Species Distribution of Non-Group D Alpha-Hemolytic Streptococci in Maternal Genital and Neonatal Blood Cultures

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At our hospital (Jefferson Davis Hospital, Houston, Tex.) since 1979, non-group D alpha-hemolytic streptococci have been isolated with increasing frequency from neonatal blood cultures with clinical findings of sepsis. A total of 47 such isolates were identified to the species level by the scheme of Facklam and were compared with 57 genital isolates from 167 maternity patients. Among the genital isolates, *S. sanguis* II and *S. MG-intermedius* accounted for 53 and 28%, respectively, and both were significantly less common in neonatal cultures (23 and 11%, respectively; $P < 0.05$). Among neonatal isolates, *S. mitis* was the single most frequent species (35%), in contrast to its rare occurrence in maternal cultures (3.4%; $P < 0.001$). The disparity between the prevalence of *S. mitis* in neonatal compared with maternal cultures suggests that this species of non-group D alpha-hemolytic streptococci may have increased virulence in neonatal hosts.

Shifts in the prevalence of etiological agents associated with bacterial sepsis in neonates have been historically documented. The predominance of beta-hemolytic streptococci, the overwhelming majority of which belong to Lancefield group A, observed in the 1930s and 1940s had disappeared until the 1970s when Lancefield group B became the single most frequently observed pathogen in many U.S. hospitals. During the past few years, several reports have indicated that other streptococci, in particular, both group D (1, 2, 5, 10, 12) and non-group D (4, 11, 14) alpha-hemolytic strains, may occasionally cause sepsis or meningitis in neonates.

We have recently reported a striking increase, at a Houston hospital with ca. 14,000 deliveries annually, of neonatal sepsis attributable to non-group D alpha-hemolytic streptococci (4). Since 1978, non-group D alpha-hemolytic streptococci have accounted for 21.7% of the isolates from the blood or cerebrospinal fluid of neonates at this hospital, with clinical findings compatible with sepsis. The prevalence of these streptococci is exceeded only by that of group B streptococci (35%). The early onset (mean age, 1.4 days) of illness in these infants suggested a maternal genital tract-to-infant vertical mode of transmission, but no epidemiological studies had been performed to document this speculation. Our previous findings and the continued frequency of these organisms as a cause of neonatal infection in our hospital prompted us to determine the prevalence of genital colonization with

non-group D alpha-hemolytic streptococci in maternity patients and to determine the species distribution of these isolates compared with that of isolates in sick neonates.

MATERIALS AND METHODS

Specimen sources. Between January, 1979, and August, 1982, 47 non-group D alpha-hemolytic streptococci were isolated from one or more peripheral blood cultures of 44 neonates with maternal risk factors predisposing to or clinical symptoms of sepsis, or both. Blood cultures were inoculated into routine medium (Fisher Scientific Products Co., Orangeburg, N.Y.) and processed in a standard fashion. Subcultures onto 5% sheep blood and chocolate agars were made at 24 h, 7 days, or whenever visible growth occurred. Periodic subcultures of uninoculated bottles were performed to exclude media contamination. Since 1976, no changes in blood culture media employed in the nurseries have been made. Isolates resembling streptococci were identified by Gram stain; catalase activity; inoculating sodium hippurate broth; bile esculin medium, and 6.5% NaCl broth; and testing with bacitracin and optochin disks. Isolates were identified as non-group D alpha-hemolytic streptococci if alpha hemolysis occurred on sheep blood agar and if the above techniques excluded groups A, B, and D streptococci as well as *S. pneumoniae*. Because some non-group D alpha-hemolytic streptococci may be bile esculin positive, Lancefield precipitin testing was done on these isolates to distinguish them from the nonenterococcal group D streptococci (13).

The medical records of the 44 neonates from whom the 47 non-group D alpha-hemolytic streptococci were isolated were reviewed in detail. All infants were housed at Jefferson Davis Hospital, Houston, Tex.

Between August, 1981, and February, 1982, genital cultures from 167 maternity patients in the same hospital were obtained after informed consent. Cotton-tipped swabs were rotated against the lower vaginal wall, inoculated into selective broth medium (Todd-Hewitt broth containing a final concentration of 5% defibrinated sheep erythrocytes and 15 µg of nalidixic acid per ml and 8 µg of gentamicin per ml), incubated overnight at 37°C, and subcultured onto 5% sheep blood agar plates. Plates were inspected after overnight incubation, and non-group D alpha-hemolytic streptococci were identified as described above. Five maternal cultures contained more than one type of alpha-hemolytic streptococci by morphology and were also isolated and speciated. Patients receiving antimicrobial agents were excluded from the study.

Species identification. Non-group D alpha-hemolytic streptococcal isolates from mothers and neonates were stored in Todd-Hewitt broth at -70°C before species identification. The scheme of Facklam (8) was employed to differentiate isolates on the basis of physiological reactions in arginine, esculin, litmus milk, mannitol, sorbitol, inulin, lactose, arabinose, and raffinose. Starch hydrolysis and 5% sucrose agar reactions of the isolates were also determined.

RESULTS

A total of 57 (34%) of the 167 maternity patients had non-group D alpha-hemolytic streptococci isolated from vaginal cultures. Five women had two types of alpha-hemolytic streptococci isolated; two of them had the same species, and the other three had *S. sanguis* II and *S. MG-intermedius*. The species distribution of these maternal isolates is summarized in Table 1 and compared with that of the 44 neonatal isolates. The two most common species from maternal cultures were *S. sanguis* II (53%) and *S. MG-intermedius* (28%). Isolation of *S. salivarius*, *S. sanguis* I, *S. mitis*, *S. uberis* and *S. mutans* was uncommon, and no isolates were identified as *S. morbillorum* or *S. anginosus-constellatus*. In contrast, *S. mitis* was the single most frequently isolated species in neonatal blood cultures (35%). Three infants with *S. mitis* sepsis had dual infections, two with *S. MG-intermedius* and one with *S. sanguis* II. Including or excluding these patients with dual infections, *S. mitis* was identified significantly more often in neonatal than in maternal cultures ($P = 0.0016$, chi-square test). The two most common species in maternal cultures, *S. sanguis* II and *S. MG-intermedius*, accounted for 23 and 11% of neonatal isolates, respectively, both being significantly more common in maternal cultures ($P < 0.05$, chi-square test). Of the neonatal isolates, no statistical difference was noted in the prevalence of *S. sanguis* II and *S. mitis* ($P > 0.05$, chi-square test). No other significant differences in species prevalence were detected.

Among the 44 neonates from whom the blood culture isolates were obtained, maternal his-

tories and clinical findings were similar to those previously reported (4). Maternal obstetrical risk factors associated with neonatal infection (prolonged [>24 h] rupture of membranes, maternal fever, chorioamnionitis, and premature [<37 weeks of gestation] onset of labor) were common (44.2%). Of the neonates, 88% had symptoms or signs compatible with sepsis at the time of their positive blood cultures, and all but one culture was positive at or before 72 h of incubation. None of these patients had meningitis, and the mortality was only 2.3%.

DISCUSSION

In recent years, an increasing number of reports have indicated that streptococci other than Lancefield groups B and D are able to cause sepsis (4) and meningitis (11, 14) in neonates. In our hospital, non-group D alpha-hemolytic streptococci have been isolated frequently since 1978 from the blood cultures of neonates with signs of sepsis. The reason(s) for the increasing prevalence of such neonatal isolates remains unknown, but information regarding the particular species isolated might provide some insight into the virulence of certain strains, especially if the species distribution is compared with that of maternal genital isolates.

Although there is little information pertaining to the species distribution of alpha-hemolytic streptococci isolated from genital cultures of women, Facklam (8) reported that *S. mitis* was found in 11.5% of cultures from gynecology patients, a prevalence consistent with that reported here (3.4%). The species distribution of neonatal blood culture isolates has not been done previously, but Ruoff and Kunz (15) and

TABLE 1. Species distribution of non-group D alpha-hemolytic streptococci

Species	No. (%) of maternal isolates	No. (%) of neonatal isolates ^a
<i>S. sanguis</i> II	30 (53) ^b	10 (23) ^{b,c}
<i>S. MG-intermedius</i>	16 (28) ^d	5 (11) ^d
<i>S. salivarius</i>	4 (7)	0
<i>S. sanguis</i> I	3 (5)	8 (18)
<i>S. mitis</i>	2 (3.4) ^e	15 (35) ^{c,e}
<i>S. uberis</i>	1 (1.8)	1 (2)
<i>S. mutans</i>	1 (1.8)	0
<i>S. morbillorum</i>	0	1 (2)
<i>S. anginosus-constellatus</i>	0	4 (9)

^a Number of isolates from which percentages were calculated was 44, since three neonates with *S. mitis* each had another species isolated.

^b Chi square = 8.8, $P < 0.005$.

^c Chi square = 1.4, $P > 0.05$.

^d Chi square = 3.9, $P < 0.05$.

^e Chi square = 17.1, $P < 0.001$.

Hardy et al. (9) have indicated that *S. mitis* accounted for 26 and 40%, respectively, of blood culture isolates from older patients. Assuming that the mode of transmission to neonates with non-group D alpha-hemolytic streptococcal sepsis is vertical from the maternal genital tract, a hypothesis strongly supported by the early age at onset of symptoms, one would expect a similar prevalence of individual species in maternal and neonatal cultures. Our findings indicate a significant disparity, most marked for *S. mitis* but also noted for *S. MG-intermedius* and *S. sanguis* II. What is not known is the prevalence of species among isolates from mothers and their clinically well neonates; this type of epidemiological evidence will be required before conclusions can be made regarding the pathogenic potential of certain species for neonates. If *S. mitis* is shown to be of similar prevalence among isolates from healthy neonates and from maternity patients, then its frequent occurrence in neonates with bacteremia would suggest enhanced virulence for certain neonatal hosts.

The heterogeneity of organisms designated as viridans streptococci has made their classification difficult. Species identification has been attempted previously by serological (6) and physiological (8) methods as well as those which define cell wall components (6) or the presence of a chromophore (3). Some investigators have used bacteriocin fingerprinting techniques (7). Serological methods have failed to provide sufficient accuracy (6, 8), and methods which determine the presence or absence of rhamnose and ribitol teichoic acid (6) are impractical for clinical laboratories. In this country, the most widely used and practical scheme is that described by Facklam (8). On the basis of physiological requirements, he suggested 10 species: *S. mutans*, *S. salivarius*, *S. uberis*, *S. sanguis* I, *S. sanguis* II, *S. MG-intermedius*, *S. mitis*, *S. anginosus-constellatus*, *S. morbillorum*, and *S. acidominimus*.

Employing the Facklam scheme, we detected a significantly different species distribution of non-group D alpha-hemolytic streptococci isolated from maternal genital compared with that of neonatal blood culture isolates. However, if the species were determined by either the method of Colman and Williams (6) or chromophore detection (3), both *S. mitis* and *S. sanguis* II would be classified as *S. mitior*, and these differences would disappear. Both *S. mitis* and *S. sanguis* II are deficient in rhamnose and rich in ribitol teichoic acid and are the only species which produce a chromophore. Despite these differences in proposed species identification methods, we believe that the Facklam scheme is a useful tool for defining the epidemiology of

human infection caused by non-group D alpha-hemolytic streptococci. It is our hope that additional studies on the species identification of clinically significant isolates, as well as those of normal flora, will increase our understanding of the epidemiology and pathogenesis of human infections associated with these organisms.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant AI 13249 from the National Institute of Allergy and Infectious Diseases. C. J. Baker is the recipient of Research Career Development Award 1 K04 AI00323 from the National Institute of Allergy and Infectious Diseases.

We appreciate the secretarial assistance of Dixie Hargraves, the technical assistance of Marcia A. Rench, and the helpful review of the manuscript by Ralph D. Feigin.

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