Molecular Characterization of Human-Colonizing *Streptococcus agalactiae* Strains Isolated from Throat, Skin, Anal Margin, and Genital Body Sites

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Received 3 March 2008/Returned for modification 15 April 2008/Accepted 7 July 2008

*Streptococcus agalactiae* carriage was evaluated by sampling four body sites in a group of 249 healthy individuals including both sexes and a wide range of ages; the aims were to study the population structure of colonizing strains by multilocus sequence typing (MLST) and to evaluate their diversity by serotyping, Smal macrorestriction analysis, and PCR screening for genetic markers of highly virulent clones for neonates. The prevalences of carriage were 27% in women and 32% in men. The major positive body site was the genital tract (23% in women and 21% in men); skin, throats, and anal margins were also positive in 2%, 4%, and 14%, respectively. These human-colonizing strains belonged mostly to serotypes III (24%), Ia (21%), V (18%), and Ib (17%). Twenty-three sequence types (STs) were identified. The MLST characteristics of the strains isolated from a single anatomic site—genital (vagina [women] or from a sample of the first urination after arising from a night’s sleep [men]), throat, skin, or anal margin—suggest a body site colonization specificity for particular STs: strains of STs 2, 10, 19, and 196 were isolated only from genital sites; strains of STs 1, 8, and 23 were isolated more frequently from throat flora; and strains recovered only from anal margin samples were more closely related to strains isolated from throats than to those from genital sites. Most strains of STs 1, 8, and 23—STs that are increasingly described as being responsible for adult infections—did not carry any markers of strains virulent for neonates, suggesting that the virulence of these strains is probably associated with other genetic determinants. In addition, the genetic diversities of the strains varied between STs: STs 2, 8, 10, 10, 23, and 196 were the most diverse; STs 1 and 19 were more homogeneous; and ST 17 strains formed three distinct groups.

*Streptococcus agalactiae* is the pathogen most commonly responsible for maternofoetal and neonatal infections, and serogroup III sequence type (ST) 17 strains of this species have been identified as more likely to cause these infections (3, 17, 24–26, 28).

*S. agalactiae* infections in nonpregnant adults have been reported increasingly since the 1970s (5, 7, 10, 14, 20, 21, 23, 26, 27). The major clinical forms consist of skin, soft tissue, and bone infections; bacteremia; urinary tract infections; pneumonia; and peritonitis. More rarely, *S. agalactiae* is responsible for septic arthritis, meningitis, and endocarditis. Risk factors have been identified (16) and include an age of over 60 years and diagnoses of diabetes, cancer, decubitus acutus, AIDS, long-course corticotherapy, chronic renal disease, cirrhosis, and neurological vessel disorders. However, cases of invasive *S. agalactiae* infections in immunocompetent nonpregnant adults have recently been reported (7, 20, 21, 23). The physiopathology of these infections is not well understood and may be linked in part to *S. agalactiae* strains with particular properties.

Reports on the genetic characteristics of the strains associated with infections in adults showed that they are diverse and belong mostly to serotypes Ia, III, and V (5, 14) and clonal complexes 1, 9, 17, 19, and 23 (17).

The portal of entry of these infections in adults is not often well documented. A better knowledge of the genetic characteristics of the *S. agalactiae* strains colonizing various body sites would contribute to a better understanding of the origin of infections in adults. Although the molecular features of *S. agalactiae* strains isolated during infectious diseases have been described in many cases, little is known about the molecular characteristics of human-colonizing strains. Therefore, we screened for *S. agalactiae* carriage in 249 healthy individuals of both sexes; the individuals were living at home and covered a range of ages, from the start of the teenage period until old age. Genital, skin, throat, and anal margin carriage was sampled by self-swabbing. The strains isolated were serotyped. The population structure of the strains was investigated by multilocus sequence typing (MLST). The genetic diversity of isolates was evaluated by Smal macrorestriction analysis and PCR screening for five previously described genetic markers of *S. agalactiae* strains able to invade the central nervous system (CNS) of neonates. Genetic features of colonizing strains isolated from the various anatomic sites were studied.

**MATERIALS AND METHODS**

**Population studied.** The objective was to enroll 25 to 30 healthy females and 25 to 30 healthy males in each of the five following age ranges: 11 to 24 years, 25 to 40 years, 41 to 54 years, 55 to 70 years, and ≥70 years. After approval of the study by the local ethical committee, 249 French individuals of ages from 11 to
S. agalactiae strain isolation. Self-swabbing was conducted in the morning before washing. Volunteers were instructed to swab or sample the following body sites or products: (i) for both sexes (swabbing), the throat, the interdigital spaces on the feet, and the anal margin; (ii) for females (swabbing), the vulva for teenagers (up to 16-year-old) girls and the vagina for adults; and (iii) for men (sampling), the first urination after arising from a night’s sleep (herein referred to as first urine), which was used to identify S. agalactiae colonization of the meatus urinarius, the preputial gland, and the end of the urethra. Swabs were transmitted to the laboratory within 12 h. Swabs and urine samples (1 ml) were placed in Todd-Hewitt broth supplemented with gentamicin (8 μg/ml) and incubated for 24 h at 37°C. The selection broths were then incubated with CO2 at 37°C. Vitek2 g-positive identification cards (Laboratoire bioMérieux, Marcy l’Etoile, France) and incubated for 24 h under aerobic conditions, and incubated for 48 h under anaerobic conditions at 37°C. Each morphotype was then subcultured on streptob agar medium (Laboratoire AES, Combourg, France) and incubated for 48 h under anaerobic conditions at 37°C. Each morphotype of Streptococcus was typed using MLST according to the procedure described by Jones et al. (17). A tree based on the use of an unweighted-pair group method using average linkages was drawn from allelic profile data by use of Phylodendron and the entire group B Streptococcus (GBS) MLST database (http://pubmlst.org/sagalactiae/).

Detection of genetic markers of virulent S. agalactiae strains. Some genetic markers are significantly more frequent in strains able to invade the CNS of neonates than in other strains, namely, the GBSSI group II intron downstream from the C5a-peptidase scpB gene (3), IS1548 in the hydF gene (25), and three prophagic DNA fragments (28). PCR was used to test the presence of these markers as previously described (3, 25, 28).

Statistical data. Chi-square tests and Fisher’s exact test (two tailed) were used to test associations, and a P value of 0.050 was considered significant.

RESULTS

Population studied. A total of 249 individuals were enrolled in the study, including 133 females and 116 males. Consistent with the study design, we recruited 25 to 30 individuals of each sex in each age class except one (Table 1). For those of ≥70 years of age, fewer men than expected were recruited (n = 10). The 249 enrolled individuals included 137 individuals unrelated to other participants and 112 individuals belonging to 47 family groups as follows: 37 partner pairs (29 with no child, 2 with one child, 4 with two children, and 2 with three children), and 3 monoparental groups (1 group with a parent and a child and 2 groups with a parent and two children), and 7 pairs of brothers and/or sisters. Therefore, these family groups included 37 partner pairs, 37 parent-child pairs, and 19 sibling groups.

Prevalence of carriage. 104 S. agalactiae strains were isolated from 73 of the 249 individuals (29%). The strains were isolated from 36 of the 133 females (27%) and 37 of the 116 males (32%). Two morphotypes were observed for an anal margin sample from one male (M113).

(i) Prevalence of carriage according to sex and age. The prevalence of carriage varied according to sex and age (Table 1). For women, the prevalence was between 20% for those aged ≥70 years and 41% for those of ages between 41 and 54 years. The prevalence for those of ages between 41 and 54 years (41%) was significantly higher than those for other age groups (20 to 27%) (P = 0.048). For men, the prevalence was more variable (Table 1). Carriage was rare (4%) for those of ages between 11 and 24 years and increased with age to 48% for those of ages between 25 and 40 years and 54% for those of ages between 41 and 54 years. The prevalence was significantly higher for those of ages between 25 and 40 years and between 41 and 54 years than for those of ages between 11 and 24 years, between 55 and 70 years, and of ≥70 years (P < 0.001). The prevalence was significantly higher for women than for men only for those of ages between 11 and 24 years (14 versus 4%) (P = 0.029).

(ii) Prevalence of carriage according to the site of isolation. The prevalence of carriage varied according to the site of isolation (Table 1). The major positive body site was the genital tract (23% from vagina in women, 21% from first urine in
men), followed by the anal margin (14%), the throat (4%), and skin (2%).

The prevalence of carriage in various body sites did not differ significantly between men and women of ages of ≥25 years (Table 1). By contrast, for those of ages between 11 and 24 years, vaginal carriage was frequent in women (21%), whereas genital carriage was rare (4%) in men (P < 0.001). The frequency of carriage at nongenital sites did not differ significantly according to sex or age.

Carriage in family groups. (i) Partner pairs (37 cases). Carriage was found for 18 partner pairs (49%) in at least one partner. Carriage was observed for both partners in 11 of the 18 cases (61%), for only the female in 2 cases (11%) and for only the male in 5 cases (28%).

(ii) Relations between parent and child (37 cases). Carriage was identified for four parents and none of their children were carriers. One child (female, 14 years of age) carried S. agalactiae, but her parents were S. agalactiae negative.

(iii) Relations between siblings (19 cases). Carriage was identified for four females of 12, 14, 15, and 18 years of age, all having one or two siblings. No carriage was found for the siblings of these carriers.

Carriage and body site of colonization. Forty-seven (21 females, 26 males) of the 73 carriers were positive at a single body site (64%). Carriage was rarely present in more than two body sites (5%).

(i) Female carrier group. Screening of the vagina/vulva, the anal margin, and the throat for 133 women detected S. agalactiae in 35 individuals (26%), accounting for 97% of the 36 carriers. For cases in which there was a single positive site of carriage, it was the vagina/vulva in 15 cases, the anal margin in 2 cases, the throat in 3 cases, and the skin in 1 case. For 14 cases, two body sites were positive (vagina and anal margin). One female was positive at her vagina, throat, and skin.

(ii) Male carrier group. Samples of the first urine, the anal margin, or the throat were S. agalactiae positive for 35 of the 116 men (30%), accounting for 95% of the 37 carriers. For cases of a single positive site, carriage was in the first urine in 14 cases, at the anal margin in 7 cases, in the throat in 3 cases, and on the skin in 2 cases. For eight cases, two body samples were positive (first urine and anal margin). For the remaining males, the carriage was positive for three body samples (first urine, anal margin, and throat in two cases and first urine, anal margin, and skin in one case).

Serotypes of human-colonizing strains. Ninety-four of the 104 human-colonizing strains (90%) were typeable. The major serotypes were serotype III (25 strains; 24%), Ia (22 strains; 21%), V (19 strains; 18%), and Ib (18 strains; 17%). Strains belonging to serotypes II (n = 4; 4%) and IV (n = 6; 6%) were rare.

(i) Partner pairs with carriage in both partners (11 cases). For 10 (91%) of the partner pairs with carriage in both partners, the serotypes of strains isolated from the partners were similar. The strains isolated from the partners of one pair were of different serotypes (serotype Ib from male M115 and serotype V from female F87).

(ii) Individuals with multisite carriage (26 cases). For 25 (96%) of the individuals with multisite carriage, all strains isolated from different body sites were of the same serotype. For the remaining individual, M219, a nontypeable strain, was isolated from the first urine and a serotype IV strain was isolated from the anal margin sample. The two morphotypes isolated from the anal margin swab of M113 both belonged to serotype III.

PFGE characteristics of human-colonizing strains. Fifty-one pulsotypes were obtained with the 104 strains.

(i) Carriage by independent carriers (51 cases). Forty-six pulsotypes were obtained with the 51 strains.

(ii) Partner pairs with carriage in both partners (11 cases). Strains isolated from both partners in nine partner pairs (82%) were of similar pulsotypes in each partner pair, with each partner pair having a different pulsotype. For one partner pair (M82, F94), the strains from the partners were both of serotype V and of different pulsotypes but were closely related (20% dissimilarity). For the 11th partner pair (M115, F87), the two strains were of different serotypes and in distantly related pulsotypes (90% dissimilarity).

(iii) Individuals with multisite carriage (26 cases). For 24 individuals (92%) in the multisite-carriage category, the strains isolated from all positive sites in the body gave similar pulsotypes. For individual M219, who had strains of different serotypes, and for individual M165, who had strains of serotype Ib, the strains from the first urine were of a pulsotype distantly related to those of the strains isolated from other positive sites (70% dissimilarity).

The two morphotypes isolated from the anal margin swab from individual M113 showed similar pulsotypes.

Genetic diversity of the human-colonizing strains. To determine the population structure of the strains and their genetic diversity, we used MLST. We first excluded redundant strains: strains were considered redundant when the isolates from both partners in partner pairs and/or from several body sites of an individual exhibited similar serotypes and pulsotypes. According to these criteria, there were 66 independent strains among the 104 isolates. Twenty-three STs were identified by MLST for the 66 strains (Fig. 1), and 45 strains (68%) clustered into eight major STs. Two major phylogenetic groups, groups I and II, were identified. Group I was homogeneous and corresponded to clonal complex CC 23. Group II comprised two subdivisions: a homogeneous subgroup (II 1) that corresponded to CC 17, and a heterogeneous subgroup (II 2) that included the six remaining major STs, which were STs 1, 2, 196, 19, 8, and 10.

(i) MLST and anatomical origin of strains. For this analysis, we included only the 36 strains isolated from a single anatomic site to ensure that they represented the considered anatomical origin (Table 2). The 12 strains belonging to STs 2, 196, 19, and 10 were all isolated only from genital sites (vagina and first-urine samples). STs 1, 8, and 23 were better represented among strains isolated only from throat (4/6; 67%) than among strains isolated from genital sites (vagina and first-urine samples). The seven strains isolated only from the anal margin samples and the six strains isolated only from throat samples were similarly distributed among STs.

(ii) MLST and serotype (Fig. 1). ST 1 was significantly associated with serotype V (P = 0.001), ST 8 with Ib (P < 0.001),
FIG. 1. Tree based on MLST and an unweighted-pair group method using average linkages (UGPMA) of the 66 unrelated S. agalactiae strains and PFGE diversity according to ST. Footnotes: 1, strains were given a code corresponding to the carrier code; 2, ST designation; 3, PCR result for GBSil1 group II intron downstream from the C5a-peptidase scpB gene; 4, PCR result for IS1548 in the hylB gene; 5, PCR result for the three prophage DNA fragments. NT, nontypeable.
and ST 23 with Ia (P < 0.001). ST 17 and ST 19 were significantly associated with serotype III (P < 0.001).

(ii) MLST and virulence markers of strains able to invade the CNS of neonates (Fig. 1). ST 17 was significantly associated with positivity for the GBSII group II intron downstream from the scpB gene (P < 0.001), and ST 19 with positivity for IS1548 in hydB (P < 0.001) and for prophage DNA fragments F7 and F10 (P values of <0.001 and <0.004, respectively). In CC 9, containing ST 8 and ST 10, three of the four strains belonging to ST 10 had a diverse set of virulence markers and, by contrast, five of the seven strains belonging to ST 8 contained none of the virulence markers studied. Most strains of STs 1, 8, and 23 did not carry any of the genetic markers studied.

(iv) MLST and PFGE (Fig. 1). PFGE diversity within each of the eight major STs was investigated. STs 2, 196, 8, 10, and 23 exhibited substantial diversity of PFGE patterns, with 23 pulsortypes among the 26 strains. STs 1 and 19 exhibited the lowest diversity, with maximal percentages of dissimilarity of 40% for ST 1 and 20% for ST 19. ST 17 exhibited a particular pattern, with six strains clustering in three distantly related pulsortypes (90% dissimilarity).

DISCUSSION

The S. agalactiae colonization rates reported in diverse populations vary from 2 to 30% (1, 2, 4, 6, 8, 9, 11, 12, 15, 19, 22). Using a procedure recommended for the detection of S. agalactiae from lower vaginal and rectal samples (4, 6) and self-swabbing, we obtained a carriage rate (29%) consistent with, although slightly higher than, values reported by others; this validates the procedure used and demonstrates the efficiency of self-swabbing for S. agalactiae detection.

Data concerning the course of S. agalactiae colonization are divergent. In view of the high rates of anorectal colonization, it has been suggested that the primary site of infection is the lower gastrointestinal tract and consequently that genital carriage merely reflects contamination from the anorectum (1, 4, 9). However, S. agalactiae colonization of genital sites may be equally high, and a high prevalence of carriage among sexually active populations has been described (12, 19); these observations led to the idea that vaginal colonization and sexual contact are major factors for the transmission of S. agalactiae (19). The apparent discrepancies between the various findings may be consequences of the specificities of the populations studied. In particular, college students (2, 4, 12, 19), pregnant women (1), and elderly women in menopause may present different characteristics depending on their gastrointestinal and genitourinary tract conditions and their sexual activity. Our study, assessing S. agalactiae colonization in a larger healthy population, which was representative of both sexes and a wide range of ages, brings additional data. First, in the youngest group (<18 years of age), colonization was rare in males although notable in females, with vaginal colonization twice as frequent as anal colonization, and anal colonization was in all cases associated with vaginal colonization. Second, various observations are informative for the three median-age classes (25 to 70 years of age): (i) the vaginal colonization rate was higher than the anorectal rate; (ii) genital colonization was highest (54%) in 41- to 54-year-old men; and (iii) the prevalence of carriage among partner pairs was high, and strains isolated from most partners were closely related.

Third, colonization was less prevalent among men in the oldest age class (>70 years of age) than among the younger-age classes. Fourth, in accordance with previously published data (12, 19), anal carriage was not observed in family members who have no sexual contact (parent/child, child/child), despite presumably sharing food and food-related utensils. These observations are consistent with sexual contact playing an important role in the spread of S. agalactiae. Nevertheless, one of the limitations of our study is that we did not evaluate either the sexual activity of the carriers or rectal carriage. Therefore, other explanations of the variations in carriage rate between sexes and according to age are possible, such as different ecological conditions in genital tracts for bacterial colonization depending, for example, on the hormonal status of the individuals.

S. agalactiae is responsible for pneumonia and adult invasive infections (5, 7, 10, 14, 20, 21, 23, 26, 27) including bacteremia, endocarditis (sometimes associated with vertebral infections), endophthalmitis, and meningitis in patients for whom the genital tract is unlikely to be the portal of entry (7, 13, 20). By comparing MLST characteristics of strains from a single anatomic site, we found that strains isolated from the throat differed from genital strains and belonged mostly to STs 1, 8, and 23, three STs previously described as being involved in infections of nonpregnant adults (17). These observations suggest that the throat could be a portal of entry for adult invasive infections; nevertheless, given the small number of isolates we studied, this notion requires confirmation by further studies including larger numbers of throat carriage strains.

We report the molecular characteristics and genetic diversity of human-colonizing strains. Most were equally distributed among the four major serotypes involved in invasive cases of disease (Ia, Ib, III, and V) (5, 17, 20, 26). The major STs that we identified for colonizing strains have also been identified as major STs for strains isolated during infectious diseases (17). Indeed, 68% of colonizing strains belonged to STs 1, 2, 8, 17, 19, 23, and 196, STs that have been associated with neonatal and/or adult infections (17). Using MLST data and genetic characteristics as assessed by PFGE and PCR for several markers of horizontal transfer (introns, insertion elements, and prophagic elements), we found substantial differences in the diversities of strains according to their STs as follows and as shown in Fig. 1: (i) in STs 1 and 19, the strains showed a low
level of genetic diversity, which may indicate the recent emergence of clones or highly selective environmental conditions; (ii) in STs 2, 8, 10, 23, and 196, the strains showed a high genetic diversity, and these strains therefore may have been subject to various ecological conditions and consequently display genome diversification; and (iii) in ST 17, most of the colonizing strains were positive for the GBSi1 group II intron downstream from the Csa-peptidase gene scpB but could be classified into one of three distant PFGE groups that may result from different genetic events or ecological pressure. By contrast to STs 2, 10, 17, 19, and 196, most of the strains of STs 1, 8, and 23 did not carry any markers of strains responsible for neonatal meningitis (Fig. 1). This suggests that strains of these STs may have mechanisms different from those involved in the invasion of the CNS of neonates.

In conclusion, our study suggests a possible body site colonization specificity of the various phylogenetic groups (STs) that compose the S. agalactiae species and shows differences of genetic diversification between STs. Further studies should be conducted to elucidate how the body site environments may have selected bacteria having a particular fitness.

ACKNOWLEDGMENT
This work was supported by the Centre Hospitalier Universitaire, Tours, France.

REFERENCES