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Screening Pregnant Women for Group B Streptococcal Colonization

Summary: The recovery rates of group B streptococcus (GBS) from anorectal swabs (RS) and vaginal swabs (VS) that were enriched were compared to the routine method to determine the optimal procedure. Separate RS and VS were collected from women attending antenatal clinics. RS and VS were placed in 2 ml enrichment and selective broth. Swabs were inoculated onto colistin/nalidixic acid agar (CNA) upon arrival in the laboratory and onto 5% sheep blood agar (SBA) and CNA after 24 h enrichment. The routine method consisted of a VS sent in transport medium and inoculated in the laboratory onto SBA (no enrichment). The overall GBS colonization rate was 24% (64/264). Of the 64 GBS carriers, 77% were colonized in the vagina and 89% were colonized in the anorectum. The anorectum was the only site of colonization in 24% of the women, whereas the vagina was the only site of colonization in 11% of cases. Enrichment increased the detection of GBS from both RS (55 versus 42; $P < 0.025$) and from VS (49 versus 27; $P < 0.001$). Of the 64 cases, enriched RS detected 86%, enriched VS detected 77% and the standard VS detected only 41%. Enriched RS and enriched VS collectively detected 99% of cases. SBA was better than CNA for subculture of the enrichment broth because of a higher recovery rate (98–100% versus 80–82%; $P < 0.01$) and the fact that the hemolysis on SBA made it easier to differentiate GBS from enterococci. The data confirm that optimal screening of pregnant women for GBS should include a combined RS/VS swab placed in enrichment broth that is then subcultured onto SBA after 24 h incubation.

Introduction

Streptococcus agalactiae (group B streptococcus, GBS) is a leading cause of perinatal infection. Neonates born to mothers who are colonized at delivery with GBS are the ones at risk of disease. Recommendations for the prevention of early-onset GBS infection using intrapartum chemoprophylaxis were recently released by the Centers for Disease Control and Prevention (CDC) [1] and jointly by the Canadian Pediatric Society and the Society of Obstetricians and Gynecologists of Canada [2]. The use of a single swab of the lower vagina and the anorectum transported to the laboratory in a selective broth medium and subcultured onto a selective solid medium was recommended for optimal recovery of GBS.

We wished to evaluate these recommendations in a prospective study to determine: (i) the recovery rate of GBS using an enrichment and selective broth transport medium (Todd-Hewitt broth supplemented with 15 μg/ml of nalidixic acid and 10 μg/ml of colistin) incubated for 24 h before subculturing onto solid media, in comparison with the regular prereduced anaerobically sterilized (PRAS) transport medium cultured directly onto solid media, (ii) the advantages and disadvantages of using a selective solid agar medium (Columbia CNA containing 15 μg/ml of nalidixic acid and 10 μg/ml of colistin) in comparison with 5% sheep blood agar (SBA) and (iii) if an anorectal swab alone is sufficient to detect all GBS-colonized women.

Materials and Methods

Pregnant women who attended the antenatal clinic at St. Boniface General Hospital in Winnipeg, Canada, from May 1995 through April 1996, had one anorectal and two vaginal swabs taken at 26–28 weeks of gestation. Vaginal and rectal swabs were obtained by sterile cotton-tipped swabs inserted 1–2 inches beyond the vaginal vestibule and the anal orifice, respectively. One vaginal swab was transported in prereduced anaerobically sterilized (PRAS) transport medium and, upon receipt in the microbiology laboratory, was subcultured onto 5% sheep blood agar (SBA) as per our standard protocol (Standard). The second vaginal and the anorectal swabs were each transported separately in enrichment and selective broth (ESB) composed of Todd Hewitt broth supplemented with 15 μg/ml of nalidixic acid, and 10 μg/ml of colistin, as described by *Fenton* and *Harper* [3]. Upon receipt in the laboratory each swab was mixed well in the ESB and then removed. The ESB was subcultured onto Columbia CNA containing 15 μg/ml of nalidixic acid and 10 μg/ml of colistin (direct). The ESB was incubated for 24 h at 37°C (enriched) and then plated onto SBA and CNA media. All solid media were incubated at 37°C and examined for GBS colonies at 24 and 48 h before being discarded as negative. *S. agalactiae* (GBS) was identified by colonial morphology, a negative PYR test (Sigma Chemical Company, St. Louis, MO), and a positive latex agglutination for GBS using the Patho DX commercial kit (Diagnostic Products Corporation, Los Angeles, CA).

Fisher's exact test (two-tailed) was used to compare proportions. A P value of less than 0.05 was considered to be significant.

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Table 1: Recovery rate of group B streptococcus (GBS) by the new methods in comparison with the standard method.[a]

Method of culture	Number of GBS-positive patients detected by the new method	Number of GBS-positive patients detected by the Standard method	P value
Direct (unenriched) vaginal	27 (3)[b]	26 (2)	NS[c]
Enriched vaginal	49 (23)	26 (0)	<0.0001
Direct (unenriched) anorectal	42 (18)	26 (2)	<0.0001
Enriched anorectal	55 (29)	26 (0)	<0.0001
Enriched anorectal and enriched vaginal	63 (37)	26 (0)	$<10^{-8}$

[a]Total number of GBS-positive patients detected by any method = 64 patients; [b] number in parenthesis = number of GBS-positive patients detected only by this method; [c]NS = not significant.

Results

A total of 274 patients was studied. Of these, 70 patients (25.5%) were positive for GBS by at least one of the five methods (standard vaginal, direct vaginal, enriched vaginal, direct anorectal and enriched anorectal). Six GBS-positive and four GBS-negative patients were excluded from the comparative analysis because one or more of the five test methods was not done. Of the remaining 264 patients, 64 (24.2%) were GBS positive by one or more of the five methods.

Group B streptococcus was detected by the direct (unenriched) vaginal and the standard vaginal methods in 27/64 (42%) and 26/64 (41%) patients, respectively. This indicated that direct plating of the enrichment and selective broth prior to incubation for 24 h (i.e. ESB used as a transport medium) was of no added value. The direct (unenriched) anorectal method detected 42/64 (66%) GBS-positive patients. Enriched anorectal and enriched vaginal methods detected 55/64 (86%) and 49/64 (77%) of GBS-colonized women, respectively (P is not significant). Enrichment (incubation of the ESB for 24 h at 37°C) resulted in an increase in the detection of GBS by both anorectal swabs (55 versus 42; $P < 0.025$) and by vaginal swabs (49 versus 27; $P < 0.001$). Enriched anorectal swabs detected 15 GBS-positive women that were not detected by the unenriched (direct) anorectal swabs, whereas the latter method detected 2 GBS-positive women that were not detected by the former method (15 versus 2; $P < 0.01$). Similarly, enriched vaginal swabs detected 22 GBS-positive women that were not detected by unenriched (direct) vaginal swabs, whereas no isolates were detected by the latter method only (22 versus 0; $P < 0.0001$). Of the 64 GBS-positive patients, 15 (24%) were colonized only in the anorectum and seven (11%) were colonized only in the vagina. Table 1 shows the frequency of GBS-positive patients detected by the standard vaginal method in comparison with the other four methods.

Of the 70 GBS-positive women, the results of SBA and CNA inoculated from 55 enriched anorectal and 50 enriched vaginal swabs were available. Of the 55 positive enriched anorectal swabs, 54/55 (98%) and 44/55 (80%) were detected on these two media, respectively ($P < 0.01$). Of the 50 positive enriched vaginal swabs, 50/50 (100%) were detected on SBA and 41/50 (82%) on CNA ($P < 0.01$). All 12 experienced technologists involved in the study preferred SBA over CNA for the detection of GBS because the readily detectable beta-hemolysis usually produced by GBS on SBA facilitated its differentiation from enterococci.

Discussion

The colon is the major reservoir of GBS. Female genital colonization with GBS is probably secondary to contamination from the anorectum [4, 5]. Early-onset GBS infection (occurring in newborns within the first 6 days of life) occurs only in newborns whose mothers are colonized with GBS in the vagina and/or anorectum [6]. In one study, women at parturition with negative vaginal but positive rectal GBS colonization had a 17% rate of vertical transmission of GBS to their newborn infants [6]. In the USA, 5 to 40% of all pregnant women are colonized with GBS in the vagina or anorectum [5–7]. Vertical GBS transmission rate ranges from 17%, in newborns of lightly colonized mothers, to 65% in those of heavily colonized mothers [6]. Of all infants born to colonized parturients, 1–2% will develop early-onset invasive GBS infection [8].

Studies from the 1970s to the 1980s showed that culture of the lower vagina and anorectum and the use of selective culture media increased the recovery rate of GBS [4–10]. Despite the data suggesting that prevention strategies could impact on GBS colonization and disease in newborns, the screening/prophylaxis recommendations have been controversial and not widely adopted [11–13]. The initial American and Canadian recommendations [1, 2, 14] focused primarily on risk factors and did not recommend intrapartum prophylaxis for women who carried GBS but who had no identifiable risk factors. Since up to 25% of GBS disease in newborns occurs where there are no identifiable risk factors, this approach was viewed as unsatisfactory. Additionally, intrapartum antimicrobial prophylaxis based solely on the presence of risk factors regardless of maternal GBS colonization status has been widely practiced, putting further into question the value of performing prenatal screening. There was also concern about the cost-effectiveness of GBS screening when the results of such screening did not impact on patient care. Subsequent American guidelines [12] recommend two algorithms for

prevention of early onset GBS disease in neonates. One employs prenatal screening at 35–37 weeks of gestation, with intrapartum chemoprophylaxis given to GBS carriers as well as for any pregnant women with risk factors. Current American [12] and Canadian [15] guidelines clearly indicate that the currently available rapid GBS screening tests are unsuitable and that culture using anorectal/vaginal sites and enrichment broth is the preferred approach. The cost-effectiveness of this approach has been supported by the CDC review of the literature [12]. The second algorithm is based on risk factors only aiming at those patients who may not have adequate prenatal care and hence no prenatal screening, as well as for those patients for whom prenatal screening results are unavailable. Despite these older studies and the current GBS prevention recommendations for screening pregnant women, few laboratories have incorporated selective enrichment broth into their procedures for GBS screening [14–16].

Our study provides prospective data to confirm the significant increase (Table 1) in detection of GBS colonization in pregnant women when the enrichment method is used for samples from both vagina and rectum (63/64) compared to the standard unenriched vaginal swabs (26/64) or enriched rectal swabs only (55/64). Although 2/64 GBS-positive samples were detected by the direct (unenriched) anorectal method and negative by the enriched method, this was probably due to overgrowth of other enteric bacteria over the 24 h enrichment. If screening for GBS were based on the unenriched vaginal swabs it is understandable why obstetricians would not widely adopt or rely on prenatal screening results, as over half of the carriers would be missed. Since the colon is the major reservoir of GBS, with the vagina likely to be colonized secondary to contamination from the anorectum [4, 5], one might have predicted that a single anorectal swab may have been sufficient for screening for GBS colonization. Indeed, the enriched anorectal swab was the single best method for detecting GBS colonization. However, our data demonstrate the value of sampling both the vagina and anorectum, since 24% of colonized women were rectal carriers only and 11% were vaginal carriers only.

Subculture of ESB to CNA compared to SBA missed 20% (11/55) and 18% (9/50) of GBS from enriched anorectal and enriched vaginal swabs, respectively. Additionally, the technologists overwhelmingly preferred SBA over CNA since hemolysis helped differentiate GBS from enterococci. Thus, subculture of ESB to SBA is recommended for optimal detection of GBS.

In conclusion, our data clearly demonstrate that the enriched vaginal/rectal swab method is highly superior to the unenriched method for detecting GBS, that SBA is superior to CNA for subculture of the enrichment and selective broth, and that although the enriched anorectal swab is the single best method for detecting GBS-colonized pregnant women, enriched anorectal swabs should be combined with enriched vaginal swabs for optimal recovery of GBS. From a practical point of view, albeit not addressed in our study design, a single enriched swab of the vagina and anorectum is more convenient, less costly and may be as efficient for recovering GBS as two separate swabs. Transportation of swabs in a regular transport medium such as PRAS appears to be acceptable as long as these swabs are incubated in enrichment and selective broth for 24 h before subculturing onto SBA. Although no diagnostic test can be 100% sensitive and specific, the enrichment approach makes screening for GBS carriage much more reliable, and as such should warrant wider compliance with the American [12] and Canadian [2, 17] screening recommendations recently proposed.

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