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Plasmid Profiling of Members of the Family *Enterobacteriaceae*, Lactobacilli, and Bifidobacteria To Study the Transmission of Bacteria from Mother to Infant

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Plasmid profiles of isolates of the family *Enterobacteriaceae*, lactobacilli, and bifidobacteria cultured from vaginal, oral, and rectal swabs collected from women soon after admission to a maternity hospital were compared with those of strains detected in the feces of their infants. Lactobacilli inhabiting the vaginas of the mothers did not appear to colonize the infant digestive tract, but evidence for the transmission of fecal isolates of the *Enterobacteriaceae* and bifidobacteria from mother to infant was obtained in four out of five cases. Many of the bifidobacteria isolated were plasmid-free but could be distinguished with biochemical profiles.

The manner in which the intestinal tracts of human infants become colonized by a microflora has continued to be of interest since the first observations on the subject by Tissier (4, 17). The source of the bacteria that initially colonize the infant intestinal tract has usually been assumed to be the maternal vagina (1, 12, 13, 15). Such an assumption, however, has not been supported by scientific data, since discriminatory tests permitting the comparison of bacterial strains isolated from maternal and infant sources have not been available (2). Plasmid profiling has been shown to be a useful technique for distinguishing between strains of bacteria in medical, industrial, and environmental studies (5, 6, 8, 9). We have used this technique to determine the source of bacterial strains present in the feces of human infants.

MATERIALS AND METHODS

Mothers and infants. Five mothers were recruited for the study on admission to the delivery suite at the Princess Anne Hospital, Southampton, United Kingdom; each gave informed consent for the collection of deep vaginal, rectal, and oral swabs at the first examination after admission and before the standard procedures of vaginal douching and application of antiseptic creams. Shortly before delivery, a lotion consisting of 0.015% chlorhexidine acetate and 0.15% cetrimide was applied liberally to the vulval and anal area. One of the mothers received an enema before delivery. The infants, all female, were full term and delivered vaginally. All but one of the infants were suckled at the breast throughout the period of the study. The other infant never received breast milk. The first stool passed by each infant and additional samples collected at 10 and 30 days after birth underwent bacteriological examination. The consent of the local Ethical Committee was obtained for this study.

Bacteriological procedures. Swabs and fecal specimens were added to preweighed bottles of Carey-Blair transport medium (Oxoid Ltd., Basingstoke, United Kingdom). The samples were maintained at refrigerator temperature (home or laboratory refrigerator or insulated container with chilled packs for transportation) until delivered to the laboratory. The time elapsed between collection of specimens and bacteriological examination seldom exceeded 6 h. Swabs were used to directly inoculate selective media for the isolation of members of the family *Enterobacteriaceae* (MacConkey agar; Oxoid), lactobacilli (Rogosa agar; Oxoid) and bifidobacteria (BIM agar as described by Muñoa and Pares [11] but with the concentration of iodoacetic acid reduced to 0.0124 g/liter). Fecal samples from the infants were diluted in distilled water, and 100-μl volumes of each dilution were spread plated to the selective media listed above. Swabs and feces were also used to inoculate liquid media for enrichment of bacterial types. The media used were brain heart infusion (Oxoid) for *Enterobacteriaceae*, MRS broth (Oxoid) for lactobacilli, and BBM medium as described by Cole and Fuller (3), but omitting nalidixic acid and rifampin, for bifidobacteria. Enterobacterial cultures were incubated aerobically at 37°C for 24 h, while lactobacilli and bifidobacteria were cultured anaerobically at 37°C for 48 h. Smears were prepared from each fecal specimen and stained by the Gram method. When present, at least seven colonies of *Enterobacteriaceae*, lactobacilli, and bifidobacteria were picked from swab and fecal cultures. In the case of fecal cultures, colonies were picked from plates inoculated with the highest dilution of fecal material giving growth (i.e., the numerically dominant types). Bacterial isolates were stored at −20°C.

Identification of bacteria. Enterobacterial isolates were identified with API 20E strips. Lactobacilli were identified with API 50CH strips (API Systems SA, La Balme les Grottes, Montalieu-Vercieu, France) as described previously (16). Bifidobacteria were distinguished from lactobacilli on morphological and biochemical bases (3) and identified by using morphological and fermentation test criteria described by Scardovi (14). Biotypes of plasmid-free bifidobacteria were determined with ATB 32A strips (API Systems).

Plasmid profiling. DNA extracts were prepared from enterobacterial strains by the boiling method described by Maniatis and colleagues (10). Extracts of DNA were prepared from lactobacillus and bifidobacterium isolates as follows. (i) A 30-ml volume of MRS broth (BBM broth for bifidobacteria), prewarmed and prereduced at 37°C, was

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inoculated with 1 ml of stationary-phase cells cultured in the same medium. (ii) The culture was incubated anaerobically at 37°C for 5 to 6 h until the bacteria were in the logarithmic phase of growth. (iii) The cells were harvested by centrifugation, washed in 10 ml of 0.01 M Tris buffer (pH 8.2), and suspended in 2.6 ml of the same buffer. (iv) The cells were lysed by the addition of 1 ml of lysozyme solution (6 mg/ml) and incubation for 25 min at 37°C, followed by the addition of 0.4 ml of sodium lauryl sulfate solution (10% solution in TE buffer [10]). (v) Chromosomal DNA was denatured by the addition of 160 μl of 3 N sodium hydroxide solution and gentle rocking of the preparations for 3 min. The preparations were neutralized by the addition of 1.5 ml of 2 M Tris buffer (pH 7.2). (vi) A cleared lysate was prepared by the addition of 0.95 ml of 20% sodium lauryl sulfate in TE buffer, followed immediately by the addition of 1.65 ml of 5 M sodium chloride solution. The lysates were left on ice at 4°C overnight. The preparations were centrifuged at 27,000 × *g* for 30 min at 5°C, and the clear supernatant was retained. (vii) The cleared lysates were incubated at 37°C after the addition of 0.4 ml of ribonuclease solution (2 mg/ml), and proteins were then removed by the addition of 8 ml of phenol saturated with TES buffer (10) and centrifugation at 9,750 × *g* for 30 min. Two extractions were then made with chloroform-isoamyl alcohol (24:1) with centrifugation at 1,935 × *g* for 5 min. (viii) DNA was precipitated by the addition of 0.4 ml of 3 M sodium acetate solution and 16 ml of cold (−20°C) ethanol. The preparations were held at −20°C overnight. (ix) The precipitated DNA was collected by centrifugation at 27,000 × *g* for 30 min at −10°C and dissolved in 100 μl of TE buffer. (x) The DNA was concentrated by using the Geneclean kit, following the instructions of the manufacturer (BIO 101 Inc., La Jolla, Calif.). Electrophoresis was carried out with 0.7% agarose gels and Tris-borate buffer (10) in a GNA-100 gel electrophoresis apparatus (Pharmacia, Uppsala, Sweden) at 30 mA for 2 h. The pattern of DNA bands observed in a bacterial extract when gels were examined by UV transillumination was deemed to be its plasmid profile, and each profile was assigned a designation: E1 to E8 for *Enterobacteriaceae*, L1 to L4 for lactobacilli, and B1 to B4 for bifidobacteria.

Stability of plasmid profiles. Examination of plasmid profiles of representative strains of *Enterobacteriaceae* (eight strains), lactobacilli (four strains), and bifidobacteria (four strains) after storage of the bacteria at −20°C for 2 months gave plasmid profiles identical to those seen when the strains were freshly isolated.

RESULTS

Comparison of Gram-stained smears and culture results. The delay in culturing samples after collection did not appear to adversely affect the recovery of anaerobic gram-positive bacteria. Bifidobacteria were isolated from all of the fecal specimens in which bacilli with coryneform or branching morphology were observed in smears.

Bacteria isolated from maternal swabs. Bacteriological examination of swabs collected from mothers identified the likely sources of lactobacilli (vagina) and *Enterobacteriaceae* and bifidobacteria (intestine) that would be available to colonize the intestinal tracts of infants (Table 1).

Bacteria isolated from infant feces. Bifidobacteria were cultured from the first fecal specimen passed by one infant. *Enterobacteriaceae* were present in the feces of all of the infants at days 10 and 30 after birth at a population level of about 10^9/g. Bifidobacteria were numerous in the feces of four out of five infants at day 10 and those of two out of five infants at day 30. Lactobacilli were rarely encountered and, when present, were in low numbers (Table 2).

TABLE 1. Bacteria detected in maternal samples

Swab	No. of swabs[a] containing bacteria of indicated group		
	Enterobacteriaceae	Lactobacilli	Bifidobacteria
Oral	0	1	0
Vaginal	1	5	1
Rectal	5	1	5

[a] Total number of swabs from each source = 5.

Plasmid profiles and identification of bacteria. Plasmid profiling permitted discrimination between strains of *Enterobacteriaceae*, lactobacilli, and bifidobacteria isolated from maternal and infant sources (Fig. 1). Eight plasmid profiles were detected among isolates of *Enterobacteriaceae*, four among lactobacilli, and four among bifidobacteria. Plasmid-free isolates were rare among the enterobacterial isolates (one *Proteus mirabilis* strain and one *Escherichia coli* strain). Isolates of *Lactobacillus crispatus*, *Lactobacillus acidophilus*, and *Lactobacillus leichmannii* harbored plasmid DNA, but plasmid-free bifidobacterial strains were encountered frequently. Plasmid-free bifidobacteria were discriminated according to their biochemical profiles expressed in numerical form according to the instructions of the manufacturer of ATB 32A strips (API Systems). Three biochemical profile types were detected: 4-536-0737-05, 7-526-2735-15, and 7-506-3335-05. Differences in profile numbers reflected differences in colors of solutions in cupules for urease, arginine dihydrolase, β-glucosidase, β-glucuronidase, glutamic acid decarboxylase, indole, nitrate, leucyl glycine arylamidase, and tyrosine arylamidase reactions. They do not necessarily reflect the results of the same biochemical tests performed by conventional bacteriological techniques. All bifidobacteria isolated in this study belonged to the species *Bifidobacterium longum* when examined by conventional bacteriological methods.

Comparison of bacterial strains isolated from mothers and their infants. Plasmid or biochemical profiles of isolates of bacterial groups were compared when they were present in specimens collected from both mother and infant.

Mother-infant pair 1. All isolates of *E. coli* from the maternal rectal swab were of plasmid profile E1. Infant feces at both 10 and 30 days after birth contained *E. coli*; all the isolates belonged to plasmid profile E2.

Mother-infant pair 2. The maternal rectal swab contained *E. coli* of profile E3 and bifidobacteria of numerical profile 4-536-0737-05. The vaginal swab contained *L. crispatus* of profile L1. Infant feces at 1 and 10 days after birth contained bifidobacteria of numerical profile 7-526-2735-15. Feces col-

TABLE 2. Bacteria detected in infant feces

Day at which fecal sample taken	No. of samples containing bacteria of indicated group (bacterial count)[a]		
	Enterobacteriaceae	Lactobacilli	Bifidobacteria
1	0	0	1 (<3.0)[b]
10	5 (8.9–9.6)	2 (<3.0–3.0)	4 (6.1–10.4)
30	5 (8.7–9.6)	1 (3.7)	2 (7.9–8.9)

[a] Total number of fecal samples taken on each day = 5. Bacterial counts are expressed as $\log_{10}$ CFU per gram of feces.
[b] Detected by enrichment only.

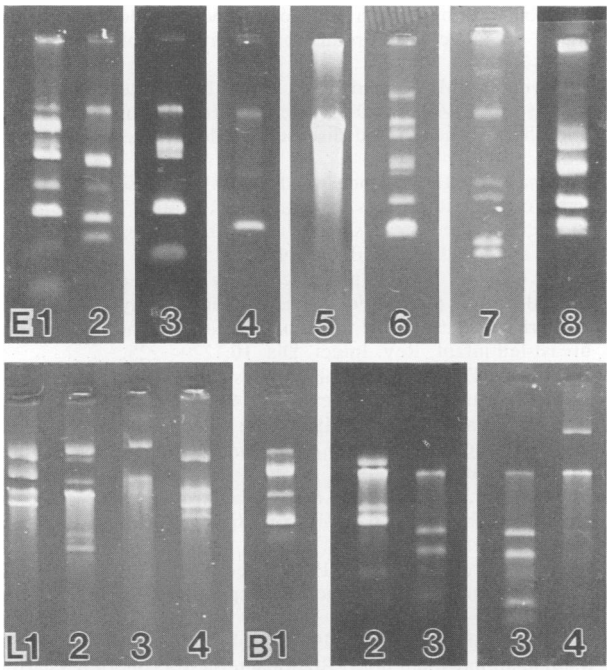

FIG. 1. Plasmid profiles (E1 through 8) of strains of *Enterobacteriaceae* are shown in the upper panels. Plasmid profiles of lactobacilli (L1 through 4) and bifidobacteria (B1 through 4) are shown in the lower panels.

lected at 10 days also contained *E. coli* of plasmid profile E3, *Enterobacter cloacae* of profile E4, and *L. acidophilus* of profile L2. Cefotaxime and pivampicillin were administered to the mother before the collection of the 30-day fecal specimen. Infant feces at 30 days harbored a microflora radically different from that at 10 days: bifidobacteria were no longer present (as determined by negative culture and the absence of cells with typical morphology from a Gram-stained fecal smear), a different *E. coli* strain was present (profile E5), and *L. leichmannii* of profile L3 was detected.

Mother-infant pair 3. The maternal rectal swab contained *E. coli* profile type E6. Two bifidobacterial types were also detected: plasmid profile B1 (five of seven isolates) and numerical profile 7-526-2735-15 (two of seven isolates). The vaginal swab harbored a bifidobacterial strain of numerical profile 7-506-3335-05. *Klebsiella oxytoca* of profile E7 was isolated from infant feces at both 10 and 30 days after birth. One of the bifidobacterial types present in the maternal rectal swab (7-526-2735-15) was detected in infant feces at 10 days. Bifidobacteria were absent from the 30-day infant specimen.

Mother-infant pair 4. The rectal swab from the mother and the feces of the infant (10 and 30 days) contained plasmid-free isolates of *E. coli*. Since plasmid-free strains of this species were encountered but once in this study, and since all of the strains had the same API 20E biotype, we assumed that all isolates were of the same strain. The maternal rectal swab also harbored a plasmid-free bifidobacterium of numerical profile 7-526-2735-15, but infant feces at both 10 and 30 days contained bifidobacteria of plasmid profile B2. The vaginal swab contained *L. crispatus* profile L4, but the infant harbored *Lactobacillus fermentum* (plasmid-free) at 10 days of age.

Mother-infant pair 5. *E. coli* of the same plasmid profile (E8) was detected in the infant feces at 10 and 30 days as was contained in the rectal swab from the mother. Bifidobacteria of plasmid profile B3 were cultured from the maternal rectal swab and were present in the feces of the infant at 10 days (seven of seven isolates) and 30 days (six of seven isolates). An additional bifidobacterial strain was detected in the 30-day fecal specimen (profile B4; one of seven isolates). The vaginal swab harbored *P. mirabilis* (plasmid-free). The infant had not been breast fed.

The results from the investigation of the five mother-infant pairs are summarized in Table 3.

TABLE 3. Origin and persistence of bacterial strains in infant feces

Characteristic	No. of mother-infant pairs with at least one example of characteristic/total no. of pairs
Detection in infant feces	
Vaginal (maternal) lactobacilli, bifidobacteria, or *Enterobacteriaceae*	0/5
Rectal (maternal) *Enterobacteriaceae*	3/5
Rectal (maternal) bifidobacteria	2/5
Persistence in infant feces[a]	
Bacteria of maternal origin	2/4[b]
Bacteria of nonmaternal origin	4/4[b]

[a] Persistence from 10 to 30 days after birth.
[b] Mother-infant pair 2 has been omitted because of the probable influence of antibiotic treatment on the 30-day result.

DISCUSSION

Plasmid profiling of bacterial isolates, supplemented with biochemical profiling in the case of *B. longum*, permitted an assessment of the contribution made by the maternal microflora to the intestinal microflora of the infant. It is assumed that the infant digestive tract is contaminated with a miscellaneous collection of bacteria from maternal and environmental sources during the first few days of life (7, 17). The feces at 10 days after birth would, in our opinion, contain bacteria that had truly colonized the digestive tract of the infant.

The vaginal microflora of the mother was not represented among the numerically predominant bacteria of the infant feces. Although lactobacilli were present in vaginal swabs from all five mothers, only two of the infants harbored these bacteria in their feces. These isolates had different plasmid profiles or were different species from those detected in the mother. Bifidobacteria and *Enterobacteriaceae* detected in vaginal swabs were of different types from those isolated from the corresponding infant.

It is possible that the modern obstetric practice of extensive vulval cleansing serves to prevent colonization of the newborn with bacteria from the maternal vagina. Evidence of transmission of fecal bacteria from mother to infant was obtained in four out of five mother-infant pairs when observations concerning enterobacterial and bifidobacterial isolates were combined. It can be speculated that transmission of fecal bacteria occurs at the time of birth, since defecation during labor is common, but it is also possible that the bacteria were acquired during handling by the mothers following birth. The isolation of bifidobacteria from the first stool passed by one of the infants was surprising, since this sample represents meconium. Such isolations have, in the

past, been considered to represent entry of bacteria into the intestinal tract from the anus or premature rupture of the membranes (1, 7).

Bacterial types of nonmaternal origin were more likely to persist in the infant intestinal tract for the period 10 to 30 days after birth than were strains detected in maternal samples. It should be noted, however, that bacterial strains considered not to be of maternal origin could simply have been minority members of the maternal microflora that were not detected when the relatively small number of colonies were picked from bacterial cultures. These strains, subdominant in the maternal microflora, might become numerically dominant and thus more easily detected in the infant intestinal ecosystem. The factors that determine which bacterial strains colonize and persist in the infant digestive tract are not known, but physiological and immunological characteristics of the infants may be of importance.

We conclude from our study that plasmid profiling of bacteria isolated from the human body is a useful tool in studying the acquisition and stability of the normal microflora. Our results indicate that transmission of fecal bacteria from mother to infant occurs but that colonization of the infant intestinal tract by maternal bacterial strains may sometimes be transient. Further, more comprehensive, studies concerning this latter phenomenon are required.

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