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BRIEF REPORT

Improving the Sensitivity of Real-time PCR Detection of Group B Streptococcus Using Consensus Sequence-Derived Oligonucleotides

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Group B *Streptococcus* (GBS) is a perinatal pathogen and an emerging cause of disease in adults. Culture-independent GBS detection relies on polymerase chain reaction (PCR) of conserved genes, including *sip*. We demonstrate suboptimal sensitivity of the existing *sip* PCR strategy and validate an improved method based on consensus sequences from >100 GBS genomes.

Group B *Streptococcus* (GBS) colonizes the gastrointestinal tract and vagina of healthy individuals. It is an important pathogen in neonates and pregnant women and is emerging as a cause of disease in nonpregnant adult populations [1]. Laboratory detection of GBS involves culture using selective and enrichment media or, increasingly, molecular methods such as polymerase chain reaction (PCR) [2]. GBS surface immunogenic protein (Sip) is a conserved GBS-specific protein encoded by a 1311 base pair (bp) gene (*sip*) that is used as a target for real-time (RT) PCR-based detection of GBS [3]. A primer-probe set targeting a 78-bp region within the *sip* gene has been described and used in human studies for GBS detection [4–9]. In the course of laboratory investigations, we observed that the published *sip* RT-PCR method did not detect the A909 (serotype Ia) GBS strain. On examination of the A909 genome sequence (Genbank NC_007432.1), we noted a single nucleotide polymorphism (SNP; *sip* g.312C>T) in the predicted region of probe binding that we hypothesized was responsible for detection failure. Here we report the design and validation of a new primer-probe set targeting a more highly conserved region of the *sip* gene, leading to improved sensitivity of detection.

METHODS

Sequence Alignment and Oligonucleotide Design

We identified the open reading frame (ORF) encoding Sip (GenBank AIX03825.1) from GBS strain CNCTC 10/84 [10]. We aligned the short DNA reads from a set of 113 previously reported GBS draft genomes (NCBI BioProject PRJEB4456) [11] to this ORF, identified SNPs, and mapped existing and candidate primer-probe sets using Geneious (version 11.0.3; Biomatters, Ltd.). Serotype information was obtained from the available metadata. Based on the alignment, we designed a new primer-probe set (F: 5'-CAG CAA CAA CGA TTG TTT CGC C-3'; R: 5'-CTT CCT CTT TAG CTG CTG GAA C-3'; Probe: 5'-AGA CAT ATT CTT CTG CGC CAG CTT TG-3') targeting a conserved 171-bp region as a candidate for validation. For comparison, we used the previously described primer-probe set (F: 5'-ATC CTG AGA CAA CAC TGA CA-3'; R: 5'-TTG CTG GTG TTT CTA TTT TCA-3'; probe: 5'-ATC AGA AGA GTC ATA CTG CCA CTT C-3'). In addition, we designed primers to amplify and sequence the target area (F: 5'-GTC AAT TGA TAT GAA TGT CTT AGC-3'; R: 5'-GTA TTG AGA GAA ACT TTT TGG TCT-3') and full-length *sip* gene (F: 5'-ATG AAA ATG AAT AAA AAG GTA CTA TTG-3'; R: 5'-TTA TTT GTT AAA TGA TAC GTG AAC-3'). All oligonucleotides were synthesized by Integrated DNA Technologies. Probes were labeled with a 5' 6xFAM fluorescent tag and contained internal ZEN and 3' Iowa Black fluorescent quenchers.

PCR Methods

Real-time PCR reactions consisted of 10 µL of TaqMan Universal Mastermix II, 0.2 µL per primer and probe (primer stocks: 40 µM; probes: 20 µM), and 9.4 µL of DNA/water (10 ng DNA for rectovaginal samples, 2 ng DNA for vaginal lavages, with water to make reaction volume 20 µL). Amplification and detection were performed in an ABI StepOne Plus cycler with StepOne software. A positive *sip* PCR was defined as a cycle threshold of <36 with confirmation by serotype-specific PCR. For amplification of *sip* regions for sequencing, we used Q5 DNA polymerase (New England BioLabs) according to the manufacturer's instructions. Bands were sized on 1% agarose

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gels, extracted using the QIAquick Gel Extraction kit (Qiagen, Inc.), and Sanger-sequenced by Genewiz.

Validation Sample Sets

We used a set of 274 rectovaginal swabs from pregnant women and a GBS-positive subset of 91 vaginal lavage samples from nonpregnant women. Samples were grown in Lim broth overnight before DNA extraction for PCR. For the rectovaginal samples, Lim broth growth was cultured on chromogenic media and colonies confirmed using latex agglutination with ImmuLex *Streptococcus* Group B (SSI Diagnostica). For the vaginal lavage samples, culture and latex agglutination were performed only in the setting of discordance between the *sip* PCR assays. Serotype was determined using real-time PCR [12]. Sensitivity and specificity were calculated using OpenEpi [13].

RESULTS

We aligned reads from 113 publicly available GBS genomes, including both invasive and carriage isolates from European and African nations over several decades (NCBI BioProject PRJEB4456) [11], to the *sip* sequence from GBS CNCTC 10/84 [10]. We noted that the same g.312C>T polymorphism that occurred in strain A909 was also present in a minority of those genomes (6/113, 5.3%), distributed among 3 serotypes (Ia, n = 1; Ib, n = 4; II, n = 1).

We performed validation of *sip* PCR techniques in a set of rectovaginal swabs from women in late pregnancy (Table 1). The new set detected GBS in 53/274 (19%) of samples. Among these PCR-positive samples, 3/53 (5.7%) were not detected by the previously published primer-probe set. Two discordant samples were serotype Ib, and 1 was serotype IV. Among the GBS-negative samples, there was 100% concordance between the 2 assays. Thus, within the rectovaginal sample set, the sensitivity of the previously published primer-probe set was 94.3% and specificity was 100%, using the new set as the gold standard. Using culture with latex agglutination as an alternative standard, the new *sip* PCR assay had a sensitivity of 95.8% and a specificity of 96.9%, whereas the previous assay had a sensitivity of 89.6% and a specificity of 96.9%.

For further investigation of discordance, we used Lim broth cultures from vaginal lavage samples that tested GBS-positive with the new primer-probe set. Of these, 5/91 (5.5%) were negative with the previous primer-probe set. Three of those samples were serotype Ib, 1 was serotype II, and 1 had both serotypes Ia and Ib. To determine genetic changes in the 8 samples (3 rectovaginal and 5 vaginal lavage) with discordance between the 2 PCR methods, we amplified a 220-bp segment of the *sip* ORF spanning the binding sites for the prior primer-probe set for sequencing. All but 1 sample (serotype IV) produced a band of the predicted size and had the same SNP as A909, revealed by sequencing. As a control, we amplified the same region from 3 serotype Ib strains that were detected by both primer-probe sets—none had the g.312C>T SNP. The serotype IV sample that did not yield a band also did not amplify with primers targeting full-length *sip*, suggesting a deletion at 1 end of the ORF.

CONCLUSIONS

Current guidelines for GBS screening in pregnancy suggest testing in late gestation and provision of intrapartum antibiotic prophylaxis to colonized women [2]. Reliable point-of-care culture-independent detection of GBS could add flexibility to screening and might minimize missed opportunities for treatment. Because of the conserved nature of the GBS *sip* gene, real-time PCR strategies for its detection have been used in clinical studies. More recently, additional culture-independent *sip* detection strategies, including loop-mediated isothermal amplification and immunochromatography, have been described [5, 14]. Here we report that the widely used *sip* primer-probe set is suboptimal for detection of a subset of GBS isolates, likely due to a mutation in the probe-binding site that is present in ~5% of strains. Using a set of >100 GBS genomes, we designed a new primer-probe set that detected an additional 8/144 (5.6%) of GBS-positive samples missed by the old primer-probe set. This

Table 1. Test Characteristics of *sip* PCR Testing on Rectovaginal Samples From Late Pregnancy

		New PCR			
Previous PCR		Positive	Negative		
	Positive	50	0	Sensitivity	94.3%
	Negative	3	221	Specificity	100%
		Culture			
New PCR		Positive	Negative		
	Positive	46	7	Sensitivity	95.8%
	Negative	2	219	Specificity	96.9%
		Culture			
Previous PCR		Positive	Negative		
	Positive	43	7	Sensitivity	89.6%
	Negative	5	219	Specificity	96.9%

Abbreviation: PCR, polymerase chain reaction.

new strategy requires validation in larger clinical sample sets, and it is notable that 1 GBS strain that was detected by the new but not the old PCR strategy did not have the g.312C>T SNP but appeared to have a larger deletion of a portion of *sip* containing the target of the prior primer-probe set. Although not the case for that particular isolate, deletions and as yet unrecognized polymorphisms in the *sip* sequence could lead to imperfect sensitivity of the new primer-probe set as well, emphasizing the importance of refinement of culture-independent detection strategies as new genomic information becomes available.

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Potential conflicts of interest. A.J.R. has served as a consultant to Pfizer. All other authors report no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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