ID: 223

TITLE: EARLY ANTIBIOTICS TREATMENT IS ASSOCIATED WITH LESS NECROTIZING ENTEROCOLITIS IN PRETERM, VERY LOW BIRTH WEIGHT INFANTS AROUND THE WORLD: NEOMUNE-NEOINUETRINET COHORT STUDY

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CONTENT:

Antibiotic treatment is used frequently in very low birth weight (VLBW, ≤ 1500 g) preterm infants in neonatal intensive care units (NICUs). Previous studies have reported higher risk of necrotizing enterocolitis (NEC) after prolonged antibiotics (AB) use during the first weeks of life. Little is known how AB initiated soon after birth affect NEC incidence. We investigated the association between early AB treatment (AB initiated within first three days after birth) and later development of NEC, using the NEOMUNE-NeoNutriNet cohort of VLBW preterm infants from 13 NICUs in five continents (n=2829). Data were collected from birth until postmenstrual age (PMA) 37 weeks, or less in case of early discharge or death. NEC incidence was compared between infants who received AB within the first three days and those who did not, without or with statistical adjustments for NICU, gestational age (GA), birth weight, gender, delivery mode, antenatal steroids, Apgar score, type and time of enteral nutrition, and use of probiotics. In explorative analyses, PMA at NEC onset was compared between the two groups and NEC incidence was correlated with the duration of early AB treatment.

The large majority (2562/2829) of infants received AB within three days of birth (Early-AB) and 3.9% of these developed NEC. For the remaining 267 infants, not receiving AB or AB after three days or more (No early-AB), NEC incidence was 9.0%. After statistical adjustment for NICU, NEC incidence was still lower in the Early-AB group (OR 0.56; 95% CI, 0.34-0.93, P<0.05). The significance level increased further after adjustment for covariates (P<0.0001). The exploratory analyses
showed no association between early AB use and PMA at NEC onset, but prolonged AB use tended to associate with increased risk of NEC (P=0.094) after adjustment for GA and other potential confounders.

Early initiation of AB in VLBW infants was associated with less NEC compared with a minority who did not get early AB. Non-infective factors leading to preterm birth in the No-early-AB group, such as preeclampsia or fetal growth failure, may have a negative impact on gut health but possibly, early AB postpones gut bacterial colonization and thereby reduces NEC-related gut inflammatory responses.

**COI:** Prof van Goudoever is the director of the Dutch Human Milk Bank and is a member of the National Health Council. Ms. Cormack serves on scientific advisory boards for Nestlé Nutrition Institute and Danone/Nutricia. Prof Simmer is the Director of the Human Milk Bank in Perth Australia and has received support from Medela and Nestlé Nutrition Institute. Prof Sangild has received grant support from ARLA Foods, Medela, Danone/Nutricia, Biofiber-Damino, Mead Johnson Nutrition, and Nestlé Nutrition Institute. Prof Bloomfield has received travel support for invited lectures from Abbot Nutrition and Nestlé Nutrition Institute. Dr. Embleton has received speakers’ honoraria from Nestlé Nutrition Institute and Danone/Nutricia, and his department has received research support from Prolacta Bioscience and Danone/Nutricia. Other authors declare no conflicts of interest.
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TITLE: FECAL MICROBIOTA TRANSPLANTATION PROTECTS AGAINST NECROTIZING ENTEROCOLITIS IN A DONOR SPECIFIC MANNER

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CONTENT:

Prevention of necrotizing enterocolitis (NEC), a life-threatening gastrointestinal morbidity of preterm infants, remains a challenge. We recently showed promising effects of fecal microbiota transplantation (FMT) from healthy suckling donors to newborn preterm pigs as a NEC-preventive strategy. Besides ensuring clinical improvements, FMT changed the recipient gut microbiota towards anaerobicity, reducing facultative anaerobic dysbiosis and increasing bacterial diversity. It remains unclear if any eubiotic donor feces will provide similar benefits. Hence, we hypothesized that FMT, using feces from two phenotypically similar donors would result in similar recipient response.

In a randomized, controlled experiment, using cesarean-delivered, formula-fed preterm pigs (n=13-14 per group) as models for NEC-susceptible preterm infants, we compared FMT from two donor pig herds (FMT1 and FMT2). Donor material consisted of mixed, homogenized colon content from 5-8 term born, sow-reared, 12±2-days-old piglets from each herd. Pooled donor bacterial compositions were comparable at order level of taxonomic classification (e.g. 65-69% Clostridiales and 18-22% Lactobacillales). A total amount of 0.1 g donor feces suspended in sterile saline was administered by rectal route twice daily the first two days after birth. On day 5, animals were euthanized and necropsied, and colon content collected for gut microbiota analysis.

FMT2 resulted in complete NEC protection (0 vs. 23 and 46% in FMT1 and controls respectively, p<0.05 vs. CON, Figure 1A). An in vivo test of gut barrier function showed reduced intestinal permeability only in FMT2 (p<0.05, Figure 1B). Besides, diarrhea was reduced and growth rate increased in FMT2 but not FMT1 recipients relative to controls (both p<0.05). Bacterial 16S rRNA gene amplicon sequencing showed changes in beta-diversity after FMT2 but not FMT1 (weighted UniFrac, p<0.05 vs. CON by PERMANOVA, Figure 1C). Similarly, only FMT2 increased alpha diversity relative to controls (Shannon-index, p<0.05 by Kruskal-Wallis test, Figure 1D). Whereas few donor-derived obligate anaerobes colonized the recipients, FMT2 recipients had increased Lactobacillus (25 vs. 0.5 and 7%) and decreased Enterococcus (25 vs. 57 and 56%) relative abundances compared with controls and FMT1 recipients.

We compared the effect of FMT, using two similar donor pools and found clear differences in recipient clinical and microbiological responses. The results indicate that the ability of FMT to protect against NEC may be mediated by a limited number of bacteria. Identification and isolation of these bacteria could lead to the development of a safe and well-defined NEC prevention therapy.

IMAGES:
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COI: None declared
**ID:** 382  
**TITLE:** ANTAGONISTIC RELATIONSHIP BETWEEN ANTIBIOTICS AND FECAL MICROBIOTA TRANSPLANTATION PREDISPOSES TO NECROTIZING ENTEROCOLITIS IN PRETERM PIGS  
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**CONTENT:**

A significant morbidity of preterm infants is necrotizing enterocolitis (NEC), an inflammatory bowel condition involving gut barrier disruption and mucosa bacterial invasion. Antibiotics (AB) are indispensable for treating bloodstream infections in preterm infants but come at a price. Prolonged parenteral AB appears to increase the risk of NEC, whereas prophylactic enteral AB may reduce it. However, any AB treatment causes gut dysbiosis and AB resistance. Fecal microbiota transplantation (FMT) is capable of preventing NEC and may reduce AB resistance. We hypothesized that sequential enteral antibiotics and FMT would effectively prevent NEC with negligible increase in antibiotics resistance.

In a controlled 2x2 factorial experiment (n=13-16 per group), cesarean-delivered preterm pigs used as model organisms were administered enteral broad-spectrum antibiotics or saline for the first four days of life, and subsequently given rectal FMT (with documented NEC-reducing potential from healthy suckling donor piglets) or control saline. The AB cocktail consisted of 50/12.5 mg/kg/d Amoxycillin/Clavulanic acid and 50 mg/kg/d Neomycin, administered twice daily. The FMT (0.1 g feces per animal) was administered 2-3 cm into the rectum twice daily using a soft catheter. Animals were fed increasing volumes of infant formula and necropsied at nine days of age, after continuous clinical monitoring, and microbiological and immunological evaluations on days 5, 7 and 9.

Only animals receiving no AB followed by FMT treatment (CON-FMT) had reduced NEC incidence (31 vs 69% in CON-CON, p<0.05, Figure 1A). Gut pathological severity was reduced in both single treatments (AB-CON and CON-FMT, Figure 1B) compared with control (CON-CON), whereas to our surprise, the combination treatment (AB-FMT) was not. AB improved growth rate and reduced diarrhea the first 4 days of life (both p<0.05), but the effects disappeared after AB discontinuation. However, both at AB discontinuation and five days later, AB-treated animals had increased AB resistance of the gut microbiota to both the administered and unrelated AB compounds. Notably, FMT treatment after AB discontinuation reduced AB-induced Cefotaxim resistance to control levels (p<0.05, Figure 1C). Finally, AB treatment suppressed blood myeloid cell development, which was not affected by FMT (Figure 1D).

The results offer insights into the principal role of the gut microbiota after preterm birth. Importantly, FMT succeeding initial AB treatment in early life has the capacity to eliminate antibiotics resistance but may be clinically detrimental and requires further refinement. Additionally, we suggest cautious use of early enteral antibiotic treatment in preterm newborns, due to disturbance of immune system development.

**IMAGES:**  
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Figure 1. Clinical, microbiological and immunological responses to sequential enteral antibiotics treatment and rectal fecal microbiota transplantation. A. Incidence of necrotizing enterocolitis. B. Gut pathological severity index. C. Antibiotics resistance of Gram negative bacteria from colon content, expressed as number of colony-forming units on MacConkey selective agar with or without antibiotics compounds. D. Blood monocyte levels in blood collected 5, 7 and 9 days after birth. Columns with different letters denote statistically significant differences at p<0.05.
COI: None declared
ID: 405
TITLE: DEVELOPMENT OF THE GUT MICROBIOTA IN EXTREMELY PRETERM INFANTS: THE EFFECT OF PERINATAL FACTORS AND LINKS WITH NECROTISING ENTEROCOLITIS
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CONTENT:
Extremely preterm infants develop in a highly unusual environment, with routine antibiotic administration and the NICU environment contributing to altered bacterial colonization of the gut. However, the influence of other perinatal factors on the gut microbiota remains poorly understood, as does the consequent impact on clinical outcomes. We aimed to characterize the development of the gut microbiota in extremely preterm infants, and to describe the influence of a range of perinatal factors on microbial colonization. We also investigated links between the gut microbiota and later development of necrotizing enterocolitis.

We carried out a prospective longitudinal study following 89 extremely preterm infants (gestational age <28 weeks) admitted to the NICU at Queen Silvia’s Children’s Hospital in Gothenburg, Sweden. Data was collected on a wide range of perinatal factors, including gestational age, birth weight, delivery mode, Apgar scores, chorioamnionitis, maternal and infant antibiotic treatment, prenatal steroids and necrotizing enterocolitis. DNA was extracted from stool samples taken at regular intervals from birth to six weeks of age and the V3-V5 region of the 16S rRNA gene was sequenced using the MiSeq platform. We described the development of the gut microbiota and used Orthogonal Partial Least Squares to explore associations with a range of perinatal factors.

The gut microbiota of extremely preterm infants showed low diversity throughout the first six weeks of life and was dominated by facultative anaerobes belonging to the genera Staphylococcus and Enterococcus and the class Gammaproteobacteria. Obligate anaerobic commensals from the genera Bifidobacterium and Bacteroides, which are abundant in the gut of healthy term infants, were only found at low levels in a minority of infants, while obligate anaerobes from the phylum Firmicutes were slow to establish. Lower gestational age at birth and chorioamnionitis were both linked with enrichment in various Proteobacteria and delayed colonization by bifidobacteria, while babies born by caesarian section had increased prevalence of Streptococcus. High levels of Klebsiella (>88%) were noted in a third of infants (2/6) in the days prior to development of necrotizing enterocolitis.

Increased abundance of potential pathogens, along with low levels of commensals providing colonization and translocation resistance, may help explain poor clinical outcomes in extremely preterm infants. In particular, necrotizing enterocolitis was often preceded by high levels of Klebsiella. Bacterial colonization patterns were influenced by a range of factors, including gestational age at birth, birth mode and chorioamnionitis.

COI: None declared