JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1975, p. 124-128

Vol. 1, No. 2
Printed in U.S.A.

Transmission of *Lactobacillus jensenii* and *Lactobacillus acidophilus* from Mother to Child at Time of Delivery

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Received for publication 19 August 1974

The presence of *Lactobacillus jensenii* and *Lactobacillus acidophilus* has been studied in specimens from the rectum and vagina of the mother, from the mouth of the infant at the time of delivery, and from the mouth and rectum of infants six days of age. *L. jensenii* could be differentiated from other species of lactobacilli by the following combination of characteristics: production of only D-lactate, hydrolysis of arginine, and fermentation of cellobiose, galactose, and ribose, but not of lactose. *L. jensenii* and *L. acidophilus* were common inhabitants of the vagina. In spite of a contamination of the infant's mouth by *L. jensenii* and *L. acidophilus* during delivery, neither of these organisms became established in the mouth of the newborn infants.

Lactobacilli predominate among the indigenous bacteria colonizing the vaginal mucous membranes (26, 33). These organisms have been designated "Döderlein's bacillus", but they do not form a homogeneous group. Various species of lactobacilli have been isolated from the vagina, the homofermentative *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. delbrueckii*, *L. leichmannii*, *L. salivarius*, and the heterofermentative *L. fermentum* and *L. cellobiosus* (17, 27, 33). Recently a new species, *L. jensenii*, was isolated from vaginal discharges (13) and this organism is not known to colonize other sites of the human body.

The newborn infant is usually considered to derive its initial oral microbial flora from the rectovaginal region of the mother during delivery. However, a bacterial contamination of the infant's mouth is not obligate and a mouth free of bacteria is not an uncommon finding after delivery (4, 6, 15, 21, 29, 34). There are in fact few studies where the transmission of specific organisms from mother to child has been established. The same serotypes of *Escherichia coli* (11) and subtypes of group B streptococci (9) have been isolated from the throats of newborn infants and from the rectum or vagina of their mothers. It is possible that *L. jensenii* may also serve as an indicator organism to demonstrate this route of transmission.

This communication describes the lactobacillus flora of the rectovaginal region of the mother and of the mouth of the infant at the time of delivery, as well as of the mouth and rectum of six-day-old infants.

MATERIALS AND METHODS

Sampling and cultivation. Samples from the vagina and rectum of the mother and from the mouth of the infant were obtained during 13 deliveries. The pregnancy and delivery were uncomplicated. Immediately after delivery, a sterile, cotton-tipped applicator stick (Cotton Buds, Johnson & Johnson, New Brunswick, N.J.) was introduced into the mouth of the infant and streaked along the upper and lower alveolar ridges. The maternal samples were then obtained before the cord was clamped. By wearing rubber gloves, the labia were separated, and a cotton-tipped applicator stick was inserted into the distal part of the birth canal and streaked against the walls of the vagina, which were overflowed with a mixture of blood and amniotic fluid from the uteral cavity. Finally, a sample was taken from the ampulla recti with an applicator stick. After collecting each sample, the applicator stick was immediately introduced into a tube with 9 ml of a balanced salts solution containing 0.01 g of gelatin per liter (24). The tubes were kept at 4 C until plating was done within a few hours. The tube was treated in a Whirlmixer (Lab-Line Instruments, Mellrose Park, Ill.) for 20 s and 0.5 ml of the sample was cultured as a pour plate in Rogosa SL agar (Difco). The plates were incubated for 96 h under an atmosphere of 95% H_2 and 5% CO_2 in a jar provided with palladium asbestos in a copper net.

Oral and fecal samples were also obtained with cotton-tipped applicator sticks from 33 healthy infants, 6 days old. The specimens were spread over the surface of freshly prepared Rogosa SL agar plates which were incubated as previously described.

From each pour plate of Rogosa SL agar, five colonies were picked and subcultured in MRS-glucose broth (Oxoid) (5), on blood plates, and on Rogosa SL agar until pure cultures were obtained. On the surface-inoculated plates of Rogosa SL agar various

colonial types could be recognized. Three colonies of each type were picked and subcultured as described above.

Identification of strains isolated. The following reference strains were used: *L. fermentum* NCTC 1750, *L. buchneri* NCDO 110, *L. brevis* NCDO 477, *L. cellobiosus* NCDO 928, *L. leichmannii* NCIB 8117, *L. jensenii* CIP 6917, *L. acidophilus* NCTC 1, *L. salivarius* NCDO 929, *L. casei* NCTC 151, and *L. plantarum* NCDO 82 and NCDO 343. Characters of isolated strains were studied as described by Rogosa et al. (28). The basal medium contained per liter: tryptone (Difco) (10 g), Lab-Lemco (Oxoid) (10 g), yeast extract (Difco) (5 g), Tween 80 (1 ml), K_2HPO_4 (2 g), sodium acetate·$3H_2O$ (5 g), diammonium citrate (2 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), and $MnSO_4 \cdot 4H_2O$ (0.05 g) (5). Fermentation of various substrates was studied in the basal medium. The concentration of galactose was 1.2% (wt/vol) and of the other substrates 2% (wt/vol). Reduction of nitrate to nitrite was tested in the indol-nitrite medium (BBL) as described by Holdeman and Moore (20). The basal medium was supplemented with 0.3% (wt/vol) L-arginine hydrochloride and 2% (wt/vol) glucose when testing production of ammonia from arginine, with 0.5% (wt/vol) esculin and 0.05% (wt/vol) ferric citrate for the test of esculin hydrolysis, with 0.5% (wt/vol) sodium hippurate and 1% (wt/vol) glucose for the test of hippurate hydrolysis, and with 2% (wt/vol) agar and 2% (wt/vol) glucose or 4% (wt/vol) sodium gluconate for the test of gas production from glucose or gluconate. The gas production was studied in pour tubes, which were observed for gas bubbles in the agar. The ability to grow at 15 and 45 C was tested in the basal medium supplemented with 2% (wt/vol) glucose. The pH of the gluconate medium was 5.2 (M. Rogosa, personal communication) and of the other media, 6.4. All media were sterilized by filtration and inoculated from an 18-h glucose broth culture. Growth was recorded after 2, 4, 7, and 14 days, when pH of the cultures was also measured. The fermentation products from glucose were studied in the PRAS glucose medium (20) and the optical isomers of lactic acid in the glucose broth with Lab-Lemco were omitted. The fermentation products were determined as described by Carlsson (2) and L-lactic acid with L-lactate dehydrogenase (Boehringer, Mannheim, Germany) as described by Hohorst (19). The amount of D-lactate was obtained by subtracting the amount of L-lactate from the total amount of lactate. Electrophoretic mobility of lactic dehydrogenases was studied in polyacrylamide gradient gels (PAA 4/30, Pharmacia Fine Chemicals, Uppsala, Sweden). The strains were grown in MRS broth (Oxoid), and the culture was centrifuged, washed, and disintegrated as described by Yamada and Carlsson (35). The cell-free extracts were mixed with an equal volume of 20% (wt/vol) glycerol. Six extracts were applied onto each gel and the electrophoresis was run for 18 h in the GE-4 electrophoresis apparatus (Pharmacia Fine Chemicals, Uppsala, Sweden). The buffer, pH 8.35, contained 0.09 M Tris, 0.08 M borate, and 0.003 M Na_2-ethylenediaminetetraacetic acid. The gel was sliced into two halves; one was stained for L-lactate dehydrogenase and the other for D-lactate dehydrogenase as described by Gasser (12).

RESULTS

In 13 deliveries, lactobacilli could be recovered from the mothers in eleven of the rectal and in eight of the vaginal samples (Table 1). Lactobacilli were also found in seven oral samples from the infants. In one delivery, lactobacilli were not found in samples from the mother or the infant. The predominant species in the vaginas of the mothers and in the mouths of the infants were *L. jensenii* and/or *L. acidophilus*. The rectal samples from the mothers contained a variety of homofermentative and heterofermentative species. When lactobacilli were found in the mouth of an infant, the same species of lactobacilli were also isolated from its mother's vagina in all cases but one (Table 1).

L. jensenii, *L. acidophilus*, and *L. salivarius* were found in only three of the samples taken from the mouths of 33 infants, 6 days old, one in each. In feces, *L. jensenii* was found in six of these infants, *L. acidophilus* in three, *L. salivarius* in one, and *L. casei* in two infants. Bifidobacteria grew on Rogosa SL agar from 13 of the 33 fecal samples. The bifidobacteria were strictly anaerobic and produced equal amounts of acetate and lactate from glucose.

The characteristics of the reference strains of *L. jensenii* and *L. acidophilus* and of the isolates are shown in Table 2. The isolates of the heterofermentative lactobacilli had characteristics in common with *L. buchneri* and *L. brevis*, but the electrophoretic mobilities of their lactate dehydrogenase were not identical to those of the heterofermentative reference strains.

DISCUSSION

A similar lactobacillus flora was found in the vagina of the mother and in the mouth of the child at the time of delivery in 6 out of 13 cases. In most cases the predominant species in these sites were either *L. jensenii* or *L. acidophilus*. In the rectal samples from the mothers a variety of lactobacillus species was found. Thus newborn infants appear to derive their initial oral flora quite often from the mucous membranes of the mother's vagina.

However, neither *L. jensenii* nor *L. acidophilus* became established in the mouth of the infants. Six days after birth *L. jensenii* was found in one infant and *L. acidophilus* in one out of 33 infants studied. This is in accordance with a previous finding that lactobacilli are

TABLE 1. *Lactobacilli found in mothers and infants at the time of delivery*

Delivery no.	No. of isolates								
	L. acidophilus			*L. jensenii*			Other lactobacilli[a]		
	Infant	Mother		Infant	Mother		Infant	Mother	
	Mouth	Vagina	Rectum	Mouth	Vagina	Rectum	Mouth	Vagina	Rectum
1	4	5	2						4
2				5					
3					1	1			3
4	5	5	5						
5	2	3		1	2	5			
6				5	3	5			
7				4	5	5	1		
8	5	5	4						1
9					3				5
10									5
11			1			1			1
12									2
13									

[a] In rectums of the mothers *L. casei* was found in five deliveries, *L. salivarius* and *L. plantarum* in one delivery each, and heterofermentative lactobacilli in three. The lactobacillus species found in one infant's mouth was heterofermentative.

mostly transients in the mouth of children below 2 years of age. The establishment of an oral lactobacillus flora in the children coincides with the development of caries lesions and the flora is then dominated by *L. casei* (3).

It has recently been proposed that the bacterial colonization of the bathed mucosal surfaces of the mouth, nasopharyngeal area, and intestinal canal is determined by a specific adherence of the bacteria to the surfaces (8, 14, 23). Bacteria differ widely in their ability to attach to various surfaces, e.g., *Veillonella* species and *Streptococcus salivarius* attach to the tongue (23), *S. pyogenes* to the pharyngeal epithelium (8), and *Vibrio cholerae* to the epithelial cells of the small intestines (10). Organisms, which cannot attach to a surface, are washed away and the results of the present study suggest that this is the case with the lactobacilli transferred from the mother's vaginal mucose to the mouth of the infant during delivery. *L. jensenii* and *L. acidophilus* simply pass through the mouth into the gastrointestinal canal. *L. acidophilus* has long been recognized as the intestinal lactobacillus (16, 25) and its preferential site of colonization is the lower part of the ileum (18, 22). In the breast-fed infant an intestinal microflora develops with high counts of lactobacilli, which is considered to prevent a colonization by enteropathogenic bacteria (1). There is an increasing number of lactobacilli in the vagina during pregnancy (31, 33). This increase may facilitate a colonization of the infant's intestine with lactobacilli at the time of delivery.

In the adult, *L. acidophilus* also constitutes a major part of the lactobacillus flora of the oral cavity (28, 32) and of the vagina. This may indicate that similar conditions for adhesion of *L. acidophilus* to the mucosal membranes would prevail in these sites. The species *L. acidophilus* represents, however, a heterogeneous group of organisms (7, 12, 22) and it is possible that different types of *L. acidophilus* colonize these sites.

It is surprising that *L. jensenii* (13) has been overlooked in previous work on the fecal and vaginal lactobacillus flora. One reason may be that this organism only survives for a few days when kept on blood agar or on Rogosa SL agar. *L. jensenii* could be differentiated from other species of lactobacilli by the following combination of characteristics: hydrolysis of arginine, fermentation of cellobiose, galactose and ribose but not of lactose, production of only D-lactate, and the electrophoretic mobility of the D-lactate dehydrogenase. In the original characterization of *L. jensenii* (13) the organism was reported to ferment trehalose, sometimes also melibiose and raffinose, but not ribose. No isolate in the present study fermented melibiose and raffinose, a few fermented trehalose, and most isolates slowly fermented ribose. The fermentation of ribose by *L. jensenii* CIP 6917 has also been observed by S. Edwardsson (personal communication).

In conclusion, the results of this study show that *L. jensenii* is a common vaginal and intestinal inhabitant, in many cases acquired

TABLE 2. *Characteristics of reference strains and isolates of Lactobacillus jensenii and L. acidophilus*[a]

Character	*L. jensenii*		*L. acidophilus*	
	Strain CIP 6917	Isolates (n = 64)	Strain NCTC 1	Isolates (n = 59)
Hydrolysis of arginine	+	64[b]	−	0[b]
Growth at 45 C	+	57	+	27
Fermentation of				
Galactose	+	59	+	46
Lactose	−	0	+	51
Melezitose	−	4	−	0
Melibiose	−	0	−	34
α-Methyl-D(+)-glucoside	+	48	−	21
Raffinose	−	0	+	37
Ribose	+	60	−	0
Trehalose	+	9	+	59
Production of				
D-Lactate	+	64	+	59
L-Lactate	−	0	+	59

[a] All isolates of *L. jensenii* and *L. acidophilus* hydrolyzed esculin and fermented cellobiose, fructose, maltose, mannose, sucrose, and salicin. No isolate hydrolyzed hippurate, grew at 15 C, or produced gas from glucose or gluconate. No isolate reduced nitrate to nitrite or fermented arabinose, mannitol, rhamnose, sorbitol, sorbose or xylose. The physiological characters were variable within or between the two species.

[b] The figures indicate the number of isolates giving a positive reaction.

already at birth, probably from the mother's vagina and via the mouth. The ecological role of this organism as well as its way of adhering to the mucosal surfaces at these sites is an interesting field for further studies.

ACKNOWLEDGMENTS

We wish to thank the personnel of the Delivery Unit of the University Hospital of Umeå for their valuable help and Lillemor Ågren and Gunn Jacobsson for their technical assistance.

This work was supported by grants from the Swedish Medical Research Council (19X-765), Semper Fund for Nutritional Research, Swedish Nutrition Foundation, and Kronprinsessan Lovisas förening för barnsjukvård.

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