JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2006, p. 3245–3250 Vol. 44, No. 9
0095-1137/06/$08.00+0 doi:10.1128/JCM.02550-05

Loss of Catabolic Function in *Streptococcus agalactiae* Strains and Its Association with Neonatal Meningitis

Anne-Sophie Domelier,[1,2]* Nathalie van der Mee-Marquet,[1,2] Adeline Grandet,[2] Laurent Mereghetti,[1] Agnès Rosenau,[1] and Roland Quentin[1,2]*

Equipe d'Accueil 3854, Institut Fédératif de Recherche 136 "Bactéries et risque maternofoetal," UFR Médecine, Université François-Rabelais de Tours, 2 bis Boulevard Tonnellé, 37032 Tours Cedex, France,[1] and Service de Bactériologie-Hygiène, CHRU Trousseau, 37044 Tours Cedex 9, France[2]

Received 7 December 2005/Returned for modification 21 March 2006/Accepted 21 July 2006

The abilities of 151 *Streptococcus agalactiae* strains to oxidize 95 carbon sources were studied using the Biolog system. Two populations were constituted: one with a high risk of causing meningitis (HR group; 63 strains), and the other with a lower risk of causing meningitis (LR group; 46 strains). Strains belonging to the HR group were significantly less able to use four carbon sources, i.e., α-D-glucose-1-phosphate, D-ribose, β-methyl-D-glucoside, and D,L-α-glycerol phosphate, than strains from the LR group ($P \leq 0.004$). Moreover, strains in the HR group significantly more frequently possessed one of several mobile genetic elements or genome deletions previously shown to be associated with strains responsible for neonatal meningitis than strains in the LR group ($P < 0.001$). These findings suggest that genetic disruption might have occurred in virulent clones of *S. agalactiae*. Fifteen biotypes (B1 to B15) were identified from the results of oxidation of the four carbon sources, of which six (B1 to B6) included 92% of the isolates belonging to the HR group. Strains of biotypes B1 to B6 are thus 13 times more likely to be able to invade the central nervous system of neonates than strains of biotypes B7 to B15. In addition, 86% of strains recently associated with neonatal meningitis (42 strains studied) were identified as being of biotypes B1 to B6. Identification of particular *S. agalactiae* biotypes may therefore be one of the criteria to assist clinicians in assessing the level of risk of neonatal meningitis when a mother and/or her neonate is colonized with *S. agalactiae*.

Streptococcus agalactiae, a bacterial pathogen frequently carried in the normal fecal and/or vaginal flora, is a leading cause of severe bacterial infections in newborn infants (39). Phenotypic differences between the *S. agalactiae* strains causing invasive central nervous system (CNS) infections in neonates and those that colonize neonates but do not cause invasive CNS infection have been identified. These include growth characteristics and production of enzymes and capsular sialic acid (25, 27, 32). However, these differences have not been confirmed by molecular studies. Serotype III polysaccharide antigens (5) and surface protein antigens, including Rib, C alpha, C alpha-like, C beta, and the immunoglobulin A-binding protein (24, 27, 44), have also been identified as virulence factors (10), but no single factor can account for the pathogenicity of *S. agalactiae* species, suggesting that as-yet-unidentified factors contribute to pathogenicity.

Multilocus enzyme electrophoresis (MLEE) is a valuable first step towards correlating specific genotypes with particular diseases. It has been used for *S. agalactiae* species and has distinguished two strongly differentiated lineages in the species (31, 33, 40). The strains responsible for neonatal meningitis are not randomly distributed in these two phylogenetic groups, indicating differences in the virulences of strains of these groups (31, 33). Multilocus sequence typing (MLST) and other molecular methods have confirmed this phylogenetic distribution of strains (17, 20, 34, 42). One lineage, described as ET1 by MLEE by Musser et al. (31) and as division I by Quentin et al. (33), is homogenous and mainly composed of serotype III strains associated with neonatal invasive disease. The MLST ST-17 presumably corresponds to this lineage. The second lineage is highly genetically diverse and includes isolates with various capsular serotypes. According to MLST, ST-19 and ST-1 also contain different capsular serotypes and have been significantly associated with the carrier state (20). We recently demonstrated that various MLST lineages were strongly associated with the presence of mobile genetic elements in the genomes of strains, probably due to horizontal gene transfers (17). This may explain why virulent subgroups of strains able to invade the CNSs of neonates more frequently possess the insertion sequence IS*1548* in the *hylB* gene (encoding hyaluronate lyase), the group II intron GBSi1 inserted downstream from the C5a-peptidase gene *scpB*, and a unique cluster of tRNA genes at the 3′ end of the rRNA operons (15, 16, 34, 35).

Such an evolution involving horizontal genetic transfer may also involve changes in catabolic functions, which have already been reported for several species (7, 8, 45). Variations in basic housekeeping functions, such as those involved in growth, detoxification, and nutrient transport, have been found to affect the capacity for virulence of *S. agalactiae*, possibly by affecting fitness in various host environments (12, 18, 19, 25, 36, 41). We therefore hypothesized that the groups of *S. agalactiae* strains able to invade the CNS of neonates could have particular metabolic profiles, including characteristic patterns of carbon source utilization.

To investigate this point, we tested the abilities of 151 *S.*

* Corresponding author. Mailing address: Equipe d'Accueil 3854, Institut Fédératif de Recherche 136 "Bactéries et risque maternofoetal," UFR Médecine, Université François-Rabelais de Tours, 2 bis Boulevard Tonnellé, 37032 Tours Cedex, France. Phone: 33 2 47 47 81 13. Fax: 33 2 47 47 85 30. E-mail for Roland Quentin: quentin@med.univ-tours.fr. E-mail for Anne-Sophie Domelier: as.domelier@chu-tours.fr.

agalactiae strains to utilize 95 different carbon sources. The aim was to determine if *S. agalactiae* strains belonging to a population associated with a high risk of neonatal meningitis exhibited particular carbon source utilization profiles. We found that the group of strains associated with CNS infections in neonates was unable to use one or several of the following carbon sources: α-D-glucose-1-phosphate, D-ribose, β-methyl-D-glucoside, and D,L-α-glycerol phosphate. This loss of catabolic function was correlated with a high prevalence of genetic markers of virulent clones of *S. agalactiae* strains. Utilization of these four substrates defined 15 biotypes, of which 6 contained most of the *S. agalactiae* strains associated with neonatal CNS infection.

MATERIALS AND METHODS

Bacterial isolates. One hundred fifty-one strains were used in this study and stored at −80°C with the Cryobeads system (AES Laboratories, France).

One hundred nine strains of *S. agalactiae* were isolated in France between 1986 and 1990 inclusive. They were selected as having documented anatomical origins and had been phylogenetically characterized by MLEE (33). They included 54 *S. agalactiae* strains isolated from cerebrospinal fluid (CSF) samples from neonates suffering from meningitis and 55 non-CSF *S. agalactiae* strains—34 vaginal and 21 from gastric fluids—isolated from asymptomatic pregnant women and neonates, respectively. The 109 strains were classified into two populations. The first population was composed of 63 strains of various origins and of all the electrophoretic types containing strains isolated from neonatal CSF. The second population of strains was composed of 46 strains isolated from vaginal and gastric samples but belonging to electrophoretic types that did not contain strains from CSF. The first population of strains can therefore be considered to represent strains associated with a high risk of neonatal CNS infection (HR group) and the second population to represent strains associated with a low risk of neonatal meningitis (LR group).

Strains were serotyped with a commercial latex agglutination kit (Pastorex Strepto BI, BII, and BIII; Bio-Rad Laboratories) and by a previously described molecular serotype identification method (22). Twenty-seven strains were of serotype I, 17 of serotype II, 56 of serotype III, and 9 of other serotypes.

Forty-two strains isolated between 2002 and 2005 from CSF samples of neonates with meningitis were also studied. These strains were sent to our laboratory from various regions of France and were isolated on different dates; they were therefore epidemiologically unrelated and comprised serotypes I ($n = 4$), III ($n = 37$), and V ($n = 1$).

Detection of genetic markers of virulent *S. agalactiae* strains. As previously shown, strains able to invade the CNS of neonates significantly more frequently possess one or more of three genetic markers than other strains (3, 34, 35). Two are mobile genetic elements, i.e., the GBSi1 group II intron downstream from the C5a-peptidase *scpB* gene and IS*1548* in the *hylB* gene, encoding a hyaluronate lyase (3, 34). The third marker is a single and specific cluster of tRNA genes at the 3′ end of the rRNA operons that have a 200-bp deletion (in comparison with the tRNA gene clusters in strains associated with a low risk of meningitis) (35). We tested for the presence of the GBSi1 intron group II downstream from the *scpB* gene by PCR using the GBSi1.rev (5′-GTGATAGATTCCCGCCTTGA-3′) and SGB.scpB.for (5′-AGCCATATGCTGCGATCTCT-3′) primers (product size, 2,175 bp) and for IS*1548* in the *hylB* gene by PCR using the IS1548.for (5′-GCCGTCAAAGGAAATCAAGA-3′) and SGB.hyl.rev (5′-GGCGCCAGTATAAGGAACAT-3′) primers (product size, 1,112 bp) as previously described (3). The nature of the tRNA gene clusters at the 3′ end of the seven rRNA operons was determined by PCR using the T7-B2 (5′-CCTAGGAAATACACCTGTTCTCATGCC-3′) and SP6-B2 (5′-AACCTTGTCTTTTAAAGTATACCGGCT-3′) primers. The presence of the single specific tRNA gene cluster at the 3′ end of the rRNA operons was demonstrated by amplification of a single DNA fragment of 1.2 kb with these primers as previously described (35); this fragment is referred to here as tRNA 1.2.

Determination of metabolic profiles. We determined metabolic profiles with the Biolog system (Biolog Inc., Hayward, California). This system is generally used for identification and tests the ability of a microorganism to oxidize a panel of 95 different carbon sources. Tests were performed in standard 96-well microplates containing a dried film of 95 different carbon sources and one negative control. Each well contained a redox dye (tetrazolium violet) for colorimetric determination of respiration due to cells oxidizing the carbon source. We used the Biolog GP2 microplate (AES Laboratories, France), which is designed for the identification of gram-positive bacteria (29), according to the procedure recommended by the manufacturer (Biolog Inc., Hayward, California). All strains were stored at −80°C with the Cryobeads system (AES Laboratories, France). They were first cultured on sheep's blood agar (Trypticase soy agar with 5% sheep's blood; bioMérieux, Marcy l'Etoile, France) for 24 h at 37°C. One colony was picked from the primoculture with a cotton swab and subcultured on BUGB medium (Biolog universal growth medium containing 5% sheep's blood; AES Laboratories, France) by incubation overnight at 37°C. Cells were harvested using sterile cotton swabs and suspended in 20 ml of 0.85% NaCl (AES Laboratories, France). The cell density was adjusted to 20% of transmittance with a 2% range as assessed using a photometer model according to the range specified by the manufacturer. Thioglycolate (AES Laboratories, France) was added to the suspension at a final concentration of 5 mM to inhibit the production of a bacterial capsule. An aliquot of 150 μl of the suspension was immediately dispensed into each well of the GP2 microplate with a multichannel pipette. The plate was incubated at 37°C for 24 h.

Interpretation of metabolic profiles. Microplates were read at two wavelengths (590 nm and 750 nm) with a computer-controlled microplate reader. Reactions were interpreted as positive or negative by the Biolog MicroLog 3 software, release 4.20 (Biolog Inc.). Statistical significance was calculated by means of the chi-square test.

TABLE 1. Oxidation of 27 of 95 carbon sources in the Biolog system by 109 *S. agalactiae* strains[a]

Position on microplate	Carbon source	No. of strains in indicated group oxidizing carbon source (%)			*P* value[d]
		Total	HR group[b]	LR group[c]	
D1	β-Methyl-D-glucoside	66 (61)	31 (49)	35 (76)	0.004
D7	D-Ribose	47 (43)	15 (24)	32 (70)	<0.001
H10	α-D-Glucose-1-phosphate	28 (26)	6 (9)	22 (48)	<0.001
H12	D,L-α-Glycerol phosphate	67 (61)	30 (48)	37 (80)	<0.001
D8	Salicin	80 (73)	42 (67)	38 (83)	NS
B7	D-Galactose	85 (80)	46 (73)	39 (85)	NS
A8	Tween 40	1 (1)	0	1 (2)	NS
B3	Arbutin	10 (9)	3 (5)	7 (15)	NS
B4	Cellobiose	3 (3)	1 (2)	2 (4)	NS
C1	α-D-Lactose	8 (7)	5 (8)	3 (6)	NS
C2	Lactulose	7 (6)	4 (6)	3 (6)	NS
C7	D-Melezitose	1 (1)	0	1 (2)	NS
C8	D-Melibiose	1 (1)	0	1 (2)	NS
C9	α-Methyl-D-galactoside	1 (1)	0	1 (2)	NS
C10	β-Methyl-D-galactoside	1 (1)	0	1 (2)	NS
C11	3-Methyl-D-glucose	4 (4)	1 (2)	3 (6)	NS
C12	α-Methyl-D-glucose	8 (7)	2 (3)	6 (13)	NS
D3	Palatinose	8 (7)	2 (3)	6 (13)	NS
D6	L-Rhamnose	1 (1)	1 (2)	0	NS
F7	Succinic acid monomethyl ester	1 (1)	0	1 (2)	NS
F10	Succinamic acid	1 (1)	0	1 (2)	NS
G2	D-Alanine	1 (1)	1 (2)	0	NS
G6	L-Glutamic acid	2 (2)	2 (3)	0	NS
G11	2,3-Butanediol	3 (3)	2 (3)	1 (2)	NS
F5	L-Malic acid	100 (92)	58 (92)	42 (91)	NS
F9	Pyruvic acid	107 (98)	62 (98)	45 (98)	NS
H6	Adenosine-5′-monophosphate	108 (99)	62 (98)	46 (100)	NS

[a] Findings for the HR group differed from those of the LR group. Four carbon sources significantly distinguished strains of the HR group from those of the LR group.
[b] HR group: 63 strains with high risk of association with neonatal meningitis.
[c] LR group: 46 strains with low risk of association with neonatal meningitis.
[d] *P* value by chi-square test. NS, not significant.

RESULTS

Identification of carbon sources that discriminate *S. agalactiae* strains of the HR group from those of the LR group. One hundred nine strains isolated between 1986 and 1990 were

TABLE 2. Biotypes of *S. agalactiae* strains defined on the basis of oxidation of four carbon sources (D1, D7, H10, and H12)

Biotype	Oxidation of the following carbon source:				No. (%) of strains in:		
	D1	D7	H10	H12	HR group[a]	LR group[b]	Recent CSF group[c]
B1	−	−	−	−	27 (43)	2 (5)	26 (62)
B2	−	−	+	−	2 (3)	1 (2)	0
B3	+	−	−	−	3 (5)	1 (2)	0
B4	−	+	−	+	2 (3)	1 (2)	1 (3)
B5	+	−	−	+	14 (22)	4 (9)	6 (14)
B6	+	+	−	+	10 (16)	12 (26)	3 (7)
B7	+	−	+	+	2 (3)	3 (6)	0
B8	+	+	+	+	2 (3)	13 (28)	1 (2)
B9	−	+	+	−	0	1 (2)	0
B10	−	−	+	+	0	1 (2)	0
B11	−	+	+	+	0	2 (5)	0
B12	−	−	−	+	0	1 (2)	0
B13	+	−	+	−	0	1 (2)	0
B14	+	+	−	−	0	1 (2)	0
B15	−	+	−	−	1 (2)	2 (5)	5 (12)

[a] HR group: 63 strains with high risk of association with neonatal meningitis.
[b] LR group: 46 strains with low risk of association with neonatal meningitis.
[c] Recent strains: 42 strains isolated between 2002 and 2005 from CSF (neonatal meningitis).

TABLE 3. Distribution of three genetic markers (tRNA 1.2 at the 3′ end of rRNA operons, IS*1548* in the *hylB* gene, and GBSi1 downstream from the *scpB* gene) in *S. agalactiae* strains belonging to HR and LR groups and various biotypes

Genetic marker(s)	No. of strains isolated between 1986 and 1990[a]				No. of recent strains of biotypes[b]	
	Group		Biotypes			
	HR[c]	LR[d]	B1–B6	B7–B15	B1–B6	B7–B15
tRNA 1.2 or GBSi1 or IS*1548*	23	20	31	12	10	1
tRNA 1.2 and GBSi1	27	6	28	5	24	4
tRNA 1.2 and IS*1548*	0	0	0	0	1	0
tRNA 1.2 and IS*1548* and GBSi1	2	0	2	0	0	0
IS*1548* and GBSi1	2	0	2	0	0	0
Total for strains with:						
At least one marker	54	26	63	17	35	5
No marker	9	20	16	13	1	1

[a] One hundred nine strains isolated between 1986 and 1990 from CSF and vaginal and gastric fluids.
[b] Recent strains: 42 strains isolated between 2002 and 2005 from CSF (neonatal meningitis).
[c] HR group: 63 strains with high risk of association with neonatal meningitis.
[d] LR group: 46 strains with low risk of association with neonatal meningitis.

tested for 95 carbon sources: 22 of the 95 were positive and 46 negative for all strains. Of the 27 remaining substrates, 4 significantly discriminated the HR group from the LR group: these 4 substrates were β-methyl-D-glucoside, D-ribose, α-D-glucose-1-phosphate, and D,L-α-glycerol phosphate, herein referred to as D1, D7, H10, and H12, respectively, on account of their positions on the Biolog microplate (Table 1). The strains of the HR group were less able to use one or more of these four carbon sources than strains of the LR group.

Determination of the biotypes and distribution of HR and LR strains among biotypes. The 109 strains tested were classified into 15 biotypes, referred to as B1 to B15 according to their oxidation of substrates D1, D7, H10, and H12, which distinguished the HR group from the LR group (Table 2). Fifty-eight of the 63 strains of the HR group (92%) were found in six biotypes (B1 to B6) and 25 of the 46 strains of the LR group (54%) belonged to the other nine biotypes (B7 to B15). Strains associated with a high risk of meningitis were therefore significantly linked to biotypes B1 to B6 ($P < 0.001$). The strains belonging to these biotypes were 13 times more likely than strains of biotypes B7 to B15 to be associated with a high risk of meningitis (odds ratio = 13.84 [4.26 to 47.71]). The positive and negative predictive values for a strain with a biotype from B1 to B6 belonging to a group associated with a high risk of neonatal meningitis were 0.73 and 0.83, respectively. Biotype B1, composed of strains unable to utilize any of the four substrates, was the most predictive (43% of HR group strains were in B1 and only 5% of the LR group strains).

Correlation between biotypes and the presence of genetic markers of strains associated with a high risk of meningitis. We tested all strains for a copy of IS*1548* in the *hylB* gene, for the GBSi1 group II intron downstream from the *scpB* gene, and for a specific and unique tRNA gene cluster at the 3′ end of the rRNA operons (tRNA 1.2). Six genetic patterns were observed based on these three genetic markers (Table 3). The presence of one or more of the three markers was significantly more frequent in strains of the HR group (54/63 strains; 86%) than in strains of the LR group (26/46 strains; 56%) ($P < 0.001$), consistent with the previous finding that these markers are prevalent in strain populations associated with a high risk of neonatal meningitis (34, 35). Accordingly, the presence of one or more of the three genetic markers was more prevalent in biotypes B1 to B6 (63/79 strains; 80%) than in biotypes B7 to B15 (17/30 strains; 57%) ($P = 0.015$).

Biotypes and recent strains associated with neonatal meningitis. We biotyped 42 CSF strains isolated between 2002 and 2005 inclusive. Forty (95%) of these strains contained one or more of the three genetic markers (IS*1548* in the *hylB* gene, a group II intron GBSi1 downstream from the *scpB* gene, and tRNA 1.2) (Table 3). The most frequent pattern was the association of tRNA 1.2 and the GBSi1 intron downstream from the *scpB* gene. This pattern was found in 67% (28/42) of these recent strains but in only 43% (27/63) of the CSF strains of the HR group isolated between 1986 and 1990.

Thirty-six of the 42 recently isolated CSF strains (86%) were in biotypes B1 to B6. Most belonged to two biotypes: 26 were in biotype B1 (62%), and 6 were in biotype B5 (14%) (Table 2). These two biotypes were also major biotypes in the collection of 109 strains of the HR group isolated between 1986 and 1990 in France (Table 2): 27 were in biotype B1 (43%), and 14 were in biotype B5 (22%). There was therefore no significant difference in the distribution of strains among the biotypes for the CSF strains isolated in the two periods, which were separated by about 15 years ($P = 0.235$).

DISCUSSION

S. agalactiae is a major cause of serious bacterial disease in neonates. We used the Biolog system to explore the ability of

109 *S. agalactiae* strains to oxidize a panel of 95 carbon sources to find metabolic differences between a population of strains able to invade the CSF of neonates (HR group of strains) and a population associated with a low risk of meningitis (LR group of strains). These two populations were defined on the basis of anatomical origin and the results of a phylogenetic study by MLEE. MLEE has been successfully used in studies of population genetics (40) and was the first method to identify intraspecies genetic subgroups of *S. agalactiae* strains able to invade the CNS of neonates (31, 33). More recent studies of specifically CSF strains with molecular tools have confirmed the findings of MLEE (34, 35, 42). For example, one of the CSF strains identified as a high-virulence isolate by MLEE (31, 33) was confirmed by multilocus sequence typing as the ST-17 clone (20). Based on these previous classifications of the species, we believe that the two populations of strains used in this study accurately represent strains associated with a high risk of causing meningitis (HR group) and strains associated with a low risk of causing meningitis (LR group).

We found that high-risk strains (HR group) were significantly less able than low-risk strains (LR group) to use α-D-glucose-1-phosphate, D-ribose, β-methyl-D-glucoside, and D,L-α-glycerol phosphate, and none of the 95 substrates were better used by the HR group than by the LR group (Table 1). The adaptation of a pathogen's metabolism to its environment can provide it with an advantage for growth, survival, and virulence (2, 6, 11, 21). The loss of catabolic function by *S. agalactiae* strains observed here may be the result of genetic processes that have improved the organism's adaptation to conditions that generate neonatal meningitis. Catabolic losses have been observed during specialization of *Escherichia coli* (8): in this species, the loss of the capacity to use D-ribose, one of the four carbon sources we found to be used by fewer HR than LR *S. agalactiae* strains, can be a result of deletions that confer a selective advantage in minimal glucose medium (7). Genomic deletions causing loss of catabolic function may also enhance the virulence of bacteria by suppressing a product that inhibits virulence, as demonstrated for *Shigella* species. Indeed, the virulence of *Shigella* spp., a group which evolved from *E. coli*, results from the acquisition of a plasmid that encodes virulence functions and also from a deletion of the *cadA* gene, which encodes the lysine decarboxylase enzyme; this enzyme is involved in the synthesis of cadaverine, which is a potent inhibitor of *Shigella* enterotoxin activity (9, 26). Similarly, the acquisition of plasmids pP1a and pMT1 is not sufficient to explain the high virulence of *Yersinia pestis.* The rapid emergence of *Y. pestis* from *Y. pseudotuberculosis*, a species that has all the extra genes that *Y. pestis* needs for virulence, seems to be a consequence of genetic disruptions causing the loss of various metabolic and catabolic functions; such genetic disruptions are associated with the adaptation of the bacteria to diverse hosts (45). These genetic disruptions from *Y. pestis* seem to have arisen when insertion sequence (IS) elements causing significant genome rearrangements were introduced into the genome (45). We recently demonstrated that acquisition of IS elements and the GBSi1 intron by *S. agalactiae* isolates probably contributed to the emergence of virulent clones, such as the MLST clone ST-17 (17). Therefore, as suggested by work with the *Yersiniae* model (45), the acquisition by horizontal transfer of novel sequences associated with ongoing genetic disruptions responsible for the loss of catabolic function may explain the emergence of strains of *S. agalactiae* able to invade the CNS of neonates. This view is reinforced by the high prevalence of mobile elements in two genes (IS*1548* in the *hylB* gene and GBSi1 intron group II downstream from the *scpB* gene [15, 34]) and genome decay in the tRNA gene cluster (a unique and specific cluster of tRNA genes at the 3′ end of the rRNA operons [35]) that we found in the population of strains belonging to biotypes B1 to B6 (Table 3). In addition, by using the same HR and LR groups of strains, we recently demonstrated that prophagic DNA elements were significantly more prevalent in strains of HR groups than in strains of LR groups (43). Therefore, a link between a high frequency of horizontal genetic transfer and catabolic loss in *S. agalactiae* strains able to invade the CNS of neonates could be hypothesized. Nevertheless, the nature of the link between catabolic loss, the acquisition of mobile genetic elements and genomic deletions, and the fitness of the *S. agalactiae* strains remain to be elucidated, and the genetic mechanisms responsible for the catabolic loss need to be demonstrated.

We defined 15 biotypes of *S. agalactiae* on the basis of the oxidation of α-D-glucose-1-phosphate, D-ribose, β-methyl-D-glucoside, and D,L-α-glycerol phosphate assessed using the Biolog system. Six of these biotypes (B1 to B6) included HR isolates; the positive predictive value for classifying a strain in biotypes B1 to B6 was 0.73, and the negative predictive value for belonging to the HR group was 0.83. In addition, most of the recently isolated CSF strains (86%) were of biotypes B1 to B6. Several strategies have been developed for the prevention of perinatal group B streptococcus infections. One is based on the assessment of risk factors at the onset of labor (particularly delivery prior to 37 weeks of gestation, intrapartum temperature of at least 38°C, and rupture of membranes for more than 18 h) that are considered as indicative of the need for antibioprophylaxis (4). Some authors recommend prenatal culture-based screening for *S. agalactiae* colonization for all pregnant women and intrapartum chemoprophylaxis by intravenous administration of antibiotics for all women who are colonized (1, 37, 38). This approach is >50% more effective than the risk-based approach for preventing perinatal *S. agalactiae* disease (37) but may have adverse effects. Excessive exposure of the mother and child to antibiotics may lead to the emergence of more-resistant and/or more-virulent pathogens, severe allergic reactions, and, in the long term, ecological disturbance of the natural flora and even abnormal immunological development in the children (1, 14, 28, 30). It would be beneficial to restrict intrapartum and neonatal antibioprophylaxis, especially when the colonized mother has no risk factor and/or when the colonized neonate has no symptoms. New strategies using simple and practical tools, such as biotyping of strains as described here, could contribute to the accurate evaluation of the risk of meningitis and would therefore be valuable for clinical studies.

Such a tool may be also used to help in the diagnosis of neonatal infection. Indeed, such diagnosis is difficult because of the poor specificity of clinical symptoms and the difficulty of obtaining CSF from neonates (13, 23). Biotyping of *S. agalactiae* strains isolated in cases with risk factors of infection or

suspected of infection can provide additional microbiological evidence for evaluating the pathogenicity of the isolated bacteria. Biotyping would then constitute one of the elements of the decision to sample the CSF of neonates and to adapt the dosage and duration of antibiotic treatment appropriately.

The Biolog system is a simple commercial tool used for the identification of bacteria. Our results indicate that it may also be used to help evaluate the risk of meningitis when an *S. agalactiae* strain is isolated from the vagina of a pregnant woman or from the gastric fluid or blood culture of a newborn infant. Microplates specially adapted for *S. agalactiae* biotyping could be developed with the objective of reducing the duration of incubation to, for example, 4 h.

ACKNOWLEDGMENTS

This research was supported by grants from the Ministère de l'éducation nationale, de l'enseignement supérieur et de la recherche to R. Quentin and from the Centre Hospitalier de Tours to R. Quentin and N. van der Mee-Marquet.

There are no conflicts of interest resulting from arrangements, support, and consultation fees from Biolog Company.

REFERENCES

1. **Agencie Nationale d'Acréditation en Santé.** 2001. Antenatal prevention of the risk of early neonatal bacterial infection. [Online.] http://www.anaes.fr/anaes/Publications.nsf/vnPDFFile/GU_LILF-5LLJAG/$File/Antenatal_prevention.pdf?OpenElement.
2. **Bernier-Février, C., L. du Merle, E. Turlin, V. Labas, J. Ordonez, A. M. Gilles, and C. Le Bougénec.** 2004. Use of deoxyribose by intestinal and extraintestinal pathogenic *Escherichia coli* strains: a metabolic adaptation involved in competitiveness. Infect. Immun. **72:**6151–6156.
3. **Bidet, P., N. Brahimi, C. Chalas, Y. Aujard, and E. Bingen.** 2003. Molecular characterization of serotype III group B-*Streptococcus* isolates causing neonatal meningitis. J. Infect. Dis. **188:**1132–1137.
4. **Centers for Disease Control and Prevention.** 1996. Prevention of perinatal group B streptococcal disease: a public health perspective. Morb. Mortal. Wkly. Rep. Recomm. Rep. **45**(RR-7)**:**1–24.
5. **Chaffin, D. O., S. B. Beres, H. H. Yim, and C. E. Rubens.** 2000. The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon. J. Bacteriol. **182:**4466–4477.
6. **Chaussee, M. S., G. A. Somerville, L. Reitzer, and J. M. Musser.** 2003. Rgg coordinates virulence factor synthesis and metabolism in *Streptococcus pyogenes*. J. Bacteriol. **185:**6016–6024.
7. **Cooper, V. S., D. Schneider, M. Blot, and R. E. Lenski.** 2001. Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. J. Bacteriol. **183:**2834–2841.
8. **Cooper, V. S., and R. E. Lenski.** 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations. Nature **407:**736–739.
9. **Day, W. A., R. E. Fernandez, and A. T. Maurelli.** 2001. Pathoadaptative mutations that enhance virulence: genetic organization of the *cadA* regions of *Shigella* spp. Infect. Immun. **69:**7471–7480.
10. **Doran, K. S., and V. Nizet.** 2004. Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy. Mol. Microbiol. **54:**23–31.
11. **Exley, R. M., J. Shaw, E. Mowe, Y. H. Sun, N. P. West, M. Williamson, M. Botto, H. Smith, and C. M. Tang.** 2005. Available carbon source influences the resistance of *Neisseria meningitidis* against complement. J. Exp. Med. **201:**1637–1645.
12. **Fleming, K. E., J. F. Bohnsack, G. C. Palacios, S. Takahashi, and E. Adderson.** 2004. Equivalence of high-virulence clonotypes of serotype III group B *Streptococcus agalactiae* (GBS). J. Med. Microbiol. **53:**505–508.
13. **Fowlie, P. W., and B. Schmidt.** 1998. Diagnostic tests for bacterial infection from birth to 90 days—a systematic review. Arch. Dis. Child. Fet. Neonat. Ed. **78:**F92–F98.
14. **Gibbs, R. S., S. Schrag, and A. Schuchat.** 2004. Perinatal infections due to group B streptococci. Obstet. Gynecol. **104:**1062–1076.
15. **Granlund, M., F. Michel, and M. Norgren.** 2001. Mutually exclusive distribution of IS*1548* and GBSi1, an active group II intron identified in human isolates of group B streptococci. J. Bacteriol. **183:**2560–2569.
16. **Granlund, M., L. Öberg, M. Sellin, and M. Norgren.** 1998. Identification of a novel insertion element, IS*1548*, in group B streptococci, predominantly in strains causing endocarditis. J. Infect. Dis. **177:**969–976.
17. **Hery-Arnaud, G., G. Bruant, P. Lanotte, S. Brun, A. Rosenau, N. van der Mee-Marquet, R. Quentin, and L. Mereghetti.** 2005. Acquisition of insertion sequences and the GBSi1 intron by *Streptococcus agalactiae* isolates correlates with the evolution of the species. J. Bacteriol. **187:**6248–6252.
18. **Johri, A. K., J. Padilla, G. Malin, and L. C. Paoletti.** 2003. Oxygen regulates invasiveness and virulence of group B *Streptococcus*. Infect. Immun. **71:**6707–6711.
19. **Jones, A. L., K. M. Knoll, and C. E. Rubens.** 2000. Identification of *Streptococcus agalactiae* virulence genes in the neonatal rat sepsis model using signature-tagged mutagenesis. Mol. Microbiol. **37:**1444–1455.
20. **Jones, N., J. F. Bohnsack, S. Takahashi, K. A. Oliver, M. S. Chan, F. Kunst, P. Glaser, C. Rusniok, D. W. M. Crook, R. M. Harding, N. Bisharat, and B. G. Spratt.** 2003. Multilocus sequence typing system for group B streptococcus. J. Clin. Microbiol. **41:**2530–2536.
21. **Kiliç, A. O., L. Tao, Y. Zhang, Y. Lei, A. Khammanivong, and M. C. Herzberg.** 2004. Involvement of *Streptococcus gordonii* beta-glucoside metabolism systems in adhesion, biofilm formation, and in vivo gene expression. J. Bacteriol. **186:**4246–4253.
22. **Kong, F., S. Gowan, D. Martin, G. James, and G. L. Gilbert.** 2002. Serotype identification of group B streptococci by PCR and sequencing. J. Clin. Microbiol. **40:**216–226.
23. **Kumar, P., S. Sakar, and A. Narang.** 1995. Role of routine lumbar puncture in neonatal sepsis. J. Paediatr. Child Health **31:**8–10.
24. **Lachenauer, C. S., R. Creti, J. L. Michel, and L. C. Madoff.** 2000. Mosaicism in the alpha-like protein genes of group B streptococci. Proc. Natl. Acad. Sci. USA **97:**9630–9635.
25. **Mattingly, S. J., J. J. Maurer, E. K. Eskew, and F. Cox.** 1990. Identification of a high-virulence clone of serotype III *Streptococcus agalactiae* by growth characteristics at 40°C. J. Clin. Microbiol. **28:**1676–1677.
26. **Maurelli, A. T., R. E. Fernandez, C. A. Bloch, C. K. Rode, and A. Fasano.** 1998. "Black holes" and bacterial pathogenicity: a large genomic deletion that enhances the virulence of *Shigella* spp. and enteroinvasive *Escherichia coli*. Proc. Natl. Acad. Sci. USA **95:**3943–3948.
27. **Mawn, J. A., A. J. Simpson, and S. R. Heard.** 1993. Detection of the C protein gene among group B streptococci using PCR. J. Clin. Pathol. **46:**633–636.
28. **McKeever, T. M., S. A. Lewis, C. Smith, and R. Hubbard.** 2002. The importance of prenatal exposures on the development of allergic disease. Am. J. Respir. Crit. Care Med. **166:**827–832.
29. **Miller, J. M., J. W. Biddle, V. K. Quenzer, and J. C. McLaughlin.** 1993. Evaluation of Biolog for identification of members of the family *Micrococcaceae*. J. Clin. Microbiol. **31:**3170–3173.
30. **Moore, R. M., S. J. Schrag, and A. Schuchat.** 2003. Effects of intrapartum antimicrobial prophylaxis for prevention of group-B-streptococcal disease on the incidence and ecology of early-onset neonatal sepsis. Lancet Infect. Dis. **3:**201–213.
31. **Musser, J. M., S. J. Mattingly, R. Quentin, A. Goudeau, and R. K. Selander.** 1989. Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B *Streptococcus*) causing invasive neonatal disease. Proc. Natl. Acad. Sci. USA **86:**4731–4735.
32. **Nagano, Y., N. Nagano, S. Takahashi, A. Suzuki, and Y. Okuwaki.** 1989. Sialic acid levels and lag time of growth in chemically defined medium containing 200 mM phosphate among strains of various serotypes of *Streptococcus agalactiae*. J. Clin. Microbiol. **27:**2148–2151.
33. **Quentin, R., H. Huet, F. S. Wang, P. Geslin, A. Goudeau, and R. K. Selander.** 1995. Characterization of *Streptococcus agalactiae* strains by multilocus enzyme genotype and serotype: identification of multiple virulent clone families that cause invasive neonatal disease. J. Clin. Microbiol. **33:**2576–2581.
34. **Rolland, K., C. Marois, V. Siquier, B. Cattier, and R. Quentin.** 1999. Genetic features of *Streptococcus agalactiae* strains causing severe neonatal infections, as revealed by pulsed-field gel electrophoresis and *hylB* gene analysis. J. Clin. Microbiol. **37:**1892–1898.
35. **Rolland, K., L. Mereghetti, S. Watt, S. Chatellier, and R. Quentin.** 2002. tRNA gene clusters at the 3′ end of rRNA operons are specific to virulent subgroups *of Streptococcus agalactiae* strains, as demonstrated by molecular differential analysis at the population level. Microbiology **148:**1493–1499.
36. **Samen, U., B. Gottschalk, B. J. Eikmanns, and D. J. Reinscheid.** 2004. Relevance of peptide uptake systems to the physiology and virulence of *Streptococcus agalactiae*. J. Bacteriol. **186:**1398–1408.
37. **Schrag, S. J., E. R. Zell, R. Lynfield, A. Roome, K. E. Arnold, A. S. Craig, L. H. Harrison, A. Reingold, K. Stefonek, G. Smith, M. Gamble, and A. Schuchat.** 2002. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N. Engl. J. Med. **347:**233–239.
38. **Schrag, S. J., R. Gorwitz, K. Fultz-Butts, and A. Schuchat.** 2002. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. Morb. Mortal. Wkly. Rep. Recomm. Rep. **51**(RR-11)**:**1–22.
39. **Schuchat, A.** 1998. Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin. Microbiol. Rev. **11:**497–513.
40. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51:**873–884.

41. **Shelver, D., L. Rajagopal, T. O. Harris, and C. E. Rubens.** 2003. MtaR, a regulator of methionine transport, is critical for survival of group B streptococcus in vivo. J. Bacteriol. **185:**6592–6599.
42. **Takahashi, S., E. E. Adderson, Y. Nagano, N. Nagano, M. R. Briesacher, and J. F. Bohnsack.** 1998. Identification of a highly encapsulated, genetically related group of invasive type III group B streptococci. J. Infect. Dis. **177:** 1116–1119.
43. **van der Mee-Marquet, N., A. S. Domelier, L. Mereghetti, P. Lanotte, A. Rosenau, W. van Leeuwen, and R. Quentin.** 2006. Prophagic DNA fragments in *Streptococcus agalactiae* strains in association with neonatal meningitis. J. Clin. Microbiol. **44:**1049–1058.
44. **Wastfelt, M., M. Stalhammar-Carlemalm, A. M. Delisse, T. Cabezon, and G. Lindahl.** 1996. Identification of a family of streptococcal surface proteins with extremely repetitive structure. J. Biol. Chem. **271:**18892–18897.
45. **Wren, B. W.** 2003. The yersiniae—a model genus to study the rapid evolution of bacterial pathogens. Nat. Rev. Microbiol. **1:**55–64.