MAJOR ARTICLE

Genotypic Diversity and Serotype Distribution of Group B *Streptococcus* Isolated from Women Before and After Delivery

Shannon D. Manning,[1,2] Maggi A. Lewis,[1] A. Cody Springman,[1,2] Erica Lehotzky,[1,2] Thomas S. Whittam,[1] and H. Dele Davies[2]

[1]Microbial Evolution Laboratory, National Food Safety and Toxicology Center, and [2]Department of Pediatrics & Human Development, Michigan State University, East Lansing, Michigan

Background. Most studies of the dynamics of maternal group B *Streptococcus* (GBS) colonization have relied on capsular serotyping to define GBS acquisition or loss. Newer molecular methods that distinguish GBS clones may expand our knowledge and influence vaccination strategies. We used multilocus sequence typing (MLST) and GBS capsular gene cluster *(cps)* genotyping to investigate the dynamics of perinatal GBS colonization.

Methods. A total of 338 GBS isolates obtained from 212 colonized women who were enrolled in a prior prospective cohort study were serotyped and genotyped by MLST and *cps* typing before (visit 1) and 6 weeks after (visit 2) delivery.

Results. Of the 212 women, 126 were colonized at both visits, whereas 66 lost and 20 acquired GBS by visit 2. MLST of the 338 strains identified 29 sequence types marking distinct bacterial clones. A change in sequence type or *cps* and serotype occurred in 23 (18.3%) of the 126 women who were colonized at both visits. Specific sequence types were associated with GBS loss and persistence. Older maternal age and exclusive intrapartum antibiotic use were associated with persistent colonization.

Conclusions. Although most GBS-positive pregnant women were stably colonized during the peripartum period, we detected changes in capsule expression and recolonization with antigenically distinct GBS clones over time by applying MLST. Combining the epidemiologic and molecular typing data revealed host factors and clones associated with persistent colonization, as well as a clone that was more readily lost. This knowledge is useful for the development of prevention and intervention strategies to reduce the likelihood of maternal GBS colonization.

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis and meningitis and is typically characterized by the polysaccharide capsule (serotype). Maternal GBS colonization is the primary risk factor for neonatal disease [1]. Fifteen percent to 40% [2–4] of pregnant women are colonized but remain asymptomatic. Although spontaneous loss and acquisition of GBS have been observed in pregnant [2, 5, 6] and nonpregnant [7, 8] women, little is known about the host factors and bacterial characteristics that influence GBS colonization.

Most studies examining GBS colonization dynamics during pregnancy have assessed changes over time by serotyping. Although specific serotypes are associated with neonatal disease [9–11], phase variation or capsular switching has been documented within clones by multilocus sequence typing (MLST) [12, 13]. For this reason, assessing differences in clonality is more informative for determining the full extent of changes in GBS colonization. Here, we report the distribution of GBS multilocus sequence types (STs), serotypes, and capsule *(cps)* genotypes in a cohort of pregnant women and explore the dynamics of colonization before and after delivery and receipt of intrapartum antibiotic prophylaxis (IAP). Determining whether specific clones are more readily acquired or are less likely to be eradicated after receipt of IAP is important for preventing neonatal GBS disease and for identifying clones to target in GBS vaccines. This also will enhance our understanding of the colonization patterns of clones such as ST-17, which

Received 10 December 2007; accepted 9 February 2008; electronically published 6 May 2008.

Reprints or correspondence: Dr. H. Dele Davies, B240 Life Sciences Bldg., East Lansing, MI 48824 (daviesde@msu.edu).

Clinical Infectious Diseases 2008; 46:1829–37

1058-4838/2008/4612-0004$15.00
DOI: 10.1086/588296

Table 1. PCR and sequencing primers for multilocus sequence typing (MLST) that amplify DNA regions specific for 7 conserved housekeeping genes.

MLST primer	Oligonucleotide sequence (5′→3′)	Amplicon size, bp
adhP-F110	TGTGCCATACTGATTTACACG	584
adhP-R693	ACAACCGCCTGTCTTTTCT	…
atr-F8	AGCCAATTATTTCAATACAAGGAC	598
atr-R605	AAACCCATTTCATGAGTGACAATA	…
glcK-F25	GACCTCGGAGGAACGACCATTA	587
glcK-R611	TGTTCTGCGAGTTGACGTGCTACT	…
glnA-F170	CTATTGAGGGCTTTGTTCGTATCA	610
glnA-R779	AAAGCATTGTTCCCTTCATTATCA	…
pheS-F10	CAAAAACAATTAGAAGAGTTGAAAA	600
pheS-R609	ACGGAAAACACGTCCAGGAG	…
sdhA-F39	TAGCCAACATAAGGGTAACATAGC	602
sdhA-R640	CTGCAACAGGGTCACAGATAAG	…
tkt-F427	AAACCAGGCTTTGATTTAGT	652
tkt-R1078	GTTGGCTTGAAACACGACT	…

NOTE. The 7 housekeeping genes were *adhP, atr, glcK, glnA, pheS, sdhA,* and *tkt.* All primers were developed on the basis of the NEM316 DNA sequence (CAD45676 [18]), and the location is relative to the start codon of the gene of interest. Primers are labeled by the gene or locus name and location within the gene in base pairs (bp) from the start codon. F, forward; R, reverse.

frequently causes neonatal disease and has been suggested to have enhanced invasiveness [13–15].

PATIENTS AND METHODS

Study population. A total of 235 GBS strains were recovered from 1207 women who were enrolled in a cohort study that was conducted during the period 1998–2000 in Calgary, Canada, and that was approved by the University of Calgary Ethics Board [4]. Isolates were not available for 23 women. The 1207 pregnant women were enrolled at 35–37 weeks of gestation, completed a questionnaire, and provided a vaginal-rectal swab specimen for GBS culture (visit 1) [4]. A subset of these women ($n = 592$) repeated the study protocol at ~6 weeks postpartum (visit 2), as described elsewhere [16]. A total of 338 GBS strains from 212 women who were colonized with GBS during at least 1 of the 2 visits were available for molecular typing (126 of the women who were positive for GBS at both visits yielded 252 strains; 66 women were positive only at visit 1, and 20 were positive only at visit 2). Medical record reviews were conducted after visits 1 and 2 and at the time of delivery.

GBS isolation,* cps *genotyping, serotyping, and susceptibility testing. DNA isolation and a PCR-based restriction fragment–length polymorphism assay to assign *cps* genotypes, which predict GBS serotypes, are described elsewhere [17]. Serotyping, performed by the National Center for *Streptococcus* (Edmonton, Canada), using the Ouchterlony immunodiffusion method with rabbit antisera, is described elsewhere [4], as are susceptibilities to erythromycin and clindamycin [16].

MLST. PCR was completed for 7 housekeeping genes that were previously identified [14] using modified primers (table 1), permitting 1 primer set to be used for both PCR and sequencing. PCR conditions included a 10-min soak at 94°C; 35 cycles of 92°C for 1 min, at 53°C for 1 min, and at 72°C for 30 s; and a 5-min soak at 72°C. PCR products were purified and sequenced (Applied Biosystems) at the Michigan State University Genomic Research Support Technical Facility (East Lansing, MI). Consensus sequences were trimmed in SeqMan (DNA Star), and allele and ST assignments were made using the MLST database [19, 20]. A neighbor-joining tree was constructed [21] with the concatenated sequence data with use of MEGA3 [22].

Epidemiological analysis. The frequency of GBS molecular characteristics was assessed by visit and colonization dynamics. Colonization at both visits was defined as "persistent" colonization when the same GBS ST was identified at both visits 1 and 2. When the ST differed over time, the women were considered to have acquired a new clone by visit 2 after losing the visit 1 clone. Univariate analyses were performed to identify host and clinical factors associated with colonization status. Logistic regression identified predictors of (1) persistent GBS colonization versus GBS loss and (2) any ST change versus no ST change among women colonized at both visits. Adjusted ORs and 95% CIs were noted. SAS software, version 9.1 (SAS Institute), was used for all analyses.

RESULTS

Study participant characteristics and colonization dynamics. Of the 212 women in the study, most (143; 67%) were aged

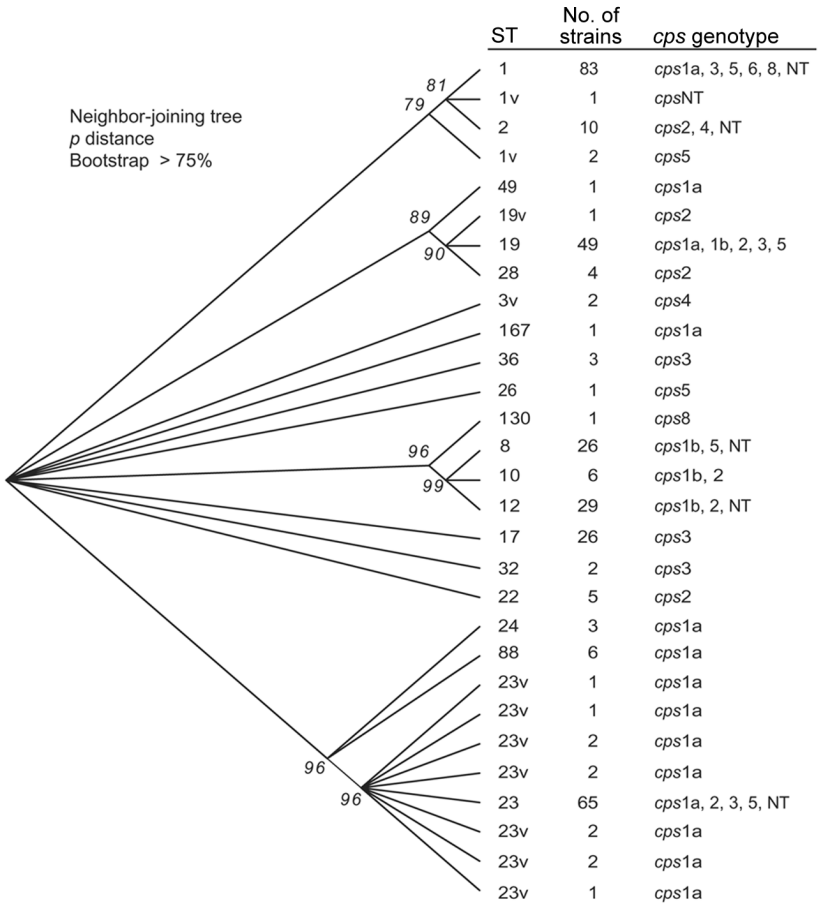

Figure 1. Distribution and frequency of group B *Streptococcus* strains, representing 29 distinct multilocus sequence types (STs), by capsular *(cps)* genotype. Dendrogram is a consensus of 500 bootstrap trees generated with the neighbor-joining algorithm with use of sequence data for 7 genes comprising 1152 codons. STs followed by a "v" represent ST variants.

26–35 years, had vaginal deliveries (189; 89%), were married (187; 88%), and had received antibiotics (201; 95%) at some point between the 2 visits. More than one-half of the women (129; 61%) received IAP during delivery, and an additional 57 women (27%) received IAP plus another antibiotic before or after delivery. Fifteen women (7%) received antibiotics during either the antepartum or postpartum period. The primary antibiotics used for IAP were ampicillin (103 [55%] of the 186 women who received IAP), penicillin (52 [28%] of 186), clindamycin (22 [12%] of 186), and cefazolin (8 [4%] of 186), although the drug combinations and dosages varied considerably.

Colonization at both visits 1 and 2 occurred in 126 (59%) of the 212 women. Sixty-six women (31%) were colonized with GBS at visit 1 but had lost GBS by visit 2, whereas 20 women (9%) had negative culture results at visit 1 but had acquired GBS by visit 2.

Genetic diversity of colonizing GBS. Application of MLST identified 29 STs among all 338 strains. Seven of the STs were composed of strains with >1 *cps* genotype (figure 1). Of the 29 STs, 18 from visit 1 and 14 from visit 2 were previously described [20], whereas another 11 were ST variants (figure 1). STs 1, 23, 19, 12, 17, and 8 together accounted for 82% of all strains. The 11 ST variants were identified among 17 strains (6 were detected twice in women colonized at both visits). Six variants were related to ST-23 with unique *atr* ($n = 3$), *glnA* ($n = 2$), *glcK* ($n = 1$), and *pheS* ($n = 1$) alleles. Another ST was related to ST-19 because of variation in *sdhA,* and 2 others were related to ST-1 because of a unique combination of previously identified alleles ($n = 1$) or variation in *glcK* ($n = 1$). A unique allele combination was also observed for the ST-3 variant (figure 1).

The frequencies of several STs varied between visits (figure 2). The frequency of ST-12 decreased between visit 1 (20 [10.4%] of 192 strains) and visit 2 (9 [6.2%] of 146 strains), although the change was not statistically significant, compared with all other STs ($P = .17$). The opposite trend was observed for the frequencies of STs 8 and 19, which increased from 6.8% to 8.9% and from 13.5% to 15.8%, respectively; neither asso-

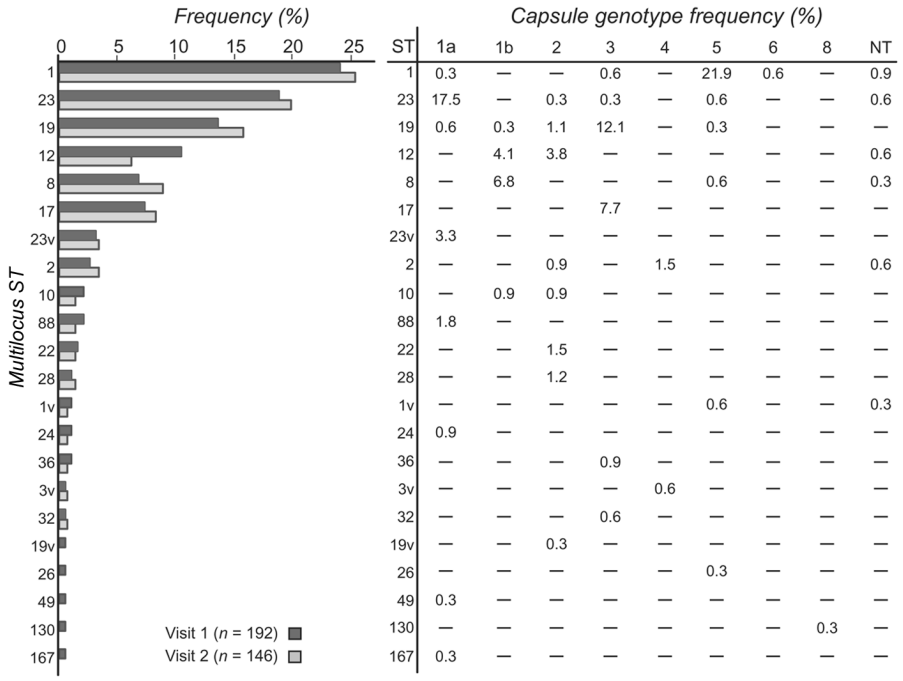

Figure 2. Distribution of multilocus sequence types (STs) and frequency of capsule *(cps)* genotypes among 338 group B *Streptococcus* strains from 212 women who were colonized during pregnancy (visit 1) and/or at 6–8 weeks after delivery (visit 2). The STs are ranked in order of decreasing overall frequency, and the proportion of some STs differed between visits. STs followed by a "v" represent ST variants; STs 23v and 1v are pooled together because of the high degree of similarity (bootstrap >96% and >79%, respectively) in the phylogenetic analyses. The overall frequency of *cps* genotypes is noted, because there were no differences in the *cps* distribution by visit; 2 ST-1 strains had *cps*5 variant genotypes but were included in the *cps*5 category.

ciation was statistically significant (figure 2). No differences were observed for any other STs or *cps* genotypes by visit.

Molecular changes among 126 women colonized at both visits 1 and 2. Comparisons of the ST, *cps* genotype, and serotype between visits revealed differences in 23 (18%) of 126 women (table 2), with a change in the colonizing ST occurring most frequently (in 13 women [10%]). Eight women had a different ST, *cps* genotype, and serotype by visit 2, whereas 5 women had a different ST, but the *cps* genotype and serotype remained the same (table 2).

A change in the *cps* genotype and/or serotype also occurred frequently (in 10 [8%] of 126 women). Five women had typeable strains at visit 1 but nontypeable strains at visit 2; the ST and *cps* genotype were identical at both visits (table 2) for all but 1 woman, who had a strain that was negative for *cps* by PCR. Capsular switching, as defined by expression of a different capsule type or a change in the *cps* genotype among strains with no ST change over time, was observed in 4 women (table 2).

Frequency of multilocus STs, capsule types, and antibiotic resistance, by colonization status. ST-12 strains occurred more frequently among women who lost GBS or a GBS clone by visit 2 (12 [15.2%] of 79 women) (figure 3), compared with women with persistent colonization (8 [7.1%] of 113 women) ($P = .07$). When stratified by *cps* genotype (figure 3), the ST-12 *cps*2 strains were lost more frequently than the ST-12 *cps*1b strains ($P = .02$, by Fisher's exact test). In addition, the combinations of ST-17 *cps*3 and ST-19 *cps*3 strains was significantly more likely ($P = .04$) to persist than to be lost, compared with all other ST and *cps* combinations (figure 3).

GBS strains (24 of 330 strains for which data were available) with intermediate or full resistance to either erythromycin or clindamycin were less likely ($P = .87$) to represent persistent ($n = 16$) or acquired STs ($n = 2$) than lost STs ($n = 6$). There was also no statistically significant difference ($P = .57$) in antibiotic resistance frequencies between visit 1 (15 [8%] of 188 strains) and visit 2 (9 [6%] of 142 strains).

Factors associated with GBS colonization and multilocus STs. Maternal age, mode of delivery, and period of antibiotic use differed by colonization status in the univariate analysis (table 3). The multivariable model identified no factors associated with an ST change, compared with no ST change, between visits, although the number of women was small. By contrast, women aged >36 years were 8 times more likely ($P = .003$) to have persistent colonization, compared with women aged <25 years (table 4). The same trend was observed

Table 2. Dynamics of group B *Streptococcus* (GBS) colonization in 23 pregnant women before (visit 1) and after (visit 2) delivery.

	Visit 1			Visit 2		
Colonization dynamic, patient no.	ST	*cps*	Serotype	ST	*cps*	Serotype
Change in ST only						
1	1	*cps*1a	Ia	23	*cps*1a	Ia
2	1	*cps*5	V	8	*cps*5	V
3	23	*cps*5	V	1	*cps*5	V
4	23	*cps*5	V	8	*cps*5	V
5	49	*cps*1a	Ia	23	*cps*1a	Ia
Change in ST, *cps*, and serotype						
6	1	*cps*5	V	19	*cps*3	III
7	1	*cps*5	V	19	*cps*3	III
8	1	*cps*NT[a]	V	23	*cps*1a	Ia
9	12	*cps*2	II	1	*cps*5	V
10	23	*cps*1a	Ia	1	*cps*5	V
11	88	*cps*1a	Ia	19	*cps*3	III
12	1	*cps*5	V	23	*cps*1a	NT
13	23	*cps*1a[b]	IV	1	*cps*5	NT
Change in serotype expression and/or *cps* genotype						
Suppression of capsular expression						
Typeable to NT						
14	1	*cps*5	V	1	*cps*5	NT
15	12	*cps*1b	Ib	12	*cps*1b	NT
16	12	*cps*1b	Ib	12	*cps*1b	NT
17	12	*cps*1b	Ib	12	*cps*NT[a]	NT
18	23[c]	*cps*1a	Ia	23[d]	*cps*1a	NT
NT to typeable: 19	1	*cps*5	NT	1	*cps*5	V
Capsular switching						
Serotype expression change						
20	17	*cps*3	III	17	*cps*3	Ia
21	23	*cps*1a	III	23	*cps*1a	Ia
Serotype expression and *cps* genotype change						
22	23	*cps*3	NT	23	*cps*1a	Ia
23	1	*cps*5	NT[d]	1	*cps*6	VI

NOTE. A change in GBS over time, which occurred in 23 (18.3%) of 126 women, was defined as any change in the multilocus sequence type (ST), capsule *(cps)* genotype, or serotype between visits 1 and 2. Allelic profiles of the 7 housekeeping genes used to classify each ST can be accessed at the MLST database [19, 20]. NT, nontypeable.

[a] PCR negative for *cps* genes.

[b] Tested repeatedly by PCR, which likely means that the serotyping result was incorrect. Because both results differed from the visit 2 results, the conclusions remained unchanged.

[c] Multilocus sequence typing variant is based on a novel *atr* allele.

[d] Because of the NT serotype at visit 1, a change in capsular expression was inferred on the basis of the *cps* change.

for women aged 26–35 years, but the association was not statistically significant (table 4). Any antibiotic use was associated with a decreased likelihood of persistent colonization (table 4); however, women who received only IAP were more likely to have persistent colonization, compared with women who used antibiotics during all other periods ($P < .001$).

DISCUSSION

Previous studies have used serotyping [2, 5] or PFGE [6] to evaluate GBS changes before and after delivery. Although specific serotypes are associated with neonatal disease [9–11], serotyping is not ideal for determining clonality, because of capsular switching [12–14] and because up to 11% of strains cannot be typed [4]. Similarly, PFGE data classify some serotypes—particularly type V—as identical even though they are not clonal [23]. Using MLST, a more sensitive tool to assess genetic differences, we gained new insights into the characteristics of colonizing GBS strains in association with different host factors. The study of GBS STs and *cps* genotypes suggests 2 hypothetical models of GBS occurrence and dynamics among

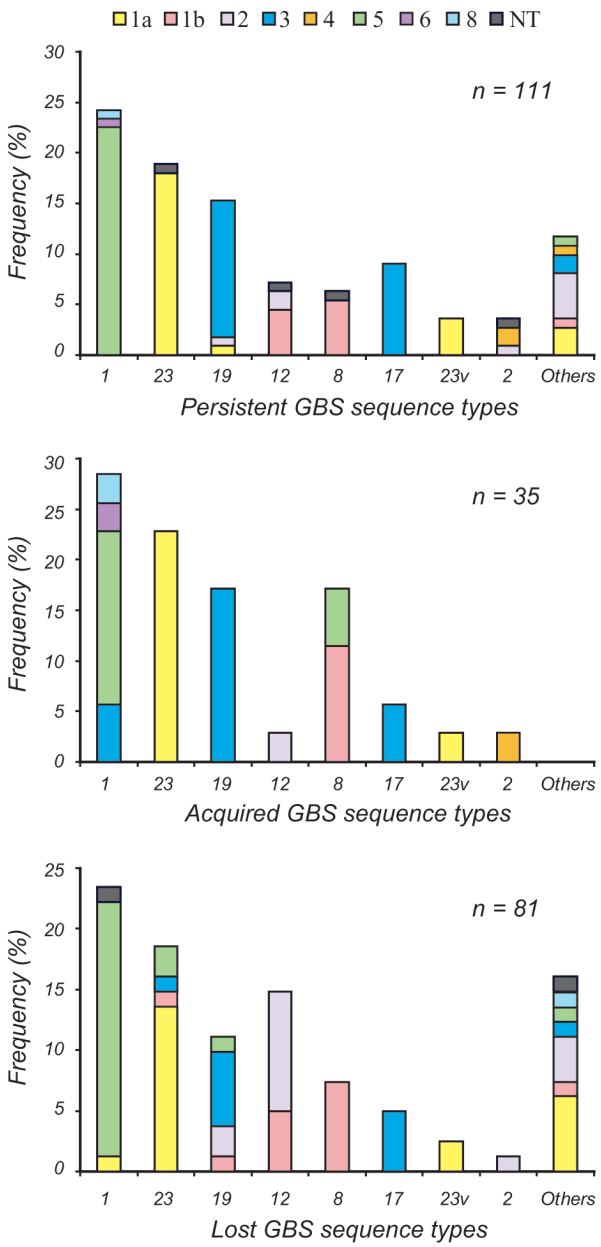

Figure 3. Distribution of multilocus sequence types, stratified by capsule *(cps)* genotypes, among 212 pregnant women who were colonized with group B *Streptococcus* (GBS) before (visit 1) and/or after (visit 2) delivery, by colonization dynamic. The sequence types are ranked in order of decreasing overall frequency, with the "others" category comprising all nonpredominant sequence types grouped together. Two women who had persistent colonization with the same GBS sequence type were colonized with different *cps* genotypes at visits 1 and 2; these cases were included in the lost and acquired distributions because of the *cps* change.

the 126 women who were colonized before and after delivery. These 2 models are based on the persistence and replacement of certain clones.

Clonal persistence model. In this model, the common GBS clone detected by MLST was constant and isolated at both visits. Under the clonal persistence model with stable phenotypes, colonizing GBS strains failed to be eradicated, because identical STs and capsular types were detected at both visits. Antibiotic use likely decreases the density of the colonizing GBS clone, but the clone rebounds at treatment cessation. In this study, this model was applicable for most women who received IAP (table 3) and 90% of the women who were colonized with GBS at both visits. The one exception is that some women can be colonized with strains of the same ST over time but have differences in capsular expression with or without a change in the *cps* genotype (table 2). In this study, most of the women who had a change only in capsular expression had typeable strains at visit 1 and nontypeable strains at visit 2; the *cps* genotype was identical in all but 1 woman. Capsule expression changes with and without *cps* genotype changes (capsular switching) also were observed. In 2 cases, capsule type III expression was documented in a *cps*1a strain, whereas capsule type Ia expression occurred in a *cps*3 strain. Because the ST remained the same in both cases, recombination within the *cps* gene cluster—particularly in *cpsH*, which was shown to confer serotype specificity in type Ia and III strains [24]—may have been responsible for the altered phenotype. Although we cannot rule out the possibility that either the serotyping or *cps* genotyping systems erroneously classified the capsule types in these cases, we suspect that such changes do occur in nature and are influenced by antibiotics and host immune factors.

Clonal replacement model. In this model, the resident strain was eliminated and replaced by new genotypes after exposure to and colonization with different GBS strains. Under the replacement model, the new resident strain was distinct in ST and capsule type. Because there are only 9 GBS serotypes, there were some cases in which the new ST from visit 2 had the same capsule as the original strain from visit 1. This situation occurred for several GBS strains expressing capsular serotype V; thus, the visit 2 strain would have been classified as identical to the visit 1 strain by serotyping and, possibly, by PFGE because of the homogenous nature of serotype V PFGE patterns [23]. However, the latter could not be directly assessed, because PFGE was not used in this study. Expression of the same capsular type by different clones suggests a survival advantage. This may be related in part to antibiotic pressures or low antibody levels against the capsular type of the colonizing strain [4]. The most common pattern in the clonal replacement model involved a clear change in the ST, *cps* genotype, and serotype between visits. This was observed in 10% of women who were colonized at both visits and implies that the original colonizing strain was eradicated and replaced with an entirely different clone. In these women, antibiotic use probably enhanced clearance of the original strain, possibly with support of host immune factors, thereby setting the stage for acquisition

Table 3. Association of host and clinical factors by group B *Streptococcus* (GBS) colonization dynamics among 212 women before (visit 1) and after (visit 2) delivery.

Characteristic	Total no. of women	No. (%) of total women				χ^{2a}	P^a
		Persistent colonization with no ST change (n =113)	Persistent colonization with any ST change (n = 13)	Acquired GBS by visit 2 (n = 20)	Lost GBS by visit 2 (n = 66)		
Age, years						8.3	.004
⩽25	36	16 (44.4)	2 (5.6)	2 (5.6)	16 (44.4)		
26–35	143	72 (50.3)	9 (6.3)	16 (11.2)	46 (32.2)		
⩾36	33	25 (75.8)	2 (6.1)	2 (6.1)	4 (12.1)		
Married						0.5	.49
No	25	11 (44.0)	2 (8.0)	4 (16.0)	8 (32.0)		
Yes	187	102 (54.5)	11 (5.9)	16 (8.6)	58 (31.0)		
Mode of delivery						3.7	.06
Vaginal	189	106 (56.1)	10 (5.3)	17 (9.0)	56 (29.7)		
Cesarean	23	7 (30.4)	3 (13.0)	3 (13.0)	10 (43.5)		
Duration of hospital stay, days						2.0	.15
⩽2	139	81 (58.3)	5 (3.6)	12 (8.6)	41 (29.5)		
⩾3	72	31 (43.1)	8 (11.1)	8 (11.1)	25 (34.7)		
Period of antibiotic use						11.1	<.001
No use	11	5 (45.5)	0 (0.0)	5 (45.5)	1 (9.1)		
Antepartum and/or postpartum	15	2 (13.3)	1 (6.7)	8 (53.3)	4 (26.7)		
Intrapartum antibiotic prophylaxis only	129	84 (65.1)	8 (6.2)	6 (4.7)	31 (24.0)		
Intrapartum antibiotic prophylaxis and other	57	22 (38.6)	4 (7.0)	1 (1.8)	30 (52.6)		
Antibiotic class[b]						1.4	.23
None	12	5 (41.7)	0 (0.0)	6 (50.0)	1 (8.3)		
Penicillin	124	80 (64.5)	10 (8.1)	5 (4.0)	29 (23.4)		
Cephalosporin	14	4 (28.6)	1 (7.1)	5 (35.7)	4 (28.6)		
Penicillin and cephalosporin	20	6 (30.0)	1 (5.0)	1 (5.0)	12 (60.0)		
Other	40	17 (42.5)	1 (2.5)	2 (5.0)	20 (50.0)		
Maternal fever during labor						0.89	.83
No	164	87 (53.0)	9 (5.5)	15 (9.1)	53 (32.3)		
Yes	48	26 (54.2)	4 (8.3)	5 (10.4)	13 (27.1)		
Ill newborn[c]						0.21	.64
No	132	71 (53.8)	8 (6.1)	14 (10.6)	39 (29.5)		
Yes	80	42 (52.5)	5 (6.3)	6 (7.5)	27 (33.8)		

[a] Mantel Haenszel χ^2 with 1 df and P for the association between colonization dynamics and each characteristic. Numbers do not add up to 212 because of missing data for some women.
[b] Refers to class of antibiotic used at any time between the 2 visits.
[c] Newborn illness not necessarily caused by GBS infection.

of new strain types. Replacement is most likely to occur after receipt of a full course of antibiotics.

In addition to the persistence and replacement models, complete elimination of the colonizing GBS strain also occurred between visits. Eradication is likely related to antibiotic use, host immune factors, and/or the molecular characteristics of the colonizing strain. In some women, it is also possible that the predominant GBS type was eliminated, allowing other types present in lower, undetectable numbers to reproduce and dominate. These dynamics cannot be distinguished from the persistence and replacement models without more extensive population sampling and additional time points.

In this study, the predominant colonizing clones (STs 1, 23, and 19) were equally represented among women with persistent colonization and women who had lost and acquired GBS (figure 3). This distribution suggests that these clones represent the most common strains in circulation or are well adapted to the vaginal mucosa. Each of the 3 major STs was associated with 1 predominant *cps* type, although multiple *cps* types were observed.

Some STs may be more difficult to eradicate by antibiotics in vivo. Strains resistant to either erythromycin or clindamycin did not persist more frequently than susceptible strains in this study. Although we did not examine it, it is possible that tol-

Table 4. Logistic regression results identifying risk factors for persistent colonization with group B *Streptococcus* (GBS) in 108 women, compared with 65 women who lost GBS between visits 1 and 2.

Risk factor	OR (95% CI)	*P*
Age, years		
⩽25	1.0	
26–35	1.8 (0.77–4.37)	.17
⩾36	8.3 (2.07–33.10)	.003
Caesarean delivery	0.8 (0.22–2.61)	.66
Period of antibiotic use between visits		
Use during other periods with or without intrapartum use	1.0	
Intrapartum use only	4.1 (1.84–9.09)	<.001
Maternal fever during labor	1.7 (0.71–3.88)	.24
Colonization with sequence type 12	0.3 (0.10–0.85)	.02

NOTE. The model was adjusted for each variable listed in the table. One hundred eight of 113 women with persistent GBS colonization and 65 of 66 women who lost GBS had all data available and were included in the analysis.

erance—and not resistance—to specific antibiotics is important for persistent colonization. This hypothesis is supported by our finding that IAP is associated with persistent colonization. Although antibiotic use at any time between visits contributed to eradication in 65 of the 66 women who lost GBS over time, use of IAP alone, compared with antibiotic use at any other time between visits, was associated with persistent colonization. This was probably attributable to the fact that IAP was given in 1 or 2 doses before delivery, whereas other antibiotics administered to treat infections before or after delivery were given for longer durations. This is consistent with prior studies demonstrating that short exposure to IAP penicillin is ineffective for eliminating GBS entirely; instead, it merely decreases the bacterial density [25, 26].

Host clearance mechanisms may also play a role in eradicating some—but not all—STs. For example, phenotypic factors that facilitate colonization could render some clones less effective at gaining entry into the bloodstream or CSF (i.e., invasion), resulting in neonatal disease. Strains of STs 1, 23, and 19 have been suggested [15] to be poor at invasion, although some members of each ST contribute to neonatal disease. In contrast, ST-17 strains have been suggested to exhibit an enhanced ability to cause invasive neonatal disease [13–15] and may not be as well suited for colonization. This is supported by the observation that ST-17 strains are closely related to strains of bovine origin [15]; thus, their ability to adapt to the human host may be decreased. ST-17 strains also only express serotype III [12, 14, 15], which is associated with lower maternal antibody levels and reduced neonatal protection [12]. We hypothesize that GBS with the type III capsule represents STs that are more difficult to eradicate; type III ST-19 and ST-17 strains combined were more likely to persist, compared with all other STs and all type III strains of different STs. This finding may partly explain why type III strains more frequently cause late-onset neonatal disease [27] and why late-onset disease frequencies have remained unchanged since the implementation of IAP protocols [28].

In the same way, some STs are likely to be more easily eradicated than others. ST-12 strains, for example, were overrepresented in women who lost GBS by the second visit. We speculate that ST-12 strains, particularly those with the *cps*2 genotype, are more susceptible in vivo to the antibiotics used during pregnancy. Supporting evidence for this includes the larger decrease in frequency of ST-12 strains by visit 2, compared with other STs, and the fact that capsule type II strains in general infrequently cause neonatal GBS disease [10, 27]. The latter observation may be related to enhanced clearance by IAP or other perinatal antibiotic use.

An interesting association also was identified between colonization dynamics and age. Although younger women were more likely to be colonized with GBS [4, 29], once colonization occurred, older women were more likely to have persistent colonization with the same GBS clone. This suggests that established clones colonizing older women represent GBS types to which these women have failed to develop immunity. This finding is corroborated by previous reports showing that older patients colonized with serotype V lack antibodies to capsular type V [30]. Consequently, the age of the patient may warrant consideration when evaluating GBS vaccination policies aiming to prevent neonatal disease.

Our study has 2 main limitations. First, GBS colonization was examined at only 2 time points. Therefore, we may have missed additional ST changes at interim time points. Although it would have been ideal to examine additional time points, the basic tenets of our observations and theories of colonization were likely to have remained unchanged. Second, this study

was not designed to examine factors associated with GBS acquisition, including sexual activity [8, 31] and dietary history [32], that may influence colonization by specific clones.

In conclusion, ST-based genotyping of GBS provided fundamental insights into the nature of maternal colonization in the context of IAP, the current cornerstone for preventing neonatal GBS disease. By characterizing GBS clones from women before and after delivery, we were able to propose colonization models and identify clones that are more likely to persist and be eradicated. In addition, we determined that IAP and older age are associated with persistent GBS colonization. These findings may prove to be useful for the future development of GBS vaccines and early- and late-onset neonatal disease-prevention programs.

Acknowledgments

We thank Dr. David Lacher, for technical assistance in developing the modified multilocus sequence typing protocol; Shari Tjugum-Holland and Dr. Annette Thelen, for DNA sequencing; and the National Center for *Streptococcus,* particularly Marguerite Lovgren, for serotyping.

Financial support. National Institutes of Health Public Health Service (AI066081).

Potential conflicts of interest. All authors: no conflicts.

References

1. Schuchat A, Wenger JD. Epidemiology of group B streptococcal disease: risk factors, prevention strategies, and vaccine development. Epidemiol Rev **1994**; 16:374–402.
2. Anthony BF, Eisenstadt R, Carter J, Kim KS, Hobel CJ. Genital and intestinal carriage of group B streptococci during pregnancy. J Infect Dis **1981**; 143:761–6.
3. Baker CJ, Barrett FF. Transmission of group B streptococci among parturient women and their neonates. J Pediatr **1973**; 83:919–25.
4. Davies HD, Adair C, McGeer A, et al. Antibodies to capsular polysaccharides of group B *Streptococcus* in pregnant Canadian women: relationship to colonization status and infection in the neonate. J Infect Dis **2001**; 184:285–91.
5. Dillon HJ, Gray E, Pass M, Gray B. Anorectal and vaginal carriage of group B streptococci during pregnancy. J Infect Dis **1982**; 145:794–9.
6. Hansen SM, Uldbjerg N, Kilian M, Sorensen UB. Dynamics of *Streptococcus agalactiae* colonization in women during and after pregnancy and in their infants. J Clin Microbiol **2004**; 42:83–9.
7. Foxman B, Gillespie B, Manning SD, et al. Incidence and duration of group B *Streptococcus* by serotype among male and female college students living in a single dormitory. Am J Epidemiol **2006**; 163:544–51.
8. Meyn LA, Moore DM, Hillier SL, Krohn MA. Association of sexual activity with colonization and vaginal acquisition of group B *Streptococcus* in nonpregnant women. Am J Epidemiol **2002**; 155:949–57.
9. Baker CJ, Edwards MS. Group B streptococcal conjugate vaccines. Arch Dis Child **2003**; 88:375–8.
10. Zaleznik DF, Rench MA, Hillier S, et al. Invasive disease due to group B *Streptococcus* in pregnant women and neonates from diverse population groups. Clin Infect Dis **2000**; 30:276–81.
11. Harrison LH, Elliott JA, Dwyer DM, et al. Serotype distribution of invasive group B streptococcal isolates in Maryland: implications for vaccine formulation. J Infect Dis **1998**; 177:998–1002.
12. Davies HD, Jones N, Whittam TS, Elsayed S, Bisharat N, Baker CJ. Multilocus sequence typing of serotype III group B *Streptococcus* and correlation with pathogenic potential. J Infect Dis **2004**; 189:1097–102.
13. Luan SL, Granlund M, Sellin M, Lagergard T, Spratt BG, Norgren M. Multilocus sequence typing of Swedish invasive group B *Streptococcus* isolates indicates a neonatally associated genetic lineage and capsule switching. J Clin Microbiol **2005**; 43:3727–33.
14. Jones N, Bohnsack JF, Takahashi S, et al. Multilocus sequence typing system for group B *Streptococcus.* J Clin Microbiol **2003**; 41:2530–6.
15. Jones N, Oliver KA, Barry J, et al. Enhanced invasiveness of bovine-derived neonatal sequence type 17 group B *Streptococcus* is independent of capsular serotype. Clin Infect Dis **2006**; 42:915–24.
16. Spaetgens R, DeBella K, Ma D, Robertson S, Mucenski M, Davies HD. Perinatal antibiotic usage and changes in colonization and resistance rates of group B *Streptococcus* and other pathogens. Obstet Gynecol **2002**; 100:525–33.
17. Manning SD, Lacher DW, Davies HD, Foxman B, Whittam TS. DNA polymorphism and molecular subtyping of the capsular gene cluster of group B *Streptococcus.* J Clin Microbiol **2005**; 43:6113–6.
18. Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of *Streptococcus agalactiae,* a pathogen causing invasive neonatal disease. Mol Microbiol **2002**; 45:1499–513.
19. *Streptococcus agalactiae* (group B streptococcus, GBS) MLST databases. Available at: http://www.pubmlst.org/sagalactiae/. Accessed November 2007.
20. Jolley KA, Chan M-S, Maiden MC. mlstdbNet-distributed multi-locus sequence typing (MLST) database. BMC Bioinformatics **2004**; 5:1–8.
21. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **1987**; 4:406–25.
22. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform **2004**; 5:150–63.
23. Elliott JA, Farmer KD, Facklam RR. Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type. J Clin Microbiol **1998**; 36:2115–6.
24. Chaffin DO, Beres SB, Yim HH, Rubens CE. The serotype of type Ia and III group B streptococci is determined by the polymerase gene within the polycistronic capsule operon. J Bacteriol **2000**; 182:4466–77.
25. Yow MD, Mason EO, Leeds LJ, Thompson PK, Clark DJ, Gardner SE. Ampicillin prevents intrapartum transmission of group B *Streptococcus.* JAMA **1979**; 241:1245–7.
26. Gardner S, Yow M, Leeds L, Thompson P, Mason E, Clark D. Failure of penicillin to eradicate group B streptococcal colonization in pregnant women. Am J Obstet Gynecol **1979**; 135:1062–5.
27. Davies HD, Raj S, Adair C, Robinson J, McGeer A. Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation. Pediatr Infect Dis J **2001**; 20:879–84.
28. Schrag S, Zywicki S, Farley M, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med **2000**; 342: 15–20.
29. Baker C, Goroff D, Alpert S, et al. Vaginal colonization with group B *Streptococcus:* a study of college women. J Infect Dis **1977**; 135:392–7.
30. Edwards MS, Rench MA, Palazzi DL, Baker CJ. Group B streptococcal colonization and serotype-specific immunity in healthy elderly persons. Clin Infect Dis **2005**; 40:352–7.
31. Foxman B, Gillespie BW, Manning SD, Marrs CF. Risk factors for group B streptococcal colonization: potential for different transmission systems by capsular type. Ann Epidemiol **2007**; 17:854–62.
32. Manning SD, Neighbors K, Tallman PA, et al. Prevalence of group B *Streptococcus* colonization and potential for transmission by casual contact in healthy young men and women. Clin Infect Dis **2004**; 39:380–8.