS in the vagina is transient and was seen mainly in young women and did not correlate with vaginal symptoms (vaginal erythema, vaginal desquamation, itching) (Romanik *et al.* 2007; Romanik *et al.* 2011; Wiechuła *et al.* 2007; Friedek *et al.* 2005; Kiely *et al.* 2011). In the authors' previous research, 27.8% of gravidas were GBS colonized with the mean age of 28 years old; our results are in accordance with other European studies (Brzychczy-Włoch *et al.* 2010; Brzychczy-Włoch *et al.* 2011; Daniels *et al.* 2011; Kunze *et al.* 2011).

In our study in the 80 pregnant women before delivery the most frequent GBS serotypes was serotype Ia and V (74%), whereas serotypes Ib, IV, and VI–VIII were not identified. Sadowy *et al.* and Brzychczy – Włoch showed in their studies that among seven observed serotypes Ia and V were identified in 37.4% and 37% GBS strains, respectively (Brzychczy-Włoch *et al.* 2010; Brzychczy-Włoch *et al.* 2011; Sadowy *et al.* 2010).

We determined EOD in one case – the infection was found to be associated with isolates of GBS serotype Ia as seen by others (Sadowy *et al.* 2010; Martins *et al.* 2007). Interestingly, serotype II in our study was observed only in one pregnant women, whereas in other researches this serotypes showed a tendency to be associated with non-invasive infections (Sadowy *et al.* 2010; Bisharat *et al.* 2005).

The differences between GBS serotypes distribution varies with geographic area, ethnic origin, virulence and antibiotics resistance of clinical isolates (Kiely *et al.* 2011; Dadvand *et al.* 2011; Ippolito *et al.* 2010; Lachenauer *et al.* 1999; Corvec *et al.* 2011). A number of studies have reported that serotype III was the most prevalent in LOD cases (Sadowy *et al.* 2010; Martins *et al.* 2007; Martins *et al.* 2011). This serotypes is also associated with meningitis; increased invasiveness of this serotype has also been suggested (Grey *et al.* 2007; Berg *et al.* 2000).

Data from Polish and German studies showed that 35% and 28% of pregnant women, respectively, were colonized with GBS serotypes III. This serotype is also very often isolated from neonates and adults in Sweden (from neonates – 62% and from adults – 29%) (Brzychczy-Włoch *et al.* 2010; Brzychczy-Włoch *et al.* 2011; Kunze *et al.* 2011; Sadowy *et al.* 2010; Berg *et al.* 2000). Additionally, serotypes III and V predominated in erythromycin – resistant GBS isolates in Southern Ireland (Kiely *et al.* 2010). Domelier *et al.* showed correlation between erythromycin resistance (*erm*) and GBS serotypes III, IV, V; *erm* serotype III strains were significantly more frequent for those isolated from vaginal carriage (30/136, 22%) and colonized neonates (3/17, 18%) than for those from EOD (0/25) (Domelier *et al.* 2008). However, in Japan, serotypes VI and VIII were the most common serotypes isolated from healthy pregnant women; these serotypes seem to be absent in central Europe (Lachenauer *et al.* 1999). Sadowy *et al.* among the seven serotypes observed in one hundred and fourteen GBS isolates from 43 centers from 28 towns during the period 1996–2005, identified serotype VI as the less prevalent (Sadowy *et al.* 2010). Serotyping shifts have been reported by Kiely *et al.* between two samplings period: 2004 and 2006. The authors observed increased in prevalence of serotype Ia (from 18.6% to 28.5%) and IV (from 7.6% to 15.2%), while serotype V decreased from 20.9% to 11.9% (Kiely *et al.*

Tab.1. Association between GBS serotypes and socioepidemiological data and neonatal outcomes.

Variable	Serological type of GBS			*p*-value*
	Ia (n=10)	III (n=5)	V (n=7)	
Age (years) (mean±SD, range)	27.3±4.81 (18–33)	33.2±6.50 (26–41)	27.0±5.03 (18–32)	0.41
Parity (median, range)	3 (1–2)	3 (1–3)	1 (1–5)	0.25
Miscarriage (median, range)	0 (0–1)	0 (0–1)	0 (0–2)	0.93
Residency (%,n)				
Rural	40 (4)	20 (1)	14.3 (1)	0.46
Small city	10 (1)	40 (2)	14.3 (1)	
Large city	50 (5)	40 (20	71.4 (5)	
GDM (n, %)	10 (1)	20 (1)	0 (0)	0.55
Diabetes mellitus (%,n)	0 (0)	20 (1)	0 (0)	0.36
Hypothyreosis (%,n)	0 (0)	20 (1)	0 (0)	0.66
Fetal weight (g) (mean±SD, range)	3377.0±405.49 (2700–3820)	3392.0±373.33 (2940–3890)	3458.57±504.56 (3030–4460)	0.51
Fetal hypotrophy (%,n)	10 (1)	20 (1)	0 (0)	0.36
Fetal makrosomia (%,n)	0 (0)	0 (0)	14.3 (1)	0.48
Apgar scores (median, range)	10 (6–10)	10 (7–10)	10 (10–10)	0.62
Blood loss during labor (ml) (mean±SD, range)	375.0±116.07 (200–500)	410.0±102.47 (250–500)	268.57±172.9 (150–600)	0.15

* Fisher's exact test for qualitative variables; Kruscal-Wallis test for quantitative variables; GBS – *Streptococcus* group

2011) in contast, according to Ippolito et al., the seroprevalence of GBS have remained relatively stable in the United States (Martins *et al.* 2011).

Currently, monovalent and polyvalent vaccines containing capsular polysaccharides of GBS conjugated with tetanus toxoid have been tested. Those vaccines are well tolerated and might be administered in both non-pregnant women in the reproductive age as well as in gravidas in the III trimester of pregnancy. After the vaccination, initially, postvaccinal immune response with IgM is induced. High level of IgM is transient. However, the serum level of IgG increases after approximately 4–8 weeks after the initial immunization and last over 26 weeks. In case of both mono- and polyvalent vaccines the 4-fold or higher rise in IgG titer was observed in 80–90% of cases. The presence of specific IgG in the mother's blood leads to the acquisition of the passive response in neonates (Kasper *et al.* 1996; Backer *et al.* 2003; Backer *et al.* 2004).

Active immunization aimed for preventing GBS colonization in mothers including not only serotypes V, II and III but also Ia GBS serotype may be an effective and safe way to prevent life threatening neonatal infections in the future, especially that the presence of specific IgG antigens in the mothers' blood creates an opportunity for acquisition of passive immunity by the neonate. Studies show that vaccinating pregnant and non-pregnant women at reproductive age is an effective way of preventing GBS infections in neonates (Melin *et al.* 2011).

CONCLUSIONS

From among four identified GBS serotypes in the population of Polish pregnant women, serotype Ia was the most dominant.

For GBS serotypes, no significant difference in the prevalence of diabetes mellitus and neonatal outcomes was observed.

Active immunization aimed for preventing GBS colonization in mothers should include not only serotypes V, II and III but also Ia in order to be an effective and safe in preventing life threatening neonatal infections.

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