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Genetic Diversity of rRNA Operons of Unrelated *Streptococcus agalactiae* Strains Isolated from Cerebrospinal Fluid of Neonates Suffering from Meningitis

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The genetic diversity of a collection of 54 unrelated *Streptococcus agalactiae* strains isolated from the cerebrospinal fluid of neonates and of 60 unrelated carrier strains was evaluated by investigating the restriction fragment length polymorphism of the rRNA gene region. Three restriction enzymes were selected for use: *Pst*I, *Hin*dIII, and *Cfo*I. Clustering analysis revealed two phylogenetic groups of strains with 40% divergence. Group I contained two clusters, A and B, and group II contained three clusters, C, D, and E. Strains of serotype Ia were mostly distributed in cluster A, and strains of serotype Ib were mostly distributed in cluster E. Serotype III isolates did not cluster. Nevertheless, 37 of the 39 isolates belonging to cluster B were of serotype III. With *Hin*dIII, two rRNA gene banding patterns characterized 38 of the 39 strains of cluster B, which represents a high-virulence group. In addition, two rRNA gene banding patterns with each enzyme and/or a pair of *Cfo*I fragments of 905 and 990 bp identified 81% of the invasive strains. On account of the genetic homogeneity of the cerebrospinal fluid strains, ribotyping is a powerful typing method for investigation of nosocomial or epidemic invasive infections only when all three enzymes are used or when *Pst*I and *Hin*dIII or *Pst*I and *Cfo*I are combined with serotyping (index of discrimination, >0.95).

Streptococcus agalactiae remains a leading cause of neonatal sepsis and meningitis, with a prevalence of 0.5 to 3.7 cases per 1,000 live births (1). *S. agalactiae* is acquired by neonates mostly from maternal vaginal carriage (1) and occasionally by nosocomial or community transmission (1, 23) or by breast milk transmission (3).

Type-specific capsular polysaccharide antigens allow *S. agalactiae* isolates to be classified into serotypes Ia, Ib, II, III, IV, and V and two candidate serotypes, NT6 and 7271 (16, 18, 21, 25). These serotypes may be further defined according to the presence of protein antigen c, R, or X (8, 24). Serotype III *S. agalactiae* comprises a major group of strains which infect neonates in the United States (1), but this serotype is also common in asymptomatic carriers. In addition, serotypes other than type III are frequently isolated in cases of early-onset disease (9).

Population genetic analysis based on multilocus enzyme electrophoresis (MLEE) revealed the clonal structure of the populations of *S. agalactiae* in the United States (22), Denmark (13), and France (27). Although two phylogenetic divisions (I and II) were identified in each study, there are some differences in the relationships among neonatal invasive strains in these countries. In the United States, 85% of the division I strains were invasive, and therefore division I was considered to be a high-virulence clone (22). In France, division I comprises half of the invasive strains, most of which belong to two electrophoretypes. These two electrophoretypes contain 67% of the division I strains. This division also may be considered a high-virulence group (27). In Denmark, no association between specific evolutionary lineage and a particular disease was observed, although putative virulence factors correlated with the genetic structure of the *S. agalactiae* population (12, 13). Restriction endonuclease (RE) analysis also argues for a genetic homogeneity of potentially virulent strains of *S. agalactiae* (7).

These findings suggest that not all *S. agalactiae* strains isolated from the vagina are able to invade the central nervous system of neonates. Identification of convenient phenotypic or genotypic markers would be advantageous for screening of potentially virulent strains among the strains present in the genital tract of pregnant women. For example, markers of isolates belonging to phylogenetic division I, with a high risk of causing infection both in the United States and in France, would be of great value.

In the present study, we further examined the collection of *S. agalactiae* strains previously studied by MLEE by Quentin et al. (27). If invasive strains of *S. agalactiae* are indeed genetically homogeneous, their rRNA genes would exhibit some specific characteristics. We therefore used ribotyping to explore the rRNA genes of unrelated *S. agalactiae* strains isolated from the cerebrospinal fluid (CSF) of neonates. The objectives of this genetic characterization were (i) to confirm that invasive isolates exhibit less genetic diversity than do carrier strains, (ii) to examine whether particular rRNA gene banding patterns are characteristic of the high-risk division I defined by MLEE and of strains isolated from CSF (27), and (iii) to determine whether ribotyping provides a sufficiently discriminatory method to investigate epidemic or nosocomial *S. agalactiae* neonatal invasive infections.

MATERIALS AND METHODS

Bacterial isolates. A total of 54 unrelated strains isolated from CSF were collected from 25 general hospitals throughout France (9, 14). The geographic

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origins of the members of this national collection of isolates have previously been described (27). All CSF strains were isolated from neonates with clinical and biological signs of meningitis (45 strains from individuals with early-onset disease and 9 from babies with late-onset disease). Fifty-nine unrelated strains isolated from asymptomatic patients were used for comparison with invasive strains; 37 strains were from the vagina of asymptomatic women, and 22 strains were from the gastric fluid of asymptomatic neonates. The type strain (NCTC 8181) was used as a reference.

All isolates were previously characterized by MLEE analysis (27) and belonged to two major phylogenetic divisions (I and II) (Fig. 1). Twenty-seven invasive strains belonged to division I, and 27 invasive isolates belonged to division II. Thirteen carrier strains were assigned to division I, and 47 carrier strains were assigned to division II.

Serotyping. Strains were typed by double diffusion and/or polystyrene-latex agglutination as previously described (9, 17, 29).

Bacterial DNA preparation. Each strain was subcultured on eight horse blood trypticase soy agar plates and incubated for 24 h at 37°C. The cultures were harvested in 15 ml of buffer (10 mM Tris-HCl, 5 mM EDTA, 1 M NaCl, 0.5% Triton X-100) and lysed by adding 150 μl of freshly prepared lysozyme (100 mg/ml) (Sigma, St. Louis, Mo.) and 150 μl of proteinase K (20 mg/ml; Sigma). The mixture was shaken overnight at 37°C to allow cell lysis. DNA was extracted and purified as previously described by Brenner et al. (6).

rRNA gene RFLP. rRNA gene restriction fragment length polymorphism (RFLP) was investigated by a method derived from those described by Grimont and Grimont (11) and Picard-Pasquier et al. (26). DNA (5 μg) was digested with 20 U of *Pst*I, *Hin*dIII, or *Cfo*I RE according to the manufacturer's instructions (Boehringer, Mannheim, Germany, or Eurogentec, Seraing, Belgium).

Restriction fragments were separated by horizontal agarose gel electrophoresis for 16 h at a constant voltage (50 V). Gels contained 0.8% agarose (Seakem ME; FMC BioProducts, Rockland, Maine) in TBE buffer (130 mM Tris, 80 mM borate, 2.5 mM EDTA [pH 8.0]) for restriction digests obtained with *Pst*I or *Hin*dIII and 1.2% agarose in TBE buffer for fragments obtained with *Cfo*I. A 1-kb DNA ladder (Life Technologies, Gaithersburg, Md.) and *Citrobacter diversus* DNA cleaved with *Mlu*I were used as molecular-size standards. Gels were stained with ethidium bromide (1 μg/ml) for 30 min. DNA fragments were vacuum transferred to a nylon membrane (GeneScreen Plus; NEN Research, Boston, Mass.) as recommended by the VacuGene vacuum manufacturer (Pharmacia, Uppsala, Sweden).

Ribosomal 16S plus 23S RNA from *Escherichia coli* (Boehringer) was used as a probe and labeled by random oligopriming with a mixture of hexanucleotides (Pharmacia), cloned Moloney murine leukemia virus reverse transcriptase (Life Technologies), and [^{32}P]dCTP (800 Ci/mmol; Amersham, Buckinghamshire, England) according to the manufacturer's instructions.

DNA was hybridized overnight at 65°C, and the membranes were then washed with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 10 min at room temperature, with 0.2× SSC containing 0.15% (wt/vol) sodium dodecyl sulfate for 5 min at 65°C, and with 0.1× SSC for 5 min at room temperature. The membranes were autoradiographed by exposure to X-Omat AR film (Eastman Kodak Company, Rochester, N.Y.).

Each different combination of rRNA gene banding patterns obtained with the different REs was defined as a distinct ribotype.

rRNA gene RFLP analysis. Autoradiographs of each gel were digitized with a video camera connected to a microcomputer (Bio-Profil; Vilber-Lourmat, Marne la Vallée, France). Numerical analysis was performed with the Taxotron package (Taxolab, Institut Pasteur, Paris, France), which includes four software programs: RestrictoScan, RestrictoTyper, Adanson, and Dendrograf. The images, in tagged image file format, were transferred to a Macintosh microcomputer, and the band migration values for each lane were determined in pixel units with RestrictoScan. Fragment sizes were calculated from migration data, with RestrictoTyper implementing the cubic spline method to schematize the electrophoretic patterns.

A similarity matrix was obtained on the basis of the Dice coefficients, i.e., the ratio of twice the number of fragments common to each pair of strains to the sum of all bands in the two strains. The relationships between rRNA gene banding patterns and between ribotypes were calculated by the unweighted pair group method with arithmetic mean (30) with the Adanson clustering program. A dendrogram of the tree description file was drawn by Dendrograf.

Index of discrimination. The single numerical index of discrimination (D) described by Hunter and Gaston was used to evaluate the discriminatory power of the typing method (15). The probability of two unrelated strains being placed into different typing groups was calculated by the following equation:

$$D = 1 - [1/N(N-1)] \sum_{j=1}^{S} n_j(n_j - 1)$$

where N is the total number of strains in the sample population, S is the total number of types described, and n_j is the number of strains belonging to the jth type. This index can be used to evaluate the discriminatory power of ribotyping (2, 4, 10) and/or serotyping (15).

RESULTS

Choice of endonucleases and reproducibility of rRNA gene RFLPs. To select REs that give a good distribution of restriction length fragments, ribotyping was initially performed on 18 *S. agalactiae* isolates from asymptomatic women with 20 different REs (*Apa*I, *Bam*HI, *Bgl*I, *Bgl*II, *Bst*I, *Cfo*I, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hin*fI, *Hpa*II, *Mlu*I, *Kpm*I, *Stu*I, *Pst*I, *Sac*I, *Sca*I, *Sst*II, and *Xho*I). *Pst*I, *Hin*dIII, and *Cfo*I gave suitable numbers of fragments covering a large range of sizes of restriction fragments and were therefore chosen for use in the isolate studies.

To evaluate the reproducibility of the method, all strains were subcultured and their DNAs were subsequently extracted and tested at least three times. The rRNA gene banding patterns were not altered by subculturing.

Gels containing 1.2% agarose in TBE buffer, instead of the 0.8% used for *Pst*I and *Hin*dIII, were required to increase the separation of *Cfo*I fragments of low molecular weight. The *Cfo*I bands under consideration ranged between 0.4 and 8 kbp.

Genetic diversity of rRNA operons of *S. agalactiae* isolates. Among all the isolates, 33 rRNA gene banding patterns were obtained with *Pst*I (P1 to P33), 27 were achieved with *Hin*dIII (H1 to H27), and 29 resulted with *Cfo*I (C1 to C29) (Fig. 2).

Determination of the relationships between rRNA gene banding patterns obtained for each RE revealed a low degree of dissimilarity (Fig. 2). When restricted with *Pst*I, the rRNA genes exhibited 56% dissimilarity for all isolates but less than 25% dissimilarity for 93% of the isolates. The rRNA genes exhibited less than 23% dissimilarity when restricted with *Hin*dIII. Greater polymorphism (36% dissimilarity) resulted from restriction with *Cfo*I.

Combining the data obtained with the three REs, 72 ribotypes were identified among the 114 strains. The genetic relationships among all ribotypes of *S. agalactiae* are represented in the dendrogram shown in Fig. 1. The analysis identified two phylogenetic groups of strains (groups I and II) with 40% divergence. Group I contained 58 isolates which were assigned to 26 ribotypes. Two clusters, A (15 strains) and B (39 strains), with 23% divergence were identified within this group. Group II was composed of 56 isolates and included 46 ribotypes. Three clusters, C (10 strains), D (20 strains), and E (18 strains), with 22% divergence were identified within this group. Group I also contained four carrier isolates not assigned to cluster A or B, and group II contained five CSF isolates and three carrier isolates not grouped in cluster C, D, or E.

Genetic variation of rRNA operons in relation to serotype and to genetic distribution of strains as defined by MLEE analysis. A total of 112 clinical *S. agalactiae* isolates were serologically typeable; the type strain is of serotype II, and one vaginal isolate was not typeable. All CSF isolates were typeable for capsular polysaccharide. Five carrier isolates that were not typeable for capsular polysaccharide were typeable for protein antigens (Table 1).

Analysis of the data revealed a correlation between genetic distributions defined by ribotypes and serotypes (Fig. 1 and Table 1). Fourteen of the 16 serotype Ia (Ia and Ia/c) strains were represented in cluster A, and 8 of the 12 serotype Ib (Ib and Ib/c) strains were represented in cluster E. The distribution of the 55 serotype III isolates was more polymorphic. Nevertheless, 37 of the 39 isolates belonging to cluster B were of serotype III or III/R. The other 18 serotype III isolates were in group II and did not cluster.

Comparison of the genetic structure of the population defined by MLEE analysis (27) and that obtained in this study

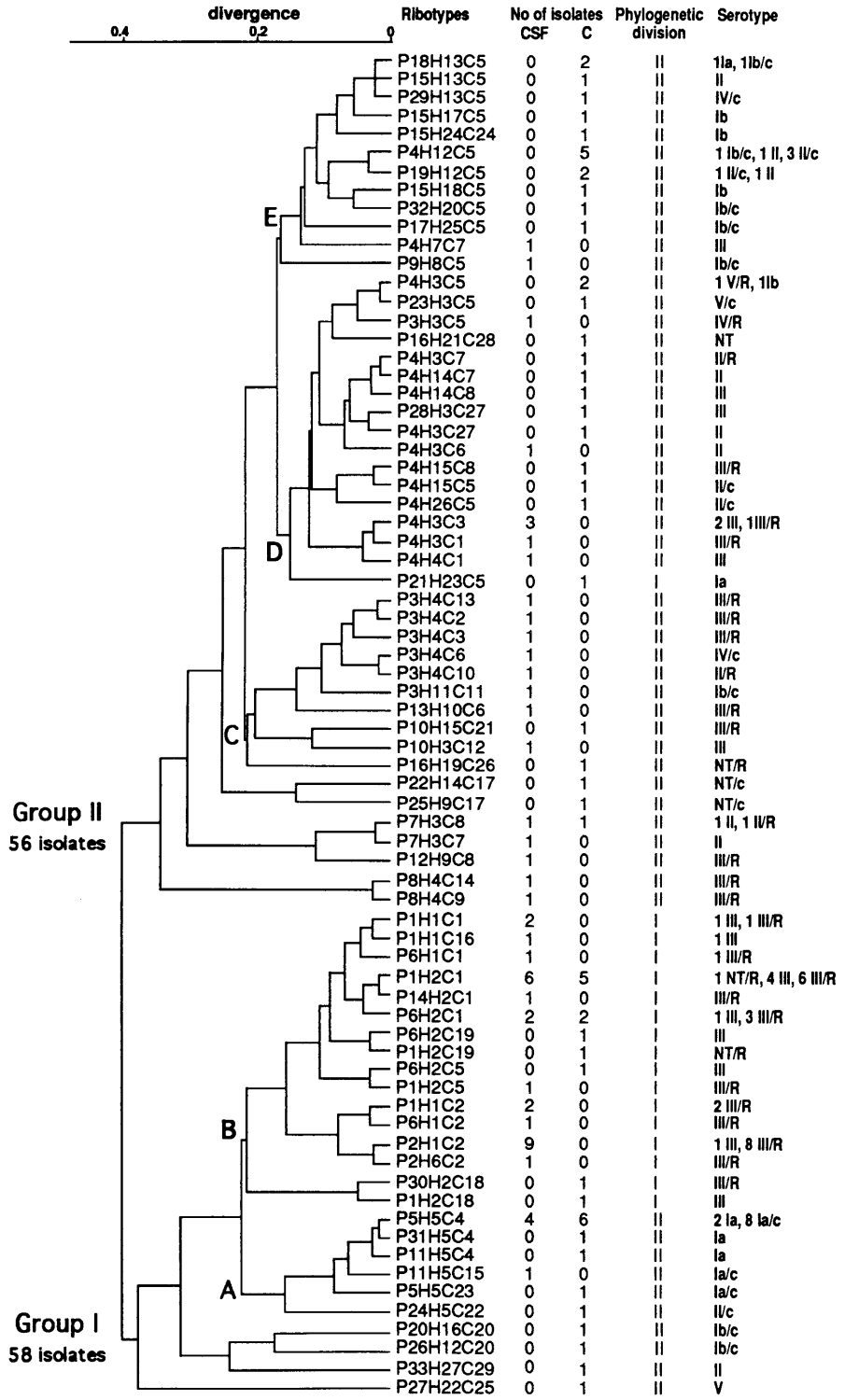

FIG. 1. Genetic relationships of the 114 *S. agalactiae* strains as estimated by clustering analysis of rRNA gene RFLP data obtained by DNA digestion with *Pst*I, *Hin*dIII, and *Cfo*I. The dendrogram was generated by the average-linkage method of clustering (unweighted pair group method with arithmetic mean). The ribotype, phylogenetic division previously defined by MLEE analysis (27), and serotype of each isolate are listed. Two groups (I and II) were identified at a distance of 0.4. There are two clusters within group I (A and B) and three clusters within group II (C, D, and E). Of the cluster B isolates, 69% are CSF strains, which represent 50% of all invasive isolates. C, carrier isolates.

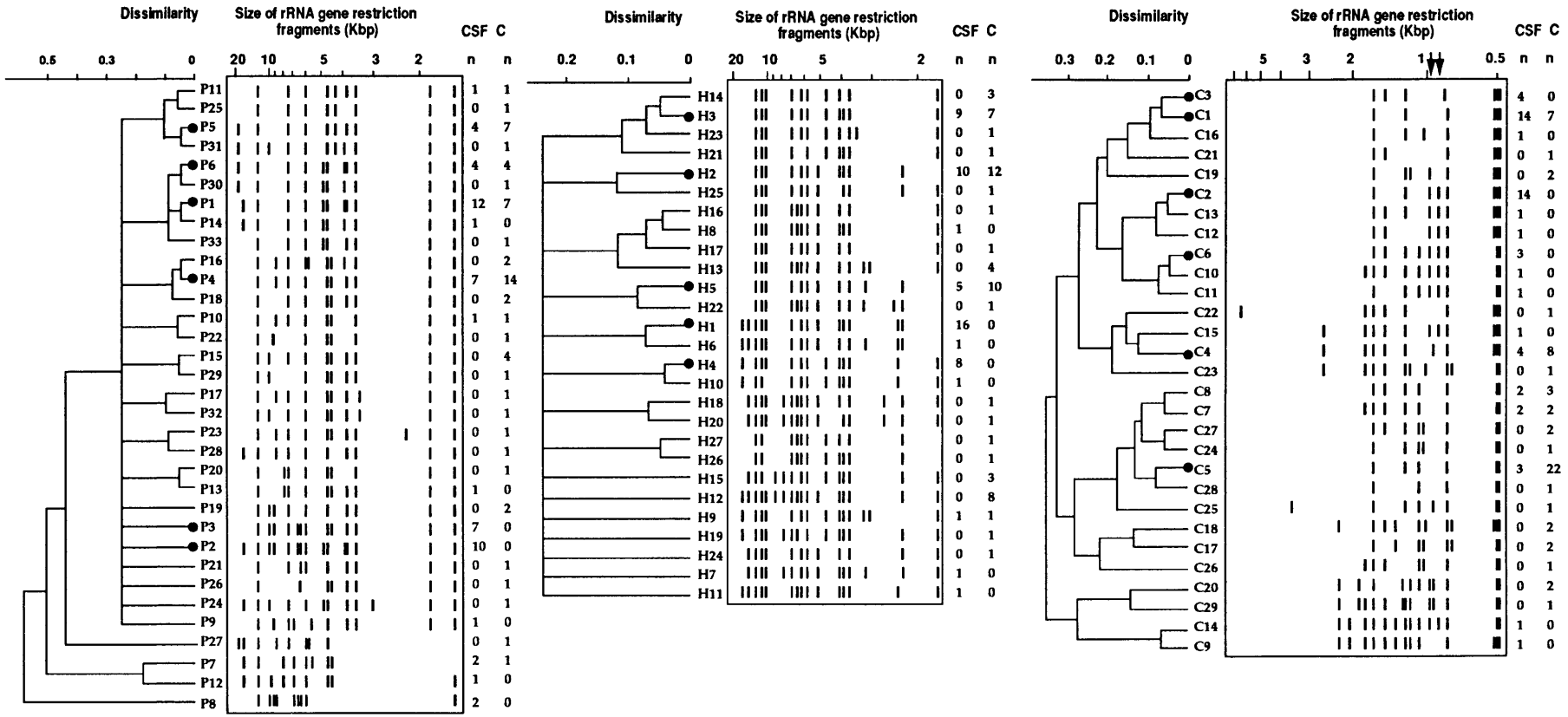

FIG. 2. Levels of similarity among rRNA gene banding patterns obtained with *Pst*I (P1 to P33), *Hin*dIII (H1 to H27), and *Cfo*I (C1 to C29). The dendrograms were generated by the average-linkage method of clustering (unweighted pair group method with arithmetic mean). Each branch of the tree faces each rRNA gene banding pattern. Most of the CSF isolates were assigned to five or six patterns (P1 to P6, H1 to H5, or C1 to C6) (●) with each RE. The nine strains isolated from cases of late-onset disease were in P1 (three strains), P2 (two strains), P3 (two strains), P9 (one strain), and P13 (one strain); H1 (three strains), H2 (two strains), H4 (one strain), H8 (one strain), H10 (one strain), and H11 (one strain); and C1 (one strain), C2 (three strains), C3 (one strain), C5 (two strains), C6 (one strain), and C11 (one strain). Downward-pointing arrows indicate *Cfo*I fragments of 905 and 990 bp. C, carrier isolates.

TABLE 1. Distribution of *S. agalactiae* isolates according to the clusters generated from rRNA gene RFLP data and according to serotype

Serotype	No. of isolates							No. of ribotypes
	Total	Cluster					Noncluster	
		A	B	C	D	E		
Ia	6	4			1	1		5
Ia/c	10	10						3
Ib	4				1	3		4
Ib/c	8			1		5	2	8
II	9				3	3	3	9
II/c	7	1			2	4		5
II/R	3			1	1		1	3
III	18		11	1	5	1		14
III/R	37		26	5	3		3	22
IV/c	2			1		1		2
IV/R	1				1			1
V	1						1	1
V/c	1				1			1
V/R	1				1			1
NT[a]	1				1			1
NT/c[a]	2						2	2
NT/R[a]	3		2	1				3

[a] NT, not typeable.

TABLE 2. Distribution of CSF and carrier isolates among the rRNA gene banding patterns in which most of the CSF strains clustered

rRNA gene RFLP	No. of CSF strains (%)	No. of carrier strains (%)	*P*
P1	12 (22.2)	7 (11.7)	0.13
P2	10 (18.5)	0 (0)	0.0006
P3	7 (13.0)	0 (0)	0.0041
P4	7 (13.0)	14 (23.3)	0.15
P5	4 (7.4)	7 (11.7)	0.45
P6	4 (7.4)	4 (6.7)	0.87
H1	16 (29.6)	0 (0)	<0.0001
H2	10 (18.5)	12 (20.0)	0.84
H3	9 (16.7)	7 (11.7)	0.45
H4	8 (14.8)	0 (0)	0.002
H5	5 (9.2)	10 (16.7)	0.24
*Cfo*I group[a]	23 (42.6)	0 (0)	<0.0001
C1	14 (25.9)	7 (11.7)	0.05
C3	4 (7.4)	0 (0)	0.03
C4	4 (7.4)	8 (13.3)	0.30

[a] The *Cfo*I group was composed of C2, C6, C10, C11, C12, C13, C14, and C15 patterns characterized by a pair of fragments of 905 and 990 bp.

(Fig. 1) showed that 39 of the 40 strains belonging to the high-virulence group defined by MLEE (phylogenetic division I) were in group B. Fifty-five of the 74 strains belonging to MLEE phylogenetic division II (27) were in group II, as defined by genetic analysis of the rRNA genes. The other 19 MLEE division II strains were in group I in this study. Their rRNA genes were therefore more related to those of strains of cluster B, which represents MLEE phylogenetic division I.

rRNA gene banding patterns as markers of high-risk group B and CSF *S. agalactiae* strains. rRNA operons of CSF isolates were less variable than those of carrier isolates. With *Pst*I, 14 rRNA gene banding patterns were identified for CSF isolates versus 26 for carrier isolates. With *Hin*dIII, 11 patterns were identified for CSF isolates versus 20 for carrier isolates. With *Cfo*I, 16 patterns were identified for CSF isolates versus 18 for carrier isolates.

Some patterns may be considered as possible markers of invasive strains. First, the rRNA genes of 38 of 39 strains belonging to group B (Fig. 1) gave patterns H1 or H2 when restricted with *Hin*dIII. Second, patterns characterized by a pair of fragments of 905 and 990 bp (C2, C6, and C10 to C15) after restriction with *Cfo*I were observed for 23 CSF strains but no carrier isolates (Fig. 2). In addition, a statistical analysis of the distribution of strains among rRNA gene banding pattern groups comprising at least four CSF isolates (Table 2) showed that patterns P2, P3, H1, H4, C1, and C3 and the pair of 905- and 990-bp *Cfo*I fragments were significantly more associated with invasive strains (χ^2 test, $P \leq 0.05$). In our CSF population, 44 of the 54 strains (81%) exhibited at least one of these markers, 23 of the 54 strains (43%) exhibited at least two of these markers, and 14 of the 54 strains (26%) exhibited three of these markers.

The distribution of the nine CSF strains isolated from cases of late-onset disease among rRNA gene groups was not significantly different from that of CSF strains isolated from cases of early-onset disease (Fig. 2).

Ribotyping as an epidemiological tool for invasive *S. agalactiae* strains. Because the CSF *S. agalactiae* isolates exhibit appreciably less genetic diversity than all the carrier isolates, and because an epidemiological tool would be an interesting means of investigating possible nosocomial or epidemic invasive infections, we evaluated the discriminatory power of ribotyping only for the 54 members of the invasive-strain class (20). The index of discrimination (*D*) was calculated for serotyping and for ribotyping with each RE, either alone or in combination (Table 3). An index of discrimination of greater than 0.95, the threshold value for interpreting typing results with confidence (15), was obtained with *Pst*I plus *Hin*dIII plus *Cfo*I ($D = 0.956$). This combination was able to define 33 ribotypes among the 54 CSF strains.

In combination with serotyping, a suitable index was obtained with *Pst*I plus *Hin*dIII ($D = 0.954$), *Pst*I plus *Cfo*I ($D = 0.953$), and *Pst*I plus *Hin*dIII plus *Cfo*I ($D = 0.964$).

DISCUSSION

S. agalactiae is the most common cause of invasive disease in neonates, originating most often from vaginal carriage by pregnant women. Nevertheless, this bacterium may also colonize the vagina and neonates without apparent clinical consequences for the newborn (1).

It is important to confirm the genetic homogeneity of invasive *S. agalactiae* populations suggested by MLEE and RE analysis (7, 22, 27) in order to formulate convenient phenotypic or genotypic criteria for identifying virulent clone families among strains present in the genital tract and for proposing appropriate epidemiological methods of investigating nosocomial *S. agalactiae* infections.

Exploration of the rRNA operons of bacteria is a valuable genetic method of studying the polymorphism of a species (4, 10, 11, 19, 28). Nevertheless, the use of dendrograms constructed from ribotyping data for phylogenetic and population genetic studies is not a fully accepted approach. Two patterns may have a fragment of a given size originating from different rRNA genes or from a different number of gene copies. Thus, dendrograms may not accurately represent taxonomic relationships among isolates (31).

A well-recognized method for studying the genetic structure of bacterial populations is MLEE analysis. The dendrogram we generated by ribotyping revealed only a population structure

TABLE 3. Numerical index of discrimination (*D*) calculated from ribotyping (with *Pst*I, *Hin*dIII, and/or *Cfo*I) alone or in association, serotyping alone, or ribotyping with serotyping of the 54 CSF strains

Typing method(s) used	No. of types	D^a
Ribotyping with *Pst*I	14	0.883
Ribotyping with *Hin*dIII	11	0.832
Ribotyping with *Cfo*I	16	0.858
Ribotyping with *Pst*I and *Hin*dIII	21	0.932
Ribotyping with *Pst*I and *Cfo*I	29	0.940
Ribotyping with *Hin*dIII and *Cfo*I	28	0.928
Ribotyping with *Pst*I, *Hin*dIII, and *Cfo*I	33	0.956
Serotyping	8	0.619
Ribotyping with *Pst*I, serotyping	24	0.929
Ribotyping with *Hin*dIII, serotyping	20	0.900
Ribotyping with *Cfo*I, serotyping	25	0.900
Ribotyping with *Pst*I and *Hin*dIII, serotyping	29	0.954
Ribotyping with *Pst*I and *Cfo*I, serotyping	34	0.953
Ribotyping with *Hin*dIII and *Cfo*I, serotyping	32	0.944
Ribotyping with *Pst*I, *Hin*dIII, and *Cfo*I; serotyping	37	0.964

[a] The typing method was discriminatory when D was ≥ 0.95 (15).

comparable to that shown by MLEE (27) when the three REs were combined so as to be able to differentiate isolates efficiently ($D > 0.95$). Specifically, both of the two approaches identified the same high-risk group of strains (MLEE division I or ribotyping cluster B). The good correlation between results obtained with the two methods indicates that the numerical analysis used here may be proposed for evaluating the genetic diversity and relationships of strains within the *S. agalactiae* population.

With this tool, *S. agalactiae* CSF isolates displayed a low diversity of rRNA operons in strains from carrier isolates and exhibited particular rRNA banding patterns. This confirms and extends earlier results (27) suggesting that some genotypes are more likely than others to cause neonatal meningitis. In accordance with this idea, a correlation between particular evolutionary lineages and putative virulence factors has been observed. Serotypes Ia, Ib, and III and protein antigen c cluster in specific lineages (27). In recent studies (12, 13), cluster analysis of a Danish collection of *S. agalactiae* strains with a combination of several markers revealed a strong correlation between genetic division and serotype, ribotype, hyaluronidase gene type and expression, presence of gene sequences hybridizing with the alpha- and beta-antigen gene probes, and expression of beta-antigen. However, no single characteristic was preferentially associated with the clinical history of the strains in these studies. Disagreement among results of various studies may be due to geographic differences in the clonal composition of the *S. agalactiae* population and/or to the selection of strains considered to be pathogens.

Although CSF isolates may be marked by particular ribotypes, and in spite of the existence of a close correlation between specific evolutionary lineage and putative virulence factors, it would be premature to propose the use of ribotyping for screening for potentially highly virulent isolates among strains colonizing women during pregnancy, for three reasons: (i) a safe and efficient method using, for example, PCR must be developed before ribotyping will be suitable for use in clinical diagnostic practice, (ii) it would be necessary to test clinical isolates of other national collections to ensure that virulent strains present particular patterns or characteristics, and (iii) genes more directly implicated in virulence than rRNA genes or genes coding for metabolic enzymes may evolve independently, more influenced by environmental and host factors.

In contrast, ribotyping should be used by reference laboratories as a valuable typing method to investigate epidemic *S. agalactiae* invasive infections. Although the use of ribotyping with *Cfo*I in combination with RE analysis of DNA should prove useful in epidemiological investigations of *S. agalactiae* infections (5), the relatively few different *S. agalactiae* clones implicated in invasive infections might restrict the conclusions of such studies (3). Evaluation of a typing method according to the criteria of typeability, reproducibility, and discriminatory power is required before it can be used for epidemiological purposes (4, 15, 20). The typeability and reproducibility of ribotyping are excellent, both being near 100%. As for all methods, the discriminatory power of ribotyping depends on the genetic diversity of the class of isolates being examined (4, 20). Indeed, within *S. agalactiae* strains, genetic diversity varies appreciably between pathogenic, carrier, and environmental strains (22, 27). Therefore, if the objective is to explore epidemic or nosocomial invasive infection, the discriminatory index should not be evaluated for populations of strains including carrier isolates or environmental isolates but rather should be tested specifically on isolates involved in invasive infections. For the class of *S. agalactiae* strains responsible for meningitis, only ribotyping with all three of the REs (*Pst*I, *Hin*dIII, and *Cfo*I) or two of the three REs (*Pst*I and *Hin*dIII or *Pst*I and *Cfo*I) in conjunction with serotyping has an appropriate level of discriminatory power, and thus this is a powerful typing method to investigate nosocomial or epidemic invasive infections.

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