JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2009, p. 1143–1148 Vol. 47, No. 4
0095-1137/09/$08.00+0 doi:10.1128/JCM.01424-08

Multilocus Sequence Types Associated with Neonatal Group B Streptococcal Sepsis and Meningitis in Canada▽

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Received 24 July 2008/Returned for modification 23 September 2008/Accepted 9 January 2009

Group B streptococci (GBS), a leading cause of neonatal sepsis and meningitis, are transferred to neonates from colonized mothers during childbirth. Prior studies using multilocus sequence typing (MLST) have found specific GBS clones (e.g., sequence type 17 [ST-17]) to be associated with neonatal disease in several geographic locations. Few population-based studies, however, have been conducted to determine the frequency of disease caused by specific GBS clones. MLST was used to assess the genetic diversity of 192 GBS strains from neonates and young children identified by population-based surveillance in Alberta, Canada, from 1993 to 2002. Comparisons were made to 232 GBS strains collected from colonized pregnant women, and all strains were characterized for one of nine capsule (*cps*) genotypes. A total of 47 STs were identified, and more than 80% of GBS strains were represented by 7 STs that have been shown to predominate in other populations. ST-17 and ST-19 were more prevalent in strains causing early onset disease (EOD) and late onset disease (LOD) than from pregnant women, whereas STs 1, 12, and 23 were more common in pregnant women. In addition, ST-17 strains and close relatives more frequently caused meningitis than sepsis and LOD versus EOD in this population of neonates. Further research is required to better understand why strains belonging to the ST-17 phylogenetic lineage are more likely to cause both LOD and meningitis and may provide clues into the pathogenesis of these conditions.

Neonatal sepsis and meningitis are frequently caused by group B streptococci (GBS), which are transmitted from mothers to newborns during childbirth (29). Up to 36% of women are colonized during pregnancy (11), and the vertical transmission rate to the newborn is ~45% (9). The use of intrapartum antibiotic prophylaxis (IAP) has significantly decreased the incidence of early onset disease (EOD) in the United States, though rates of late onset disease (LOD) remain unchanged (26, 29). The incidence of both EOD and LOD in the United States is 0.34 cases per 1,000 live births (26). Among the nine distinct polysaccharide capsules (serotypes), types Ia, III, and V more frequently cause neonatal disease (8, 25, 26, 33), and ~80% of LOD is caused by type III strains (8).

Population genetics methods have been applied to GBS strains to investigate genotypes associated with disease, assess genetic variation within genotypes, and examine the role of recombination in the generation of new genotypes. Several methods have identified specific GBS genotypes to be associated with neonatal disease. Multilocus enzyme electrophoresis, which examines variation in 11 conserved metabolic enzyme loci, grouped serotype III GBS strains from neonates into two phylogenetic lineages (24). The electrophoretic type 1 (ET-1) lineage more frequently caused disease relative to the ET-2 lineage (24). Similarly, multilocus sequence typing (MLST), which uncovers sequence variation among conserved housekeeping genes, has classified GBS strains into numerous clones, or sequence types (STs) (16). Some STs group together into clusters following phylogenetic analyses and as many as seven clusters, or clonal complexes (CCs), have been identified among clinical GBS strains (2, 3). The distribution of CCs has been shown to vary in colonizing and invasive strains (2, 3, 17, 19). The ST-17 serotype III strains were associated with neonatal disease in several populations and may have an enhanced ability to cause disease (2, 3, 16, 17, 19, 20).

This study examines the genetic diversity of GBS by using MLST of strains from neonates with invasive disease obtained via population-based surveillance in Canada, and makes comparisons to the genotypes identified among pregnant women sampled during the same time and from the same region. Evaluation of whether specific GBS genotypes are associated with disease type and clinical presentation and if the distribution of lineages varies by population is imperative, as genetic and population variability could potentially limit the effectiveness of a universal GBS vaccine.

MATERIALS AND METHODS

Study population. Two GBS collections were examined, and approval to collect the strains was granted by The University of Calgary Ethics Board. The "invasive" collection includes 192 strains recovered from neonates (n = 185) or young children between 0 and 14 years of age (n = 7) with GBS disease as described previously (6, 8). All but four cases, which were identified retrospectively (1993 to 1994), were identified through population-based surveillance in the Province of Alberta, Canada (1995 to 2002) (6, 8). The four strains isolated from neonates identified retrospectively and the seven strains from children were examined in the phylogenetic analyses but were omitted from the epidemiological analyses. The "colonizing" collection includes 232 GBS strains isolated from pregnant women in Calgary (a major metropolitan city within Alberta) during an overlapping period (1998 to 2000). Women were cultured via vaginal-rectal swabs as described previously (6), and the participating women were previously determined to be representative of pregnant women throughout Alberta (6).

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▽ Published ahead of print on 21 January 2009.

MLST and capsule (*cps*) genotyping. The MLST protocol has been described previously (22). Briefly, PCR fragments for seven housekeeping genes (*adhP*, *atr*, *glcK*, *glnA*, *pheS*, *sdhA*, and *tkt*) were amplified and sequenced. The consensus sequences were trimmed in SeqMan (DNASTAR), and alleles and ST assignments were made using the GBS MLST database (15). Twenty-eight of the invasive strains and 192 of the colonizing strains were previously characterized by MLST to assess the pathogenic potential of type III clones (7). Both the DNA isolation method and PCR-based restriction fragment length polymorphism assay, which predicts the capsule serotype, were described elsewhere (21).

Phylogenetic and epidemiological analyses. A neighbor-joining tree was constructed (28) with the sequence data using MEGA4 (32), and a phylogenetic network was applied to 46 parsimonious-informative (PI) sites in SplitsTree4 using the neighbor-net algorithm (14). STs that grouped together with >70% bootstrap support were considered part of the same GBS cluster, or CC. Recombination between STs was evaluated using the pairwise homoplasy index (PHI) (5).

The frequencies of STs, CCs, and *cps* genotypes were assessed by GBS collection, and comparisons were made between collections using the likelihood ratio χ^2 or Fisher's exact test. The Mantel-Haenszel χ^2 test was used to test for trends. Unadjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated, and logistic regression was used to simultaneously identify predictors of infection with specific GBS genotypes. SAS (v. 9.1) was used for all analyses.

RESULTS

Study population. Among the 192 invasive strains identified throughout the 10-year period, the majority (93%) were isolated from neonates with either EOD (n = 125) or LOD (n = 54). Additional strains were isolated from stillbirths (n = 6), infants between 3 and 15 months of age (n = 5), and children ages 4 and 14 years (n = 2). For epidemiologic analyses, the six stillbirths were included in the EOD category. In all cases, the primary culture site was blood (n = 163), although GBS was isolated from the cerebrospinal fluid (CSF) in 19 neonates and organ tissue in the six stillbirths and four additional cases; the latter were confirmed GBS cases.

Genetic diversity of GBS. MLST classified the 192 invasive strains into 27 STs and the 232 maternal colonizing strains into 32 STs (Fig. 1), yielding 47 unique STs. Most (n = 28; 60%) STs have been described previously (15). Fourteen represented variants of known STs, and an additional five had novel allele combinations (STs 410, 411, 412, 413, and 414) not found in the MLST database (15). Ten of the 19 variants were found in the colonizing strains (Fig. 1) described elsewhere (22), while the 9 remaining variants were from invasive strains and included 4 STs related to ST-17 (Fig. 1), 2 STs related to ST-23, and 3 STs with novel allele combinations. Among the four ST-17 variants, ST-440 and ST-436 contain a single nucleotide polymorphism (SNP) in *tkt* and *adhP*, respectively, and ST-438 and ST-437 differ by unique SNPs in *pheS*. The two ST-23 variants differ by SNPs in *sdhA* (ST-439) and *adhP* (ST-435).

Four CCs were identified and named for the predominant member in each cluster (Fig. 1). The largest complex, CC-23, contained 15 members grouping together with 95% bootstrap support, whereas the smallest cluster (CC-12) had three members grouping together with 90% support (Fig. 1). Because ST-19 has been grouped together with other STs in prior studies (3, 17, 19, 20), particularly ST-28, four STs were included in the CC-19 group despite the low (64%) bootstrap value (Fig. 1). There was no difference in the associations identified when solely ST-19 strains versus CC-19 strains were used. Thirteen singletons, or STs not part of a cluster, were identified, and all singletons were represented by fewer than seven strains (Fig. 1).

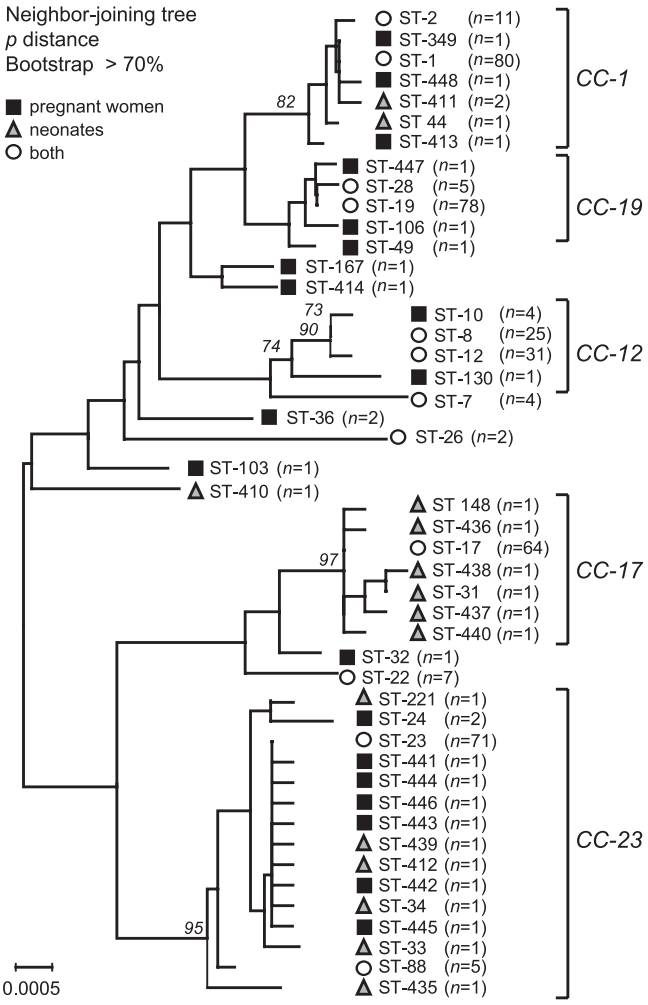

FIG. 1. Phylogenetic relationships among MLSTs from 424 GBS strains recovered from either neonates with invasive disease, colonized pregnant women, or both. The consensus tree was constructed using the neighbor-joining algorithm based on the distance matrix of pairwise differences between STs. Only those relationships with node percentages of >70% bootstrap confidence values based on 1,000 replicates are shown. Each CC, with the exception of CC-19 (64%), comprises STs that cluster with a 70% or greater bootstrap confidence value. STs formerly published (22) as variants (v) were assigned new ST designations: ST-1v is ST-448; ST-19v is ST-447; and the six ST-23 variants are STs 441, 442, 443, 444, 445, and 446.

The overall level of genetic diversity was 0.006 for all 47 STs; diversity did not differ between colonizing and invasive STs. A total of 66 variable nucleotide sites were identified in the concatenated gene sequences (3,457 bp), and 45 sites were parsimonious informative (PI). There were 43 and 45 PI sites in colonizing and invasive strains, respectively. The neighbor-net algorithm revealed that all but one of the CCs had evidence for recombination (Fig. 2). The exception was CC-17 (PHI, P = 1.0), which contained ST-17 and six other closely related STs from invasive strains (Fig. 2).

Distribution of sequence types and *cps* genotypes by GBS specimen site. Twelve STs were common to both colonizing

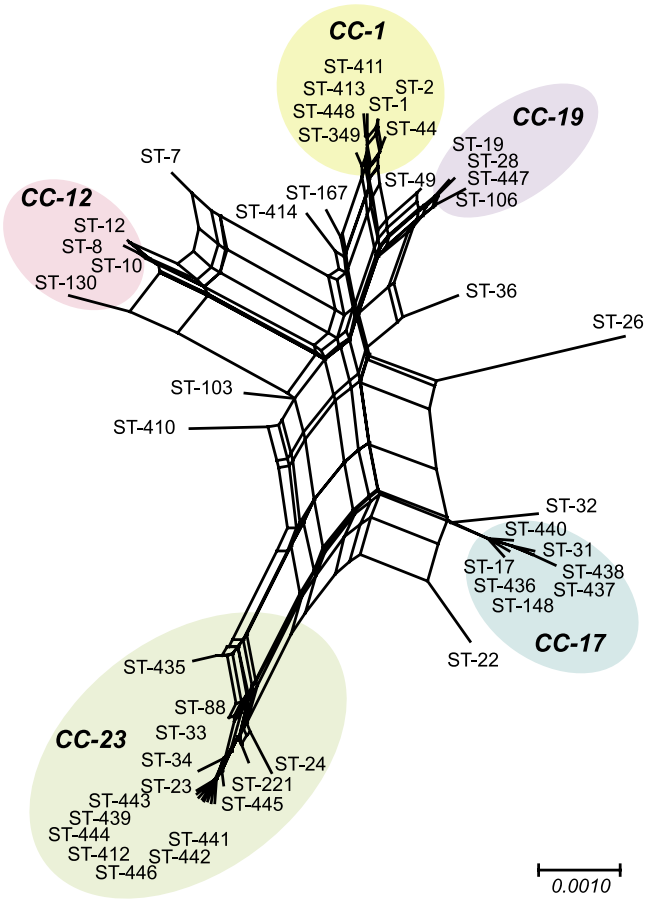

FIG. 2. Phylogenetic network applied to 45 PI sites out of the 3,457 total nucleotide sites using the neighbor-net algorithm for 424 strains of GBS representing 47 STs. The colored circles mark the CCs identified in the neighbor-joining phylogeny. STs formerly published (22) as variants (v) were assigned new ST designations: ST-1v is now ST-448; ST-19v is now ST-447; and the six ST-23 variants are STs 441, 442, 443, 444, 445, and 446.

and invasive specimens (Table 1), whereas 15 and 20 STs were unique to invasive and colonizing strains, respectively (Fig. 1). Seven of the 12 common STs accounted for 85% of strains (Table 1), with 4 STs occurring in various frequencies between invasive and colonizing strains (Table 1). Only STs 1, 12, and 17, however, remained significantly different when just the invasive cases from Calgary, the location of the pregnant woman cohort study, were examined (Table 1). The most notable difference was observed in the frequency of ST-17, which was significantly more common in all invasive strains (OR, 5.6; 95% CI, 2.87, 11.14; $P < 0.0001$) as well as invasive strains solely from Calgary (OR, 6.7; 95% CI, 2.99, 15.01; $P < 0.0001$). In contrast, both ST-1 (OR, 0.5; 95% CI, 0.30, 0.92; $P = 0.01$) and ST-12 (OR, 0.3; 95% CI, 0. 08, 0.70; $P = 0.003$) predominated in colonizing strains versus all invasive strains, and the associations were similar when only Calgary strains were assessed. Although ST-19 was not significantly more prevalent in invasive strains from Calgary (OR, 1.4; 95% CI, 0.67, 2.94; $P = 0.33$), the association was similar in the entire Alberta population (OR, 1.7; 95% CI, 1.00, 2.88; $P = 0.04$). Consequently, all invasive strains were used for the remaining analyses without stratifying by location.

Because the frequency differences identified between the STs and collections were similar when the CCs were evaluated, CCs were used for the epidemiological analyses. The same was true for the *cps* genotype (Fig. 3). Among all 424 strains, *cps3* (35%), *cps1a* (21%), and *cps5* (20%) predominated. CC-17 was the only homogeneous group (Fig. 3). The 192 invasive strains were more likely to have *cps3* relative to the 232 colonizing strains (OR, 4.2; 95% CI, 2.66, 6.48; $P < 0.0001$), whereas *cps1a* (OR, 0.6; 95% CI, 0.35, 0.97; $P = 0.03$) and *cps5* (OR, 0.5; 95% CI, 0.31, 0.89; $P = 0.01$) strains predominated in colonizing strains (Fig. 3). These results were not surprising given that the type III capsule was more common in CC-17 and CC-19 strains and the type V capsule was more common in CC-1 strains (Fig. 3) relative to strains representing all other CCs. Among the 181 EOD and LOD cases, 52% were caused by *cps3* strains of the CC-17 ($n = 53$), CC-19 ($n = 40$), or CC-23 ($n = 2$) lineage.

Association with disease status and severity. CC-17 strains were significantly more common in neonates with both EOD and LOD relative to pregnant women (Table 2), whereas CC-19 strains were only more common in EOD cases. In contrast, CC-12 strains were significantly less likely to cause EOD, while CC-23 strains were less common in LOD cases relative to the colonizing frequency in pregnant women. Although the

TABLE 1. Distribution of the predominant GBS MLSTs from neonatal invasive strains and maternal colonizing strains from Alberta, Canada[a]

ST (no. of strains) and source[b]	No. (%) of strains[c]:	
	Colonizing	Invasive
Province of Alberta (413 [232 colonizing, 181 invasive])		
ST-1 (79)*	54 (23.3)	25 (13.8)
ST-19 (77)*	35 (15.1)	42 (23.2)
ST-23 (70)	45 (19.5)	25 (13.8)
ST-17 (62)†	14 (6.0)	48 (26.5)
ST-12 (29)*	24 (10.3)	5 (2.8)
ST-8 (24)	13 (5.6)	11 (6.1)
ST-2 (10)	8 (3.4)	2 (1.1)
Others (62)	39 (16.8)	23 (12.7)
Calgary (302 [232 colonizing, 70 invasive])		
ST-1 (59)*	54 (23.3)	5 (7.1)
ST-19 (49)	35 (15.1)	14 (20.0)
ST-23 (57)	45 (19.5)	12 (17.1)
ST-17 (35)†	14 (6.0)	21 (30.0)
ST-12 (26)*	24 (10.3)	2 (2.9)
ST-8 (17)	13 (5.6)	4 (5.7)
ST-2 (9)	8 (3.4)	1 (1.4)
Others (50)	39 (16.8)	11 (15.7)

[a] A subset of invasive strains ($n = 302$; 73%) were isolated from Calgary, the location of the maternal colonization study, and therefore were analyzed separately.

[b] The χ^2 test P value (*, $P < 0.05$; †, $P < 0.00001$) highlights significant differences in the frequency of colonizing versus invasive STs in each subset.

[c] Percentages were calculated using the total number of invasive or colonizing strains as the denominator, and only the invasive strain numbers change when Calgary is assessed independently. Note that the four strains isolated during the retrospective study period (1993 and 1994) and the seven strains from children were excluded in the analysis, and, therefore, only those invasive strains that caused neonatal disease are represented.

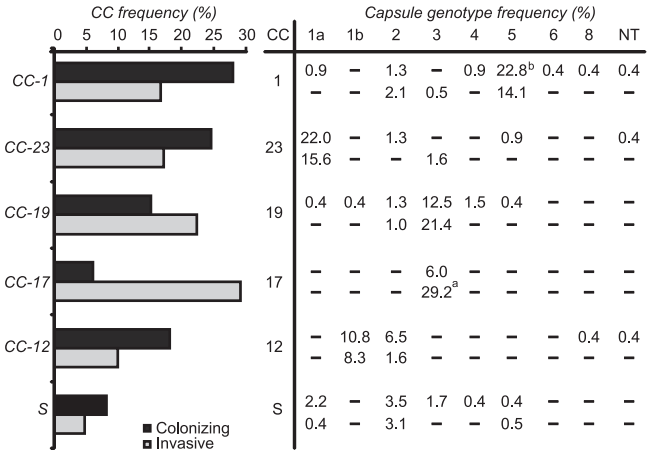

FIG. 3. Distribution of GBS CCs as determined by MLST and frequency of *cps* genotypes among all 424 GBS strains from neonates with invasive disease ($n = 192$) and women colonized during pregnancy ($n = 232$). The CCs are ranked in order of decreasing overall frequency, and the frequency of *cps* genotypes is presented for both invasive and maternal colonizing strains. Singletons (S) refer to the STs that were not associated with a CC or GBS cluster. Footnote symbol a indicates that, together, the CC-19 and CC-17 invasive strains more frequently had *cps3* than colonizing strains (χ^2, 360.71; df, 1; $P < 0.0001$). Footnote symbol b indicates that colonizing CC-1 strains more commonly had *cps5* than invasive strains (χ^2, 305.81; df, 1; $P < 0.0001$).

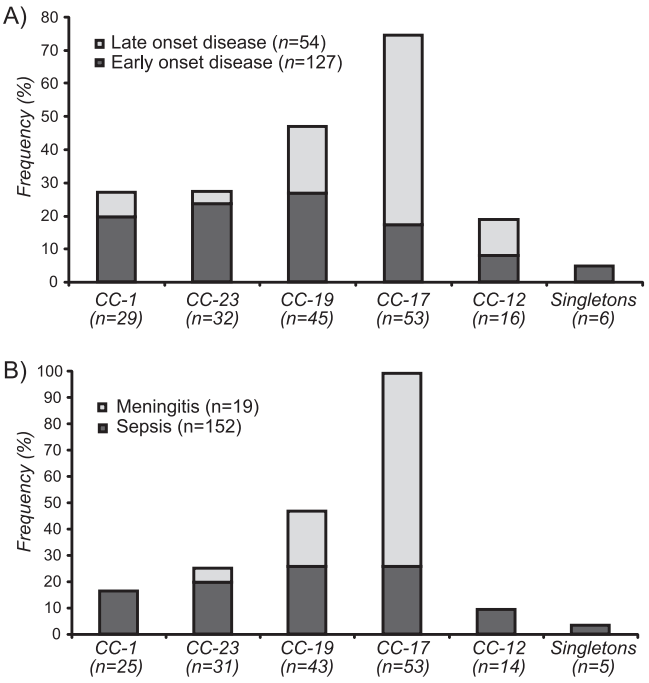

FIG. 4. Frequency of disease stratified by GBS CCs among invasive strains recovered from Alberta, Canada. The CCs are ranked in order of decreasing overall frequency, and the singleton STs represent clones not associated with any CCs. (A) Percentage of EOD and LOD cases by CC isolated from 181 of the 192 neonates with invasive disease. The 10 strains isolated from stillbirths ($n = 6$) and neonatal tissue ($n = 4$) were included in this analysis because the disease onset was known. However, the seven strains from children and the four strains identified retrospectively between 1993 and 1994 were omitted. (B) Disease severity by CC for 171 neonates with neonatal disease and GBS isolation from either blood ($n = 152$) or CSF ($n = 19$). In addition to the 7 strains from children and 4 strains from cases identified retrospectively, the 10 strains isolated from neonatal tissue and stillbirths were excluded to examine associations between disease type and CC.

additional CCs were not significantly different between groups, the direction of the association for all CCs except CC-17 and CC-19 indicated a lower prevalence in both EOD and LOD relative to maternal colonization (Table 2).

LOD was most common in patients infected with either CC-19 (20%) or CC-17 (57%) strains (Fig. 4A); however, CC-17 strains were 8 times more likely than CC-19 strains to cause LOD versus EOD (95% CI, 2.86, 22.10; $P < 0.0001$). Among only the *cps3* CC-17 ($n = 31$) and *cps3* CC-19 ($n = 11$) strains, the CC-17 lineage is still four times more likely to cause LOD relative to EOD (95% CI, 1.41, 9.94; $P = 0.003$). On the other hand, both CC-1 (95% CI, 2.35, 11.45; $P < 0.0001$) and CC-23 (OR, 8.1; 95% CI, 1.77, 50.74; $P = 0.001$) caused more cases of EOD than LOD (Fig. 4A).

In addition, CC-17 strains were significantly more likely to be isolated from cases presenting with meningitis (any CSF isolation) than sepsis (blood isolation) relative to all other GBS genotypes (OR, 8.1; 95% CI, 2.51, 27.51; $P < 0.0001$) (Fig. 4B). The result was the same for ST-17 strains only. Because neonates with LOD ($n = 11$; 20%) were more likely to have meningitis than neonates with EOD ($n = 8$; 7%) (OR, 3.6; 95% CI, 1.20, 10.31; $P = 0.007$), logistic regression was used to simultaneously adjust for clinical presentation and disease status. CC-17 infection was associated with both LOD

TABLE 2. Unadjusted associations between GBS CCs and neonatal disease status[a]

Genotype (413 strains)	Pregnant women (232 strains)		EOD (127 strains)[b]			LOD (54 strains)		
	No. (%) of strains	OR	No. (%) of strains	OR (95% CI)	*P*	No. (%) of strains	OR (95% CI)	*P*
CC-1 (94 strains)	65 (28)	1.0	25 (19)	0.6 (0.36, 1.09)	0.12	4 (8)	0.2 (0.06, 0.63)	0.08
CC-23 (89 strains)	57 (25)	1.0	30 (24)	1.0 (0.55, 1.62)	0.84	2 (4)	0.1 (0.02, 0.52)	0.0006
CC-19 (84 strains)	39 (17)	1.0	34 (27)	2.1 (1.17, 3.63)	0.007	11 (20)	1.3 (0.56, 2.82)	0.54
CC-17 (67 strains)	14 (6)	1.0	22 (17)	3.3 (1.53, 7.04)	0.0007	31 (57)	21.0 (9.20, 48.66)	<0.0001
CC-12 (58 strains)	42 (18)	1.0	10 (8)	0.4 (0.17, 0.84)	0.008	6 (11)	0.6 (0.20, 1.49)	0.22
Singletons (21 strains)	15 (6)	1.0	6 (5)	0.7 (0.24, 2.04)	0.50	0 (0)		0.08[c]

[a] Differences in the distribution of CCs were tested using the likelihood ratio χ^2 (1 df); OR, 95% CI, and *P* values were calculated relative to the combination of all other genotypes from pregnant women. "Singletons" refers to those STs not part of a CC.
[b] GBS strains identified retrospectively before 1995 ($n = 4$) were omitted even though they represented EOD cases.
[c] Fisher's exact test was used because no LOD cases were caused by singleton STs.

TABLE 3. Multinomial logistic regression results identifying factors associated with infection by two common GBS lineages among 171 neonates with EOD or LOD who presented with either sepsis or meningitis[a]

Factor	Infection with CC-17 (53 strains)		Infection with CC-19 (43 strains)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Meningitis	6.8 (2.11, 21.80)	0.001	0.8 (0.26, 2.78)	0.78
LOD	5.5 (2.53, 11.81)	<0.0001	0.7 (0.30, 1.52)	0.34

[a] Two models, which used the different CCs as the outcome of interest, were adjusted for disease type (meningitis versus sepsis), disease severity (EOD versus LOD), isolation date, and geographic location (Calgary versus all of Alberta). Gender was not included in the models because of the high frequency of cases missing this information. Furthermore, the 4 strains identified retrospectively, the 7 strains from children, and the 10 strains isolated from stillbirth or other tissue were excluded from this analysis. ORs, 95% CIs, and *P* values were calculated relative to all other GBS genotypes ($n = 93$).

and meningitis in this population of neonates, whereas CC-19 infection was not associated with either (Table 3).

DISCUSSION

The combination of population genetics and molecular epidemiologic analyses has contributed to a better understanding of the circulating GBS genotypes that predominate among neonatal disease cases as well as colonized mothers. The examination of all neonates with GBS disease in Alberta, Canada, from 1995 to 2002 has revealed that strains of the CC-17 lineage cause significantly more EOD and LOD than other GBS lineages and that CC-17 strains are more likely to cause meningitis than other genotypes, including CC-19 strains that share the same *cps* type (serotype III). These findings confirm previously reported associations between ST-17 and neonatal disease in Israel (2), Sweden (20), the United Kingdom (17), Portugal (23), and the United States (19), thereby supporting the idea that CC-17 strains have an enhanced ability to invade and cause disease. This study, however, is the first to identify a relationship between the CC-17 lineage and meningitis.

Among both invasive and maternal colonizing strains, there is a core group of seven GBS genotypes that predominate in Canada as in other populations (2, 3, 17, 20). Although each genotype was not significantly associated with colonization in this study, only the ST-17 and ST-19 lineages were more prevalent in neonates than pregnant women. It is probable that some colonizing genotypes that make up certain CCs (e.g., CC-1 and CC-23) are well adapted to the female genitourinary tract and may be more readily acquired via direct contact and subsequently transmitted to susceptible neonates during childbirth. This may explain why these genotypes also contribute to neonatal disease and were significantly more common in EOD than LOD in this study. Despite being well adapted to the vagina, it is likely that strains from these lineages are more easily eradicated by antibiotic treatment and IAP or that they contain unique virulence factor profiles and, thus, rarely cause LOD. In contrast, a higher percentage of LOD cases were caused by CC-17 strains than EOD cases, which can be explained in part by antibiotic use during childbirth. Our prior study of pregnant women revealed that *cps3* ST-17 and ST-19 clones were more likely to persist 6 to 8 weeks postpartum despite IAP (22). Therefore, if strains belonging to CC-17 are less likely to be eliminated following IAP and have an enhanced ability to invade, then it is not surprising that CC-17-associated LOD occurs frequently.

The finding that CC-17 strains are more likely to cause meningitis relative to strains of all other genotypes implies that there are differences with regard to disease pathogenesis among GBS lineages. These differences are even apparent between CC-17 and CC-19 strains, even though 95% of both lineages have the type III capsule, which was previously shown to be associated with meningitis (8). Unlike CC-17 strains, the CC-19 strains were not more likely to cause meningitis than sepsis compared with other lineages indicating that CC-17 strains have unique characteristics independent of *cps* type that contribute to more meningeal disease. This hypothesis is consistent with prior studies highlighting genetic variation between GBS clonal groups in putative virulence genes such as the fibrinogen-binding gene (*fbs*) (4, 27), serine-rich repeat region gene (*srr*) (4, 30), surface protein genes (*spb-1* and gbs2018) (4), laminin binding protein-C5a peptidase genes (*lmb-scpB*) (4), and alpha C protein genes (4).

The extensive level of recombination between STs is apparent in all but one lineage from this population. High recombination levels among colonizing lineages (e.g., CC-1 and CC-23) suggest that some genotypes are better adapted for survival in humans and are likely diversifying through recombination to generate new genotypes. The exception, however, was the neonatal disease-associated CC-17 lineage, which was comprised of STs lacking evidence for recombination. This indicates that either the STs within CC-17 have arisen by point mutation or have diverged from the common ST-17 clone independently due to selective pressures or that recombination takes place outside the human host. We hypothesize that recombination within CC-17 occurs in bovines and that the novel STs identified within CC-17 represent genotypes that are temporarily successful in that they contribute to disease, but not as well to colonization. This may explain why each MLST study of invasive GBS has STs unique to each population (2, 3, 17, 20). Support for this hypothesis includes the evidence that ST-17 has arisen from a bovine ancestor (1) and that bovine and human GBS strains are comprised of distinct bacterial populations (1, 4, 10, 31).

ST-17, however, is unique in that it has a widespread distribution and was associated with neonatal disease in several populations (2, 3, 17, 20). Therefore, ST-17 strains currently represent highly successful invasive clones, which may be related to an ability to persist following antibiotic treatment or other unique molecular characteristics. It is also possible that the distribution of ST-17 will change and another clone, perhaps one related to ST-17, will emerge among neonatal disease cases in the future. Such a phenomenon has been observed in GBS previously. For example, strains with the type V capsule, which typically represent ST-1 clones, emerged in the 1990s (12) and caused a shift in the proportion of neonatal disease attributable to other serotypes (13). Large-scale studies will be required to characterize GBS genotypes from multiple human and bovine populations collected during different time periods to examine trends.

Assessment of the genetic diversity of GBS strains isolated from neonates with invasive disease offers new insights into the

genetic backbone of those GBS strains most important for disease development. CC-17 strains commonly caused neonatal disease in Canada between 1995 and 2002 and elsewhere during the same period and may contribute to more severe disease. In addition, because ST-17 strains were shown to persist in women following IAP (22) and more frequently caused LOD and meningitis in this population, genotyping methods that rapidly identify the ST-17 lineage and its close relatives may be required if GBS is isolated during pregnancy. Such a genotyping method has been described (18) and may be utilized to identify neonates at risk of developing LOD and meningitis.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI066081 from the National Institutes of Health.

We thank Katherine Schaeffer for technical assistance, Shari Tjugum-Holland from the Michigan State University Research Technology Support Facility for DNA sequencing, and Nicola Jones of the John Radcliffe Hospital for new ST designations.

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