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# Typing of Group B Streptococci: Comparison of Pulsed-Field Gel Electrophoresis and Conventional Electrophoresis

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**The *Sma*I restriction endonuclease digestion patterns of chromosomal DNAs from 35 group B streptococci were analyzed by pulsed-field gel electrophoresis (PFGE). Nineteen different patterns and four possible variants were identified. Twenty-four isolates were previously analyzed by conventional electrophoresis of *Hin*dIII-digested and/or *Bgl*II plus *Eco*RI double-digested chromosomal DNA. Although interpretations by both methods were essentially the same, PFGE identified as variants two isolates that were previously classified as the same isolate. More importantly, PFGE of the chromosomal DNA of group B streptococci digested with *Sma*I generated more easily defined patterns, since fewer and better separated bands were obtained, whereas digestion with *Hin*dIII or *Eco*RI plus *Bgl*II typically generated 100 or more bands. *Sal*I digestion also yielded easily evaluable results, although the *Sal*I fragments were somewhat smaller than those generated by *Sma*I. In our hands, PFGE patterns were more easily discerned and interpreted than were patterns previously generated by conventional electrophoresis.**

Group B streptococci (GBS) are a major cause of mortality and morbidity in the neonatal period, frequently causing bacteremia and meningitis (1). In adults, GBS occur preferentially in certain groups, such as diabetics, pregnant and postpartum women, and patients who are immunocompromised, and can cause serious local infections or disseminated disease (1, 5, 11, 12, 13, 14, 16, 18). The modes of transmission and certain aspects of the pathogenesis of early- and late-onset GBS infections in neonates have been defined through classical epidemiological techniques and the use of markers such as the antibiotic susceptibilities of isolates, serotyping, and phage typing (2, 3). A simple and reliable technique for distinguishing isolates at the subspecies level would be very useful for defining outbreaks and investigating patients who are sources of late-onset disease. In the study described here we performed pulsed-field gel electrophoresis (PFGE) of chromosomal DNAs from a collection of GBS isolates to assess the potential utility of this technique as a tool for investigating the epidemiology of GBS infections.

## MATERIALS AND METHODS

**Bacterial strains.** Thirty-five isolates from the collection at the Baylor College of Medicine were tested; 24 isolates have been described previously (3). Nine isolates (designated with the prefix C) were from pregnant women in Mexico City and were kindly provided by Jose Luis Arredondo Garcia. Two strains (CNCTC 1/82 and CNCTC 10/84) were prototype IV and V strains, respectively, obtained from the Postgraduate Medical Institute and the Streptococcus Reference Laboratory, Prague, Czechoslovakia, and were kindly provided by Jarmila Jelinkova. The strain designation by source and serotype was initially unknown to the individual performing the chromosomal analysis by PFGE. Table 1 shows isolate designation, serotype, restriction endonuclease digestion pattern (REDP) by conventional gel electrophoresis as defined in a previous report (3), as well as special epidemiological features of particular strains.

**Serotyping.** Serotyping was done by the capillary precipitin method described by Lancefield (4) and others (3, 17) by using antisera prepared in rabbits. Certain antisera required absorption with appropriate strains to achieve type specificity (4, 17).

**Chromosomal analysis by PFGE.** Genomic DNA was prepared by described previously methods (7, 8). Briefly, GBS were grown overnight in 5 ml of brain heart infusion broth at 37°C. The cells were harvested and suspended in 2 ml of PIV buffer (1 M NaCl, 10 mM Tris hydrochloride [pH 7.6]). One milliliter of this suspension was mixed with 1 ml of 1.6% low-melting-temperature agarose (InCert Agarose; FMC Corp., Marine Colloids Division, Rockland, Maine) in warm water and was then pipeted into a plug mold (Bio-Rad Laboratories, Richmond, Calif.) and allowed to solidify. Plugs were placed in 10 ml of fresh lysis solution (6 mM Tris hydrochloride [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine, 20 μg of RNase per ml, 1 mg of lysozyme per ml), and the mixture was incubated overnight at 37°C. This solution was replaced with 10 ml of ESP (0.5 mM EDTA [pH 9 to 9.5], 0.5% sodium lauroyl sarcosine, 50 μg of proteinase K per ml), and the mixture was then incubated overnight at 50°C with shaking. The plugs were washed three times with 10 ml of TE (10 mM Tris-HCl [pH 7.5], 0.1 mM EDTA) and stored at 4°C.

On the basis of the results of previous work with gram-positive cocci (6, 7), we used *Sma*I for digestion of GBS DNA. This was done by incubating a small slice of an agarose plug in 200 μl of $H_2O$ with 2 μl of *Sma*I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and 25 μl of reaction buffer for 12 to 24 h at 37°C. The slices were washed with 1 ml of TE for at least 1 h at 37°C, melted at

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TABLE 1. Sources and characteristics of the GBS isolates used in the present study

| Isolate designation | Serotype | REA pattern[a] | PFGE[b] | Source[c] |
|---|---|---|---|---|
| 18, 22 | Ia | $A_1$ | a | Houston; mother-infant pair; 18R; 22B; EOS |
| 15 | Ia | $A_1$ | a′ | Houston; neonatal sepsis; B; EOS |
| 10, 25 | Ia | $A_2$ | b | Houston; mother-infant pair; B; EOS |
| C10 | Ia | NP[d] | c(a″) | Mexico; V |
| 2 | Ia/c | $B_1$ | d | Houston; neonatal sepsis; B; EOS |
| C40 | Ia/c | NP | e | Mexico; V |
| C41 | Ia/c | NP | e′ | Mexico; wound exudate |
| 26, 27, 28, 29, 30, 31, 32 | Ib/c | $C_1$ | f | Houston; neonatal outbreak (10); LOS |
| 7 | Ib/c | $C_4$ | g | Houston; neonatal sepsis; B; EOS; recent immigrant from Nigeria |
| C2 | Ib/c | NP | h | Mexico; endometrium |
| C21 | Ib/c | NP | h′ | Mexico; V |
| 17 | II | $D_2$ | l | Houston; neonatal sepsis; B; EOS |
| 23 | II | $D_3$ | m | Houston; adult bacteremia; B |
| C9 | II | NP | n | Mexico; V |
| C6 | II | NP | o | Mexico; urine |
| 6, 16 | III | $E_1$ | q(r′) | Houston; mother-infant pair; V (isolate 16); CSF (isolate 6); LOS |
| 901, 969 | III | $E_3$ | r | Palo Alto, Calif.; recurrent neonatal sepsis |
| 13, 20 | III | $E_4$ | s | Houston; mother-infant pair; V (isolate 13); CSF (isolate 20); EOS |
| C1 | III | NP | t | Mexico; V |
| C7 | III | NP | u | Mexico; urine |
| CNCTC 1/82 | IV | NP | v | Czechoslovakia; prototype type IV strain |
| 3, 11 | V | $F_7$ | w | Houston; mother-infant pair; V (isolate 3); B (isolate 11); EOS |
| CNCTC 10/84 | V | NP | x | Czechoslovakia; prototype type V strain |

[a] REA, restriction enzyme analysis by conventional electrophoresis (3).
[b] *Sma*I digestion pattern analyzed by PFGE. Patterns marked with a prime or a double prime were interpreted as probable variants (e.g., a, a′, and a″). When this interpretation was made subsequent to the initial classification, the initial name is given first and the pattern which it resembles is given in parentheses.
[c] B, blood; V, vagina; CSF, cerebrospinal fluid; R, rectal; EOS, early-onset sepsis; LOS, late-onset sepsis.
[d] NP, not performed.

65°C for 10 min, and loaded into the wells of 1.2% agarose gels (Sea Plaque GTG agarose; FMC) in 0.5× TBE buffer (10× of TBE is 0.89 M Tris, 0.025 M EDTA, and 0.89 M boric acid). Gels were processed with a contour-clamped homogeneous electric field device (CHEF DRII; Bio-Rad) and variable pulse times over 30 h at 200 V. Gels were stained with ethidium bromide; this was followed by 6 to 24 h of destaining in distilled water. The gels were then photographed with UV light. When discrepancies in the interpretations were observed by PFGE compared with those determined by conventional electrophoresis (3), isolates were reserotyped and then reprocessed by PFGE. Selected isolates were also digested with a battery of other enzymes, including *Not*I, *Sfi*I, *Xba*I, *Eag*I, *Rgr*II, *Sal*I, *Spe*I, and *Sst*I.

## RESULTS

**Serotype I.** Within serotype I, we observed two very different REDPs among the six serotype Ia isolates studied (Fig. 1 and Table 1) and two patterns that might represent variations of one of the two REDPs. The three almost identical isolates (isolates 15, 18, and 22) included a pair of isolates from a mother and her infant (early-onset sepsis) which had identical patterns (PFGE pattern a), and an isolate from a blood culture (isolate 15) from an unrelated neonate; the pattern of isolate 15 showed one additional fragment of ~60 kb (PFGE pattern a′). These three isolates had REDPs that were identical by conventional electrophoresis (3). Among the other three isolates, there was a pair from a mother and her infant (isolates 10 and 25) which had identical

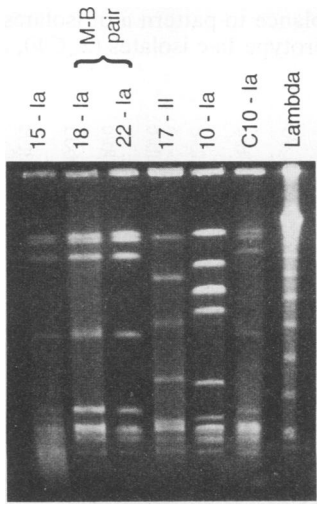


FIG. 1. PFGE of *Sma*I-digested chromosomal DNAs from serotype Ia and II GBS. Isolates 15 and 18 both showed PFGE pattern a; isolate 22 had a pattern similar to those of isolates 15 and 18 (a′), and isolate C10 had a pattern possibly related to those of isolates 15 and 18 (a″). The other two isolates had different patterns. The molecular size markers are bacteriophage lambda concatamers. M-B pair, pair of isolates from a mother and her infant.

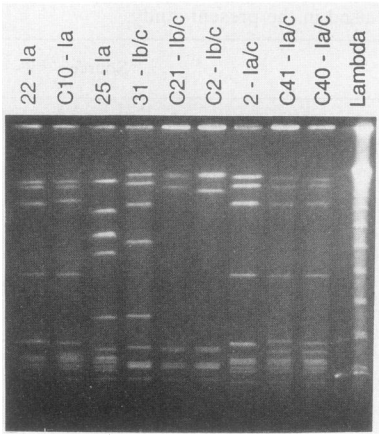


FIG. 2. PFGE of *Sma*I-digested chromosomal DNAs from serotype Ia, Ib/c, and Ia/c GBS. Isolates 22 and C10 showed PFGE patterns which appeared to be similar but not identical [a and c(a″), respectively], as did the pairs C21 and C2 (patterns h and h′, respectively) and C41 and C40 (patterns e and e′, respectively). The other isolates had different patterns.

patterns by PFGE; this pattern (designated pattern b) differed markedly from that seen with isolates 15, 18, and 22. Strain 10 was isolated from the blood culture of a woman who was febrile on postpartum day 1, and strain 25 was isolated from the blood of her full-term male infant who was evaluated for onset of fever and respiratory distress on the second day of life. When isolates 10 and 25 were studied by conventional electrophoresis (3), the patterns were also thought to be identical. The sixth serotype Ia isolate (isolate C10) was a vaginal isolate from a pregnant woman in Mexico. Compared with the first three serotype Ia isolates (isolates 15, 18, and 22), there was an increase in the size of one of the large fragments (Fig. 1 and 2) as well as differences in two of the small fragments that were best visualized on gels with good resolution for small fragments (data not shown); this pattern was classified as pattern c(a″) to emphasize its resemblance to pattern a of isolates 15, 18, and 22.

The three serotype Ia/c isolates (2, C40, and C41) studied

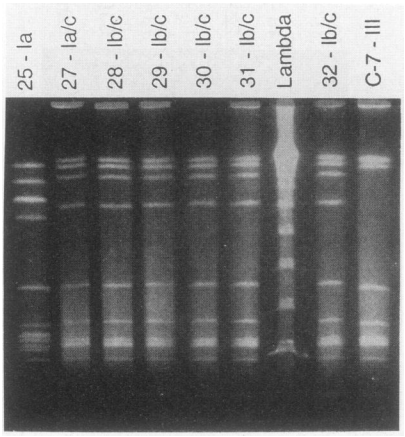


FIG. 3. PFGE of *Sma*I-digested chromosomal DNAs from serotype Ia, Ia/c, Ib/c, and III GBS, including isolates 27 to 32, which had identical PFGE patterns (pattern f), from an outbreak in a neonatal intensive care unit (10). The bands on this gel are not as well separated as the bands in Fig. 2 (shorter electrophoresis time).

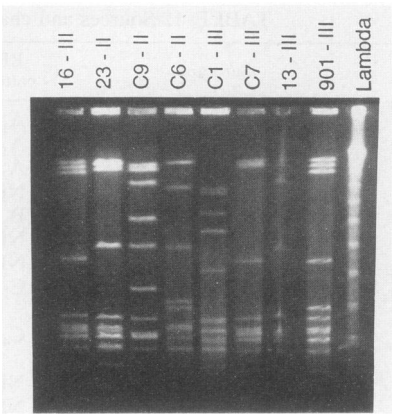


FIG. 4. PFGE of *Sma*I-digested chromosomal DNAs from serotype II and III GBS. All isolates except isolates 16 and 901 had different PFGE patterns. The patterns of isolates 16 and 901 appeared to be similar but not identical [patterns q(r′) and r].

had similar REDPs that differed in only two to four bands (Figure 2). These patterns were somewhat similar to those of the serotype Ia isolates 15, 18, and 22.

We observed three REDPs among the 10 Ib/c isolates (see Fig. 2 and 3 for selected isolates) and 1 isolate which we designated as a possible variant (isolate C21) because of its similarity to the pattern of isolate C2. Seven of these isolates (isolates 26 to 32) were from infants involved in an outbreak of late-onset disease in a neonatal intensive care unit in Houston previously described by Noya et al. (10). The restriction patterns of the outbreak strain and the other strains were markedly different.

**Serotype II.** The four serotype II isolates yielded four distinctly different patterns, three of which are shown in Fig. 4. One of the isolates from Houston (isolate 23) had some resemblance to serotype III isolates (Fig. 4), but there was

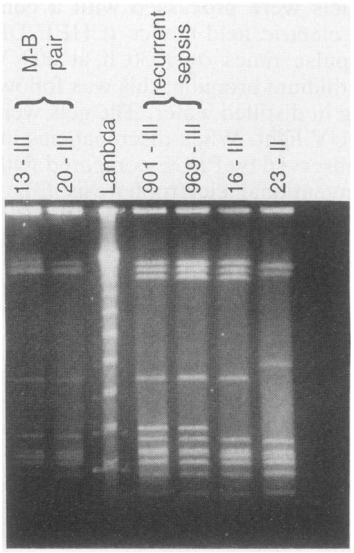


FIG. 5. PFGE of *Sma*I-digested chromosomal DNAs from serotype II and III GBS. The isolates from a mother and her infant (M-B pair; isolates 20 and 13) had identical PFGE patterns, as did isolates 901 and 969 recovered from the same individual. The pattern of isolate 16 was similar but not identical to those of isolates 901 and 969 (Fig. 4).

marked polymorphism among the serotype II isolates themselves. Two of these strains were studied and also classified as being different by conventional electrophoresis (3).

**Serotype III.** We studied eight serotype III isolates (Fig. 4 and 5). One pair of isolates from a mother and her infant (isolates 13 and 20, Fig. 5) had identical REDPs, as did two blood culture isolates from an infant with recurrent sepsis (isolates 901 and 969). A second pair of isolates from a mother and her infant (isolates 6 and 16) also had identical patterns that were different from the other serotype III patterns. Two Mexican serotype III strains, C1 and C4, each had a unique pattern.

**Serotypes IV and V.** The prototype strains of serotypes IV and V had unique patterns that were different from those of all other isolates studied (data not shown). One pair from a mother and her infant (isolates 3 and 11), initially classified as nontypeable (3) but recently reclassified as serotype V, had the same unique REDP.

For some isolates, *Sma*I generated patterns with only a few large fragments plus a number of smaller ones, with few fragments in the intermediate region (100 to 400 kb), and it was sometimes difficult to see all fragments on a single gel. Therefore, we also examined the patterns obtained with a number of other restriction enzymes. Only *Sal*I yielded an evaluable pattern; *Sal*I fragments were smaller and better seen when the gel was electrophoresed at a short pulse time (2 to 6 ms). Digestion with *Sal*I confirmed the identities of the REDPs of isolates 26 and 27 and isolates 10 and 25 and confirmed the dissimilarities of isolates 16 (serotype III) and 17 (serotype II) (data not shown). Other restriction enzymes used for this purpose either cut too infrequently (*Not*I, *Sfi*I, *Eag*I, *Rgr*II, *Spe*I) or too frequently (*Xba*I) to be useful.

## DISCUSSION

In order to adequately study the molecular epidemiology and pathogenesis of GBS infections, a simple, efficient, and reliable method of characterizing strains within serologic types is needed. Bacteriophage typing is a time-honored method (15) that has been used to help elucidate the epidemiology and pathogenesis of this group of organisms, but it is cumbersome, requires specific reagents, and, at least in one epidemic situation, gave inconsistent results (10). Plasmid pattern analysis has been successfully used for this purpose in other organisms, but plasmids are uncommon in GBS; hence, the value of plasmid pattern analysis is limited for elucidating the epidemiology and pathogenesis of GBS isolate. Recently, Denning et al. (3) demonstrated the value of conventional electrophoresis and restriction enzyme analysis in studying the relationship among strains at the chromosomal level, and this has been confirmed by others (2). The drawbacks of this technique relate mainly to difficulty in discerning the restriction patterns obtained; this is a result of the numerous and tightly clustered DNA bands seen on the gels. Although adjacent lanes can readily be compared, it is difficult to evaluate distinctly spaced lanes and it is particularly difficult to make even preliminary assessments when organisms are represented on different gels. Ribotyping also has been used as an epidemiological tool in an attempt to prove the role of breast milk as a vehicle for the transmission of GBS in late-onset neonatal GBS infections (2). However, ribotyping has not been rigorously tested as a subspecies typing scheme. Moreover, results of our own studies (unpublished data) with enterococci have shown that ribotyping fails to distinguish many strains that have markedly different REDPs by PFGE.

In the present study, we found that PFGE of GBS chromosomal DNAs yields reproducible and easily readable patterns. Although the patterns had fewer bands and less diversity than we have previously seen in enterococci (6–8), concordance and diversity were generally found when expected. In particular, isolates that were interpreted as having the same pattern by conventional electrophoresis of *Hin*dIII- or *Eco*RI plus *Bgl*II-digested chromosomal DNAs generally had the same or a closely related pattern by PFGE (Table 1). PFGE patterns, however, were easier to analyze because of the presence of a fewer number of bands that were spaced further apart. Both of these techniques evaluate total genomic DNA, and patterns could be influenced by the acquisition of a plasmid. With PFGE, a plasmid that remains extrachromosomal will not generally be present on the gel if it has no sites for the restriction enzyme being used. With enzymes such as *Sma*I, which have infrequent recognition sites in DNA with a low G+C content, the lack of a recognition site in a plasmid is quite possible. If recognition sites are present, the resulting linear band(s) appears as an additional band(s) on the gel. Since plasmids are often less than 100 to 150 kb, the band(s) will generally be in the lower range of the gel, the region visualized less well on most PFGE gels. This is one reason why we interpreted isolates with a difference of only one or a few bands, particularly in the lower region, as representing possible variants of each other. A plasmid which integrates into the chromosome will result in a change in one band and may yield new bands, again, depending on whether it has restriction sites for the restriction enzyme used. With conventional electrophoresis, which relies on digestion with enzymes with frequent recognition sites, additional DNA (e.g., plasmids) will almost certainly be cleaved and will thus add to the many bands already present. These may or may not be well seen, and the effect on interpretations would be variable.

It was interesting that some serotype Ia isolates (e.g., isolates 10 and 22) resembled serotype Ia/C isolates (e.g., isolates 2, C40, and C41). Multilocus enzyme analysis has also shown that isolates of different serotypes may have the same electropherotype, indicating that they belong to the same clone (9).

The similarity, although not identity, of some epidemiologically unrelated strains (e.g., isolates 18 and C10) may reflect the spread of a single clone over a large geographic area. In such instances, differences of one or two fragments may be a clue to the lack of the direct spread of a strain. However, a change in a fragment might also occur while a strain is infecting an individual, e.g., by mutations or by acquisition of a plasmid or a transposon. Thus, we generally classify isolates with patterns that differ by two bands as possible variants of each other, to emphasize a possible relationship, rather than as different, which might lead us to overlook a possible outbreak. When a single clone is truly widespread geographically, it may be impossible for electrophoresis of chromosomal DNA to verify that an outbreak has occurred, since even epidemiologically unrelated isolates will have similar or identical REDPs. In such instances, however, no technique is likely to be useful and investigation would have to rely on classical epidemiology.

Although more work needs to be done with PFGE to define its place for subspecies strain differentiation, it is clear that it has distinct advantages over some of the previously used typing schemes, primarily in that the bands and patterns are easily and clearly visualized. Further comparative studies between these different techniques will be important for defining their relative roles as epidemiological tools.

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