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Evaluation of the Granada Agar Plate for Detection of Vaginal and Rectal Group B Streptococci in Pregnant Women

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Granada medium was evaluated for the detection of group B streptococci (GBS) in vaginal and rectal swabs compared with selective Columbia blood agar and selective Lim broth. From May 1996 to March 1998, 702 pregnant women (35 to 37 weeks of gestation) participated in this three-phase study; 103 (14.7%) of these women carried GBS. In the first phase of the experiment (*n* = 273 women), vaginorectal specimens were collected on the same swab; the sensitivities of Granada tube, selective Columbia blood agar, and Lim broth were 31.4, 94.3, and 74.3%, respectively. In the second and third phases (*n* = 429 women), vaginal and rectal specimens were collected separately; the sensitivities of Granada plate, selective Columbia blood agar, and Lim broth (subcultured at 4 h on selective Columbia agar in the second phase and at 18 to 24 h in Granada plate in the third phase) were 91.1, 83.9, and 75%, respectively, in the second phase and 88.5, 90.4, and 63.5%, respectively, in the third phase. There were no statistically significant differences in GBS recovery between the Granada agar plate and selective Columbia blood agar, but the Granada plate provided a clear advantage; the characteristic red-orange colonies produced overnight by GBS can be identified by the naked eye and is so specific that further identification is unnecessary. The use of the Granada tube and Lim broth did not result in increased isolation of GBS. In conclusion, the Granada agar plate is highly sensitive for detecting GBS in vaginal and rectal swabs from pregnant women and can provide results in 18 to 24 h.

In 1996, the Centers for Disease Control and Prevention (CDC) published guidelines (9) for the prevention of perinatal group B streptococcus (GBS) disease, one of the most common causes of neonatal sepsis throughout the world. The CDC recommended two prevention strategies as follows: (i) intrapartum antibiotic prophylaxis for women identified as GBS carriers at 35 to 37 weeks of gestation and for women who go into labor or develop ruptured membranes at <37 weeks of gestation or (ii) intrapartum antibiotic prophylaxis for women who are at risk at the time of labor.

A multicenter study performed in the Barcelona area between 1994 and 1996 (19) disclosed that GBS was the leading cause of neonatal infection, with an incidence of 1.48 per 1,000 live births and a mortality of 8.7%. Among 103 women with microbiologically confirmed perinatal sepsis, only 25% had been studied for GBS carrier status during pregnancy, 54% presented no obstetric risk factors during labor, and only 10.7% received intrapartum antimicrobial prophylaxis. Thus, in May 1997, the Catalan Society for Obstetrics and Gynecology, for Pediatrics, and for Infectious Diseases and Clinical Microbiology (8) recommended the first CDC strategy with some modifications. The Spanish Society for Obstetrics and Gynecology agreed with these recommendations in September 1998 (27).

A basic point for implementing this prevention strategy is the identification of GBS carriers. The CDC advocates the use of selective broth medium (Todd-Hewitt broth supplemented with colistin and nalidixic acid) incubated for 18 to 24 h and subcultured onto sheep blood agar plate. A new medium for GBS isolation, known as new Granada medium, was described recently (10a, 10b). This selective and differential medium, which utilizes the unique ability of GBS to produce a red-orange pigment, detects and identifies GBS in a single step. The chemical nature of this pigment, best produced under anaerobic conditions, has not been established. In Granada medium serum, proteose peptone and folate pathway inhibitors (such as methotrexate) enhance pigment production and starch stabilizes the pigment (24).

This study compares several culture media and procedures, including Granada agar plate, Granada medium tube, selective Columbia blood agar, and selective Lim broth, for the detection of GBS in vaginal and rectal specimens from pregnant women.

MATERIALS AND METHODS

From May 1996 to March 1998, 2,535 vaginal and rectal samples from 702 women in the 35th to 37th week of pregnancy, examined in the Obstetrics Clinic of the Hospitals Vall d'Hebron, were submitted to the hospital microbiology laboratory for the detection of GBS.

The study consisted of three phases according to the methods used for sample collection and processing. The participants in the first phase, from May 1996 to May 1997, included 273 pregnant women. Vaginal and rectal samples were collected on the same swab, and three samples were obtained from each subject, for a total of 819 swabs. Each of the three swabs was processed separately. In the consulting room, one swab was immediately placed in a tube of Granada agar medium (Biomedics SL, Madrid, Spain), and another was immediately placed in Lim broth (Todd-Hewitt broth, 1% yeast extract, 15 μg of nalidixic acid/ml, and 10 μg of colistin/ml). These media, together with the third swab (modified Amies transport medium; Eurotubo, Barcelona, Spain), were sent to the laboratory. The third swab was used to inoculate a selective Columbia agar plate (Columbia agar, 5% human blood, 15 μg of nalidixic acid/ml, and 10 μg of colistin/ml). The Lim broth was incubated in a 37°C bath for 4 h and subcultured to a selective Columbia agar. Both plates of selective Columbia agar were incubated at 35 to 37°C in an atmosphere of 5% CO_2 and evaluated at 24 and 48 h. The Granada tube was incubated in a 37°C bath for 48 h and examined periodically for signs of red-orange colonies.

The second phase of the study, from June to October 1997, included 262 women. Vaginal and rectal samples were collected separately and in duplicate by using different swabs, for a total of 1,048, which were sent to the laboratory. One vaginal and one rectal swab were placed separately in selective Lim broth,

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TABLE 1. Number of GBS-positive cultures detected in the second phase of the study in 262 vaginal and 262 rectal specimens

Specimen	No. of positive GBS cultures detected by:			No. of women colonized
	Granada plate	Selective Columbia	Selective Lim broth	
Vaginal	19	19	16	22
Rectal	32	28	26	34
Total	51	47	42	36

TABLE 3. Number of GBS-positive cultures detected in the third phase of the study in 167 vaginal and 167 rectal specimens

Specimen	No. of positive GBS cultures detected by:			No. of women colonized
	Granada plate	Selective Columbia	Selective Lim broth	
Vaginal	22	21	17	24
Rectal	24	26	16	28
Total	46	47	33	32

incubated, and subcultured under the conditions described for the first phase. The second swabs, rectal and vaginal, were inoculated separately, in random order, onto both selective Columbia plate and Granada agar plate (proteose peptone, 25 g; soluble starch, 20 g; morpholinepropanesulfonic acid [MOPS], 11 g; Na_2HPO_4, 8.5 g; glucose, 2.5 g; sodium pyruvate, 1 g; $MgSO_4$, 0.2 g; methotrexate sodium salt, 6 mg; crystal violet, 0.2 mg; colistine sulfate, 5 mg; metronidazole, 10 mg; horse serum, 50 ml; agar 10 g; and water, 1,000 ml). Granada plate was incubated under anaerobic conditions and examined at 24 and 48 h.

The third phase, from November 1997 to March 1998, included 167 women. Sample collection and processing were carried out in the same manner as that described for the second phase but with the following modifications to the Lim broth: (i) 5% horse serum was added, (ii) subculturing was carried out after 18 to 24 h of incubation instead of after 4 h, and (iii) subcultures were plated on Granada agar instead on selective Columbia blood agar.

Quantitative growth was evaluated in the Granada and Columbia plates as follows. Over 80 CFU was defined as heavy colonization, between 80 and 20 CFU was defined as moderate, and under 20 CFU was defined as light.

GBS was identified in Columbia blood agar by the presence of beta-hemolytic and nonhemolytic colonies showing a positive CAMP or coagglutination test (Phadebact Strep B test; Boule Diagnostics). The presence of red-orange pigmented colonies in Granada medium is characteristic of GBS; nevertheless, identification was confirmed by the CAMP test or by antigen detection in all cases. Women whose GBS reports were positive were sent immediately to the delivery room.

Statistical methods. The McNemar test for correlated percentages was used to compare the culture media.

RESULTS

Of the 702 pregnant women studied, 103 (14.7%) were found to be colonized by GBS. In the first phase of the study, 35 of 273 women (12.8%) were found to be GBS carriers. In this phase, GBS was recovered from 11 specimens in Granada tube, from 33 in Columbia blood agar, and from 26 in selective Lim broth, giving sensitivities of 31.4, 94.3, and 74.3%, respectively. The differences between Columbia agar and Lim broth were not significant, but there were statistical differences between these media and the Granada tube (P = 0.0000075 and P = 0.0023, respectively).

In the second phase of the study, 36 (13.7%) of the 262 women studied were found to be colonized. Of these, 20 harbored GBS in the rectum and vagina, 2 harbored GBS only in the vagina, and 14 harbored GBS exclusively in the rectum. Of the 56 samples (Table 1) from which GBS was isolated, 51 were positive by Granada agar plate, 47 were positive by Columbia agar, and 42 were positive by Lim broth, resulting in sensitivities of 91.1, 83.9 and 75%, respectively. Separate analyses of vaginal and rectal specimens indicated no differences among the three media. Analysis of the total GBS showed no differences between Granada and Columbia plate (P = 0.39) and significant differences between Granada plate and Lim broth (P = 0.03) (Table 2).

In the third phase, GBS was identified in 32 (19.2%) of the 167 women studied: 20 from rectal and vaginal specimens, 4 from vaginal specimens only, and 8 from rectal specimens only. Table 3 shows the 52 GBS-positive isolates—46 in Granada agar plate, 47 in Columbia blood agar, and 33 in selective Lim broth—with sensitivities of 88.5, 90.4, and 63.5%, respectively. There were no significant differences among the three media in GBS detection from vaginal specimens, whereas Granada and Columbia plates were more sensitive than Lim broth for the rectal specimens (P = 0.04 and P = 0.004). Analysis of the total GBS (Table 4) showed no differences between Granada agar plate and Columbia blood agar (P = 1) and significant differences between Granada and selective Lim broth (P = 0.009).

The second and third phases of the study combined showed that of the 429 pregnant women, 68 (15.8%) harbored GBS: 40 in the vagina and rectum, 6 only in the vagina, and 22 (32.4%) only in the rectum. In the 6 women with exclusively vaginal detection, colonization was heavy in 3, moderate in 2, and light in 1, whereas in the 22 with exclusively rectal detection, colonization was heavy in 10, moderate in 7, and light in 5. For 21 samples, there were discrepancies between Granada agar plate

TABLE 2. Comparison in the second phase of the study between Granada agar plate and selective Columbia agar[a] and between Granada agar plate and selective Lim broth[b]

Method and result	Results by Granada plate	
	No. GBS positive	No. GBS negative
Columbia		
GBS positive	43	4
GBS negative	8	469
Lim broth		
GBS positive	40	2
GBS negative	11	471

[a] No differences were found between Granada agar plate and Columbia (P = 0.39).
[b] Significant differences were found between Granada agar plate and Lim broth (P = 0.03).

TABLE 4. Comparison in the third phase of the study between Granada agar plate and selective Columbia agar[a] and between Granada agar plate and selective Lim broth[b]

Method and result	Result by Granada plate	
	No. GBS positive	No. GBS negative
Columbia		
GBS positive	42	5
GBS negative	4	283
Lim broth		
GBS positive	29	4
GBS negative	17	284

[a] No differences were found between Granada agar plate and selective Columbia (P = 1).
[b] Significant differences were found between Granada agar plate and Lim broth (P = 0.009).

and Columbia blood agar: GBS grew only in Granada agar in 12 cases (5 vaginal and 7 rectal samples and grew only in Columbia agar in 9 cases (4 vaginal and 5 rectal samples). These 21 GBS-positive samples isolated on only one of the agar plates presented the following characteristics: 13 (9 isolated from Granada plate and 4 from Columbia) showed light growth (range, 1 to 10 CFU; mean, 4.6), 4 (2 isolated from Granada and 2 from Columbia) showed moderate growth (range, 20 to 50 CFU; mean, 27.5), 1 showed heavy growth (100 CFU) on Granada plate, and 3 nonhemolytic GBS-positive samples grew on Columbia plates, while Granada plates were considered negative because the growth showed no production of pigment. Of these 21 samples, 11 were positive by selective Lim broth.

Samples from the obstetrics clinic were delivered to the microbiology laboratory at 2:00 p.m. Results positive by Granada plate were detected the following morning (between 18 and 22 h later) for all cases. In this medium, GBS red-orange colonies were easy to recognize, even when colonization was light or when GBS were mixed with other microorganisms. All the pigmented colonies were confirmed as GBS by CAMP or by a coagglutination test.

DISCUSSION

This comparative study was conducted to determine the feasibility of implementing Granada medium as the standard procedure for GBS detection in our laboratory. This evaluation was made up of three phases with different protocols in an attempt to achieve better detection. The poor results obtained during the first phase with Granada tube prompted us to focus in the second phase on Granada plate. The unsatisfactory results obtained during the second phase with Lim broth motivated us to change the conditions for the third phase.

In our study, Granada plate sensitivity ranged from 88.5 to 91.1%, selective Columbia sensitivity ranged from 83.9 to 94.3%, and Lim broth sensitivity ranged from 63.5 to 75%. Thus, none of these media was totally sensitive, indicating that for optimum GBS detection, the use of more than one medium is advisable.

Granada tube sensitivity was as low as 31.4%. An inherent drawback of Granada medium (tube and plate, differing only in the proportion of agar [3 and 10 g/liter, respectively]) is its poor stability. Once prepared, the medium must be used within 3 weeks, and stability is severely disturbed by environmental changes. In the first study, tube inoculation was carried out in the obstetrics clinic, where the tubes were removed from the refrigerator early in the day, maintained at room temperature, and used during the course of the morning. Unused tubes were stored at 4°C. This type of handling was probably the main reason for the poor results obtained by Granada tube. A new evaluation performed with tubes maintained at 4°C until inoculation (not feasible in our obstetrics clinic) is necessary to assess the sensitivity of Granada tube under optimum conditions. Since GBS can be preserved for up to 4 days in transport medium (26), we decided to discontinue Granada tube inoculation in the obstetrics clinic and to begin the evaluation of Granada plate inoculated in the laboratory.

In our experience, Granada agar plate was as sensitive as Columbia agar plate and had the advantage (20) of providing overnight results by examination with the naked eye. The red-orange colonies produced by GBS on Granada agar are clearly apparent, even when there is only a single colony or very few colonies or when GBS is mixed with other microorganisms (mainly other streptococci and *Proteus* spp.). The colonies are so characteristic and unique that identification by antigen detection or the CAMP test is unnecessary. In contrast, identification of suspected GBS colonies is mandatory when selective Columbia plate is used; and, when there are few colonies or when GBS is mixed with other flora, subculture is required prior to identification. Thus, another advantage of Granada plate is reduced cost (20) for laboratory personnel and reagents.

Screening was performed at 35 to 37 weeks because colonization status at delivery can be accurately predicted and because this time period (30) allows an adequate length of time for culture to be performed and results to be reported (16). However, we cannot ignore the fact that a percentage of women deliver between 38 and 39 weeks. Thus, the use of a medium such Granada, which reports GBS colonization status in 24 h, assures antibiotic prophylaxis based on screening results in women who deliver shortly after 37 weeks.

The discrepancies between Granada and Columbia plates were due mainly (62%) to light colonization. Since the two media were plated with the same swab and the order of inoculation was random, in cases of light GBS colonization there might have been no remaining GBS on the swab when the second plate was inoculated. Three other discrepancies were due to strains of GBS that do not produce hemolysis and pigment. Granada medium always gives negative results with these types of strains. Pigment is produced by 93 to 98.5% of GBS strains isolated from human clinical specimens (13, 18, 21) and by only 35% of strains from bovine sources (21). There is a high correlation between the capacity to produce pigment and the capacity to release hemolysin (13, 21, 22, 28), since the genes that determine these properties are in contiguous loci of the chromosome (29).

We found selective Lim broth to be less sensitive than Granada agar plate or selective Columbia agar, particularly for rectal specimens. By the end of the second phase, we suspected that the poor results obtained with selective Lim broth were due to an excessively short incubation time (4 h). We had used a short incubation period so that the subculture could be evaluated the following morning and the results could be delivered within 48 h. Nevertheless, when we switched to an 18-h incubation period (third phase), the sensitivity of selective Lim broth showed no change. This finding contrasts with those reported by the CDC (9) and other groups (4, 6, 14, 25), who recommend the use of selective broth and its subculture after 18 to 24 h onto sheep blood agar plate as the most sensitive procedure for detecting GBS. However, our results are similar to those published by Dunne et al. (12), who reported that the sensitivities of direct plating onto selective blood agar or selective Todd-Hewitt broth are equivalent. The competitive growth of rectal and vaginal microorganisms, especially *Enterococcus faecalis* (12), can suppress the growth of GBS in Lim broth; in fact, we frequently found moderate to heavy growth of several microorganisms, particularly *Streptococcus* species, in the Lim broth subcultures of specimens that were positive for GBS by Granada and/or Columbia plates and negative by Lim broth.

Overall, 14.7% of women in the 35th to 37th week of pregnancy were colonized by GBS vaginally and/or rectally. Colonization differs among ethnic groups and geographic areas and with age. Reported data range in the United States from 10 to 30% (2, 7, 11, 23) and in Spain from 7 to 12% (1, 5, 10, 15, 18); in Israel the reported incidence is 4% (17). Our study showed that 32.4% of women harbored GBS only in the rectal area, consistent with previously reported data (3, 7, 11). Such epidemiological information is the basis for the creation of guidelines in each country to prevent perinatal GBS disease.

In conclusion, our experience indicates that Granada agar

plate is highly sensitive for detecting GBS in vaginal and rectal specimens from pregnant women and has the valuable advantage of providing results in 18 to 24 h.

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