MICROBIOLOGY

Evaluation of four chromogenic media for the isolation of Group B Streptococcus from vaginal specimens in pregnant women

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Summary

Direct culture on four commercial chromogenic media, selective for the detection of Group B Streptococcus (GBS), were compared with the conventional pre-enrichment Centers for Disease Control and Prevention (CDC) method for the ability to isolate GBS in 242 pregnant women's self-collected vaginal/perineal swabs. The sensitivities and specificities for direct culture on to chromogenic agar were 92% and 100% for StreptSelect (Bio-Rad Laboratories), 86% and 100% for Brilliant GBS (Thermo-Fisher Scientific), 94% and 100% for CHROMagar Strept (CHROMagar, Dultur Diagnostics), 86% and 100% for ChromID Strepto B (Biotrin), CDC recommendation of broth pre-enrichment then culture on blood containing selective agar had a sensitivity and specificity of 90.0% and 100% respectively. The chromogenic agar tested produced comparable results to the pre-enrichment CDC method.

Key words: Chromogenic agar, GBS, Group B Streptococcus, Streptococcus agalactiae

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INTRODUCTION

Group B Streptococcus (GBS) can cause serious infections in neonates, specifically early-onset sepsis, which can lead to severe morbidity and mortality. Vaginal/gastrointestinal colonization during pregnancy increases the risk of neonatal infection; however, screening and prophylactic treatment of the positive mother can reduce the risk of neonatal infection.1-3 Routine screening of pregnant women at 34–36 weeks gestation for GBS has significantly reduced the occurrence of GBS associated early-onset neonatal sepsis.4,5 Currently the Centers for Disease Control and Prevention (CDC) recommends placing a vaginal/rectal swab into an enrichment broth (Lim broth with colistin (10 μg/mL) and nalidixic acid (15 μg/mL)) incubated overnight. The enrichment broth was inoculated onto blood agar with colistin and nalidixic acid (CNA) when screening for GBS. This was the method used in our laboratory at the time of this study. The manufacturers claim the current newer and improved versions of chromogenic agar selective for GBS have the ability to detect non-haemolytic strains of GBS, which was a problem for the early agar. Recent studies comparing performance of chromogenic agar with enrichment culture have shown comparable sensitivity to methods without an enrichment step.6,7 The use of selective chromogenic media, without broth enrichment, may have acceptable sensitivity and specificity while reducing the turnaround time to identification of GBS by up to 24 hours.

Therefore we compared direct culture of vaginal/perineal swabs on to four chromogenic media (StreptSelect, CHROMagar, Brilliant GBS, ChromID Strepto B) for the presumptive identification of GBS to the current CDC recommended broth enrichment procedure and subculture on colistin-nalidixic acid agar with 5% sheep blood (CNA).8 The aim was to find a more efficient method that is as sensitive and specific as the CDC recommended method.

MATERIALS AND METHODS

From February 1 to May 2015 self-collected vaginal/perineal swabs from 242 patients were screened for GBS. Decoys swabs were used with Amity gel transport media. When self-collecting the swabs for GBS screening our patients were instructed to wash the vagina, then the perineum using the swab. Patient swabs were emulsified in 0.5 mL, 0.9% saline and vortexed for 5 s then 50 μL was inoculated onto whole plates of four chromogenic screening media: StreptSelect (Bio-Rad Laboratories, USA); Brilliant GBS (Thermo-Fisher Scientific, USA); Strept (CHROMagar, Dultur Diagnostics, USA); ChromID Strepto B (Biotrin, France). The remaining 250 μL of saline was transferred into Lim broth (10μg/mL, colistin). The plates and Lim broth were incubated at 35–37°C in air for 24 h (manufacturer’s recommendation), then 5 μL of Lim broth was inoculated onto CNA (CHROM, Australia), and incubated at 35°C in air for 18 h. All suspect colonies from all media were identified by the Vitek MS MALDI-TOF (bioMérieux). Isolates of GBS from any of the media tested was considered a true positive.

The Wilson score method without continuity correction was used to calculate confidence intervals. To eliminate media inhibition as a cause of false negatives rather than media sensitivity, the isolates were subcultured onto the media on which they had previously failed to grow.

RESULTS

A total of 242 prenatally self-collected vaginal/perineal swabs were tested for the presence of GBS. Of those, 50 (21%) were positive for GBS on at least one of the media tested. Brilliant GBS, StreptSelect and CHROMagar had sensitivities which were ≥92%, compared to the CDC recommended pre-enrichment/ CNA culture of 92% (95% confidence interval (CI) (81.16, 96.83%); ChromIDStrepto B had a lower sensitivity of 86% (95% CI 73.81, 93.05%) (Table 1). It was noted on Brilliant GBS and CHROMagar that there was a tendency for virens colonies to produce small, pale-coloured colonies that required extra testing as per the manufacturer’s recommendations.

All positive GBS isolates subcultured onto media where they initially failed to grow, resulted in subsequent growth, indicating the discrepancy was due to low numbers of organisms.

DISCUSSION

CDC guidelines for GBS screening were updated in November 2010. They included mention of new chromogenic agar to aid in the detection of GBS. Prior to this, detection of non-haemolytic strains (approximately 4% of total GBS isolates) were unreliable. As a consequence, the CDC recommended the use of an enrichment broth subcultured on blood agar with CNA when screening for GBS.

This trial intended to compare our existing method, Lim broth/CNA, which conformed to the CDC guidelines,9 of screening for GBS from antenatal patients with a proposed new procedure utilising direct inoculation onto four types of chromogenic media. Brilliant GBS had the highest sensitivity and specificity of inoculation compared to Strept and ChromID Strepto B produced similar results compared to pre-enrichment with CNA inoculation. No one method detected all positives.

One patient was GBS positive on subculture after Lim broth enrichment but negative by direct inoculation onto any chromogenic media. This probably reflects very low numbers of GBS present on the initial swab. However, there were four other swabs that were positive on the chromogenic agar but negative by the Lim broth/CNA method. Vaginal swabs were emulsified into 0.5 mL of saline solution (manufacturers recommendation). Therefore, low numbers of GBS were diluted across the four chromogenic agar plates, reducing recovery rates. The same patient using StreptSelect and CHROMagar were all from low yield specimens with less than four colonies isolated on positive plates. There were three false negative samples on ChromID Strepto B which had large yields on all other media tested (>10 CFU). This is consistent with the lower sensitivity of this agar found in this study.

Brilliant GBS suppressed the normal flora in 51% of all samples, which was at least 10% more than the other chromogenic agars tested. It was noted on this agar that Streptococcus faecalis and E. faecium formed false positive colonies which previously inoculation showed a 1.5% (total n = 15,404) increase in GBS isolation. Although our study size was small, the retrospective analysis of long term data using a similar method to Group B demonstrates direct inoculation onto Brilliant GBS produces comparable results to the conventional pre-enrichment CDC method.

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References


