Carriage of Streptococcus agalactiae in Women and Neonates and Distribution of Serological Types: a Study in Brazil

LESLIE C. BENCHETRIT, 1* SERGIO E. L. FRACALANZZA, 1 HELOISA PEREGRINO, 1 ALIPIO A. CAMELO, 2 AND LEONARDO A. L. R. SANCHES²

Institute of Microbiology¹ and Gynecological Clinics of the University Hospital,² Federal University, 21941, Rio de Janeiro RJ, Brazil

Received 23 October 1981/Accepted 7 January 1982

The prevalence of group B streptococcal carriage was evaluated in nonpregnant women and in mothers and their offspring. The overall carriage rate of group B streptococci at one site was 18.2%. Streptococci were recovered from one or more of the sites sampled in 25.6% of mothers and 15.4% of newborn infants. The maternal genital carriage rate was 18.6%, and acquisition of the organism from the mother was assessed by serological typing of group B streptococcal isolates in the mother-infant pairs. A cervical carriage rate of 16.3% was seen in nonpregnant women.

Streptococcus agalactiae, the Lancefield group B streptococcus, has been recognized over the last few years as the main etiological agent of serious neonatal disease (1, 3, 7, 13, 17). Vaginal carriage of group B streptococci (GBS) is common among pregnant women (3, 10), and their newborns are frequently contaminated with the organism. It has been documented that the early-onset type of group B streptococcal disease occurs within the first days and even hours of the baby's life (3, 19) and that acquisition of the organism is from the maternal birth canal during delivery (4, 10). However, not all infants carrying GBS develop illness and most remain asymptomatic (3). It is also commonly recognized that the normal cervical-vaginal microflora of nonpregnant women includes S. agalactiae (6). In addition, it has been suggested that in women carrying GBS in their genital tracts, one of the most likely reservoirs for the organism is the ano-rectal region (2, 9).

This study was undertaken to assess the cervical carriage of GBS in nonpregnant women and carriage of the organism in mothers and their offspring at the time of delivery.

MATERIALS AND METHODS

Clinical material. Nonpregnant women examined were from the Gynecological Clinics at the University Hospital, Federal University. Parturient women included in the investigation were enrolled in the Fernando de Magalhães Maternity Hospital, Rio de Janeiro. Single swabs from the cervix were taken with the aid of a sterile speculum from 257 ambulatory nonpregnant women between the ages of 18 and 67 years. Vaginal and anal cultures (a single culture for each site) were obtained from 86 parturient women between the ages of 16 and 52 years on admission to the labor and delivery area. Cultures were taken with a swab

from 78 newborn infants from the external ear canal and anus immediately after birth and before routine cleansing. Seventy-eight mother-baby pairs were thus studied as cultures could not be obtained from eight neonates.

Bacteriological studies. Swabs were incubated aerobically at 35°C for 18 h in 1 ml of a selective medium consisting of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.), 5% sheep blood, nalidixic acid (15 μg), and gentamicin sulfate (8 μg) (5). The broth was then subcultured onto plates containing tryptose blood agar base (Difco) and 5% sheep blood. The plates were examined for hemolytic activity after incubation for 18 h at 35°C in normal atmosphere. Negative plates (those with no beta-hemolytic colonies) were routinely reincubated and reobserved at 48 h. Beta-hemolytic streptococci (one colony for each positive site) were picked from the plates and grown for 18 h at 35°C in 4 ml of Todd-Hewitt broth.

Serological classification of streptococci. Grouping of the streptococcal isolates was carried out by the nitrous acid procedure described by El Kholy et al. (8), and the streptococci used for the preparation of nitrous acid extracts were from the 4-ml broth cultures. Group B streptococcal isolates were typed by the capillary precipitin method using hot-hydrochloric acid extracts from the sediment of 100-ml Todd-Hewitt broth cultures (12, 16, 18). Type Ic strains were also identified by double diffusion in agar gel (15). Grouping and typing sera were prepared in our laboratory.

RESULTS

During the 16-month period of the study (March 1980 to June 1981), cultures were obtained from 422 individuals, 164 of whom (86 parturient women and 78 newborn infants) were cultured at more than one site. Of the remaining 258 persons (nonpregnant women), a vaginal culture was obtained from one who had undergone surgery of the genital tract in 1979, and

TABLE 1. Multiple carriage of GBS by parturients and newborn infants

Group of popula- tion examined	No. of persons examined	No. of persons with culture sites positive	No. of cultures	No. of persons with positive cul- tures at:		
			positive	Vagina ^b	Anus	Ear
Parturients ^a	rients ^a 86		31	16	15	
Newborn infants	78	12	20		9	11

^a This number includes the 78 women whose newborn infants were cultured.

^b Site of specimen.

TABLE 2. Distribution of GBS serological types in the populations examined

Population	No. of persons examined	No. of strains recovered of serological type:						No. of cultures	
ropulation		Ib	Ic	II	III	Ibc ^a	NT ^b	positive	
Nonpregnant women	258°	6	4	16	11	0	6	43	
Parturients	86^d	9	10	5	3	1	3	31	
Newborn infants	78 ^e	5	6	2	4	0	3	20	

^a Contains the antigen Ibc only.

^b NT, Not typable.

^c One vaginal and 257 cervical cultures.

^d Each woman had one vaginal and one anal culture obtained.

" Ear canal and anal cultures were taken from each baby.

TABLE 3. Distribution of GBS by serological type and clinical source of specimen

Clinical source of specimen	No. of individuals examined	No. of cultures	No. of strains recovered of serological type:						
			Ib	Ic	II	III	Ibc ^a	NT ^b	
Cervix	257°	42	5	4	16	11	0	6	
Vagina	87^d	17	5	6	1	2	Ö	3	
Anus	164e	24	7	6	5	3	1	2	
Ear	78 ^f	11	3	4	1	2	0	1	

^a Contains the antigen Ibc only.

^b NT, Not typable.

^c Nonpregnant women only.

^d One 59-year-old woman who underwent hysterectomy in 1979 and 86 parturients.

^e Eighty-six parturients and 78 babies.

f Babies only.

cervical cultures were obtained from the other 257

Sites of isolation of group B streptococci. About 18% of the entire study population had at least one positive culture. In parturients, the prevalence of GBS carriage was 25.6% (Table 1). Twenty-two mothers and 12 newborn babies had at least one site culture positive for GBS. Five mothers with positive vaginal cultures gave birth to babies who did not have GBS isolated at the time of delivery. Among 22 colonized mothers, 9 had the organism at the two sites and 13 had the organism at one site (vagina or anus, Table 1). GBS were isolated from one or more of the culture sites in 12 of 78 infants cultured immediately after birth. Ear-canal cultures (11 isolates) were slightly more positive than anal cultures (9

isolates). Four of the infants had GBS recovered from only one site (ear or anus). Eight infants had GBS at the two sites. All infants studied represented live births, and none developed clinical disease. Cervical carriage rate was 16.3% for the nonpregnant women (Tables 2 and 3).

Serological type distribution of group B streptococcal isolates. The distribution of serological types of group B streptococci was as follows: Ib, 21.3%; Ic, 21.3%; II, 24.4%; III, 19%. There were 12 nontypable strains and 1 Ibc isolate. The serological types of GBS isolates are summarized in Tables 2 and 3 according to the groups of population and the culture sites, respectively. Types II and III were most frequent in cervical cultures of nonpregnant women, and together were isolated from 27 of 257 individuals. Eleven culture-positive infants were born to mothers who had vaginal cultures positive for GBS. Paired maternal-baby serological types were identical in these 11 cases. Of these 11 mothers, 8 yielded the same GBS types from the vagina and from the anus.

DISCUSSION

In the current investigation, cervical carriage of GBS among nonpregnant women was not significantly different from that reported for the prevalence of vaginal colonization with the organism among normal (college) women (6). GBS of serotypes II and III were the most prevalent among nonpregnant women in our population and have also been the serotypes most commonly isolated from similar individuals (6). The correspondence of serological type distribution in 11 mothers and their newborns in our study strongly suggests acquisition of GBS from the maternal genital tract before birth or at the time of delivery. Carriage of the same serological type in the vagina and at the ano-rectal region by 8 of the 11 mothers also suggests that the rectal site may have been a reservoir for contamination of the vagina, as previously reported (2, 9). Contamination at more than one site was seen in 8 of our newborn infants, and GBS were isolated from the ear canals of 11 of 12 infants. This observation is consistent with the view that contamination of the amniotic fluid with GBS may occur and results in the isolation of the organisms from the external ear canal of neonates (4, 10, 11, 14).

The purpose of this communication was to present our experience in the urban area of Rio de Janeiro with documented cases of group B streptococcal carriage in women and newborn infants and to compare these data with those from numerous studies performed mainly in countries of the northern hemisphere.

Baker and Barrett (4) previously isolated GBS as part of the vaginal flora of 25.4% of parturient women. The organism has been isolated from the vagina in 4.6% of women at the time of delivery (11), and the isolation rates from neonates has varied from 10% (11) to 26.2% (4). Ferrieri et al. (10) found that GBS were present in 8.3% of mothers in labor and 3.4% of newborns. Pass et al. (14) found that GBS occurred in 12.5% of cultures in infants. In their study, 19% of mothers carried the organism as part of the vaginal flora. GBS of serotypes Ib, Ic, II, and III were the most prevalent among the parturients and their offspring in Ferrieri's study (10). Types II (up to 35%) and III (up to 38%) were also most commonly isolated from mothers in labor and neonates (1, 4, 11).

It is important to note at this point that, by sampling at least two sites in the 78 mother-infant pairs examined, we did not show a significant difference in the carrier rates or even serological types between our population groups and those studied in other geographical areas of the temperate zones. Our investigation tends to indicate that carriage of GBS in the perinatal period may be as common in the large developing country of Brazil as elsewhere.

ACKNOWLEDGMENTS

This investigation was supported by research grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (400559/79, to L.C.B.), Financiadora de Estudos e Projetos, and Conselho de Pesquisa da Universidade Federal do Rio de Janeiro.

We thank the medical and nursing staff of the Gynecological Clinics and Maternity Hospital for their cooperation in collecting specimens. Richard R. Facklam kindly provided bacterial strains and protocols for the production of grouping and typing antisera in rabbits. We thank Patricia Ferrieri, Dwight R. Johnson, and Pamela Petersen for their help in the typing program.

LITERATURE CITED

- 1. Anthony, B. F., and D. M. Okada. 1977. The emergence of group B streptococci in infections of the newborn infant. Annu. Rev. Med. 28:335-369.
- Badri, M. S., S. Zwaneh, A. A. Cruz, G. Mantilla, H. Baer, W. N. Spellacy, and E. M. Ayoub. 1977. Rectal colonization with group B streptococci: relation to vaginal colonization of pregnant women. J. Infect. Dis. 135:308

 312.
- Baker, C. J. 1977. Summary of the workshop on perinatal infections due to group B streptococci. J. Infect. Dis. 136:137-152.
- Baker, C. J., and F. F. Barrett. 1973. Transmission of group B streptococci among parturient women and their neonates. J. Pediatr. 83:919-925.
- Baker, C. J., D. J. Clark, and F. F. Barrett. 1973. Selective broth medium for isolation of group B streptococci. Appl. Microbiol. 26:884-885.
- Baker, C. J., D. K. Goroff, S. Alpert, J. R. Evrard, B. Rosner, and W. M. McCormack. 1977. Vaginal colonization with group B streptococcus: a study in college women. J. Infect. Dis. 135:392-397.
- Eickhoff, T. C., J. O. Klein, A. K. Daly, D. Ingall, and M. Finland. 1964. Neonatal sepsis and other infections due to group B beta-hemolytic streptococci. N. Engl. J. Med. 271:1221-1228.
- El Kholy, A., L. W. Wannamaker, and R. M. Krause. 1974. Simplified extraction procedure for serological grouping of beta-hemolytic streptococci. Appl. Microbiol. 28:836-839.
- Embil, J. A., T. R. Martin, N. H. Hansen, S. W. MacDonald, and F. R. Manuel. 1978. Group B beta-hemolytic streptococci in the female genital tract: a study of four clinic populations. Br. J. Obstet. Gynecol. 85:783-786.
- Ferrieri, P., P. P. Cleary, and A. E. Seeds. 1977. Epidemiology of group B streptococcal carriage in pregnant women and newborn infants. J. Med. Microbiol. 10:103-114.
- Franciosi, R. A., J. D. Knostman, and R. A. Zimmerman. 1973. Group B streptococcal neonatal and infant infections. J. Pediatr. 82:707-718.
- Lancefield, R. C. 1934. A serological differentiation of specific type of bovine haemolytic streptococci (group B). J. Exp. Med. 59:441-458.
- McCracken, G. H., Jr. 1973. Group B streptococci: the new challenge in neonatal infections. J. Pediatr. 82:703-706.
- 14. Pass, M. A., B. M. Gray, S. Khare, and H. C. Dillon, Jr.

- 1979. Prospective studies of group B streptococcal infections in infants. J. Pediatr. 95:437-443.
- Rotta, J., R. M. Krause, R. C. Lancefield, W. Everly, and H. Lackland. 1971. New approaches for the laboratory recognition of M types of group A streptococci. J. Exp. Med. 134:1298-1315.
- Swift, H. F., A. T. Wilson, and R. C. Lancefield. 1943. Typing group A haemolytic streptococci by M-precipitation reactions in capillary pipettes. J. Exp. Med. 78:127–133.
- Wilkinson, H. W. 1978. Group B streptococcal infection in humans. Annu. Rev. Microbiol. 32:41-57.
- 18. Wilkinson, H. W., R. R. Facklam, and E. C. Wortham. 1973. Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). Infect. Immun. 8:228-235.
- 19. Yow, M. D. 1975. Epidemiology of group B streptococcal infections. Prog. Clin. Biol. Res. 3:159-166.