JCM
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Evaluation of the Cepheid Xpert GBS Assay for Rapid Detection of Group B Streptococci in Amniotic Fluids from Pregnant Women with Premature Rupture of Membranes

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The Xpert GBS real-time PCR assay for the detection of group B streptococci (GBS) in antepartum screening samples was evaluated on amniotic fluid samples collected from 139 women with premature rupture of membrane at term. When any intrapartum positive result from the Xpert GBS or culture was considered a true positive, the sensitivities of the Xpert GBS and culture were 92.3% and 84.6%, respectively. This assay could enhance exact identification of candidates for intrapartum antibiotic prophylaxis.

Streptococcus agalactiae, also known as group B streptococcus (GBS), is the leading infectious cause of neonatal morbidity and mortality. Maternal GBS colonization is one of the most important risk factors for early onset GBS disease (EO-GBSD). Mother-child transmission can occur during membrane rupture or delivery. The reported rate of GBS vaginal colonization among pregnant women ranges from 4% to 36% in Europe (1). The risk of EO-GBSD increases in cases of preterm delivery, maternal fever, and premature rupture of membrane (PROM) more than 12 h before delivery. Intrapartum antibiotic prophylaxis (IAP) reduces significantly the incidence of EO-GBSD (2). It is still debated whether this IAP will favor colonization by antibiotic-resistant bacteria (3, 4). In France, the strategy to identify women for targeted IAP is based on universal antenatal screening with vaginal culture at 35 to 37 weeks gestation (5). GBS culture remains the gold standard for the detection of GBS colonization. However, its turnaround time (TAT) varies from 18 to 72 h, which makes it not adapted for intrapartum screening.

Term PROM is defined as the spontaneous rupture of membranes more than 12 h at term before the onset of regular uterine contractions. PROM at term affects 8 to 10% of pregnant women (6). When PROM is confirmed, active management with labor induction or expectant management is possible. One criterion for expectant management is GBS-negative status while pregnant women with GBS-positive term PROM should be offered antibiotic prophylaxis and induction of labor (6). However, it has been well documented that results of antepartum GBS screening culture do not always accurately predict intrapartum GBS status (7, 8). A nucleic acid amplification test (NAAT) may be able to identify women who are positive at the time of delivery. The Xpert GBS (Cepheid) has shown to be an accurate and easy-to-use PCR for the detection of GBS DNA from vaginal or rectal specimens (8, 9). With Xpert GBS intrapartum screening, significant decreases in neonatal infections and the length of stay (LOS) were demonstrated (47% fewer hospitalization days in neonatology/90% fewer days in the intensive care unit [ICU]) (10).

The objective of our study was to validate the Xpert GBS assay directly on amniotic fluids collected from pregnant women with rupture of membranes at term gestation before the onset of labor.

Our prospective study was conducted at Antoine Béclère Hospital (Clamart, France), a university hospital with a level III maternity center, from May 2011 through May 2012. We included 139 women with PROM that occurred at ≥37 weeks of gestation.

Amniotic fluid samples were collected by obstetricians, with a sterile pipette from liquid flowing onto the sterile speculum; the fluid was placed in sterile containers and transferred within 30 min to the laboratory. One hundred microliters of amniotic fluid samples was cultured on Columbia blood agar (bioMérieux) and incubated at 37°C in an anaerobic atmosphere from 18 to 24 h. Beta-hemolytic colonies and suspect nonhemolytic colonies were identified as GBS by using a latex agglutination test (Pro-Lab Diagnostics). GBS colonization was defined as positive in the case of GBS growth on the plates. Swabs were soaked for 1 min in the amniotic fluids and then directly transferred into the Xpert GBS cartridge and broken off at the scored mark. The cartridge was introduced into the GeneXpert system (Cepheid), which integrates the DNA extraction, amplification, and detection. The sample preparation time was 5 min. The TAT was 50 min for negative results and 32 min for positive results. The result was immediately passed on to obstetricians.

The overall GBS PCR test yield was 100% (no invalid or error results). Out of the 139 amniotic fluid samples, 12 (8.6%) were found positive by the Xpert GBS assay (Table 1). Cycle thresholds for positive samples ranged between 27.1 and 39.3. The comparison of Xpert GBS assay versus culture results of amniotic fluids showed a sensitivity of 90.9% and a specificity of 98.4%. We obtained one specimen that was negative by PCR and positive by culture. We initiated further investigation, cultured the strain,

Received 2 January 2013 Returned for modification 17 January 2013
Accepted 31 January 2013
Published ahead of print 6 February 2013
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doi:10.1128/JCM.03356-12

TABLE 1 Detection of GBS in 139 amniotic fluids by culture and Xpert GBS assay

GBS culture	Results of Xpert GBS assay		Total
	Positive	Negative	
Positive	10	1	11
Negative	2	126	128
Total	12	127	139

extracted the DNA, and performed a sequencing analysis. A faint band appeared on the gel after PCR with sequencing primers, confirming this sample was GBS positive. After quantification by culture of the initial amniotic fluid, we showed only 10^2 CFU/ml; this very low quantity is certainly the explanation of this result. We also obtained two samples that were positive by PCR, with a cycle threshold of 39.3, and negative by culture. When any intrapartum positive result from the Xpert GBS assay or culture was considered a true positive of GBS colonization, the sensitivities of the Xpert GBS assay and standard culture were 92.3% (12/13) and 84.6% (11/13), respectively.

Of the 139 enrolled women, 133 underwent GBS culture screening at 35 to 37 weeks gestation (Table 2). Among the 6 women without available culture status, 5 were negative by PCR and 1 was positive by PCR. The antenatal GBS colonization rate by culture and the intrapartum GBS colonization rate by PCR were 8.3% (11/139). However, 54.6% (6/11) of those women found to be GBS positive in amniotic fluid by the Xpert GBS assay did not have GBS detected in antepartum culture. No GBS invasive disease in mothers and newborns studied were observed during the study period compared to the previous year, May 2010 to May 2011, in which we observed 1 bacteremia among the women.

To the best of our knowledge, this is the first validation of the Xpert GBS assay in amniotic fluids. In our study, the results of the NAAT intrapartum and antepartum culture were discordant in 9% of women overall. The PCR assay has allowed withholding IAP in half of the women with an antepartum-positive GBS screening. At this time, the Xpert GBS assay is the only method available for identification of PROM women at risk that allows the clinicians to prescribe the IAP and to potentially avoid the delivery of a GBS-colonized neonate from women who would otherwise not be determined to be at risk.

In summary, the Xpert GBS assay is a rapid and accurate method for GBS detection in amniotic fluids. This tool could enhance the exact identification of candidates for IAP and be helpful for management of women with PROM.

TABLE 2 Comparison of Xpert GBS assay and GBS culture on amniotic fluid samples versus GBS culture screening at 35 to 37 weeks gestation in women with PROM ≥37 weeks gestation

Documented results of antepartum GBS culture	Results of intrapartum Xpert GBS assay (PCR) and GBS culture[a]				Total
	PCR +/ culture+	PCR+/ culture−	PCR−/ culture+	PCR−/ culture−	
Positive	4	1	1	5	11
Negative	5	1	0	116	122
Not available	1	0	0	5	6
Total	10	2	1	126	139

[a] +, positive; −, negative.

ACKNOWLEDGMENT

We declare that we have no conflicts of interest.

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