Selective *versus* Non-selective Culture Medium for Group B Streptococcus Detection in Pregnancies Complicated by Preterm Labor or Preterm-Premature Rupture of Membranes

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The objective of this study was to identify group B streptococcus (GBS) colonization rates and compare detection efficiency of selective *versus* non-selective culture media and anorectal *versus* vaginal cultures in women with preterm labor and preterm-premature rupture of membranes (PROM). A prospective cohort study of 203 women was performed. Two vaginal and two anorectal samples from each woman were collected using sterile swabs. Two swabs (one anorectal and one vaginal) were placed separately in Stuart transport media and cultured in blood-agar plates for 48 hours; the other two swabs were inoculated separately in Todd-Hewitt selective media for 24 hours and then subcultured in blood-agar plates. Final GBS identification was made by the CAMP test. A hundred thrity-two cultures out of 812 were positive. The maternal colonization rate was 27.6%. Colonization rates were 30% for preterm PROM and 25.2% for preterm labor. Todd-Hewitt selective medium detected 87.5% and non-selective medium 60.7% GBS-positive women. Vaginal samples and anorectal samples had the same detection rate of 80.3%. Anorectal selective cultures detected 75% of carriers; 39% of GBS-positive women were detected only in selective medium. A combined vaginal-anorectal selective culture is appropriate for GBS screening in this population, minimizing laboratory costs.

Key Words: *Streptococcus agalactiae*, culture media, premature rupture of membranes, preterm labor.

Streptococcus agalactiae, or group B streptococcus (GBS), is an important infectious agent in perinatology; it is the predominant bacteria in early-onset neonatal sepsis [1,2]. GBS can be acquired during labor or in utero by transmission from maternal-vaginal or anorectal-colonized mucosa. Prematurity is also a risk factor, and mortality is higher in preterm than in term newborns [3].

Reported maternal colonization rates are quite variable, but generally range from 20-30% [4,5]. The differences in colonization rates depend on the particular population and especially on the laboratory methods used to identify GBS. Brazilian data are scarce [6,7], but are in general agreement with literature reports from other countries.

Since maternal colonization at delivery is the main risk factor for neonatal disease [4], microbiological techniques must be designed in order to maximize detection rates. The use of selective media containing antibiotics is reported to be both sensitive and also the most adequate method for detection [5]. The anatomic site of sampling is also important, and anorectal and vaginal cultures are recommended for detection in pregnant women [8].

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We analyzed culture methods for the detection of GBS in pregnancies complicated by preterm labor (PTL) or preterm premature rupture of membranes (PPROM), comparing Todd-Hewitt selective medium with non-selective medium and anorectal with vaginal samples.

Material and Methods

During the 12-month period from February 2003 to January 2004, all pregnant women (total 203) with preterm labor or preterm premature rupture of membranes attended at the Maternity Ward of the Campinas State University, located in Campinas, in the state of São Paulo, Brazil were enrolled and included in the study. All participating women signed an informed consent. Socio-demographic, clinical and obstetrical data were collected at the time of admission and retrospectively from patient's charts.

Samples were collected from the proximal third of the vaginal introitus and from inside the anus through the anal sphincter. Two vaginal and two anorectal swabs were obtained from each woman. Two swabs (one vaginal and one anorectal) were separately placed in two tubes containing Stuart transport media and then cultured in non-selective 5% blood-agar plates for 48 hours. The other two swabs were immediately inoculated into two tubes containing Todd-Hewitt selective broth medium (manufactured by PROBAC, Brazil), enriched with gentamicin and nalidixic acid, incubated for 24 hours, and then subcultured for 24 hours in non-selective 5% blood-agar plates. The final identification of GBS in all samples was made by the CAMP test, which is based on the production of an arrow-shaped hemolysis zone in conjunction with a *Staphylococcus aureus* colony in a blood-agar plate. Four

types of culture were then identified: selective anorectal, non-selective anorectal, selective vaginal and non-selective vaginal. A woman was considered colonized if any of the four cultures were positive.

Detection rates for each type of culture medium and each sampling site were calculated. Chi-square or Fischer's exact test were used to detect significant differences, and a P value < 0.05 was considered statistically significant.

Results

One hundred thirty two (16.2%) GBS-positive cultures were obtained from the 812 specimens collected from the 203 pregnant women. Fifty-six women (27.6%) had at least one of the cultures positive. Table 1 shows the clinical and obstetric characteristics of the women enrolled in the study. White race and low education level were significantly associated with colonization.

Colonization rates for women in preterm labor were 25.2%, and for women with preterm premature rupture of membranes it was 30%. Sixteen women (7.8%) had all of the four cultures positive. Selective anorectal cultures were positive in 42 patients (20.7%) and selective vaginal cultures were positive in 38 (18.7%). Non-selective anorectal cultures were positive in 24 patients (11.8%) and non-selective vaginal cultures in 28 (13.8%). Table 2 shows the comparison of the selective *versus* non-selective cultures. There was discordance in the results for 29 women. Twenty-two (39)% of the colonized women were detected only in the selective-media cultures.

Table 3 shows culture results by site of sampling. Discordance between results was observed in 22 women. However, the number of colonized women detected by each of the cultures was the same (45 each, or 80.3% of all colonized women). However, these 22 women were not the same in each group.

Selective media detected 87.5% of the 56 colonized women, compared to 60.7% detected by non-selective cultures. There was no difference in the detection rate between anorectal and vaginal samples (80.3%).

Discussion

Screening of group B streptococcus colonization is recommended for all pregnant women between 35 and 37 weeks, and in situations of risk of preterm delivery, which are preterm labor and preterm premature rupture of membranes [5]. Antibiotic prophylaxis of colonized women, during labor, greatly reduces the risk of neonatal disease [9].

It has been reported that GBS isolation is 20% to 40% greater when combined vaginal and anorectal cultures are collected [8,10-12]. A significant proportion of women were found to have only one of these sites colonized; this proportion was 18% to 24% higher in anorectal compared to vaginal samples [11,12]. In an analysis of 651 specimens, the combination of anorectal and vaginal cultures reduced the number of false-negative results, allowing detection of 97.8% of GBS carriers [13]. Since there are no published data comparing the risk of neonatal disease by colonized anatomic site, it is recommended that both vagina and rectum be cultured [5].

In our study, 11 patients were detected only in vaginal and another 11 only in anorectal cultures; the number of carriers detected would have been the same if only one of these sites was sampled. However, anorectal-selective medium detected 75% of GBS carriers.

Todd-Hewitt selective medium enriched with gentamicin and nalidixic acid inhibits growth of Gram-negative bacteria, and it has greater sensitivity when compared to other non-selective media, such as blood or Granada agar [14]. Twenty-two cases, or 39% of all colonized women in this study, were detected only in the samples incubated in Todd-Hewitt medium. The proportion of colonized women detected by the selective medium was 87.5%, and 15 affected patients would not have been detected if only non-selective medium had been used; the detection rate would have fallen 30%. This finding supports the current view that selective medium is fundamental to maximize detection rates and should be employed by laboratories involved in screening of pregnant women [5].

In the clinical scenario, which is where this screening was done, a critical issue is the time needed to obtain reliable results from the cultures, which is 48 hours when using the methods recommended by CDC. Preterm labor and premature rupture of membranes are high-risk situations for early-onset sepsis, for which adequate intrapartum antibiotic prophylaxis is necessary. Rapid identification tests would be more suitable, since reliable and immediate results would be available, avoiding unnecessary antibiotics prescription. The only FDA-approved rapid test is a real-time PCR assay (IDI-Strep B®), which has high sensitivity and specificity [15].

A study evaluating maternal colonization rates in 34 reports from developing countries found a GBS-prevalence rate of 12.7%. However, when considering only those studies in which adequate laboratory methods were used (use of selective culture medium and collection of vaginal specimens), this rate increases to 17.8%. Inappropriate microbiological methods were used on almost half of the patients included in this analysis [16].

The economic costs of universal maternal screening must be considered. We believe that given the enormous amount of money spent in intensive care units to care for infected newborns, and later to treat long-term disabilities caused by neonatal disease, the cost-benefit ratio favors culture screening. The Centers for Disease Control and Prevention estimated that $300 million dollars were spent in a year to treat almost 7,500 cases of early-onset GBS disease [17]. There is no published evaluation of this issue in developing countries. In women with preterm labor, which had a prevalence of 25%

Table 1. Clinical and obstetric characteristics and maternal colonization by group B Streptococcus

Variable	Colonized N	Non colonized N	Total	RR (CI 95%)	P
Age					
< 19	12	26	38	1.7 (0.7–2.02)	0.541
≥ 19	44	121	165		
Race					
White	36	69	105	1.65 (1.08–2.69)	0.027
Other	20	78	98		
Low level of education*					
Yes	37	73	110	1.70 (1.03–2.81)	0.031
No	17	69	86		
Gestational age					
<32 weeks	23	55	78	0.90 (0.57–1.41)	0.632
≥ 32 weeks	33	92	125		
Diagnosis					
PPROM	30	70	100	1.38 (0.87–2.18)	0.178
PTL	26	77	103		
Primigravid					
Yes	23	52	75	0.84 (0.54–1.32)	0.452
No	33	95	128		
Mean birth weight (g)	2147	2361		1.44 (0.88–2.36)	0.134
± SD	± 762	± 744			

RR = Risk Ratio CI = Confidence Interval; PPROM = Preterm premature rupture of membranes; PTL = Preterm labor; SD = Standard deviation; *less than elementary school completed.

Table 2. Group B Streptococcus detection results by type of culture medium

	Positive	Negative	Total
Non selective	34 (16.7%)	169 (83.3%)	203 (100%)
Selective	49 (24.1%)	154 (75.9%)	203 (100%)
Total	83 (20.4%)	323 (79.6%)	406 (100%)

Table 3. Group B Streptococcus culture results by anatomic site

	Positive	Negative
Vaginal	45 (22.2%)	158 (77.8%)
Anorectal	45 (22.2%)	158 (77.8%)
Total	90 (22.2%)	316 (77.8%)

GBS-positive mothers, initiating penicillin treatment before culture results would result in overtreatment of three out of four patients. This combined strategy of culturing women at risk while prescribing antibiotics has not been evaluated on a cost/benefit basis, which involves not only culture and antibiotic costs, but also pediatric care after birth. In our country, penicillin is a low-cost antibiotic.

We found that selective culture medium yielded the highest detection rates, with no difference between anorectal and vaginal samples. The proposed combined anorectal and vaginal swab directly inoculated into a tube containing Todd-Hewitt medium would cost about two U.S. dollars in Brazilian currency per patient. We believe that this low cost would be feasible even for universal screening at our maternity. This strategy was previously compared through delayed inoculation after sampling in transport media and gave better results, with enhanced detection rates [18].

Conclusions

The use of Todd-Hewitt selective medium yielded greater detection rates than non-selective medium. There was no difference in detection rates between vaginal and anorectal samples, however selective anorectal cultures detected more colonized women than the other three types of cultures.

From a practical standpoint, a single combined vaginal-anorectal sample incubated in selective medium would be appropriate. This strategy would minimize laboratory costs without compromising maximum detection capacity, which is crucial for the prevention of early-onset neonatal disease.

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