RESEARCH BRIEF

Intestinal Colonization Among Very Low Birth Weight Infants in First Week of Life

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Objective: To study intestinal colonization patterns in very low birth weight infants in the first week of life in a neonatal intensive care unit.

Methods: Meconium/stool specimens were obtained on days 1, 3, 5 and 7 from 38 very low-birth-weight infants in a level III neonatal intensive care unit.

Results: On day 1, 45% had sterile guts, and by day 3, all infants were colonized. *E. coli*, *K. pneumoniae* and *Enterococcus fecalis* were predominant organisms. *Lactobacilli* was found in one isolate and *Bifidobacteria* was not detected during the study period. There was an association between formula feeding and *E. coli* colonization.

Conclusions: Very low birth weight infants admitted in neonatal intensive care units have abnormal intestinal colonization patterns.

Key words: *Intestine, Colonization, Neonate, Very low birth weight*.

Very low birth weight (VLBW) preterm infants in Neonatal Intensive Care Units (NICUs) do not follow the normal pattern of colonization followed by healthy, breast-fed, term infants [1-3]. Modern-day laboratory techniques have enabled a more detailed understanding of the aberrant colonization pattern in preterm infants [4-6]. There is a paucity of reports on intestinal colonization patterns from NICUs in developing countries. The type of environmental flora, bacterial load and antibiotic usage being different in these countries, one would expect colonization patterns to differ from developed countries. This study describes the longitudinal pattern of intestinal colonization in preterm infants.

METHODS

This was a prospective study conducted over a one-year period in the level III NICU of a tertiary care institute in Northern India. The Institute Ethics Committee approved the study protocol. Consecutive newborn infants with a birth weight less than 1500 grams, admitted to the NICU within 8 hours of birth, were eligible for inclusion. We excluded patients with any gastrointestinal malformation; any major malformation that might limit life expectancy to less than 7 days, and anticipated NICU stay of less than 7 days. We obtained written, informed parental consent before enrolment. The post-enrolment exclusion criteria were unanticipated discharge or death prior to 7 days of NICU stay.

Stool/ meconium specimens were obtained for culture on day 1 (24 ± 6 h), day 3 (72 ± 6 h), day 5 (120 ± 6 h) and day 7 (168 ± 6 h) after birth. The stool/meconium sample (rectal swab, in case of non-passage of stools) containing approximately 1 g of stool was taken on a sterile cotton swab, transported in a screw-capped sterile container with Cary-Blair medium to the microbiology laboratory, and processed immediately. For aerobic culture the sample was plated on McConkey agar and blood agar. For anaerobic culture, it was plated on blood agar and de Man, Rogosa, Sharpe (MRS) agar for lactobacillus (HiMedia Laboratories Pvt. Ltd., Mumbai, India), and incubated under anaerobic conditions. The laboratory identified species using conventional biochemical tests. The data collected included maternal and neonatal demographic details, mode of delivery, risk factors of sepsis, intrapartum antibiotics, postnatal antibiotics, details of feeding and stooling pattern, necrotizing enterocolitis and organisms colonizing the stool/ meconium/rectal swab.

RESULTS

We enrolled 38 neonates (26 males) and followed them up until 7 days in the NICU. The mean (SD) birth weight was 1082 (261) g (range 550-1480 g); gestational age 30.4(2.5) weeks (range 26-37 weeks), and median 5 minute Apgar score was 8 (range 6-9). Seventeen (45%) participants were small for gestational age. The median number of doses of antenatal steroids given was 1 (range

0-4); 15 (37%) were delivered by cesarean section; 9 (24%) after prolonged rupture of membranes (>18 hours) and 7 after preterm onset of labor. All mothers had received intra-partum antibiotics, and 23 (60.5%) neonates received antibiotics within the first 7 days. Intrapartum antibiotics included ampicillin alone to 15 (39.5%); ampicillin with metronidazole with erythromicin to 21 (55.3%); ampicillin with gentamicin to 1 and ampicillin with gentamicin with metronidazole to 1 mother.

Seventeen neonates (45%) had sterile meconium/ rectal swab cultures on the first day of life (***Table* I**). No subject had sterile stool/rectal swab cultures on days 3 and 5 of life; one culture was sterile on day 7. On day 1 of life, 34.2% and 21% neonates were colonized with one and two bacterial species, respectively. On day 3 of life, 37%, 32%, 29% and 3% were colonized with 1, 2, 3 and 4 bacterial species, respectively. On day 5, 45%, 50% and 5% were colonized with 1, 2 and 3 bacterial species, respectively. On day 7, 55%, 32% and 11% were colonized with 1, 2 and 3 species, respectively. In the entire study period, we found *Lactobacillus* in only one sample and *Bifidobacteria* could not be isolated from any sample. *E. coli* was the major colonizer. When we longitudinally followed each subject, we found a marked variability in the type of colonizing organisms.

Enteral feeding was started in 24 (63.2%), 30 (78.9%), 32 (84.2%) and 33 (86.8%) neonates by days 1, 3, 5 and 7, respectively. Exclusive breast milk feeding was strongly encouraged but preterm formula milk was started when breast milk was not available or insufficient.

TABLE I ORGANISMS ISOLATED FROM STOOL IN FIRST WEEK OF LIFE

Organisms	*Day 1*	*Day 3*	*Day 5*	*Day 7*
Escherichia coli	6	25	26	27
Klebsiella pneumoniae	9	23	14	16
Acinetobacter anitratus	2	4	4	2
Pseudomonas aeruginosa	0	1	0	0
Proteus vulgaris	0	1	1	1
Gram Negative Bacteria (non-classified)	1	0	0	0
Enterococcus fecalis	7	14	9	1
Staphylococcus aureus	3	4	6	4
Coagulase Negative *Staphylococcus*	0	0	0	1
Micrococcus	0	1	0	0
Diphtheroides	0	1	1	4
Lactobacillus spp.	0	0	0	1

E. coli colonized neonates with a significantly higher cumulative volume of formula milk feed by day 5 and this trend continued until day 7 (***Web Table* I**). There was also a significantly higher number of gavage feeds among colonized neonates on day 5.

***Web Table* II** shows the antibiotic use pattern in neonates having *E-coli* or *K. pneumonia* colonization. We did not find a significant association between administration of these antibiotics until the above days and colonization with *E. coli* and *K pneumonia* on those days. Three infants in the study developed necrotizing enterocolitis. None of them were colonized with *Lactobacillus* or *Bifidobacterium* during the first 7 days. The number developing NEC was too small to allow meaningful statistical comparisons with colonization patterns.

DISCUSSION

This study shows that in an Indian level III NICU, the gut colonization pattern found in VLBW infants is different from that seen in normal term infants. The single largest colonizer was *E. coli*, followed by *Klebsiella pneumoniae* and *Enterococcus fecalis*. Oligo-colonization, a progressive decrease in the number of colonizing species, paucity of lactic acid bacteria and a predominance of potentially pathogenic bacteria characterized this pattern.

The paucity of organisms could be attributed to evaluating colonization only up to the first 7 days of life and administration of broad-spectrum antimicrobial agents to almost two-thirds of neonates in our study. A lower intestinal biodiversity in preterms has been reported earlier [7-10]. The lack of normal flora predisposes to overgrowth of potentially pathogenic species, especially under antibiotic pressure, which in turn impairs gut immunity and mucosal function [11,12]. Dysbiosis and oligo-colonization have been shown to be associated with NEC [13].

Colonization with *Lactobacillus* and *Bifidobacteria* increases from after the first week of life; whereas, we evaluated neonates only in the first 7 days. This, along with the use of formula milk and antibiotics could account for the paucity of colonization with these organisms. Other studies suggest that *Lactobacilli* are present in smaller numbers in low birth weight infants and are often absent when such infants receive antibiotic therapy [14,15].

The clinical implication of this study is that VLBW infants in an NICU environment are colonized with potentially pathogenic organisms within the first week of life itself. This abnormal colonization may not only be

WHAT THIS STUDY ADDS?

- Very low birth weight infants in an Indian neonatal intensive care unit have oligocolonization of the gut in the first week of life.
- Bacteria cultured from their stool samples are potentially pathogenic.

associated with diseases in the neonatal period (such as sepsis, NEC) but also with post-neonatal diseases (such as atopy, inflammatory bowel disease, irritable bowel syndrome etc).

A limitation of this study was that we were unable to identify the strain of the bacterial species cultured in stool or perform quantitative assays of the colonizing organisms. In addition, we did not determine the resistance pattern of the organisms in stool. The setting in which this study was conducted may limit the generalizability of the findings. The absence of a control group of healthy breastfed hospital delivered infants in our study precludes comparison between VLBW infants and "Normal" infants.

We conclude that preterm very low birth weight neonates have oligocolonization of the gut in first week of life, and the colonizing bacteria are potentially pathogenic.

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