ORIGINAL ARTICLE

Identification of pregnant women at risk of *Streptococcus* group B colonisation

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Abstract

OBJECTIVES: The aims of the study were: 1) to evaluate the prevalence of recto-vaginal group B streptococci (GBS) colonisation using Todd Hewitt Browth – recommended by the Centers for Disease Control (CDC) – and Granada medium; 2) to establish the sensitivity and specificity of Granada medium for the detection of GBS colonisation; 3) to evaluate each vaginal Gram stained swab for bacterial vaginosis (BV) using Nugent criteria and for determining the amount of polymorphonuclear (PMN) leucocytes.

METHODS: Eighty pregnant women between 35 and 40 gestation weeks hospitalised in the Department of Gynecology and Obstetrics, Medical University of Silesia, Poland, were included in the study. Two specimens were collected from each patient: one from the posterior vaginal fornix (Gram stain) and one from both vagina and anus to detect GBS colonisation. Each vaginal Gram stained swab was evaluated for BV using Nugent criteria as well as for PMN leucocyte count. To detect GBS colonisation, the liquid Todd Hewitt Broth, subsequently subcultured to blood agar and direct inoculation onto Granada medium, were used. Isolated GBS were identified by morphological features and by serological (Slidex Strepto-Kit, bioMérieux) and biochemical (rapid ID 32 Strep, bioMérieux) testing.

RESULTS: GBS colonisation was observed in 22 (27.8%) patients in both used media. Only in one case were GBS detected in Todd Hewitt Broth and not detected in Granada medium. The sensitivity and specificity of Granada medium were established as: 95.65% and 100%, respectively, compared with Todd Hewitt Broth recommended by CDC. Nugent criteria demonstrated 6.25% of cases of BV; in one case both BV and GBS colonisation were detected.

CONCLUSIONS: The selective Granada medium may be used concurrently with liquid Todd Hewitt Broth as a screening tool for prenatal group B streptococcal colonisation in pregnant women.

Abbreviations:

GBS	- Group B streptococci
BV	- bacterial vaginosis
CDC	- Centers for Disease Control
BA	- Columbia agar with 5% sheep blood
AAP	- American Academy of Pediatrics
ACOG	- American College of Obstetricians and Gynecologists
PMN	- polymorphonuclear

INTRODUCTION

Group B streptococci (GBS) are a frequent etiological agent of serious infections in neonates, infants, pregnant and parturient women (Heath *et al.* 2004; Zaleznik *et al.* 2000; Centers for Disease Control and Prevention 1996; 2002; Romanik & Martirosian 2004; Peltier 2003). The risk factors for infection are: preterm birth, premature rupture of membranes, body temperature ≥ 38°C during delivery, chorioamnionitis, urinary tract infection and the absence of antibodies and bacterial capsular antigens in the mother and child (Centers for Disease Control and Prevention 1996; 2002; Peltier 2003). The rate of neonatal mortality due to early onset GBS infections is 10.6%, whereas 8% of deaths are caused by late onset central nervous system complications (Romanik & Martirosian 2004).

The reservoir and source of GBS infection in neonates is primarily the mother's gastrointestinal tract, as well as her urogenital system and colonisation by this bacteria is often transient or intermittent (Romanik & Martirosian 2004). Pregnancy creates conditions conducive to rapid multiplication of streptococci in the changed vaginal environment, especially in the case of a coexistent inflammatory condition (Romanik & Martirosian 2004; Romanik *et al.* 2007). GBS colonisation in pregnant women is associated with *Candida* spp. infection and cytolytic vaginosis and in this case change in the vaginal ecosystem may lead to symptoms such as itch, burning, pruritus, dyspareunia (Romanik & Martirosian 2004; Romanik *et al.* 2007). On the contrary, detection of GBS in female genitourinary specimens during a routine gynecological examination is not causally related to vulvovaginal symptoms (vaginal discharge, local discomfort, itch). The change in vaginal microenvironment may lead to bacterial vaginosis (BV). The correlation between GBS colonisation and the change in the vaginal ecosystem associated with BV or the amount of polymorphonuclear (PMN) leucocytes in stained Gram method smears (as a sign of inflammation) has not been determined yet.

The risk of GBS transmission to a neonate during delivery is estimated at 70% (Centers for Disease Control and Prevention 1996, 2002). In 1996 CDC (Centers for Disease Control), AAP (American Academy of Pediatrics) and ACOG (American College of Obstetricians and Gynecologists) developed guidelines concerning the prophylaxis of perinatal GBS infections during pregnancy and delivery (Centers for Disease Control and Prevention 1996; 2002; Romanik & Martirosian 2004). Following these guidelines, GBS screening tests are performed in many countries on all pregnant women. However, there are some differences especially in growth media used (Todd Hewitt Broth versus blood sheep agar), the way and location in which swabs are taken (vaginal, cervical, rectal), the time when the swabs should be taken (first, second or third trimester of pregnancy) and others (Blanckaert *et al.* 2003; Kowalska *et al.* 2003; Wilk *et al.* 2003; El Aila & Tency 2010). These factors can influence the determined rate of GBS colonisation and also appropriate antibiotic treatment during delivery (Centers for Disease Control and Prevention 1996; 2002; Romanik & Martirosian 2004). Granada medium is a commercially available selective agar with granadaene which allows GBS isolation and rapid visual identification because of orange-red carotenoid pigmented colonies (De La Rosa-Fraile 2003).

The aims of the study were: 1) to evaluate the incidence of recto-vaginal GBS colonisation using the CDC-recommended Todd Hewitt Broth and Granada medium; 2) to establish the sensitivity and specificity of Granada medium for the detection of GBS colonisation; 3) to evaluate each vaginal Gram stained swab for bacterial vaginosis using Nugent criteria and for determining the amount of polymorphonuclear leucocytes.

MATERIAL AND METHODS

A group of 80 pregnant women between 35 and 40 gestation weeks hospitalised in the Department of Gynecology and Obstetrics, Medical University of Silesia, Tychy, Poland, was included in the study. The studied pregnant women had unruptured fetal membranes and had not been treated with antibiotics for at least two weeks before the test. Two specimens were collected from each patient: from the posterior vaginal fornix (in order to perform a direct Gram stain test) and, subsequently, from the vagina and the rectum, in order to perform a GBS isolation.

Vaginal microflora was assessed using the Nugent criteria. PMN leucocytes count in direct vaginal swabs was also performed. BV was diagnosed based on a result of 7 to 10 points according to the Nugent system (Nugent 1991).

GBS colonisation tests were conducted in compliance with CDC recommendations, using Todd Hewitt Broth (bioMérieux, France) and then subcultured onto Columbia agar with 5% sheep blood. Additionally, solid selective differentiation Granada medium (bioMérieux, France) was used, onto which specimens collected from the vaginal vestibule and the rectum were inoculated directly.

Isolated GBS were identified based on their morphological features as well as serological (Slidex Strepto-Kit bioMérieux, France) and biochemical (rapid ID 32 Strep bioMérieux, France) tests.

The dependencies between the investigated features were assessed by means of Shapiro–Wilk and χ^2 tests, using the Statistica 8.0 computer software. Hypotheses with 95% probability, which correspond to a coefficient $p<0.05$, were accepted as statistically significant.

The clinical study had been approved by the Medical University of Silesia Bioethical Committee (KNW-6501-48/II/06/08).

RESULTS

GBS colonisation was found in 23 (28.7%) of the studied women. In 22 cases growth of GBS was observed in both microbiological media (Todd Hewitt Broth and Granada agar). In one case growth of group G *Streptococcus* was observed in Todd Hewitt Broth. Granada agar sensitivity and specificity were established at 95,65% and 100% relative to Todd Hewitt Broth recommended by CDC (Table 1).

The medical history of the pregnant women included in this study showed no risk factors of GBS infection in neonates, such as preterm birth, the time of fetal membrane rupture ≥ 18 hours or body temperature during delivery ≥ 38 °C. In one case early sepsis was diagnosed in the neonate.

Based on the results of the GBS colonisation test, the pregnant women were divided into two groups:

- the investigated group – 23 women colonised by GBS
- the control group – 57 women not colonised by GBS

The age of the women in both groups was comparable (Shapiro–Wilk test p=0.2157), however, GBS colonisation was found mainly in younger women, in the age range 25–35 years. The mean age of GBS colonised and uncolonised patients was similar (Table 2).

The analysis of other demographic variables (place of residence, parity and obstetric history) did not reveal statically significant differences between both groups (Table 3).

The assessment of vaginal microflora according to the Nugent criteria showed pathologies (4 to 10 points) in 6.25% of the women studied and also no association with diabetes and vaginal PMN leucocytes was observed.

DISCUSSION

The presence of GBS in the gastrointestinal tract and in the vagina is observed mainly in young women up to the age of 30 and is usually asymptomatic, which was confirmed in our research (Centers for Disease Control and Prevention 1996; 2002).

According to references, GBS are found in 15–30% of women between 35 and 37 gestation weeks, if the

Tab. 1. Sensitivity and specificity analysis of the microbiological media used in the study (Todd Hewitt Broth and Granada agar).

GBS growth	true positive	true negative	false positive	false negative	Sensitivity (%)
Granada medium	22	57	0	1	95.60%
Todd Hewitt broth	23	56	1	0	100%

Tab. 2. Age analysis of the studied women.

Group	N	Mean (years)	Min	Max	SD	*p*-value
Investigated	23	28.65	18	41	5.54	0.68
Controls	57	28.54	16	40	5.27	

Tab.3. Demographic and clinical analysis of the investigated women.

Variables		PREGNANT WOMEN with GBS colonisation N (%)	PREGNANT WOMEN without GBS colonisation N (%)
Place of residence	City (population > 150 000)	12 (27.27)	32 (72.73)
	Town (population < 150 000)	4 (16.0)	21 (84.0)
	Village	7 (63.6)	4 (36.4)
Parity	Nulliparas	11 (26.2)	31 (73.8)
	Paras	12 (31.6)	26 (68.4)
Obstetric history	Miscarriages	3 (37.5)	5(62.5)
	Without complications	20 (27.8)	52 (72.2)
Diabetes in mother	Type I	1(100)	0 (0)
	Gestational diabetes	2 (40)	3 (60)
Leucocytes in vaginal specimens	None	17 (30.9)	38 (69.1)
	Present	5 (22.7)	17 (77.3)
Delivery	Vaginal birth	12 (23.5)	39 (76.5)
	Caesarean section	11(37.9)	18 (62.1)
	TOTAL	**23 (100%)**	**57 (100%)**

* statistically significant difference ($p<0.05$)

microbiological test has been performed following CDC recommendations (Centers for Disease Control and Prevention 1996; 2002).

The type of microbiological medium used, the methodology of the test and the specimen collection site have a considerable influence on the determined incidence of GBS colonisation (Blanckaert 2003; Tazi *et al.* 2008; 2009; Tamayo *et al.* 2004).

CDC recommends the use of selective liquid Todd Hewitt Broth for direct inoculation of the tested specimen and then its incubation at the temperature of 35–37 °C in a 5% CO_2 enriched atmosphere for 18–24 hours. Then it must be transferred on solid blood agar with 5% defibred sheep blood.

Granada agar is a selective medium often recommended for performing GBS colonisation screening tests in pregnant women (El Aila & Tency 2010). It allows for rapid direct identification of GBS, forming an orange to red-coloured colony. Granada agar can also be used in detecting asymptomatic bacteriuria caused by GBS in pregnant women (Gupta & Briski 2004).

Tamayo *et al.* used two solid media, Columbia agar with 5% sheep blood (BA) and Granada, to determine the incidence of asymptomatic urinary tract infection in pregnant women. GBS growth on Granada medium was observed in 103 of 105 infected women compared to only 50 when the culture was performed on BA (Tamayo *et al.* 2004). The authors pointed out that Granada agar has limited diagnostic value in cases of low GBS concentration in urine (10^3–10^4 CFU/ml urine) and excessive growth of other bacteria (*Staphylococcus* spp., *Escherichia coli*, *Enterococcus* spp.). Granada agar must be incubated in anaerobic conditions, under which GBS produces a characteristic dye. This requirement increases the cost of the microbiological test (Tamayo *et al.* 2004).

The antibiotic treatment, which is currently recommended by CDC for women during delivery, may not prevent the occurrence of the late complication of GBS infections in neonates, or it may be the cause of allergic reactions in mothers. Active immunisation aimed at preventing GBS colonisation in mothers may be an effective and safe way to prevent life-threatening neonatal infections in the future. The presence of specific IgG antigens in the mother's blood creates an opportunity for the acquisition of passive immunity by the neonate. Studies show that vaccinating pregnant and non-pregnant women at reproductive age can be an effective way of preventing GBS infections in neonates (Kasper *et al.* 1996; Backer *et al.* 2003; Shen *et al.* 2001).

Considerable differences in the established incidence of GBS colonisation – from 3.3% to 19% in GBS screening tests performed in Poland on pregnant women – were described (Kowalska *et al.* 2003; Wilk *et al.* 2003; Kraśnianin *et al.* 2009). Usually specimens collected from pregnant women have been inoculated onto solid Columbia medium with 5% defibred sheep blood. However, according to references, this medium shows the lowest sensitivity in detecting GBS colonisation and asymptomatic urinary tract infections in women. Selecting the proper medium for conducting GBS colonisation screening tests is particularly important, as obtaining significant GBS bacteriuria and growth of GBS on solid medium (not previously passaged from liquid medium) is the basis of diagnosing GBS colonisation (Centers for Disease Control and Prevention 1996; 2002).

The present research showed high sensitivity and specificity of this medium in detecting GBS colonisation, and in our opinion Granada agar medium can be used as an alternative to Todd Hewitt Broth. Similar results were obtained by other authors (Tazi *et al.* 2008, 2009; Tamayo *et al.* 2004; Gupta &Briski 2004).

CONCLUSIONS

Selective Granada medium can be used concurrently with Todd Hewitt Broth for determining GBS colonisation in screening tests on pregnant women.

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Małgorzata Romanik – study conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript, final approval, revising for important intellectual content
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Gayane Martirosian – study conception and design, drafting the manuscript, final approval, revising for important intellectual content
Ryszard Poręba – study conception and design, drafting the manuscript, final approval, revising for important intellectual content.
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