

Standardization and Enrichment of Culture Medium Improve Detection of Group B Streptococci during Prepartum Screening

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Abstract

The identification and treatment of infections by the streptococci group B (GBS), or *Streptococcus agalactiae* in pregnancy, are meant to prevent potential clinical disorders such as endometritis and neonatal sepsis in early onset (EOD) and late (LOD).

The purpose of the study was to analyze the methods of collection and cultivation of GBS in all microbiology laboratories of two Italian regions, Piedmont and Valle d'Aosta, in search of a correlation that could explain the remarkable rate variability of outcomes of the test.

The study team analyzed 28491 test from 70 public and private laboratories of microbiology, and crossing data with regional registries of births, there was a positive test by an average of 14.6%, range between 2.4% and 22.6%.

Further analysis has revealed that the laboratories used an enrichment broth culture showed a positive test of the 15.49% of the cases ($\pm 95\%$ CI: 14.78 to 16.23%; range 10.7 to 22.60%), while the samples analyzed without enrichment broth showed a positivity of 10.53% ($\pm 95\%$ CI: 10 to 11.08%; range from 2.40 to 20%) ($p < 0.0000001$).

The results show that the breach of the guidelines of microbiology generate a high rate of false negatives and how the enrichment broth can be the gold standard for the culture of GBS during pregnancy.

Keywords: Group B streptococci; Prepartum Screening; Broth-enriched culture

Introduction

Many women are colonized in the genital and gastrointestinal tracts with Group B *Streptococcus* (GBS), or *Streptococcus agalactiae*, but remain free from symptoms [1]. However, pregnant women colonized with GBS are at increased risk to develop clinical disorders, such as urinary tract infections, endometritis and neonatal GBS sepsis, which occurs in two to four infants per 1000 live births [2,3]. The presentation of neonatal GBS diseases differs according to age at onset [4], with a bimodal distribution of cases by age, and two main syndromes have been recognized: early-onset disease (EOD) and late-onset disease (LOD) [5]. EOD usually occurs during labor or within the first few days after delivery and can affect the woman, the baby or both [1]. EOD is acquired from the mother by the ascending route in the uterus during labor or by direct contact at delivery. The clinical features are fulminant pneumonia, meningitis or sepsis. LOD can occur after the first week of life and may be acquired from the mother at birth or later from other individuals. It is characterized by

bacteremia, meningitis and septic arthritis [6]. Krémery and Paradise observed GBS meningitis in LOD after 14 days of hospitalization in five of 101 cases [7].

Screening for GBS in pregnant women and targeted intrapartum antibiotic prophylaxis in whom identified as carriers are key elements to prevent EOD [8-10]. However, methods of specimen collection and analysis may affect the reliability of the results and subsequent treatment [11-13]. Too many cases of neonatal sepsis caused by GBS still occur in newborns of women not undergoing antibiotic prophylaxis: indeed, the lack of compliance with guidelines for specimen collection and culture may lead to false negative results.

In 2007, we performed GBS screening cultures on 28491 pregnant women of Piedmont and Aosta valley, and we found a rate of positive cultures which ranged between 2.4% and 22.6% in single laboratories. The purpose of the study was to investigate methods of GBS collection and culture in all the laboratories of these two Italian regions, in search of a correlation which could explain the considerable rate variability.

Material and Method

In 2007 all birth files were analyzed through a birth registration database. It was not necessary to seek the advice of the ethics committee because the study did not affect the normal care procedures.

Screening vaginal or vaginorectal swabs and culture results were performed at the 35th gestational week to all pregnant women who gave birth in Piedmont or Aosta Valley hospitals.

In spring 2008 we sent by post to all 70 public and private microbiological laboratories of Piedmont and Aosta Valley a file card regarding clinical tests performed in 2007 for GBS search in pregnancy and usually culture methods. The requested items for each laboratory were:

1. Sample technique and number of samples per year.
2. Typology of culture procedures: selective, chromogenic or broth.
3. Incubation atmosphere of cultures.
4. Identification systems.
5. Lay out of antibiotics sensitive tests.
6. Use of internal and external quality check.
7. Report standard: qualitative, half-quantitative, quantitative.

Four months after posting the file card, we performed a phone reminder to the laboratories which did not reply. A last reminder was posted two months later.

Results

We contacted 70 laboratories, 23 did not reply at all. Among the 47 responders, 16 provided partial results: five returned not interpretable data for the scope of research, four did not have a microbiology section, three laboratories sent all data except positivity percentage, and four only survey data, omitting answer to further inquiries; evaluable but partial data from these 16 labs were 6316.

Thirty-one labs provided all requested data, accounting for 22175 tests, 57.5% of the 38565 births recorded in Piedmont and Aosta Valley in 2007. Overall, 28491 (22175+6316) evaluable tests were collected: (24.36%) for vaginal, and (75.64%) for vaginorectal swabs. The average positivity percentage for GBS stands at 12.7% (CI ± 95%: 12.2-13.1) with a huge variability within each laboratory (range 2.4-22.6%). In 4183 cases (14.6%), GBS culture was positive.

During data analysis, a lack of homogeneity related to search methods for GBS in the different laboratories has been evidenced. In particular, reasons for such variability were:

1. Routine use of selective culture composed by Columbia agar + 5% blood + colistin-nalidixid acid (CNA), was related to 78% of the laboratories, accounting for 84% of swabs analyzed; 13% of laboratories utilized non selective culture with addition of 5% sheep blood (on 7% of total swabs); only 9% of the laboratories made use of chromogenic culture (9% of total swabs);
2. 35% of the laboratories, which analyzed 44% of total swabs, resorted to broth enrichment over that time;
3. 67% made use of CO₂ to 10% in controlled atmosphere (on 75% of swabs); 24% of the laboratories (on 19% of swabs) utilized incubation in anaerobiosis; 9% of the laboratories used incubation in ambient atmosphere (on 6% of swabs).

The percentages of positivity detected, be compared with other data analyzed, showed significant variability, resulting in then use, or not, of the enrichment broth. This variability has allowed us to divide the laboratories, on the basis of three bands for positivity rate that are reported in the following three tables: (Tables 1-3).

Lab. n°	(1) tipo	% vag. swabs	%vag./rect. swabs or vag+rect swabs	Total invest ig.	% pos	(2) Mediu m	Enrich m. broth	(3) Incub ation atmo sphere	(4) ID
10	a	20%	80%	1731	4.39 %	a + c	no	a	a
18	c	0%	100%	945	6.90 %	a	no	a	b
24	c	81%	19%	420	7.38 %	c + d	no	c	b
30	c	32%	68%	296	5.84 %	a	no	Data received	not
31	c	32%	68%	135	6.30 %	a	no	a	b
37	c	81%	19%	415	6.40 %	a	no	c	b
43*	c	0%	100%	415	2.40 %	a + b	no	a	a + b
57*	c	97%	3%	667	2.00 %	a + c	no	a	a + b
Tot. e%		36%	64%	5024	4.95 %		no 100%		

a: S.O.C. (Operational Structure Complex); b: S.O.S.(Operational Structure Simple)
c: Sector of the general Lab. of Clinical pathology

Table 1: Laboratories with positivity <10% in the investigation of screening.

Lab. No.	(1) tip o	% vag. swabs	%vag./ rect. swabs or vag+rect swabs	Total investi g.	% pos	(2) Mediu m	Enric m. broth	(3) Incub ation atmo sphere	(4) ID
1	c	95%	5%	1147	11.4 3%	a	yes	a	a
4	a	75%	25%	1717	10.0 7%	a + c	yes	b	a + b
8	b	0%	100%	23	14.6 0%	a	no	b	a + b
9	c	0%	100%	2668	14.2 0%	a + c	no	a	a
12	b	0%	100%	1009	12.8 0%	a	no	a	b
15	b	0%	100%	876	14.2 7%	a + c	no	a	b

17	c	0%	100%	946	14.0 0%	a	yes	a	b
19	c	0%	100%	180	12.2 0%	a + c	no	a	a
28	b	20%	80%	158	13.8 0%	a + c	yes	a	b
38	a	0%	100%	800	11.0 0%	b	no	c	b
41	b	46%	54%	435	12.0 0%	a	yes	a	a + b
48*	c	94%	6%	237	14.3 5%	b	yes	a	b
49*	c	75%	25%	112	12.0 0%	b	no	b	b
67*	c	91%	9%	110	10.0 0%	a	no	b	b
Tot. e%		29%	71%	10418	12.6 1%		no 55.46 %		

a: Blood agar Columbia CNA ; b: Chromogenic media
c: Blood agar; d: other culture media

Table 2: Laboratories with positivity between 10% and 15% in the investigation of screening.

La b. no	(1) tipo	% vag. swab s	%vag./ rect. swabs or vag +rect swabs	Total invest ig.	%	Lab . no	(1) tipo	% vag. swa bs	%vag./ rect. swabs or vag +rect swabs
6	b	99%	1%	683	15.60 %	a	no	b	b
11*	c	0%	100%	200	20.00 %	a	yes	a	a
14	b	0%	100%	602	16.80 %	a + c	no	b	a + b
25	c	19%	81%	1263	19.00 %	c	yes	a	a
34	b	80%	20%	842	17.30 %	c + d	yes	a	b
42	b	6%	94%	1534	19.20 %	a	yes	a	b
55*	c	0%	100%	490	20.00 %	a	no	a	b
61*	c	87%	13%	145	22.60 %	a	yes	a	a + b
70	c	1%	99%	974	19.42 %	a	yes	b	b
Tot. e%		27%	73%	6733	20.05 %		no 26.3 0%		

a-18-24 h incubation time at 35-37°C in CO₂; b-18-24 h incubation time at 35-37° anaerobically

c-18-24 h incubation time at 35-37° aerobically

Table 3: Laboratories with positivity >15% in the investigation of screening.

GBS cultures of laboratories which made use of broth enrichment had a GBS positivity of 15.49% (CI ± 95%: 14.78-16.23%; range 10.7-22.60%). Analyzed samples without broth enrichment showed a positivity of 10.53% (CI ± 95%: 10-11.08%; range 2.40-20%) (Figure 1).

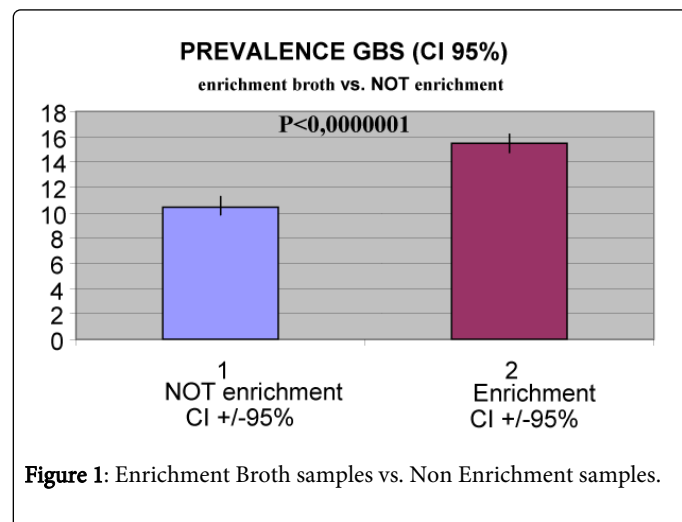


Figure 1: Enrichment Broth samples vs. Non Enrichment samples.

The difference between the two methods was statistically significant ($p < 0.0000001$) with χ^2 test.

No significant statistical evidence was noted for other investigated indicators.

Conclusion

Prevention of EOD is primarily focused on the identification of risk factors and carrying out of cultural screening of women in childbirth [1] and on the efficacy of intrapartum antimicrobial prophylaxis thereafter [10].

The Centers for Disease Control and Prevention (CDC) developed consensus guidelines to reduce the rate of GBS neonatal disease [11]. These guidelines suggested two alternative approaches: the first concerned antibiotic prophylaxis for patients with one or more risk factors for GBS neonatal transmission; the second was based on universal screening of all pregnant women between 35 and 37 gestation weeks through vaginorectal cultures. The key element of a screening-based prevention strategy is a careful identification of colonized parturients who should then be targeted with intrapartum antimicrobial prophylaxis. Infants born to women prenatally identified as GBS carriers, indeed, had 29 times the risk of EOD than infants born to women with negative cultures. Prematurity, rupture of the membranes ≥ 18 hours before delivery and intrapartum fever were about seven times more likely to be complicated by EOD. The guidelines also suggested some modifications encouraging the use of antibiotics with narrower spectrum to reduce induction of resistance and to improve the predictive value of screening cultures.

To detect the incidence and existing policies for prevention of GBS infection in Europe, a questionnaire was sent to all members of the European Society for Paediatric Infectious Diseases and to all delegates

of the European Association of Perinatal Medicine in 1999. The reported incidence of GBS colonization among pregnant women in Europe ranged from 1.5 to 30% [12].

The efficacy of cultural methods for GBS search is often dependent on several variables of accuracy operations which include

1. Period in which the survey was conducted. The most suitable to provide sensibility and specificity in detection women, who remain colonized until delivery is between 35th and 37th gestation week.
2. A preanalytical phase characterized by accuracy of sample collection. This can be carried out with only one swab, taking together vaginal and rectal specimens, or with two distinct swabs. Double swabbing improves the efficacy of microbiological survey. Such swabs, put in non-nutritive culture (Amies or Stuart's) keep viability of GBS for four days at room temperature. Badri et al. demonstrated that the higher incidence of positive rectal swabs in comparison with vaginal cultures suggests that the gastrointestinal tract is the primary site of GBS colonization and vaginal colonization may represent a contamination [14].
3. Analytical phase, whose efficacy, in part conditioned by used materials, depends particularly on adherence to protocols, as well as accuracy of operators who conduct the microbiological survey. Utilizing one swab for both sample taking, or two swabs, one for each place, does not influence diagnostic efficacy of the survey. The site of GBS isolation does not represent significance for clinical management of an eventually positivity. For both swabs is it recommendable utilizing a single enrichment broth and selective culture, as both sites present a resident microbial population, whose growth could inhibit GBS, with consequent false negative results.
4. Post analytical phase, consisting in submitting GBS isolated strains to antimicrobial susceptibility testing.

Several studies have documented that the accuracy of prenatal screening cultures in identifying intrapartum colonization status can be enhanced by collection of cultures between 35 and 37 gestation weeks, swabbing the lower vagina and rectum and using selective enrichment broth to maximize the isolation of GBS [11].

A previous study by Vergani et al. [8] demonstrated that the optimal strategy to prevent GBS disease could not be established. They compared neonatal mortality and morbidity rates associated with EOD in three periods characterized by different prevention strategies. From 1/1987 to 12/1990, no screening for GBS during pregnancy, nor standardized chemoprophylaxis; from 1/1991 to 12/1994, antibiotic prophylaxis only with risk factors for GBS; from 1/1995 to 12/1999, universal screening for GBS with vaginorectal cultures and chemoprophylaxis for women with positive results or risk factors. In fact, the adoption of universal screening is associated with a reduction in rate of EOD compared with the risk factor policy. The results of this study show that the wide difference of results can be attributed to GBS culture technique, particularly to non-enriched broth. A false negative vaginal swab, therefore, can be misleading in intrapartum antibiotic prophylaxis decision making. Pinto et al. reported that from 92 infants with EOD, prenatal culture for GBS colonization was performed only in 22 women [15]. Of the 18 negative cultures for GBS, appropriate selective culture media had been used in only six patients and only three of these were obtained within six weeks before delivery. In 15 cases, GBS swabs were collected more than six weeks before delivery or obtained using suboptimal culture technique. Both the timing of

specimen collection and a correct analytical process according to guidelines appear mandatory to improve the reliability of screening test.

A recent Italian experience by Berardi et al. pointed out that during the study period, 2003-2005, confirmed EOD occurred 30 times [16]. Maternal cultures were available for 22 cases, 17 screened mothers were GBS negative and five positive. During 2003, optimal culture methods were available from only one of 26 laboratories, but at the end of the study, the number raised to 12/26. It's important to emphasize that among 17 screened negative mothers, culture methods were optimal in only one of 17 subjects.

In a recent study, Pulver et al. concluded that out of 45 women who delivered infants with EOD, 79% cultures obtained during routine prenatal care were negative [17]. The possible explanations for negative screens are attributable to inappropriately collection technique, but microbiological culture methods were not described. Many factors, such as improper conservation, culture technique, sample transportation and intermittent elimination of bacterium in carriers can influence the negativity of a recto-vaginal swab [18]. Van Dyke et al. highlighted that screening more than five weeks before childbirth, collection of specimens, processing of cultures, transient colonization of GBS, reporting and recording of screening results are part of factors which may contribute to false negative results [19].

Our results show how most of the "false negative" cultures are related to non-observance of microbiological guidelines, particularly as far as broth enrichment is concerned. Standardization of microbiological methods in the different realities is warranted, in order to get more comparable data, and to improve reliability of results.

New scenarios are now opening through the availability of rapid screening test based on NAAT (nucleic acid amplification test), whose preliminary results are very encouraging [20,21]. At this moment, however, broth-enriched culture of vaginorectal specimens as prepartum screening is still the golden standard for the prevention of neonatal GBS infections. Rapid NAAT methods can be an alternative during peripartum for women who did not undergo such a screening [22].

However, to date, it is not possible to come to a definitive conclusion, as you will need to perform a new study to demonstrate the difference in GBS culture positive by both methods in the same settings for the same laboratory conditions, as the same methodology of sample collection and the simultaneous treatment of the same.

It is also important to remember that, the development of vaccines against GBS in the next future could essentially modify the epidemiology of GBS carriers and the clinical approach to pregnant women and newborns.

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