Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 61 (2008) 369–372
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Bacteriology

Comparison of BD GeneOhm real-time polymerase chain reaction with chromogenic and conventional culture methods for detection of group B *Streptococcus* in clinical samples

Dawn Smith, John D. Perry*, Larissa Laine, Angela Galloway, F. Kate Gould

Microbiology Department, Freeman Hospital, Newcastle-upon-Tyne NE7 7DN, UK

Received 29 November 2007; accepted 18 March 2008

Abstract

A total of 200 antenatal high vaginal swabs were screened for the presence of group B *Streptococcus* (GBS) using a conventional culture method (recommended by Centers for Disease Control and Prevention). Screening was also performed by using a new chromogenic agar, chromID Strepto B, and by using the BD GeneOhm StrepB real-time polymerase chain reaction (PCR), which was performed directly on swabs without enrichment. Using a combination of all methods, we detected GBS in 101 samples. A total of 82 samples (81.2%) were positive using PCR, and 83 samples (82.2%) were confirmed as positive by culture (any method). PCR was more sensitive for detection of GBS than direct culture using any method ($P < 0.0005$). PCR was also more sensitive than any single enrichment method, but this difference was not statistically significant. With culture as a "gold standard", the PCR method showed a sensitivity of 77.1% and a positive predictive value of 79.3%. Of the culture-positive samples, significantly, more GBSs were detected by direct plating on chromID Strepto B than on selective sheep blood agar (67.5% versus 57% respectively, $P < 0.02$). After selective enrichment, 92.8% of GBS were isolated on chromID Strepto B compared with 89.2% isolated on sheep blood agar.

Keywords: Group B streptococci; *Streptococcus agalactiae*; Chromogenic agar; Polymerase chain reaction

1. Introduction

Group B streptococcal disease remains a leading infectious cause of morbidity and mortality among newborns. Asymptomatic carriage of group B streptococci (GBSs) in the maternal genitourinary tract or gastrointestinal tract commonly leads to colonization of the neonate. In a small proportion of cases, estimated at 1.8 cases per 1000 live births in the United States, colonization leads to onset of invasive disease (Zangwill et al., 1992). In an effort to reduce this rate of disease, the Centers for Disease Control and Prevention (CDC) recommended universal screening of pregnant women to detect vaginal and rectal colonization by GBS at 35 to 37 weeks of gestation (Schrag et al., 2002). If GBS is detected, systemic intrapartum antibiotic therapy is then initiated to eradicate colonization. This strategy has been associated with a 33% reduction in early-onset invasive disease in the United States, with 1.2 cases per 1000 live births reported between 2003 and 2005, after the implementation of new guidelines (CDC, 2007).

Culture involves overnight enrichment in a selective broth medium followed by subculture onto blood agar to isolate GBS colonies demonstrating typical β hemolysis (Schrag et al., 2002). This process typically requires 2 days to provide results. Recently, alternative methods have become commercially available for the detection of GBS colonization. These include a novel chromogenic agar, chromID Strepto B (formerly Strepto B ID), which highlights GBSs as red colonies. This medium uses multiple chromogenic substrates and is incubated aerobically, which distinguishes it from other media, such as Granada medium, that rely on pigment production under anaerobic conditions (de la Rosa et al.,

* Corresponding author. Tel.: +44-191-2231226; fax: +44-191-2231224.
E-mail address: jdp@blueyonder.co.uk (J.D. Perry).

0732-8893/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.diagmicrobio.2008.03.007

1992). In a preliminary study, chromID Strepto B was shown to have at least an equivalent sensitivity to Granada medium (Perry et al., 2006).

Real-time polymerase chain reaction (PCR) methods that offer the ability to detect GBS colonization within 1 h of sample receipt have also been developed (Davies et al., 2004). One such method, marketed by BD GeneOhm, has received Food and Drug Administration (FDA) approval for detection of GBS from clinical samples and generates results in less than 1 h. The aim of this study was to assess the use of these 2 new methods in comparison with conventional culture methods for the isolation of GBS from vaginal swabs.

2. Materials and methods

2.1. Culture media and identification tests

Preprepared culture media were kindly provided by bioMérieux, Basingstoke, UK. These included chromID Strepto B plates (chromID; ref: 43461), sheep blood agar plates supplemented with 10 mg/L of colistin and 15 mg/L of nalidixic acid (CNA) (ref: 43071), sheep blood agar plates without antibiotics (ref: 43041), and Todd–Hewitt broth supplemented with 10 mg/L colistin and 15 mg/L nalidixic acid (ref: 42116). Slidex Strepto kits and rapid ID 32 STREP kits were purchased from the same source.

2.2. Patient samples

Universal prenatal screening for GBS colonization is not recommended by authorities in the United Kingdom; however, high vaginal swabs received for routine culture from pregnant women are routinely screened for GBS in our laboratory. A total of 200 consecutive high vaginal swabs from distinct pregnant women were included in this study. In all cases, swabs were tested within 24 h of collection. Rectal swabs were not available.

2.3. PCR technique

All reagents, buffers, and other consumables needed for the PCR were provided in kit form by BD GeneOhm (Becton Dickinson, Oxford, UK). Each swab was placed into 1 mL of PCR sample buffer, and the plastic stem was cut using aseptic precautions. This was left to stand for 5 min before a high-speed vortex for 15 s. After vortex, 50 μL of cell suspension was transferred into a lysis tube. The lysis tube was vortexed at high speed for 5 min, then centrifuged for 6 s to bring the contents to the bottom of the tube. The tubes were then heated at 95 °C for 2 min and, afterward, placed on a cooling block. One positive control tube, 1 negative control tube, and a master mix tube for each specimen were then placed on the Cepheid SmartCycler® cooling block. The tubes were inoculated with 25 μL of diluent before 1.5 μL of each lysate was added to the corresponding master mix tubes. The tubes were closed and centrifuged for 10 s. The tubes and controls were then vortexed for 10 s while on the cooling block at 2 to 8 °C. After a run was created with the BD GeneOhm StrepB assay protocol, each reaction tube was placed in the corresponding I-CORE module. Results were available after a 41-min run.

2.4. Culture methods

As described previously, 50 μL of sample was transferred to the lysis tube for PCR. At the same time (i.e., after vortexing), the same volume of the sample buffer was then also inoculated directly onto chromID and CNA blood agar and spread to obtain isolated colonies. The remaining sample buffer (approximately 850 μL) was then inoculated into 10 mL of selective Todd–Hewitt broth. The solid media were incubated at 37 °C in aerobic conditions and read after 24 and 48 h. The Todd–Hewitt broth was subcultured after 24 h of incubation onto chromID and sheep blood agar. The plates were incubated at 37 °C in air. These were then read after 24 and 48 h of incubation for the presence of GBS.

2.5. Identification

Any red colonies on chromID and any β-hemolytic colonies on either CNA or sheep blood agar were regarded as presumptive GBS. Such colonies were subcultured onto sheep blood agar to obtain a pure culture and confirmed as GBS using latex agglutination for group B antigen and biochemical identification using rapid ID 32 STREP. Suspect colonies that did not prove to be GBS were identified using appropriate API strips. In cases where culture was positive and PCR produced a negative result, the PCR was repeated using the GBS isolate to ensure the presence of the PCR target.

2.6. Statistical methods

The various methods were compared with each other for statistical significance using McNemar test.

3. Results

With a combination of culture and PCR, 101 samples (50.5%) showed evidence of colonization with GBS, with 83 samples positive by culture and 82 samples positive by PCR (Table 1).

3.1. Comparison of culture methods

Of the 83 isolates, 56 (67.5%) were recovered as red colonies after direct plating onto chromID compared with 47 (57%) isolates recovered by direct plating on CNA ($P < 0.02$). The isolation rate of GBS was increased after enrichment using any medium ($P < 0.0005$), and 77 isolates (92.8%) were recovered as red colonies on chromID compared with 74 isolates (89.2%) recovered on blood agar. One strain of GBS that was not recovered using blood agar was shown to be nonhemolytic when subcultured from chromID onto sheep blood agar. Various hemolytic colonies