Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of *Streptococcus agalactiae* (group B streptococci) in pregnant women

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Abstract

Development of rapid and sensitive detection methods for group B streptococci (GBS) in pregnant women remains useful in order to adequately identify pregnant women at risk of transferring GBS to their neonate. This study compared the CDC recommended sampling and culture method with two qPCR methods for detecting GBS colonization.

For a total of 100 pregnant women at 35–37 weeks of gestation, one rectovaginal ESwab each was collected. Eswab medium was inoculated into Lim broth, incubated for 24 h and plated onto chromID™ Strepto B agar (ChromAgar). DNA was extracted with the bioMérieux easyMAG platform, either directly from the rectovaginal ESwab or from Lim broth enrichment culture. Two different qPCR formats were compared, i.e. the hydrolysis probe format (Taqman, Roche) targeting the *sip* gene and the hybridization probe format (Hybprobe, Roche) targeting the *cfb* gene.

Both qPCR techniques identified 33% of the women as GBS-positive. Only one culture-positive sample was qPCR-negative. qPCR directly on the sample significantly increased the number of women found to be GBS-positive (27%) compared to culture (22%). Moreover, the sensitivity of qPCR after Lim broth enrichment (33%) was again significantly higher than qPCR after DNA extraction directly from the rectovaginal swabs (27%).

In conclusion, for prenatal screening of GBS from rectovaginal samples of pregnant women, our results are in accordance with CDC guidelines, which suggest using qPCR after Lim broth enrichment in addition to conventional (culture-based) detection. qPCR after Lim broth enrichment further increased the percentage of GBS-positive women, as detected by direct qPCR, from 27 to 33%, although the bacterial inoculum was low for these subjects.

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Keywords: Group B streptococci; qPCR; Lim broth; Chromagar; Pregnant women

1. Introduction

Group B streptococci (GBS), i.e. *Streptococcus agalactiae*, have remained the leading cause of bacterial sepsis and meningitis in neonates for the last two decades, despite a broadly implemented screen-and-treat policy. In Belgium,
13–25% of pregnant women are colonized with GBS, and GBS is responsible for 38% of early neonatal infections (Guidelines from the Belgian Health Council, 2003). The incidence of invasive neonatal GBS infection is reported to range from 0.5 to 3.0 per 1000 live births, with 4%—10% mortality associated with early-onset infections (Centers for Disease Control and Prevention, 2010; Schrag et al., 2000). GBS disease has two clinical presentations, both of which can cause significant morbidity and mortality: 1) early-onset disease, defined by onset within the first 6 days of life, accounting for 85% of neonatal GBS infections; and 2) late-onset disease, which occurs after the 6th day of life, with most infections occurring before 3 months of age (Baker and Edwards, 1995). Early-onset neonatal infection usually results from vertical transmission during delivery, or acquisition in utero just before labor, whereas late-onset disease is considered to be caused predominantly by horizontal transmission. Among colonized neonates, 1–3% develop disease, almost always in the form of early-onset infection within 24 h after birth (Davies et al., 1999). Guidelines from the Centers for Disease Control and Prevention (CDC) recommend that all women should be screened at 35–37 weeks of gestation and that those women found to be colonized with group B streptococci should receive intrapartum intravenous antibiotic prophylaxis either with penicillin G or ampicillin (Centers for Disease Control and Prevention, 2010), effective in reducing the incidence of early-onset neonatal GBS infections (Boyer and Gotoff, 1986). The standard method for diagnosis of group B streptococcal colonization comprises culture of combined vaginal and rectal samples in a selective broth medium, with subculture onto solid media, which is time-consuming, requiring 48–72 h (Schuchat, 1998).

Recently, alternative methods have become commercially available for the detection of GBS colonization. These include a novel chromogenic agar, i.e. chromID Strepto B (formerly Strepto B ID) agar or ChromAgar, which highlights GBS as red colonies after aerobic incubation (Dundas et al., 2008); Perry et al., 2006; Tazi et al., 2008), and Granada agar, which relies on pigment production revealed only under anaerobic conditions (de la Rosa et al., 1992). In addition, real-time PCR (qPCR) assays have been described. Ke et al. (Ke et al., 2000) developed a qPCR method based on amplification of a cfb gene fragment, and Bergh et al. (Bergh et al., 2004) established a qPCR targeting the sip gene. In this study, we compared the sensitivity of both qPCR formats, directly from samples and after Lim broth enrichment, with that of an optimized culture method, as published previously (El Aila et al., 2010).

2. Materials and methods

2.1. Study design

One-hundred consecutive women between 35 and 37 weeks of gestation, attending the prenatal clinic at Ghent University Hospital (with an average of 1200 deliveries per year) were enrolled in the study from June 2009 to January 2010. The study was approved by the Ethics Committee (IRB protocol nr 2007/096) of Ghent University Hospital. All women provided informed consent prior to collection of one rectovaginal Eswab per participant.

2.2. Collection and culture of specimens

Rectovaginal samples were collected using nylon flocked swabs that were submerged into 1 ml of ESwab transport medium (ESwab, Copan Diagnostics, Brescia, Italy), which was then further used. We used 200 μl of Eswab medium for DNA extraction, 200 μl for Lim broth and 50 μl for culture. Rectovaginal sampling was carried out by midwives by rotating an ESwab against the vaginal wall at the midportion of the vault. Subsequently, the swab was carefully withdrawn to prevent contamination with microflora from the vulva and introitus and the swab was inserted 1.5—2 cm beyond the anal sphincter and gently rotated to touch the anal crypts. All samples were transported to the Laboratory of Bacteriology Research within 4 h.

Direct plating was carried out by inoculating 50 μl from the ESwab transport medium onto ChromAgar (chromID™ Strepto B agar, BioMérieux, Marcy l’Étoile, France). The ChromAgar plates were incubated at 37 °C for 18–24 h in aerobic conditions in the dark. Another 200 μl was inoculated into tubes with 5 ml of Todd-Hewitt broth with 1% yeast extract, 15 μg/ml nalidixic acid and 10 μg colistin/ml (Lim Broth, Becton Dickinson, Erembodegem, Belgium), which were incubated aerobically at 37 °C and subcultured onto ChromAgar after overnight incubation. ChromAgar was examined for pale pink to red, round and pearly colonies.

2.3. DNA extraction

For DNA extraction, volumes of 200 μl were taken from: i) the ESwab transport medium of the rectovaginal sample; and ii) incubated Lim broth tubes. DNA extractions from the sample and from the Lim broth were performed using the NucliSENS easyMAG platform (BioMérieux) according to the manufacturer’s instructions (Dundas et al., 2008). Briefly, 200 μl of the ESwab transport medium, modified Amies transport medium, from the rectovaginal ESwab or 200 μl from the incubated Lim broth was added to 1800 μl for Lim broth was added to 1800 μl of elution buffer. Nucleic acids were eluted in 100 μl of elution buffer.

2.4. qPCR assays

GBS nucleic acid detection was based on two different qPCR assays, one targeting the cfb gene which encodes the CAMP factor (Ke et al., 2000) and the other targeting the sip gene which encodes the surface immunogenic protein Sip (Bergh et al., 2004). Primers and probes for sip and cfb genes were as described previously (Bergh et al., 2004; Ke et al., 2000). For detection of the sip gene, one hydrolysis probe (TaqMan Probe) was used for the qPCR assay (Bergh et al., 2004). The qPCR was
performed in a 20 µL reaction volume on the LightCycler® 1.2 instrument using the LightCycler® Taqman Master kit (Roche Diagnostics, Mannheim, Germany). Each reaction contained reagents to final concentrations of 0.5 µM of each primer and 0.2 µM of the hydrolysis probe, and contained 5 µl of DNA extract. Thermal cycling parameters consisted of 95 °C denaturation for 10 min, followed by 50 amplification cycles of 5 s denaturation at 96 °C, 10 s annealing at 58 °C and 1 s extension at 72 °C.

Detection of the cfb gene was done with a pair of fluorescently labeled adjacent hybridization probes, i.e. STB-F and STB-C (Ke et al., 2000). qPCR was performed in a 20 µL reaction volume on the LightCycler® v1.2 instrument using the LightCycler® FastStart DNA Master HybProbe kit (Roche Diagnostics). Each reaction contained reagents to final concentrations of 0.4 µM of each primer, 0.2 µM of each hybridization probe and 5 µl of DNA extract. Thermal cycling parameters consisted of 10 min at 95 °C for denaturation and activation of the Taq polymerase, followed by 45 cycles of 10 s denaturation at 95 °C, 10 s annealing at 55 °C and 5 s extension at 72 °C.

Tenfold serial dilutions of a DNA extract of type strain LMG 14694T were run to produce a standard curve. The lower limit of detection of the assays was determined as 100 copies of GBS genomic DNA per PCR reaction.

Positive controls (highly purified DNA from 5 × 10^8 GBS cells) and negative controls (HPLC purified water) were included in each run. Samples with discordant results (positive by qPCR and negative by standard culture) were investigated further by DNA sequence analysis of the amplification product.

2.5. Statistical methods

The sensitivities of qPCR assays and culture methods were compared by McNemar’s test and the Mann—Whitney U test, with p values of <0.05 considered as significant.

3. Results

During the last decade, several studies have demonstrated the utility of real-time PCR (qPCR) for rapidly and reliably detecting GBS colonization in (pregnant) women, as summarized in Table 1. Here we compared Lim broth with subculture on ChromAgar, which we had previously shown to be more sensitive than Lim broth and colistin nalidixic acid or Lim broth and Granada agar (El Aila et al., 2010), with two qPCR formats, both with and without Lim broth enrichment, for their ability to detect GBS in rectovaginal swabs from pregnant women at 35–37 weeks of gestation.

Among a total of 100 pregnant women tested, 66 were negative by both culture and qPCR, while 22 were positive by culture, 27 by direct qPCR and 33 by qPCR after Lim broth enrichment. The only sample that was positive by culture and negative by qPCR was culture-positive only after enrichment (Table 1, row: this study), similar to previous findings (Bergh et al., 2004). For the 21 samples positive for both culture and qPCR, both DNA directly from the sample and DNA extracted after Lim broth enrichment were qPCR-positive.

4. Discussion

4.1. Hybridization probes versus hydrolysis probe-based qPCR

To our knowledge, this is the first study comparing two different real-time PCR (qPCR) detection chemistries targeting two different genes for detection of group B streptococci (GBS), i.e. hybridization probe-based qPCR targeting the cfb gene and hydrolysis probe-based qPCR targeting the sip gene. We found complete agreement between the two PCR assays. The identical results with both assays can be taken as indicative of the robustness of the qPCR approach in general and of the fact that both PCR targets are adequate for detection of GBS. The only difference between the two assays was the Cq value. The cutoff Cq value for qPCR based on the cfb gene is ≥40, whereas the cutoff Cq value for qPCR based on the sip gene is ≥35. When the Cq values were in the range of 15–20, the difference in the Cq value between the two assays was 0 or 1; when Cq values were in the range of 20–30, the difference was 2–3.

4.2. Prevalence established by culture and qPCR

Although the prevalence of colonization in our study on the basis of Lim broth enrichment and subculture onto ChromAgar (22%) is in agreement with that reported in the literature on the basis of culture-based prevalence studies (El Aila et al., 2009; Hakansson et al., 2008; Schuchat and Wenger, 1994), we found a significantly higher number of women to be positive when direct qPCR was used (27%), in agreement with other studies that reported increases of 12%–21% of GBS-positives using direct qPCR (Davies et al., 2004; Convert et al., 2005; Rallu et al., 2006).

The number of GBS-positives established by qPCR after Lim broth enrichment (33) compared to subculture after Lim broth enrichment (22) is also in agreement with most other studies. Goodrich et al. (Goodrich and Miller, 2007) established GBS colonization for a total of 200 women after enrichment for 4 h in Lim broth, followed by culture, StrepB Analyte Specific Reagent (LightCycler qPCR) and the BD-StrepB test; they found colonization rates of 26.5, 29.5 and 30.0%, respectively. Rallu et al. (Rallu et al., 2006) reported that Lim broth-enhanced scpB PCR identified 37% of a total 605 rectovaginal specimens as GBS-positive, whereas subculture identified only 16% as positive. Block et al. (Block et al., 2008) found a higher rate of S. agalactiae-positives with carrot-broth-enhanced qPCR (33.0%) than with carrot broth culture and subculture (29.6%). However, Scicchitano et al. (Scicchitano and Bourbeau, 2009) found that both culture and the BD GeneOhm StrepB assay, both after Lim broth enrichment, detected 95.3% of the total number of GBS-positives.

The single sample for which culture was positive only after incubation for 48 h in Lim broth enrichment; even then, only a few colonies were observed after subculture. This indicates the presence of a very low inoculum, which may have been
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<th>C -/P+</th>
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Table 1
Results of different studies on qPCR tests for detection of GBS from vaginal, rectal and rectovaginal samples.
picked up only by chance by the culture, and which may have been missed during DNA extraction. We also started from cultured colonies to apply DNA extraction and qPCR so as to exclude the possibility that this strain was missed by the PCR approach due to primer mismatches. Strong amplification was observed, indicating that this strain was clearly picked up by both PCR approaches.

The lower sensitivity of culture might possibly be explained by the presence of antagonistic microorganisms such as enterococci, which inhibit or overgrow the GBS (Dunne and Holland-Staley, 1998). Also, antibiotics and feminine hygiene products have been shown to inhibit growth of GBS (Ostroff and Steaffens, 1995). Furthermore, inappropriate storage and transport conditions of the samples could give false-negative culture results (Rosa-Fraile et al., 2005). Moreover, non-viable organisms may be potential causes of discrepancy between negative culture and positive PCR.

In addition to greater sensitivity and less demanding sample storage and transport, as offered by PCR, qPCR provides the additional advantage of quantification, which may add important information, since heavy colonization with GBS has been shown to correlate with a higher risk of neonatal sepsis (Boyer et al., 1983). We found that samples with the highest Cq values, which can be assumed to have the lowest bacterial load, were also those that were culture-negative, indicative of a lower bacterial load, i.e. the mean Cq value for the 21 culture/PCR-positive samples was 21.5 (CI: 18.7–24.3), strongly indicative of higher bacterial load, whereas that for the 12 culture-negative PCR-positive samples was 26.5 (CI: 22.3–30.8), strongly indicative of a lower bacterial load in the latter (p < 0.05). For the 12 samples that were positive only by qPCR, the mean Cq value for the six samples that were also positive when DNA was extracted directly from samples was 22.6 (CI: 15.4–29.8), whereas the Cq value of the six samples that were only positive when DNA was extracted from Lim broth after enrichment was 30.3 (CI: 27.7–32.9), strongly indicative of lower bacterial load in the latter (p < 0.05). The initial bacterial load for the last six samples was probably so low that, even after enrichment, culture remained negative. This low initial load is also indicated by the fact that direct qPCR was negative; moreover, Cq values of qPCR after enrichment were high, meaning that the signal became positive only later, as seen for very low loads (in this case: even after enrichment). Fig. 1 presents the Cq values observed for these three categories of samples.

### 4.3. Direct qPCR vs Lim-broth-enhanced qPCR

Quantitative PCR may also offer the advantage of reduced time-to-results, making it useful as an intrapartum screening method (Bergseng et al., 2007; Davies et al., 2004; Gavino and Wang, 2007). However, in our study, qPCR after Lim broth enrichment detected GBS in 33 women, i.e. six more than detected by qPCR directly from rectovaginal swab (p < 0.05; McNemar’s test), indicating that combining qPCR with prior Lim broth enrichment significantly increases sensitivity compared to qPCR directly on the sample, in agreement with other studies. Maloney et al. (Maloney et al., 2004) reported that BD GeneOhm StrepB detected more GBS-positive women after Lim broth enrichment (no detailed data available). Block et al. (Block et al., 2008) concluded that broth-enhanced qPCR was more sensitive than direct swab PCR, although precise data were not provided. Hutchens and Schreckenberger (Hutchens and Schreckenberger, 2006) reported that the IDI-StrepB Cepheid StrepB SmartCycler assay had a sensitivity of 85.7% when performed directly on swab specimens compared to sensitivity after enrichment culture.

In conclusion, when detecting GBS directly from rectovaginal swabs, the use of qPCR irrespective of the qPCR assay (hybridization probe qPCR targeting the cfb gene or

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**Fig. 1.** Cq values of GBS-qPCR-positive rectovaginal samples using the hybridization probe assay (cfb gene). X-axis: C+: culture-positive, C−: culture-negative; D+: qPCR-positive, directly from sample, D−: qPCR-negative, directly from sample; Lim+: qPCR-positive only after Lim broth enrichment. Y-axis: Cq value of qPCR.
hydrolysis probe qPCR targeting the sip gene) increased the number of GBS-positive women compared to culture, and sensitivity was further increased by combining Lim broth enrichment with qPCR.

The use of overnight incubated Lim broth enrichment prior to qPCR significantly increases the sensitivity of GBS detection, but reduces the speed — as achieved when direct qPCR is carried out. This result warrants further studies to indicate which of the two techniques, i.e. intrapartum screening with a rapid technique (direct qPCR) or antepartum screening with what is currently the most sensitive approach (broth-enriched qPCR), is clinically most relevant.

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