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Original Article

Detection of *Streptococcus agalactiae* by immunochromatography with group B streptococcus-specific surface immunogenic protein in pregnant women

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ABSTRACT

Background: Infection with *Streptococcus agalactiae* (Group B streptococcus: GBS) is a significant cause of morbidity and mortality in neonates. Screening for GBS is mainly done by culture-based methods, but a reliable result may take several days to obtain and culture is difficult to perform at institutions without a laboratory. We evaluated an immunochromatography method for rapid detection of GBS-specific surface immunogenic protein (Sip) using anti-Sip monoclonal antibodies.

Materials and methods: A total of 377 cervical and vaginal swabs collected during weeks 35–37 of gestation were inoculated into GBS medium F and incubated. Growth of microorganisms and production of red/orange pigment were assessed by observation. Then culture extracts were subjected to immunochromatography and were also inoculated onto chromID Strepto B (STRB) medium, after which isolates were serotyped and characterized by PCR.

Results: Of the 377 samples, 54 (14.3%) were positive for GBS by immunochromatography after incubation in GBS medium F. On the other hand, GBS was isolated from 58 (15.4%) of the 377 samples by culture with GBS medium F and STRB medium. Ten of the 58 isolates were non-pigmented and 4 of these were not detected by immunochromatography. The sensitivity, specificity, positive predictive value, and negative predictive value of immunochromatography were 93.1% (54/58), 100% (319/319), 100% (54/54), and 98.8% (319/323), respectively.

Conclusions: Immunochromatography was comparable to culture on STRB medium for detecting GBS, indicating that this method could be used clinically for GBS screening in pregnant women even at small institutions.

1. Introduction

Streptococcus agalactiae (Group B streptococcus: GBS) is a significant cause of infectious morbidity and mortality in neonates. Among pregnant women, the reported GBS colonization rate ranges widely from 3.2% to 19.0% [1–3] and GBS is responsible for 1.8 neonatal infections per 1000 births annually [4]. GBS infection can be acquired by the neonate from the mother at the time of

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delivery. The Centers for Diseases Control and Prevention (CDC) guidelines for prevention of GBS infection (updated in 2002) recommend that all pregnant women be screened for GBS carriage during weeks 35–37 of gestation [5], with intrapartum antibiotic prophylaxis being provided for GBS carriers. In 2011, the CDC reported that the incidence of early-onset GBS infection in the United States had recently declined to 0.34–0.37 per 1000 live births compared with 1.7 per 1000 live births in the early 1990s [6].

To improve detection of GBS, guidelines recommend that vaginal and rectal swabs be inoculated into selective enrichment medium, such as Lim broth or modified Granada medium, after which the colour change indicating growth of GBS is awaited according to the manufacturer's instructions [6]. Development of media with a reliable colour indicator for GBS has improved the precision of culture tests and facilitated processing of specimens by clinical laboratories with limited technical capacity [7]. Microorganisms that grow in selective enrichment culture are streaked onto an appropriate agar plate (e.g. 5% sheep blood agar), and suspected GBS colonies are subjected to further identification tests, such as the latex aggregation test, CAMP test, or conventional tests. While employing these methods improves the sensitivity of detection, it requires several days to complete all the procedures for identification of GBS by culture. In addition, non-hemolytic, non-pigmented GBS have been reported in 1–4% of pregnant women [8–12]. Since culture may fail to detect non-hemolytic GBS, alternative methods have been developed recently such as the DNA probe test and PCR [13–15]. However, these methods have the disadvantages of being more expensive and requiring trained technicians.

Immunochromatography has been employed for rapid detection of various microorganisms [16,17]. Recently, an immunochromatography method was developed for detection of GBS that targets surface immunogenic protein (Sip) [18]. To the best of our knowledge, there has been no clinical investigation of this method among pregnant women. Therefore, we performed the present study to evaluate the performance of this rapid immunochromatography method for identifying GBS in clinical specimens from pregnant women.

2. Materials and methods

2.1. Collection of specimens and culture

Kitasato University Hospital is a teaching hospital with 1033 beds located in Kanagawa, Japan. This prospective cohort study was performed between August 2014 and February 2015, with 333 cervical swabs and 44 vaginal swabs being collected from 377 pregnant women at 35–37 weeks of gestation for routine GBS screening as part of standard management of pregnancy. The swabs were transported to the Laboratory Department of Kitasato University Hospital, inoculated into GBS medium F (Fuji Pharma Co., Ltd., Tokyo, Japan), and incubated at 35 °C for 24 h (Fig. 1). Growth of microorganisms and production of red/orange pigment were confirmed macroscopically. After incubation in GBS medium F, swabs were transferred to a solution containing 250 μl of 0.5 M NaOH-0.02% Triton X-100 and let stand for 3 min at room temperature, following which 250 μl of 0.6 M TAPS containing 0.15 M HCl-0.02% Triton X-100 was added for neutralization. GBS medium F obtained from overnight culture was also subcultured on chromID Strepto B (STRB) medium (Sysmex bioMérieux, Co., Ltd., Tokyo, Japan) at 35 °C for 24–48 h [18]. GBS strains and other microorganisms isolated after subculture on STRB medium were identified by using MALDI Biotyper (Bruker Corporation, MA, USA). All other reagents used were of the highest grade commercially available.

2.2. Immunochromatography

Extracts obtained from GBS medium F were subjected to immunochromatography. GBS-specific Sip antigen is expressed by GBS strains of all serotypes. Immunochromatography was performed by using a nitrocellulose membrane strip with anti-Sip monoclonal antibody (clone R6E8) [18] immobilized at the test line and anti-mouse IgG antibody [19] immobilized at the control line. In brief, a test sample developed in the sample pad at the proximal end of the test strip reaches the conjugate pad, where colloidal gold-conjugated anti-Sip monoclonal antibody (clone S6E8) [18] reacts with Sip antigen in the sample. Then complexes migrate toward the distal end of the test strip. At the test line, immobilized anti-Sip antibody traps the complexes and forms sandwich immune complexes, creating a red line. Colloidal gold-conjugated anti-Sip monoclonal antibody (clone S6E8) that has not reacted with Sip antigen passes the test line and reaches the control line, where it reacts with immobilized anti-mouse IgG antibody and forms a second red line [18,19].

2.3. Serotyping

Serotyping of GBS (serotypes Ia to IX) was performed by multiplex-PCR, as described elsewhere [20].

2.4. Statistical analysis

Statistical analysis was done with the $\chi 2$ test and Fisher's exact test, as appropriate, and $p < 0.05$ was considered to indicate significance.

3. Results

The mean age of the 377 pregnant women was 34 years (S.D. ± 5.2). The GBS-positive rate in relation to age is shown in Table 1, demonstrating that GBS was detected in women of all ages except the youngest group. Growth of microorganisms was detected in 254 of the 377 specimens (67.4%). Using GBS medium F plus immunochromatography, GBS was detected in 54/377 specimens (14.3%). On the other hand, GBS was detected in 58/377 (15.4%) specimens by culture with GBS medium F plus STRB medium. Among those 58 GBS isolates, 48 produced red/orange pigment, 6 were negative for pigment production, and 4 were negative by enrichment culture. Immunochromatography detected all 48 of the pigment-producing strains, as well as 4 out of 6 pigment-negative strains and 2 out of 4 enrichment culture-negative strains. Thus, four out of 58 GBS isolates were not detected by immunochromatography (Fig. 1). The sensitivity, specificity, positive predictive value, and negative predictive value of immunochromatography were 93.1% (54/58), 100% (319/319), 100% (54/54), and 98.8% (319/323), respectively (Table 2). All isolates were susceptible to penicillin (data not shown).

The GBS serotype was Ia in 11/58 isolates (19.0%), Ib in 3 (5.2%), II in 7 (12.1%), III in 21 (36.2%), IV in 2 (3.4%), V in 8 (13.8%), and VI in 6 (10.3%). The relation between the age of the pregnant women and the GBS serotype is shown in Fig. 2, revealing that multiple serotypes were detected at most ages.

4. Discussion

We evaluated a rapid immunochromatography method for detection of GBS in specimens after overnight enrichment culture. This method does not require special equipment and was able to detect GBS-specific Sip antigen in only 15 min with a high sensitivity and specificity, and could also detect non-pigmented GBS. In

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