Group B streptococcal bacteriuria during pregnancy as a risk factor for maternal intrapartum colonization: a prospective cohort study

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Abstract

Purpose. Current evidence is inconclusive regarding the intrapartum administration of chemoprophylaxis, merely based on the presence of group B streptococcal (GBS) bacteriuria of any colony count, in the prevention of early-onset neonatal GBS infection. The aim of this study was to assess whether GBS bacteriuria is a risk factor for intrapartum colonization (IPC) regardless of urinary concentration or the results of late third-trimester rectovaginal screening cultures (RVSCs).

Methodology. Six hundred and eight pregnant women, with urine specimens cultured between May 2011 and May 2013, were enrolled in this prospective cohort study. RVSCs were available for 582 women and intrapartum rectovaginal cultures for 246.

Results. The prevalence of GBS bacteriuria and positive RVSCs was 10.8 and 16.5 %, respectively. The frequency of IPC was 15.9 % (39/246). Sensitivity, specificity, positive and negative predictive values of urine culture and of RVSC in predicting GBS IPC were 41, 94.7, 59.3 and 89.5 %, and 76.9, 95.4, 76.9 and 95.4 %, respectively. GBS bacteriuria was significantly associated with IPC, overall [relative risk (RR) 5.6] and in women with negative RVSC (RR 8.5), with bacteriuria <10^c.f.u. ml^{-1} (RR 5.9) or when both circumstances coexisted (RR 8.9). The urinary colony count was <10^c.f.u. ml^{-1} in 13 of the 16 women with GBS bacteriuria and IPC.

Conclusion. GBS bacteriuria is a risk factor for IPC, irrespective of urinary GBS concentration or of colonization status at late gestation. Therefore, microbiology laboratories should search, and report, GBS of any colony count in urine from pregnant women, and not only in the presence of ≥10^c.f.u. ml^{-1} as the 2010 CDC guidelines recommend.

INTRODUCTION

*Streptococcus agalactiae* or Lancefield group B *Streptococcus* (GBS) is the leading cause of early-onset infectious disease among newborn infants in developed countries. GBS are Gram-positive cocci, generally β-haemolytic, whose main virulence factors are the polysaccharide capsule (which interferes with phagocytosis) and the production of haemolysin. In healthy adults, the primary reservoir of GBS is the gastrointestinal tract, which is the main source of colonization in pregnant women. Rectovaginal carriage can be transient, intermittent or persistent [1]. According to published data, the rate of vaginal or rectal colonization by GBS among pregnant women in Europe ranges from 16 to 22 % [2]. Early-onset infections are acquired vertically through exposure of the foetus to the micro-organism when passing through the birth canal. Colonization by GBS occurs, in the absence of intrapartum antibiotic prophylaxis, in approximately 60 % of infants born to colonized mothers and, in the absence of preventive measures, an estimated 1–2 % of them will develop early-onset infection, characterized by pneumonia, sepsis or meningitis [3].

Keywords: group B *Streptococcus* bacteriuria; colony count; third-trimester rectovaginal colonization; risk factor; intrapartum colonization.
Intravenous administration of prophylactic treatment with penicillin or amoxicillin, at the time of labour onset, to all GBS-carrier pregnant women and to those presenting risk factors (prematurity, prolonged rupture of membranes, intrapartum fever, previous delivery of an affected baby) and whose colonization status is unknown, is considered the most effective approach to preventing early-onset disease in the newborn; actually this is the strategy currently recommended by the CDC and also by the European Guidelines elaborated by a group of experts from representative European countries [4]. In order to identify colonized women, universal GBS antenatal screening of rectovaginal samples must be performed between 35 and 37 weeks of gestation [5, 6]. Intrapartum antibiotic prophylaxis is also recommended for women with GBS bacteriuria during pregnancy, as this condition is regarded as a sign of heavy genital tract colonization. The CDC 2002 guidelines prompted microbiology laboratories to report the isolation of any colony count of GBS in urine [5], while the recommendation of the 2010 CDC revised guidelines is to screen and report urine samples only for the presence of GBS at concentrations of $\geq 10^4$ c.f.u. ml$^{-1}$ in pure or mixed culture, arguing that it is unclear whether identification of low colony-count bacteriuria is cost-effective [6]. In spite of that, in a consensus document published in 2013, the Spanish Societies of Obstetrics and Gynaecology, Neonatology, Infectious Diseases and Clinical Microbiology still advocate reporting the presence of GBS in urine at any concentration and considering all women with GBS bacteriuria during the current pregnancy, regardless of bacterial count, candidates to receive antibiotic intrapartum prophylaxis without the need for screening for GBS colonization in the late third trimester [7]. The same advice is given by the European guidelines, although without referring to a definitive urinary GBS count threshold [4].

Nevertheless, some authors state that there is a lack of evidence to recommend intrapartum chemoprophylaxis to a woman simply for having had GBS bacteriuria detected during pregnancy, given that in a considerable percentage of cases she will be not colonized at the end of gestation [8, 9]. Indeed, in a study conducted at our laboratory in 2006, only 60 % of pregnant women with GBS bacteriuria were found to have positive rectovaginal screening cultures (RVSCs) at 35–37 weeks’ pregnancy and, interestingly, there were no significant differences when results were stratified by GBS colony count [8]. As far as we are aware, only one study to date has gauged the accuracy of GBS bacteriuria in predicting intrapartum colonization (IPC), but this work only included women that had GBS isolated from urine samples (without providing data about the colony count) and, moreover, their colonization status at late gestation was unknown [10]. Therefore, we considered it pertinent to undertake a study aimed at assessing the actual risk of IPC among women with documented GBS bacteriuria at any time of pregnancy and at any concentration, but having negative RVSCs at the end of the third trimester of gestation in order to evaluate whether administration of intrapartum chemoprophylaxis is justified in those cases.

The objective of this prospective cohort study is to investigate whether pregnant women presenting GBS bacteriuria during pregnancy are at an increased risk of intralabour GBS colonization, irrespective of the colony count in urine samples or of the results of RVSCs performed at 35–37 weeks’ gestation.

**METHODS**

**Patients and design**

Between May 2011 and May 2013, 608 pregnant women (average age 32 years, range 20–46 years) whose urine samples had been sent to the microbiology laboratory of the Hospital Verge de la Cinta (Tortosa, Spain), for systematic research of asymptomatic bacteriuria or because of clinical suspicion of urinary tract infection and who gave written informed consent were initially enrolled in this prospective cohort study. The final cohort consisted of 246 women who gave birth at the Hospital Verge de la Cinta and who had GBS rectovaginal cultures performed on admission for delivery. The results of RVSCs at 35–37 weeks’ gestation were available for 237 of those 246 women and, overall, for 582 of the 608 originally recruited.

**Microbiological methods**

Urine samples and rectovaginal swabs were stored refrigerated for up to 24 and 48 h, respectively, from their collection until being processed following the standard microbiologic procedures established at our laboratory for GBS detection: (1) rectal and vaginal specimens, obtained at 35–37 weeks’ pregnancy or at the time of labour onset, were collected using Dacron swabs that were immediately inserted into Amies transport medium. Specimens were inoculated into antibiotic-supplemented Todd–Hewitt selective enrichment broth (bioMérieux, Marcy l’Étoile, France) that was incubated for 18–24 h at 35–37 °C, followed by subculture on Granada agar plates (Biomedics, Madrid, Spain). Agar plates were incubated for 24–48 h at 35–37 °C under an anaerobic atmosphere. (2) Urine specimens: 10 µl of urine was inoculated onto CPS chromogenic agar plates using a calibrated agar loop and another 10 µl was inoculated onto a Granada agar plate. CPS plates were incubated for 18–24 h at 35–37 °C in an aerobic atmosphere, and Granada plates were incubated for 24–48 h at 35–37 °C in an anaerobic atmosphere.

A sample was considered positive for GBS if it grew the characteristic orange-to-red colonies on Granada plates or if it yielded turquoise, violet or pale pink colonies that were able to agglutinate with GBS-specific antisera (Streptokit, bioMérieux, Marcy l’Étoile, France) on CPS medium.

**Statistical analysis**

The results of categorical variables were expressed as percentages and their corresponding 95 % confidence interval (CI). Differences in proportions were compared using
either the chi-square test or Fisher’s exact test. Differences were considered significant at $P \leq 0.05$. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both antenatal RVSCs and urine cultures were calculated to evaluate their accuracy in predicting GBS colonization at the time of delivery. The association between the presence of GBS bacteriuria or positive RVSCs and IPC by GBS was assessed by means of relative risk (RR) and was considered significant if the entire 95% CI was above 1.

**RESULTS**

**Occurrence of GBS bacteriuria and GBS rectovaginal carriage at 35–37 weeks’ gestation**

The prevalence of GBS bacteriuria was 10.8% (66/608; 95% CI 8.5–13.6%) among the 608 pregnant women initially enrolled in this study, and 11% (27/246; 95% CI 7.4–15.6%) among the 246 with complete data. Only six women in the latter group presented urinary bacterial counts $\geq 10^4$ c.f.u. ml$^{-1}$. Sixty-four of the 66 women presenting urinary colonization had GBS isolated from the sample collected for systematic research of asymptomatic bacteriuria, while in the remaining two, GBS were recovered from a subsequent specimen referred to the laboratory because of a suspected urinary tract infection.

Overall, the prevalence of rectovaginal GBS carriers at 35–37 weeks’ gestation was 16.5% (96/582; 95% CI 13.6–19.8%) and 16.4% (39/237; 95% CI 12–21.8%) for those women who finished the study and for whom this result was available, respectively. Given that we could not find statistically significant differences between the prevalence of GBS bacteriuria or of late third-trimester GBS rectovaginal colonization among pregnant women initially recruited for the study and those who finished it, we assume that the latter were representative of the whole.

**Accuracy of urine culture and RVSCs in predicting intrapartum GBS colonization**

The results obtained from urine cultures and rectovaginal cultures performed at 35–37 weeks’ gestation and at time of delivery in the 246 pregnant women making up the final cohort are shown in Fig. 1.

Sensitivity, specificity, PPV and NPV of urine culture, relative to GBS colonization at delivery, were 41, 94.7, 59.3 and 89.5%, respectively, when considering a positive result to be any colony count of GBS; and 7.7, 98.6, 50 and 85%, respectively, when the threshold was established at $\geq 10^4$ c.f.u. ml$^{-1}$. The corresponding values for prenatal RVSCs were 76.9, 95.4, 76.9 and 95.4%. Global figures, as well as those stratified on the basis of rectovaginal status colonization at 35–37 weeks’ gestation for urine cultures and on the basis of the presence of GBS bacteriuria for RVSCs, are summarized in Table 1.

**Relative risk of GBS IPC in women presenting bacteriuria during pregnancy or in women presenting positive RVSC**

The overall frequency of IPC by GBS in the final cohort was found to be 15.9% (39/246; 95% CI 11.5–21%). Rectovaginal cultures performed at labour were positive in 59.3% (16/27) of women experiencing GBS bacteriuria and in 10.5% (23/219) of those not experiencing GBS bacteriuria during pregnancy; therefore, GBS bacteriuria at any time during the current pregnancy increased by 5.6-fold (95% CI 3.9–8.9) the risk of intrapartum colonization by GBS. Stratified analysis of data demonstrated that the increase in risk was significant, regardless of GBS carriage status at 35–37 weeks’ gestation or of GBS colony count in urine samples (Table 2). In fact, in the subset of women with negative RVSCs, GBS bacteriuria increased by 8.5-fold the likelihood of GBS IPC and 8.9-fold when considering only those with GBS urinary colony count $< 10^4$ c.f.u. ml$^{-1}$.

The overall RR of IPC in women with positive rectovaginal cultures at late gestation was 17.1 (95% CI 8.8–33), increasing to 21.2 (95% CI 9.2–48.7) in those without GBS bacteriuria during the current pregnancy, while the figure in women having had GBS isolated from urine samples was 3.4 (95% CI 1.3–9) (Table 3).

**DISCUSSION**

Although some authors have questioned the strategy based on universal maternal GBS culture screening and systematic intrapartum antibiotic administration to all colonized pregnant women to prevent invasive early-onset GBS disease, arguing that these practices are not supported by conclusive evidence [11, 12], numerous studies have demonstrated a dramatic decline in the incidence of this clinical entity following the implementation of the above-mentioned recommendations [13–17]. Nevertheless, considerable controversy has arisen concerning the specific recommendation for including maternal GBS bacteriuria at any point during pregnancy, and particularly for any bacterial count, as an indication for intrapartum prophylaxis. It should be noted that most works evaluating the role of GBS bacteriuria as a risk factor for early-onset neonatal infection are derived from women with significant bacteriuria [18–20]. Moreover, one study which found an increased risk among infants born to women with low colony-count GBS bacteriuria might have had a problem of biased selection [21]. Indeed, the 2010 CDC revised guidelines (unlike the 2002 CDC guidelines [5] and other national guidelines [7, 22]) states that a laboratory should report GBS in urine specimens when present at concentrations of $\geq 10^4$ c.f.u. ml$^{-1}$ in pure culture or mixed with another organism. The reasons adduced to justify this proposal are the lack of information about how much additional disease is prevented by searching for low colony-count GBS bacteriuria and the increase in workload that it represents [6]. On the other hand, in a previous study carried out in our health region we demonstrated that, although GBS bacteriuria was associated with rectovaginal
colonization at 35–37 weeks’ gestation even at concentrations of GBS <10⁴ c.f.u. ml⁻¹, up to 40 % of women presenting GBS bacteriuria were not colonized at the end of gestation [8].

The current work – aimed at clarifying the impact of GBS bacteriuria on maternal IPC and, consequently, on newborn colonization – is, to the best of our knowledge, the first prospective study that provides analysis of data stratified by GBS urinary count and by results of late third-trimester RVSCs. The results presented here prove that isolation of any colony count of GBS from urine during gestation is significantly associated with GBS IPC, both in rectovaginal carriers at 35–37 weeks’ pregnancy and in non-carriers, and, accordingly, all women presenting GBS bacteriuria should receive intrapartum chemoprophylaxis.

The prevalence of GBS bacteriuria among the study population of pregnant women (10.8 %, with 2.5 % presenting significant bacteriuria) was comparable to that reported by other authors [23, 24]. Likewise, the prevalence of rectovaginal GBS colonization at 35–37 weeks’ gestation (16.5 %) was similar to that found by other investigations conducted in our geographical setting [2, 25] and in a previous study conducted in our health region in 2006 [8].

With regard to the accuracy of prenatal cultures in predicting intrapartum GBS colonization, rectovaginal prepartum

Table 1. Accuracy of urine culture and of RVSCs at 35–37 weeks’ gestation in predicting IPC by GBS

<table>
<thead>
<tr>
<th>Urine culture (+ any GBS colony count)</th>
<th>Sensitivity (95 % CI)</th>
<th>Specificity (95 % CI)</th>
<th>Positive predictive value (95 % CI)</th>
<th>Negative predictive value (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subset positive 35–37 weeks’ RVSC</td>
<td>43.3 % (25.6–61.1 %)</td>
<td>88.9 % (68.4–109.4 %)</td>
<td>92.9 % (79.3–106.4 %)</td>
<td>32 % (13.7–50.3 %)</td>
</tr>
<tr>
<td>Subset negative 35–37 weeks’ RVSC</td>
<td>33.3 % (2.5–64.1 %)</td>
<td>95.8 % (92.9–98.6 %)</td>
<td>27.3 % (0.95–53.59 %)</td>
<td>96.8 % (94.3–99.3 %)</td>
</tr>
<tr>
<td>Total</td>
<td>41 % (25.6–56.5 %)</td>
<td>94.7 % (91.6–97.7 %)</td>
<td>59.3 % (40.7–77.8 %)</td>
<td>89.5 % (85.4–93.6 %)</td>
</tr>
<tr>
<td>Urine culture (+ GBS ≥10⁴ c.f.u. ml⁻¹)</td>
<td>7.7 % (1.6–20.9 %)</td>
<td>98.7 % (95.8–99.7 %)</td>
<td>50 % (11.8–88.2 %)</td>
<td>85 % (79.8–89.3 %)</td>
</tr>
<tr>
<td>35–37 weeks’ RVSC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subset positive urine culture</td>
<td>81.2 % (62.1–100.4 %)</td>
<td>88.9 % (68.4–109.4 %)</td>
<td>92.9 % (79.4–106.4 %)</td>
<td>72.7 % (46.4–99.1 %)</td>
</tr>
<tr>
<td>Subset negative urine culture</td>
<td>73.9 % (56–91.9 %)</td>
<td>95.8 % (92.9–98.6 %)</td>
<td>68 % (49.7–86.3 %)</td>
<td>96.8 % (94.3–99.3 %)</td>
</tr>
<tr>
<td>Total</td>
<td>76.9 % (63.7–90.2 %)</td>
<td>95.5 % (92.6–98.4 %)</td>
<td>76.9 % (63.7–90.2 %)</td>
<td>95.5 % (92.6–98.4 %)</td>
</tr>
</tbody>
</table>
Table 2. Relative risk of IPC by GBS in women presenting bacteriuria during the current pregnancy (global data and data stratified by colony count of GBS in urine specimens and by the result of third-trimester RVSCs

<table>
<thead>
<tr>
<th>Frequency of IPC</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td></td>
</tr>
<tr>
<td>Any urinary GBS colony count</td>
<td></td>
</tr>
<tr>
<td>&lt;10^4 GBS c.f.u. ml⁻¹</td>
<td>59.3% (16/27) 5.64 (3.9–8.9)</td>
</tr>
<tr>
<td>≥10^4 GBS c.f.u. ml⁻¹</td>
<td>61.9% (13/21) 5.89 (3.53–9.83)</td>
</tr>
<tr>
<td>Negative GBS bacteriuria</td>
<td>50% (3/6) 4.76 (1.96–11.57)</td>
</tr>
<tr>
<td>Negative RVSC</td>
<td></td>
</tr>
<tr>
<td>Any urinary GBS colony count</td>
<td></td>
</tr>
<tr>
<td>&lt;10^4 GBS c.f.u. ml⁻¹</td>
<td>27.3% (3/11) 8.5 (2.5–29.5)</td>
</tr>
<tr>
<td>Negative GBS bacteriuria</td>
<td>3.2% (6/187) 8.91 (2.17–36.52)</td>
</tr>
<tr>
<td>Positive RVSC</td>
<td></td>
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<tr>
<td>Any urinary GBS colony count</td>
<td></td>
</tr>
<tr>
<td>&lt;10^4 GBS c.f.u. ml⁻¹</td>
<td>92.9% (13/14) 1.37 (1.1–1.86)</td>
</tr>
<tr>
<td>Negative GBS bacteriuria</td>
<td>68% (17/25) 1.35 (0.98–1.85)</td>
</tr>
</tbody>
</table>

screening showed a PPV and a NPV (76.9% and 95.4%, respectively) that were equivalent to those communicated by other authors, ranging from 67 to 87% for PPV and from 88 to 96% for NPV [8, 26–31]. The PPV and NPV of GBS bacteriuria during the current pregnancy for our cohort were 59.3 and 89.5%, respectively. Edwards et al., in the only study that, as far as we are aware, has addressed this issue, reported a PPV of 61%, although they could not estimate the NPV since they only included women known to have had bacteriuria [9]. The variations in the predictive values of prenatal cultures could be attributed to differences in sample collection and culture methods, as well as to differences in the prevalence of GBS colonization among distinct populations [2]. In this sense, it is appropriate to point out that, as between 5 and 8% of GBS are non-haemolytic and non-pigmented [32], they could remain undetected in Granada plates but not in CPS chromogenic agar, which may have led to an underestimation of the rate of intrapartum rectovaginal colonization in our series.

Notwithstanding that the RR and the accuracy of late third-trimester RVSCs in anticipating IPC was higher than that of GBS bacteriuria (as noted by Edwards et al., even though they only performed rectovaginal screening at the end of gestation to women without GBS bacteriuria [10]), it is worth highlighting that the NPV of rectovaginal cultures dropped from 94.5 to 72.7% when considering only the subset of women having a positive urine culture in the current pregnancy. This could be explained by the intermittent nature of genital GBS colonization and by the greater probability of becoming colonized by 35–37 weeks’ gestation and delivery in women having had bacteriuria during pregnancy (which is a surrogate for heavy colonization [6]). Furthermore, the results of the present study demonstrate that, overall, maternal GBS bacteriuria during the current gestation increases the likelihood of IPC 5.6-fold and, what is more relevant, that GBS bacteriuria remains a significant risk factor for genital colonization at delivery in women with negative RVSCs (RR 8.5), colony count <10^4 c.f.u. ml⁻¹ (RR 5.9) or when both circumstances coexist (RR 8.9). In fact, 13 of the 16 women (81.2%) presenting GBS bacteriuria and intrapartum colonization had a urine colony count <10^4 c.f.u. ml⁻¹, whereas urine was the only positive antenatal sample in three of the 39 women (7.7%) found to be colonized at delivery, with two of them exhibiting colony counts as low as 10^3 and 1.5.10^3 c.f.u. ml⁻¹. The lower frequency of intrapartum colonization and the lower RR found in the subset of women with urinary GBS counts above 10^4 c.f.u. ml⁻¹ in comparison with the group of women with <10^4 c.f.u. ml⁻¹, although apparently paradoxical, could be the result of the eventual influence of antibiotic treatment (frequently prescribed to pregnant women with high-degree GBS bacteriuria) on IPC status.

Regarding the increased workload that exhaustive searching and identification of low-count GBS bacteriuria in all pregnant women means for a clinical microbiology laboratory, it could be simplified by inoculating urine samples from pregnant women on Granada agar plates, as well as on the usual non-selective culture medium. The main advantage of this medium is that it allows easy visual identification of pigmented GBS (red- to orange-coloured colonies), especially when the specimen contains a low number of GBS or when GBS is accompanied by other micro-organisms [23, 33].

Finally, we would like to note that assessment of the actual impact of the recommendations derived from the results presented here for the decrease in the occurrence of invasive early-onset GBS neonatal infection is beyond the scope of our research. On the other hand, it is worth mentioning that the relatively low PPV of antenatal cultures in identifying colonization at the time of labour implies that a
considerable number of women (7.7% in our cohort) would receive unnecessary antibiotic prophylaxis. Although currently there is no evidence to suggest an association between intrapartum antibiotic exposure and an increase in non-GBS sepsis or in infections due to ampicillin-resistant Gram-negative bacteria among newborn infants [6, 34, 35], continued surveillance of this subject is mandatory and it would be advisable to implement intrapartum antimicrobial prophylaxis based on universal intrapartum GBS screening using a rapid real-time PCR testing method, as advocated by the European consensus conference (4).

In conclusion, the results of the present study provide evidence to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures to support the recommendations urging laboratories to search and report GBS of any colony count in urine cultures.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References


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