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CONCISE ARTICLE

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Optimisation of Prenatal Group B Streptococcal Screening

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Abstract The purpose of the study presented here was to confirm the high yield of group B streptococci (GBS) on Granada medium for the detection of pregnant GBS carriers and to compare the results with those obtained using standard Columbia blood agar at two participating centers in Belgium. Culture results of the vaginorectal swabs obtained at the two centers were also compared. A total of 1,142 samples (838 in Leuven and 304 in Bonheiden) obtained from consecutive pregnant women were cultured onto both media. Of all GBS carriers 84.7% were detected on Columbia blood agar and 93.4% on Granada agar (*P*<0.01, McNemar test). The addition of Granada agar was responsible for a 15% higher rate of detection of GBS carriers. As a result of this study, both participating hospitals will use a combination of Granada agar with Columbia blood agar for optimal GBS screening in the future.

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Introduction

Group B streptococci (GBS) remains a leading cause of serious early-onset neonatal infection in spite of major efforts to detect pregnant carriers [1, 2]. Early-onset GBS disease is caused by the transmission of bacteria from the mother's genital or anorectal mucosae to the newborn either during delivery or in utero after rupture of the membranes [1, 3]. Early-onset GBS disease presents as a systemic illness within the first 7 days of life and is accompanied by high rates of morbidity and mortality. Intrapartum antibiotic prophylaxis has been shown to be the most effective strategy to prevent GBS infection [3, 4].

The first step in the prevention of early-onset GBS disease in the newborn is the detection of GBS colonization in pregnant women. In 1996, the Centers for Disease Control and Prevention (CDC) still recommended two types of prevention strategy, one of which was a culture-based approach and the other a clinical risk-based approach [3], but the CDC now accepts the use of a culture-based approach in combination with preventive measures in the presence of some well-defined risk factors [1]. This currently accepted strategy was developed because a recent CDC-sponsored multistate study demonstrated that a strict culture-based approach is more than 50% more effective than a strict risk-based approach in preventing perinatal GBS disease [5].

Several reports have shown that media of Islam type, such as Granada agar and GBS medium, have equal or better sensitivity for GBS than the CDC-recommended culture in selective broth medium followed by subculture on sheep blood agar [6, 7, 8, 9]. Granada agar is a selective and differential medium for rapid detection of beta-hemolytical GBS. The production of an orange carotenoid pigment, a unique characteristic of beta-hemolytic GBS, is enhanced by the use of methotrexate in this medium [7]. Granada agar has the advantage of detecting and identifying GBS in a single step, which makes it more accurate, easier to use and cheaper than the enrichment broth technique [8].

Claeys et al. [10] showed a higher rate of GBS detection with Granada compared with TSA blood agar. The purpose of our study was to confirm the high yield of GBS on Granada agar and to compare the results with the currently used Columbia blood agar. Moreover, the results of culture from the vaginorectal swabs obtained at the two participating centers (University Hospitals of Leuven and Imelda Hospital of Bonheiden) were compared.

Materials and Methods

In this study, the STARD guidelines were taken into account wherever possible [11].

The University Hospitals of Leuven comprise a tertiary teaching hospital with 1,897 hospital beds. There are 30 obstetricians (10 board certified and 20 residents) and 2,150 deliveries per year. The Imelda Hospital of Bonheiden is a secondary teaching hospital with 453 hospital beds. There are 9 obstetricians (6 board certified and 3 residents) and 1,000 deliveries per year. In these two hospitals a GBS screening protocol was introduced for pregnant women in which culture-based screening was combined with risk-based screening for patients for whom no culture result was available. In Leuven the protocol was introduced in September 2001, whereas in Bonheiden it was implemented in 2000 and updated in September 2001.

The sample collection protocol requires that combined vaginorectal swabs be obtained from women after 35 weeks of gestation. These samples should then be sent to the hospital laboratory in either Amies or charcoal-free Stuart transport medium together with the request to screen for GBS only. During our prospective study, which was conducted from November 2001 up to and including May 2002 in Leuven and from January 2002 up to and including May 2002 in Bonheiden, we analyzed 1,142 consecutive swabs (838 in Leuven and 304 in Bonheiden).

Since no commercial Granada medium was available in Belgium at the start of the study, we prepared the Granada agar as described by de la Rosa et al. [7], with pH 7.45 +/- 0.1 adjustment as the only modification. The Granada medium was made as follows: 10 g of agar (Difco, Belgium), 25 g of proteose peptone no.3 (Becton Dickinson, Belgium), 20 g of soluble starch (Merck, Belgium), 11 g of morpholinopropanesulfonic acid (MOPS) hemisodium salt (Acros, Belgium) and 8.5 g of anhydrous Na_2HPO_4 (Merck) were dissolved in 1000 ml of water by heating gently and stirring several times. After dissolution was complete, the pH was adjusted to 7.45 +/- 0.1 with NaOH (Merck). The solution was autoclaved at 121°C for 15 min and subsequently allowed to cool to 50–55°C. Ten milliliters of a filter-sterilized solution containing 250 g of glucose (Difco) per liter, 100 g of sodium pyruvate (Acros) per liter, 20 g of anhydrous $MgSO_4$ (Merck) per liter, 0.6 g of methotrexate sodium salt (Wyeth-Lederle, Belgium; 100 mg/ml) per liter, 0.02 g of crystal violet (Merck) per liter; 0.5 g of colistin sulfate (Alpha Pharma, Belgium) per liter, and 1 g of metronidazole (Certa, Belgium) were then added. Fifty milliliters of sterile horse serum (Gibco, Belgium) was added. The medium was then poured into plates. Contrary to the recommendation made by de la Rosa et al. [7], we found pH adjustment to be very important in order to obtain good pigmentation.

The Columbia blood agar used in this study was a homemade agar based on the method of Murray et al. [12] using horse blood instead of sheep blood.

The vaginorectal swabs were cultured onto two homemade agar plates, i.e., Columbia blood agar and Granada agar. Since the two media were inoculated with the same swab, inoculation was alternated first onto Columbia blood agar, which was incubated in 5% CO_2, and then onto Granada agar, which was kept under anaerobic conditions. Both agar plates were screened for *Streptococcus agalactiae* after 24 and 48 h. Only beta-hemolytic colonies appearing on Columbia blood agar were analyzed with Streptex (Murex Biotech, UK) to identify Lancefield group B streptococci. Orange-pigmented colonies on Granada agar were diagnostic for GBS; thus, no further identification test was necessary [7, 9].

All swabs were processed within 16 h of collection. Both hospital laboratories utilized the same agar plates (all made in Leuven) and protocol, and the results obtained by the two laboratories using the two culture media were compared. In addition, the culture yields obtained from the vaginorectal swabs at each of the two centres were compared. All statistical procedures (McNemar, Fisher's exact test) were performed using the Analyse-it software program for Microsoft Excel (Microsoft, USA).

Nine months after implementation of the new screening protocol in Leuven, a small and anonymous inquiry was performed among the 30 obstetricians to test their compliance with the hospital's GBS prevention guidelines and sampling procedures. Included in the inquiry were questions about how they usually take the screening samples.

Results and Discussion

During the study all vaginorectal swabs were cultured onto Columbia blood agar as well as Granada agar plates. Both agar plates were screened for *Streptococcus agalactiae* after 24 and 48 h. The second reading (48 h) of the Columbia blood agar and Granada agar plates revealed an additional GBS yield of 3% and 21%, respectively, compared to the first reading (24 h). The higher yield of GBS on Granada after the second reading is due to enhanced pigment production after longer incubation.

The distribution of positive cultures on the two media was identical at the two centres (Table 1). Of all GBS-positive cultures, 84.8% were detected on Columbia blood agar and 93.4% on Granada agar ($P<0.01$, McNemar test). Since this difference is statistically significant, we conclude that Granada agar is superior to Columbia blood agar for the detection of GBS. Moreover, by adding Granada agar to Columbia blood agar (i.e., using both media) we observed a 15% higher yield of GBS. This supplementary yield could be explained by the inhibitory effects of the Granada agar on the other commensal vaginorectal flora.

Table 1 Comparison of positive GBS culture results obtained at two Belgian hospitals using two screening media

Medium	University Hospitals Leuven (*n*=150)	Imelda Hospital (*n*=79)	Total (*n*=229)
CBA	10 (6.7%)	5 (6.3%)	15 (6.6%)
GA	23 (15.3%)	12 (15.2%)	35 (15.3%)
CBA and GA combined	117 (78%)	62 (78.5%)	179 (78.2%)

CBA, Columbia blood agar; GA, Granada agar

In contrast with a previous study by Claeys et al. [10], in which all samples positive on blood agar were also positive on Granada medium, our study demonstrated that 6% of the samples positive on Columbia blood agar did not show up on Granada agar (Table 1), even though some of them showed massive growth. The difference between the findings of these two studies is difficult to explain since the Columbia media used in the present study has the same microbiological characteristics as the TSA blood agar used by Claeys et al. [10]. Hence, we decided to continue to use a combination of both Granada and Columbia blood agar in order to obtain maximal GBS detection rates.

It should be noted that non-hemolytic GBS organisms, which account for 1–2% of all GBS strains [13], will still be missed on Granada agar, because the genes for pigment production and hemolysis induction are linked to each other. Thus, non-hemolytic GBS will only be found if a search for non-hemolytic colonies is conducted.

Although we observed an identical distribution of positive cultures on both culture media in the two centres, the overall detection rate of GBS carriers was higher in Bonheiden compared to Leuven. In Leuven, only 150 of the 838 samples (17.9%; 95%CI, 15.4–20.6%) were positive, while in Bonheiden 79 of 304 (26%; 95%CI, 21.2–31.4%) were positive ($P<0.005$, Fisher's exact test). Both percentages of GBS-positive culture results are within the 10–30% range of colonization of pregnant women described by the CDC [1].

The 8% higher detection rate of GBS carriers observed in Bonheiden compared to Leuven is unlikely to be explained by a different patient population, since there is only a 40 km distance between the two hospitals. One possible explanation is that a more rigorous sample collection technique may have been used by obstetricians in Bonheiden than in Leuven. Although participating obstetricians reached a consensus at the beginning of the study to take a combined vaginorectal swab for GBS and to use either Amies or charcoal-free Stuart transport medium, the smaller number of obstetricians in Bonheiden (9 instead of 30 in Leuven) may have accounted for better adherence to the sampling technique protocol. Indeed, it has been well established in the literature that there are two critical points in the collection of samples for GBS screening [1, 3]: (i) it is necessary to collect a combined vaginorectal swab, not only a vaginal swab; (ii) it is important to use Amies or charcoal-free Stuart transport medium with the swab.

The small inquiry in Leuven, which was completed by 90% of the obstetricians (9/10 board certified and 18/20 residents), revealed that 90% of the pregnant women with a planned vaginal delivery were screened. The inquiry also revealed that 30% (8/27) of the obstetricians took a vaginal sample only instead of the recommended vaginorectal swab and that 11% (3/27) used a dry swab instead of the recommended swab with Amies or charcoal-free Stuart transport medium. Numerous variables, such as the amount of deliveries per obstetrician and the age of the obstetrician, were not taken into account.

During this study, one case of early-onset GBS disease occurred in Leuven, and antenatal GBS screening had not been performed in that particular case. In Bonheiden there was no case of early-onset disease.

From this study, we conclude that the use of a combination of Granada and Columbia blood agar and an adequate sample (vaginorectal swabs in transport medium) are necessary for optimal GBS screening. Good communication between the laboratory and the obstetricians is also imperative.

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