CONCISE COMMUNICATION

Analysis of Restriction Fragment Length Polymorphisms of the Insertion Sequence IS1381 in Group B Streptococci

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Group B streptococci (GBS) are typed by capsular polysaccharide type. IS1381, an insertion sequence previously described in *Streptococcus pneumoniae,* was cloned from GBS strain A909. The presence of multiple copies of IS1381 in A909 suggested that IS1381 analysis might be an effective subtyping tool. IS1381 was found by Southern blot analysis to be present in 18 (72%) of 25 of unrelated GBS isolates tested. IS1381 analysis allowed discrimination between strains that contain IS1381 with a discriminatory power >99%. Eight of 8 sets of epidemiologically related isolates containing IS1381 give identical or nearly identical patterns of IS1381 insertion. For 2 maternal/infant sets, a single additional insertion was seen in 1 strain, suggesting that an additional insertion occurred between maternal colonization and infection of the infant. Insertion patterns of IS1381 are an effective tool for subtyping GBS.

Group B streptococci (GBS) are the leading cause of sepsis and meningitis in neonates in the United States. Typing is done by serotyping for capsular polysaccharide; types most commonly isolated from humans are Ia, Ib, II, III, and more recently, types IV, V, and, occasionally VI, VII, and VIII [1]. Subtyping methods allow for discrimination between unrelated strains of the same capsule type. Nongenetic subtyping methods are of limited use. Several genetic analyses have been developed for subtyping GBS, including studies of restriction fragment length polymorphisms of ribosomal RNA genes [2–4], a variety of other genes [5], and of total chromosomal DNA (restriction enzyme analysis [REA]) [3, 6–8]. Polymerase chain reaction (PCR) testing using random primers (RAPD) has been recently applied to GBS [9, 10]. These genetic analyses have a high discriminatory power (>0.90).

Insertion sequences (ISs) have also been used. A previous study identified an IS in GBS, IS1548, and found that it was present in most isolates from adult patients with endocarditis, but in few isolates from women vaginally colonized by GBS [11]. We have identified an IS (IS1381) in GBS. IS1381 had previously been described in *Streptococcus pneumoniae* [12], where it is present in multiple copies. We hypothesized that IS1381 might also be present in multiple copies in GBS and could be used for subtyping. We determined the prevalence and pattern of insertion of IS1381 from 15 independent GBS isolates and from 10 sets of epidemiologically linked maternal-infant strains by using Southern blot analysis.

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Materials and Methods

Bacterial strains and plasmids. Nonpaired GBS strains were obtained from a library of GBS isolates contracted by the National Institutes of Health and maintained by Dr. Craig Rubens [13]. Epidemiologically linked strains were collected by 2 of the authors (P.F. and S.H.), and were characterized by P.F. as described elsewhere [14]. A909-F1 is a mutant of A909, which was generated by use of the transposon Tn917. PT7-Blue was obtained from Novagen (Madison, WI).

DNA techniques. The transposon insertion site was cloned by digestion of pBluescriptSK+ and A909-F1 DNA with *Hin*dIII, ligation of digested A909-F1 DNA and plasmid DNA, and transformation of the ligation reaction into DH5-a. Transformants were screened by colony hybridization by use of Tn917 as a probe with the Genius kit (Boehringer-Mannheim, Mannheim, Germany). A single positive clone (pJAP101) was isolated. Primers were synthesized by Gibco/BRL (Gaithersberg, MD). Primer sequences are IS reverse primer, ATTTTTTTAGGACGATTGATTGATTTT; IS ITR primer, GATTAGAGTTACTGCGAAAC. PCR was done by use of reagents from Sigma (St. Louis) and done in a GeneAmp 2400 thermocycler (Perkin-Elmer, Norwalk, CT) per the manufacturer's protocol. Samples were heated to 94°C for 1 min; then 28 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 2 min; then 72°C for 7 min. DNA sequence analysis was done by use of equip-

ment and reagents from the ABI-Prism system according to the manufacturer's protocol. Computer analysis was done by use of Genetics Computer Group software (University of Wisconsin, Madison).

Restriction enzyme digestion and Southern blot hybridization were done by use of the Genius kit (Boehringer-Mannheim). The probe for Southern blot analysis was generated by digesting pJAP101 with *Dra*I and *Hin*dIII, isolating a 564-bp internal fragment of IS1381 by agarose gel electrophoresis, and labeling the fragment with digoxigenin by using the Genius kit. Hybridization was done in 5× standard saline citrate (SSC) with 0.1% sodium sarcosine, 0.02% SDS, 50% formamide, and 2% blocking agent from the Genius kit at 42°C overnight. The blot was washed twice with 2× SSC with 0.1% SDS at 25°C for 15 min, and then twice with 0.1 × SSC with 0.1% SDS at 68°C for 15 min. Bound probe was detected, as described in the Genius protocol, by autoradiography. The sequence of IS1381 from GBS is available from Genebank accession number AF064785.

Results

Cloning of IS1381 from GBS strain A909. Identification of an IS in the GBS strain A909 was incidental to cloning of DNA flanking the Tn917 insertion in a transposon mutant of A909 (A909-F1). A909-F1 is a fibronectin adherence mutant isolated from a library of Tn917 mutants. DNA flanking the transposon insertion in A909-F1 was cloned into pBluescript SK+ (pJAP101). Southern blot hybridization analysis of A909 DNA and A909-F1 DNA digested with *Eco*RI revealed that sequences homologous to pJAP101 hybridized to multiple fragments (figure 1*A*). Subsequent nucleotide sequence analysis of pJAP101 revealed a region homologous to the 3′ portion of ISL2. These results suggested that pJAP101 contains a partial insertion sequence.

We cloned the remaining 5′ portion of the IS by using PCR with A909 DNA as template and one primer consisting of known nucleotide sequence of the IS (IS reverse primer) and a second primer consisting of the inverted terminal repeat (IS ITR primer). A 450-bp product was recovered by use of pT7 blue (pJAP104). Nucleotide sequence analysis of pJAP104 revealed that we had cloned the remaining 5′ portion of the IS. By using the sequences from these 2 clones ,we generated a putative sequence for the entire IS. Subsequent homology searches revealed that the putative IS from A909 had a high degree of homology to IS1381 (94%), a newly submitted IS from *S. pneumoniae.*

Analysis of IS1381 insertions in the chromosome of multiple GBS strains. If IS1381 was present in most strains of GBS and gave distinct Southern blot patterns for unrelated strains, then IS1381 would be a useful subtyping tool. To test the prevalence of IS1381 in GBS clinical isolates, we used DNA from 15 unrelated GBS clinical isolates from 6 different serotypes for digestion with *Eco*RI and Southern blot analysis (figure 1). IS1381 was present in 10 (67%) of 15 strains tested. When we combined these data with that from the epidemiologically linked strains that demonstrate IS1381 in 8 of 10 unrelated sets of strains, the total prevalence of IS1381 in the strains tested was 18 (72%) of 25. These results demonstrate that IS1381 was present in a high percentage of GBS strains.

The banding pattern for each strain was unique, except for 2 sets of isolates containing only a single copy of IS1381, which contain an identical restriction fragment. Although these strains were isolated from the same hospital and are of the same serotype, they are not epidemiologically related. These results suggest that identical Southern blot patterns for single insertions of IS1381 may occur in epidemiologically unrelated isolates on rare occasions.

Fifteen of 18 strains containing IS1381 contained multiple copies. Banding patterns for each these 15 strains was unique. Furthermore, although pairs of strains hybridized to up to 2 identically sized restriction fragments, each isolate could be distinguished from all other isolates based on at least 2 fragments unique to 1 of the strains. We conclude that the IS1381 analysis in strains containing IS1381 allows for differentiation of epidemiologically unrelated strains on the basis of the number and size of restriction fragments containing IS1381 with a discriminatory power of 99.3%.

Analysis of IS1381 insertions in maternal-infant pairs. We hypothesized that epidemiologically related isolates would give identical or nearly identical IS1381 insertion patterns. To test this hypothesis, we analyzed 10 maternal-infant sets (figure 2). Five sets were collected by Dr. Hillier at hospitals at the University of Washington and the University of Pittsburgh (figure 2*A*), and 5 sets were collected by Dr. Ferrieri at the University of Minnesota (figure 2*B*). DNA was digested with *Eco*RI and IS1381 insertions analyzed by Southern blot. Eight of 10 sets of related isolates contained IS1381. Identical patterns were seen for all isolates within a set for 6 of 8 sets. Two pairs (lanes 12, 13 and lanes 14, 15) gave patterns that were nearly identical but differed by the addition of a single fragment in 1 of each pair. The additional 2 high–molecular-weight bands seen in lane 15 were also seen in lane 14, with longer exposure times. Because this result may have been caused by partial digestion of the DNA with *Eco*RI, DNA was digested on 3 different occasions; each blot gave identical results. These data indicate that for 2 of the strain sets tested, IS1381 appears to have been deleted or duplicated during the time period between the isolation of the maternal and fetal isolate.

Discussion

These results demonstrate that IS1381 is present in 72% of unrelated GBS isolates tested and that 8 of 8 sets of epidemiologically related isolates give identical or nearly identical patterns of IS1381 insertion on Southern blot analysis. In contrast, of 18 unrelated strains, distinct patterns of IS1381 inser-

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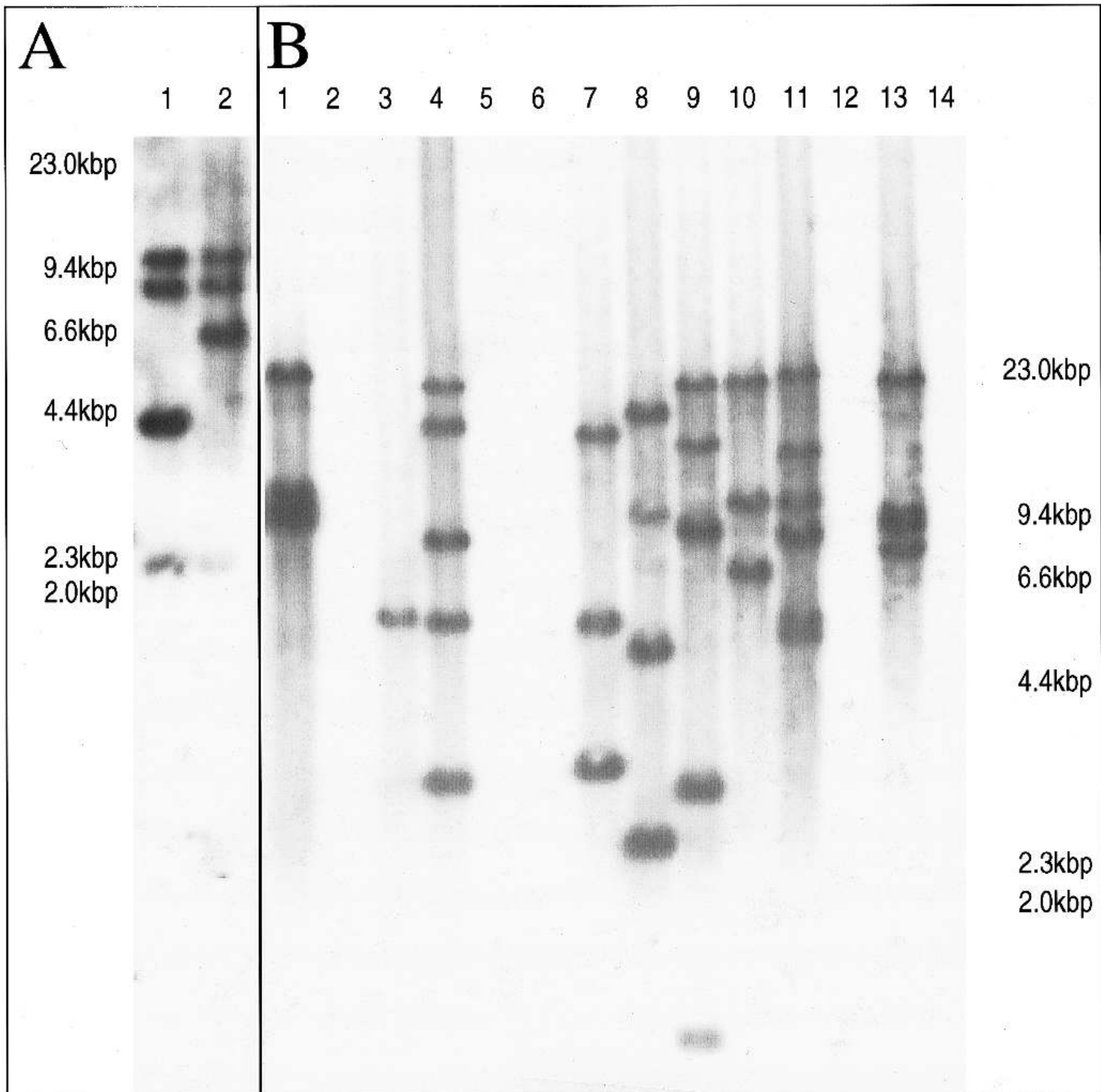

Figure 1. Southern blot analysis of DNA isolated from 14 different strains and probed with IS1381. Strains with serotypes - lane 1, O90, type Ia; lane 2, H36B, blood, type Ib; lane 3, DK15, type Ib; lane 4, DK14, type Ib; lane 5, B201, type V; l ane 6, 515, type Ia; lane 7, D136C(2), type III; lane 8, NCTC 10/84, type V; lane 9, NT6, human, type VI; lane 10, DK23, type II lane 11, M781, type III; lane 12, B523, type Ia; lane 13, M709, type Ib; lane 14, COH1, type III.

tion were seen for all isolates, except for 2 unrelated sets of strains, which contained a single identical insertion. These results suggest that analysis of insertion patterns of IS1381 is an effective tool for subtyping GBS for strains that have IS1381.

One limitation of this method is the absence of IS1381 in a significant percentage of strains (28%). However, if 2 strains are discordant for the presence of IS1381, this demonstrates that the 2 strains are unrelated. One would expect that the unrelated pairs of strains would both lack IS1381 (null result) and that the analysis would be uninformative, in ~8% of cases.

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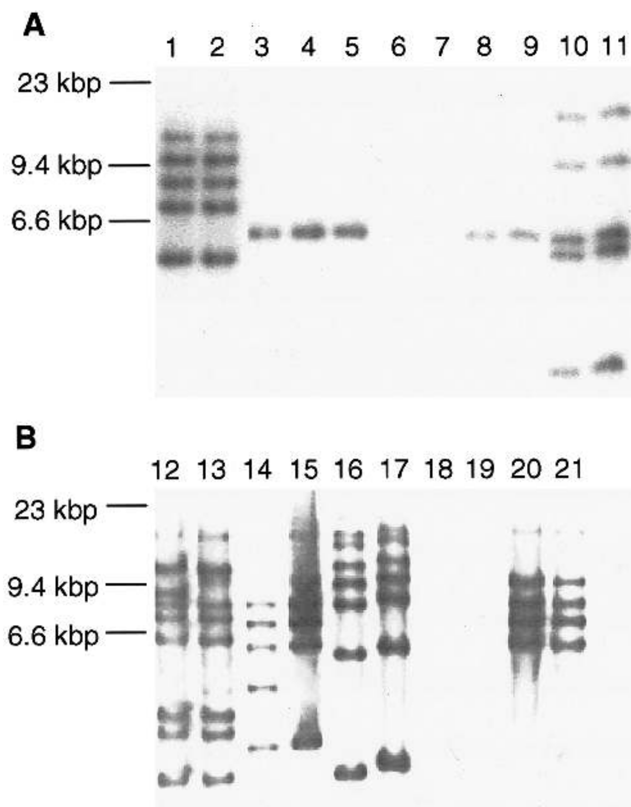

Figure 2. Southern blot analysis of epidemiologically related isolates from mothers and their infected infants probed with IS1381. *A,* Strains isolated by S.H. from the University of Washington and the University of Rochester. *B,* Strains isolated by P.F. at the University of Minnesota. Linked strains are indicated by brackets. [lane 1, HST 1586, maternal amniotic fluid, type V; lane 2, HST 1586, infant blood, type V]; [lane 3, PIA-00012 , infant spleen, type II; lane 4, PIA-00012, mother placenta, type II; lane 5, PIA-00012, infant lung, type II]; [lane 6, GBS 1911, infant blood, type Ia; lane 7, GBS 1911, mother vagina, type Ia]; [lane 8, PIA-00010, infant blood, type II; lane 9, PIA-00010, mother vagina, type II]; [lane 10, GBS 895, infant spinal fluid, typ e Ia; lane 11, GBS 895, mother, placenta, type Ia]; [lane 12, 81–410, mother, vagina, type III; lane 13, 81–412, infant, nares, type III]; [lane 14, 82–292, mother, vagina, type III; lane 15, 82–-294, infant, nares, type III]; [lane 16, 81–224, mother, vagina, type III; lane 17, 81–226, infant, nares, type III]; [lane 18, 83–285, mother, vagina, type III; lane 19, 83–287, infant, nares, type III]; [lane 20, 81–108, mother, vagina, type III; lane 21, 81–122, infant, blood, type III].

Null results should be confirmed with a positive-control strain, such as A909.

It is notable that IS1381 analysis can detect differences between epidemiologically linked strains. In 2 cases, a single additional insertion was found between 2 strains isolated at delivery from maternal and infant sources. The presence of multiple other insertions on identically sized fragments strongly suggests that these 2 strains are related. The additional IS1381-containing fragment suggests that an insertion occurred during the time the mother was colonized. This finding demonstrates that IS1381 transposes frequently, leading to rapid divergence of strains. This technique can therefore differentiate between some strains that diverged recently from a common parent. Other epidemiological techniques do not distinguish between epidemiologically linked strains.

Previously described nongenetic subtyping methods are of limited use, whereas genetic subtyping methods are highly discriminatory. Ribotyping is straightforward to perform and easily analyzed, but unrelated strains often give identical ribotyping patterns [15]. REA and pulsed-field gel electrophoresis are useful for subtyping strains of types Ia, Ib, II, and III, but of limited utility in subtyping strains of type V [6]. Restriction enzyme analysis of chromosomal DNA (REAC) is highly discriminatory and can be used to subtype any GBS strain [3, 6–8]. RAPD can also be effectively used to discriminate GBS strains of the same capsular polysaccharide type, whereas epidemiologically linked strains give identical patterns [9]. IS1381 may provide a valuable complement to these analyses. The simplicity of the analysis allows IS1381 analysis to be used as an initial screen, with null results analyzed by use of other genetic techniques, such as RAPD or REAC. Alternatively, the rapid divergence of IS1381 sequences may allow for differentiation of otherwise identical strains, and IS1381 analysis can be used as a secondary screen for strains that give identical patterns with RAPD or REAC.

In summary, IS1381 analysis provides a simple and sensitive technique for discriminating between most unrelated GBS isolates, while resulting in identical or near-identical patterns for epidemiologically linked GBS isolates. These results suggest that IS1381 analysis is a useful method for subtyping GBS strains.

References

1. Blumberg HM, Stephens DS, Modansky M, et al. Invasive group B streptococcal disease: the emergence of serotype V. J Infect Dis **1996**;173: 365–73.
2. Jensen NE, Aarestrup FM. Epidemiological aspects of group B streptococci of bovine and human origin. Epidemiol Infect **1996**;117:417-22.
3. Bingen E, Denamur E, Lambert-Zechovsky N, et al. Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. J Infect Dis **1992**;165:569–73.
4. Huet H, Martin C, Geslin P, Grimont F, Quentin R. Ribotyping of *Streptococcus agalactiae* strains isolated from vaginas of asymptomatic women. Res Microbiol **1993**;144:457–65.
5. Hauge M, Jespersgaard C, Poulsen K, Kilian M. Population structure of *Streptococcus agalactiae* reveals an association between specific evolutionary lineages and putative virulence factors but not disease. Infect Immun **1996**;64:919–25.
6. Blumberg HM, Stephens DS, Licitra C, et al. Molecular epidemiology of group B streptococcal infections: use of restriction endonuclease analysis of chromosomal DNA and DNA restriction fragment length polymorphisms of ribosomal RNA genes (ribotyping). J Infect Dis **1992**;166: 574–9.

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7. Denning DW, Baker CJ, Troup NJ, Tompkins LS. Restriction endonuclease analysis of human and bovine group B streptococci for epidemiologic study. J Clin Microbiol **1989**; 27:1352–6.
8. Takahashi S, Adderson EE, Nagano Y, Nagano N, Briesacher MR, Bohnsack JF. Identification of a highly encapsulated, genetically related group of invasive type III group B streptococci. J Infect Dis **1998**; 177:1116–9.
9. Chatellier S, Ramanantsoa C, Harriau P, Rolland K, Rosenau A, Quentin R. Characterization of *Streptococcus agalactiae* strains by randomly amplified polymorphic DNA analysis. J Clin Microbiol **1997**; 35:2573–9.
10. Limansky AS, Guardati MC, Sutich EG, et al. Characterization of clinical isolates of *Streptococcus agalactiae* by random amplified polymorphic DNA using degenerate oligonucleotides. Medicina (B Aires) **1995**; 55: 681–4.
11. Granlund M, Oberg L, Sellin M, Norgren M. Identification of a novel insertion element, IS1548, in group B streptococci, predominantly in strains causing endocarditis. J Infect Dis **1998**; 177:967–76.
12. Sanchez-Beato AR, Garcia E, Lopez R, Garcia JL. Identification and characterization of I S1381, a new insertion sequence in *Streptococcus pneumoniae.* J Bacteriol **1997**; 179:2459–63.
13. Kasper DL, Baker C, Edwards M, et al. NIH Initiative on the prevention of group B streptococcal infections in neonatal and infant populations. Technical Proposal Volume I. RFP NIH NIAID DMID 92 13., **1992**.
14. Johnson DR, Ferrieri P. Group B streptococcal Ibc protein antigen: distribution of two determinants in wild-type strains of common serotypes. J Clin Microbiol **1984**; 19:506–10.
15. Chatellier S, Huet H, Kenzi S, Rosenau A, Geslin P, Quentin R. Genetic diversity of rRNA operons of unrelated *Streptococcus agalactiae* strains isolated from cerebrospinal fluid of neonates suffering from meningitis. J Clin Microbiol **1996**; 34:2741–7.

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