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Effects of Intrapartum Penicillin Prophylaxis on Intestinal Bacterial Colonization in Infants

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Early-onset group B streptococcal (GBS) infections remain a leading cause of morbidity and mortality in infants. To prevent the vertical transmission of GBS and neonatal GBS infection, guidelines recommend intrapartum penicillin or amoxicillin prophylaxis. This intrapartum antibiotic prophylaxis (IAP) is suspected to favor colonization by antibiotic-resistant bacteria. However, the effects of this prophylaxis on the patterns of acquisition of gastrointestinal bacterial flora in infants have never been studied. We collected stool samples from 3-day-old infants born to mothers who received intrapartum amoxicillin (antibiotic-exposed group; $n = 25$) and to untreated mothers (non-antibiotic-exposed group; $n = 25$). The groups were matched for factors known to affect intestinal microbial colonization: gestational age, type of delivery, and type of feeding. Qualitative and quantitative differential analyses of the bacterial flora in stool samples were performed. Similar numbers of infants in the non-antibiotic-exposed and antibiotic-exposed groups were colonized by aerobic bacteria and amoxicillin-resistant enterobacteria (75 and 77%, respectively) ($P = 0.79$). In contrast, significantly fewer infants in the antibiotic-exposed group than in the non-antibiotic-exposed group were colonized by anaerobic bacteria, especially *Clostridium* (12 and 40%, respectively) ($P < 0.05$). Regarding intestinal bacterial colonization, the differences between antibiotic-exposed and non-antibiotic-exposed infants were remarkably few. The only statistically significant effect was the reduced initial bacterial colonization by *Clostridium* in the antibiotic-exposed group. In our study, the use of IAP did not favor colonization by β-lactam-resistant bacteria. However, further evaluations are required to highlight the potential risks of the widespread use of antibiotics to prevent early-onset GBS infection.

Early-onset group B streptococcal (GBS) infections are still a leading cause of morbidity and mortality in infants (approximately 4% of infected infants die) (29). Maternal GBS colonization is a major risk factor for early-onset disease in infants (28). The gastrointestinal tract is a reservoir for GBS, but GBS can also transiently or intermittently colonize the vagina. The vagina or rectum of 10 to 30% of pregnant women is colonized by GBS (16, 29). To prevent the vertical transmission of GBS and early-onset (within the first week of birth) GBS infection, the recently revised Centers for Disease Control and Prevention guidelines recommend intrapartum penicillin or ampicillin prophylaxis (28).

The benefits and potential risks of the widespread use of intrapartum antibiotic prophylaxis (IAP) to prevent GBS disease were recently reviewed (29, 33). A retrospective cohort study by Schrag et al. showed that IAP significantly reduces the incidence of early-onset GBS infection (29). However, some authors have suggested that this GBS prevention policy may also have adverse effects, such as an increased incidence of neonatal infections caused by pathogens other than GBS, including β-lactam-resistant strains (9, 15, 18, 20, 35, 36). Some studies have investigated the effects of antimicrobial agents on human flora (34). The impact of IAP for the prevention of perinatal GBS disease on the establishment of the bacterial gastrointestinal flora in infants has never been studied. This antibioprophylaxis may affect transmission to neonates of bacteria derived from vaginal or fecal flora.

At birth, the gastrointestinal tract is sterile, and it is rapidly colonized by bacteria originating from the mother and the environment (10, 19). The first colonizing bacteria are aerobic bacteria, such as staphylococci, enteroccoci, and enterobacteria (26). Then, anaerobic bacteria, such as *Bacteroides*, *Bifidobacterium*, and *Clostridium* species, gradually colonize the gastrointestinal tract (26). Various factors can affect intestinal colonization: gestational age (11), type of delivery (13), type of feeding (26), and antibiotic therapy (4, 5, 17). The aim of our study was to compare the patterns of acquisition of gastrointestinal bacterial flora in infants born to mothers treated with amoxicillin, the penicillin used in France for IAP, due to GBS carriage and in infants born to untreated mothers. We also determined the susceptibility to amoxicillin of isolated strains of aerobic and anaerobic bacteria.

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MATERIALS AND METHODS

Patient population. A prospective study was conducted between January 2000 and April 2000 with 50 3-year-old infants born at the Versailles Maternity Hospital (Versailles, France). Pregnant women found to be rectal and/or vaginal carriers of GBS at 35 to 37 weeks of gestation were candidates for antibiotic

prophylaxis. The prophylaxis protocol consisted of 2 g of intravenous amoxicillin at the time of labor and then 1 g intravenously every 4 h until delivery. None of these mothers should have received antibiotics within 15 days of the delivery.

Two groups of infants were studied: infants born to mothers who received intrapartum amoxicillin, referred to as the antibiotic-exposed group ($n = 25$), and infants born to mothers who did not, referred to as the non-antibiotic-exposed group ($n = 25$). The infants in both groups were matched for factors known to affect intestinal microbial colonization: gestational age, type of delivery (vaginal or cesarean section), and type of feeding (breast fed or bottle fed).

Collection and culturing of stool samples. The ward staff collected stool samples from each infant (after approval by the family) on day 3 after birth. These samples were stored at 4°C for a maximum of 6 h before being transferred to the laboratory, where they were stored at −80°C in brain heart infusion broth with 15% glycerol until they were tested (8).

For bacterial analysis, stool samples were thawed and serially diluted (10^{-2}, 10^{-4}, and 10^{-6} [wt/vol]) in a prereduced peptone liquid medium. Quantitative analysis of the flora was performed as described before (6). The dilutions were plated on various selective and nonselective media by using the automated WASP spiral system (AES Laboratory, Combourg, France). Trypticase soy agar (bioMérieux, Marcy l'Etoile, France) was used to detect all aerobes, Drigalski agar (Bio-Rad, Marnes la Coquette, France) was used for enterobacteria, D Coccossel agar (bioMérieux) was used for enterococci, and Chapman 110 agar (VWR, Strasbourg, France) was used for staphylococci. All aerobic plates were incubated at 37°C. No specific media were used to isolate GBS strains, but the presence of GBS colonies was checked on Columbia agar base supplemented with 5% sheep blood (bioMérieux).

For anaerobic bacteria, the dilutions were plated on cysteine (160 mg/liter)-Columbia agar base supplemented with 5% sheep blood and neomycin at 100 mg/liter for total anaerobes, with kanamycin at 7.5 mg/liter and vancomycin at 100 mg/liter for *Bacteroides*, and with 5% whole milk, colistin at 10 mg/liter, and neutral red at 40 mg/liter for *Clostridium*. For *Bifidobacterium*, Wilkins-Chalgren agar base containing D-glucose at 10 g/liter, kanamycin at 7.5 mg/liter, L-cysteine at 0.5 g/liter, and 0.5% (vol/vol) Tween 80 was used. All anaerobic plates were incubated at 37°C in an anaerobic chamber.

Aerobic bacteria were identified by routine laboratory methods after 24 h of incubation. Anaerobic cultures were examined after 48 h and 5 days of incubation. Bacterial counts are expressed as the $\log_{10}$ CFU per gram of feces. The count threshold was 10^3 CFU/g of feces.

Antibiotic susceptibility. Selective media with amoxicillin at 8 mg/liter and cefotaxime at 4 mg/liter were used to screen for β-lactam-resistant enterobacteria. The susceptibility of staphylococci, enterococci, and enterobacteria to antibiotics was determined on Mueller-Hinton agar by the agar diffusion method. The MICs of amoxicillin were determined by the agar diffusion method on Mueller-Hinton agar for enterococci and enterobacteria and on Wilkins-Chalgren agar for *Bacteroides*, *Clostridium*, and *Bifidobacterium* according to the NCCLS (23). Plates were incubated in an aerobic or an anaerobic chamber depending on the strain for 24 h (48 h for *Bifidobacterium*).

Statistical analysis. The prevalences of colonized infants in the two groups were compared by using McNemar's chi-square test for matched data. The Wilcoxon rank test for paired data was used to compare colonization levels between the two groups. *P* values of greater than 0.05 were considered to be nonsignificant. All of the analyses were performed with the SPSS 8.0 program.

RESULTS

Population. All 50 infants were healthy and full term. Forty-eight infants were born by the vaginal route, and 2 were born by cesarean delivery; 34 were breast fed, and 16 were bottle fed. There were no differences between the two study groups with respect to birth weight (overall [mean and standard deviation], 3,380 ± 400 g), gestational age (39.1 ± 1.0 weeks), type of delivery, type of feeding, or sex ratio. None of the infants received antibiotics after birth.

Bacterial intestinal colonization. All infants, except for one from the antibiotic-exposed group, were colonized on day 3. Regarding aerobe colonization, the number of colonized infants and the bacterial colonization levels were not statistically different between the two groups (Tables 1 and 2). In both groups, staphylococci colonized the highest number of infants (88 and 84% in the non-antibiotic-exposed and antibiotic-exposed groups, respectively; Table 1). Staphylococcal isolates were mainly coagulase negative (96 and 85% in the non-antibiotic-exposed and antibiotic-exposed groups, respectively). In both groups, no infant was colonized by GBS. Fewer infants were colonized by enterobacteria and enterococci, as three infants (12%) and seven infants (28%) were not colonized by either enterobacteria or enterococci in the non-antibiotic-exposed and antibiotic-exposed groups, respectively ($P = 0.22$). Totals of 68 and 60% of infants in the non-antibiotic-exposed and antibiotic-exposed groups, respectively, were colonized by enterococci (Table 1). All enterococcal isolates were identified as *Enterococcus faecalis*, except for one in the antibiotic-exposed group, which was found to be *Enterococcus hirae*. The occurrences of enterobacteria were similar in the two groups (Table 3). *Escherichia coli* was the most frequently isolated enterobacterial species (83 and 56% of isolates in the non-antibiotic-exposed and antibiotic-exposed groups, respectively) (Table 3). The following enterobacteria were also found: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Hafnia alvei* (Table 3). Four infants from each group were colonized by a fungus. The four infants in the antibiotic-exposed group were colonized by *Candida albicans*, whereas those in the non-antibiotic-exposed group were colonized by *C. albicans*, *Candida glabrata*, and *Saccharomyces cerevisae*.

Totals of 24 and 36% of the infants were not colonized by

TABLE 1. Number of infants colonized in non-antibiotic-exposed and antibiotic-exposed groups

Organism	No. (%) of colonized infants in the following group:		*P* value[a]
	Non-antibiotic exposed ($n = 25$)	Antibiotic exposed ($n = 25$)	
Enterobacteria	16 (64)	13 (52)	0.58
Amoxicillin-resistant enterobacteria	12 (75)	10 (77)	0.79
Enterococci	17 (68)	15 (60)	0.73
Staphylococci	22 (88)	21 (84)	1
Bacteroides	7 (28)	13 (52)	0.15
Clostridium	10 (40)	3 (12)	0.04
Bifidobacterium	12 (48)	6 (24)	0.18

[a] As determined by the McNemar test.

TABLE 2. Colonization of gastrointestinal tracts of infants in non-antibiotic-exposed and antibiotic-exposed groups

Organism	Log CFU/g of feces in the following group:				*P* value[a]
	Non-antibiotic exposed ($n = 25$)		Antibiotic exposed ($n = 25$)		
	Median	Range	Median	Range	
Enterobacteria	9.2	3.3–9.8	8.4	3.3–9.5	0.18
Enterococci	7.3	3.3–9.5	8.3	3.6–10.3	0.78
Staphylococci	7.0	4.0–9.3	6.5	3.6–8.0	0.53
Bacteroides	7.9	3.6–9.6	8.0	6.3–10.3	0.12
Clostridium	6.2	3.6–8.1	5.3	4.3–5.8	0.01
Bifidobacterium	8.5	6.9–10.3	8.2	4.3–9.5	0.1

[a] As determined by the Wilcoxon test.

TABLE 3. Occurrence of enterobacteria in the two groups

Enterobacteria[a]	No. of colonized infants in the following group:	
	Non-antibiotic exposed ($n = 25$)	Antibiotic exposed ($n = 25$)
AMX S *Escherichia coli*	4[b]	3[c]
AMX R *Escherichia coli*	11[b,d]	6[d]
Klebsiella pneumoniae	1	3
Klebsiella oxytoca		2[c,e]
Enterobacter cloacae	1	
Citrobacter freundii		1[e]
Hafnia alvei	1[d]	1[d]

[a] AMX S, amoxicillin susceptible; AMX R, amoxicillin resistant.
[b] One infant each was colonized with amoxicillin-susceptible *E. coli* and amoxicillin-resistant *E. coli.*
[c] One infant was colonized with amoxicillin-susceptible *E. coli* and *K. oxytoca.*
[d] One infant was colonized with amoxicillin-resistant *E. coli* and *H. alvei.*
[e] One infant was colonized with *K. oxytoca* and *C. freundii.*

anaerobes in the non-antibiotic-exposed and antibiotic-exposed groups, respectively ($P = 0.58$). The numbers of infants colonized by *Bacteroides* were not significantly different between the two groups (Table 1), and colonization levels were similar (Table 2). The following species of *Bacteroides* were identified: *Bacteroides uniformis*, *Bacteroides stercoris*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides fragilis*, *Bacteroides cacae*, and *Bacteroides thetaiotaomicron*. In contrast, fewer children in the antibiotic-exposed group were colonized by *Clostridium* (3 versus 10 in the non-antibiotic-exposed group) ($P < 0.05$) (Table 1). Moreover, the density of colonization by *Clostridium* spp. was significantly lower in the antibiotic-exposed group (5.3 versus 6.2 log units/g in the non-antibiotic-exposed group) ($P < 0.05$) (Table 2). All *Clostridium* isolates were identified as *Clostridium perfringens*, except for one in the antibiotic-exposed group, which was found to be *Clostridium acetobutylicum*. Six antibiotic-exposed infants and 12 non-antibiotic-exposed infants were colonized by *Bifidobacterium* spp. without statistical significance (Table 1). The following *Bifidobacterium* species were identified: *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, and *Bifidobacterium pseudocatenulatum*.

Antibiotic susceptibility. Among coagulase-negative staphylococci (CoNS), 39 and 56% were methicillin resistant in the non-antibiotic-exposed and antibiotic-exposed groups, respectively. One of the methicillin-susceptible isolates from the non-antibiotic-exposed group was also penicillin susceptible. All three *Staphylococcus aureus* isolates in the antibiotic-exposed group were methicillin susceptible (one of these was penicillin susceptible). The only isolate of *S. aureus* in the non-antibiotic-exposed group was methicillin susceptible. All isolates of enterococci were susceptible to amoxicillin (MIC, <2 mg/liter) and vancomycin. The occurrences of amoxicillin-resistant enterobacteria were similar in the two groups: 75 and 77% of isolates in the non-antibiotic-exposed and antibiotic-exposed groups, respectively ($P = 0.79$) (Table 1). Totals of 60 and 44% of *E. coli* isolates were resistant to amoxicillin (MIC, >512 mg/liter) in the non-antibiotic-exposed and antibiotic-exposed groups, respectively. All of the amoxicillin-resistant *E. coli* isolates were susceptible to amoxicillin-clavulanic acid and to cefotaxime.

In both groups, all tested isolates of *Clostridium* and *Bifidobacterium* were susceptible to amoxicillin (MIC, ≤16 mg/liter). Concerning *Bacteroides*, 80 and 83% of tested isolates were resistant to amoxicillin in the non-antibiotic-exposed and antibiotic-exposed groups, respectively.

DISCUSSION

The incidence of β-lactam-resistant gram-negative neonatal sepsis appears to be increasing (9, 15, 18, 20, 35, 36). It has been suggested that this increase is due to the widespread use of penicillin or aminopenicillin, the first-line recommended agents for IAP to prevent GBS disease. This antibiotic prophylaxis may affect the maternal bacterial flora to which an infant is exposed and thus the establishment of normal flora. It may also favor colonization by antibiotic-resistant bacteria, especially β-lactam-resistant enterobacteria. The aim of our study was to assess the impact of antibiotic prophylaxis on the vertical transmission of bacteria derived from the mother's flora to the infant.

Even if it was not the main objective of this study, our results showed that intestinal bacterial colonization was slightly delayed in both groups, especially in the antibiotic-exposed group: on day 3, 12 and 28% of infants were not colonized by either enterobacteria or enterococci in the non-antibiotic-exposed and antibiotic-exposed groups, respectively ($P = 0.22$). Moreover, one vaginally delivered infant from the antibiotic-exposed group was not colonized by any aerobic or anaerobic bacteria. Totals of 24 and 36% of infants were not colonized by any anaerobic bacteria in the non-antibiotic-exposed and antibiotic-exposed groups, respectively ($P = 0.58$). Several authors demonstrated that bacteria colonizing infants originated mainly from mothers (19, 39). However some recent studies described delayed colonization (24, 30). Nowrouzian et al., who monitored intestinal colonization by *E. coli*, found that only 42% of infants were colonized on day 3 (24). This delay in colonization compared with the findings of older studies is probably due to the strict hygienic conditions currently applied during and after delivery (1, 30). These obstetric practices (including treatment of the genital tract with disinfectants) may alter the quantitative composition of the mother's microflora and could affect and delay the colonization of infants (21, 24, 25).

Concerning aerobic colonization, the numbers of colonized infants and the bacterial colonization levels were not statistically different between the two groups, showing that the antibioprophylaxis did not alter bacterial colonization. Moreover, the prevalences of amoxicillin-resistant enterobacteria were not significantly different between the two groups (75 and 77% of isolates in the non-antibiotic-exposed and antibiotic-exposed group, respectively) ($P = 0.79$). Most studies have reported that the incidence of non-GBS sepsis (including that attributable to *E. coli*, the second leading cause of neonatal sepsis after GBS) has remained stable or even declined despite the increased use of IAP for GBS (2, 7, 15, 40). The only studies to have reported an increased incidence of β-lactam-resistant gram-negative neonatal sepsis concerned premature or low-birth-weight infants (15, 18, 33). For the latter popula-

tion, Moore et al. noted that after adjustment for gestational age and interval between membrane rupture and delivery, rates of early-onset sepsis among neonates whose mothers had received IAP and those whose mothers had not received IAP did not differ (22). Moreover, preterm infants are often more exposed to antibiotics during the prenatal and intrapartum periods for indications such as preterm labor or preterm premature rupture of membranes. This extended antibiotic exposure, which is clearly different from short-term IAP, can lead to the selection of resistant bacteria. Thus, they are more likely to be colonized by resistant enterobacteria and are at an overall greater risk of infection by these bacteria (3, 12, 27). Studies that have reported a significant association between resistant sepsis and IAP have suggested that mothers of infants with antibiotic-resistant sepsis receive an average of more than 10 intrapartum doses (35, 37), whereas no more than 1 or 2 doses are commonly given during labor to prevent GBS disease—doses unlikely to be sufficient to select a resistant strain. In addition, a recent study showed that IAP apparently had no effect on the selection of antibiotic-resistant bacteria in the vaginal flora (32).

However, antibiotic exposure is not the only factor driving colonization by resistant bacteria. The increased incidence of bacterial resistance to antimicrobial agents in both nosocomial and community settings requires careful thought. A French prospective study with 320 *E. coli* strains isolated from urinary tract infections between 1998 and 1999 showed that the prevalence of resistance to amoxicillin was 46.1% (31). Gupta et al. showed that the prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women is also increasing (14). The resistant strains isolated from urine originated mainly from the gastriointestinal tract, explaining the high rate of amoxicillin-resistant *E. coli* strains isolated in both groups (54%). CoNS were the most predominant bacteria in both groups, and the percentage of CoNS strains that were methicillin resistant was high. CoNS are increasingly important nosocomial pathogens in neonatal intensive care units (38). The resistant strains that colonize the infant intestine are probably acquired from the environment or the mother's skin flora.

Antibioprophylaxis seems to have more effects on gut colonization by anaerobic bacteria. Fewer infants were colonized by *Bifidobacterium* and *Clostridium*, but more antibiotic-exposed infants were colonized by *Bacteroides*. However, only colonization by *Clostridium* was significantly altered. These results may reflect the susceptibility of these bacteria to amoxicillin.

Our data suggest that short-term intrapartum prophylaxis does not have a major effect on initial colonization of the neonatal gut and does not favor colonization by antibiotic-resistant bacteria, especially amoxicillin-resistant *E. coli*. However, further evaluations are required to highlight the potential risks of the widespread use of antibiotics to prevent early-onset GBS infections.

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