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Minimally Invasive Diagnostic and Prognostic Biomarkers in Neonates with Necrotizing Enterocolitis, Single Centre Experience

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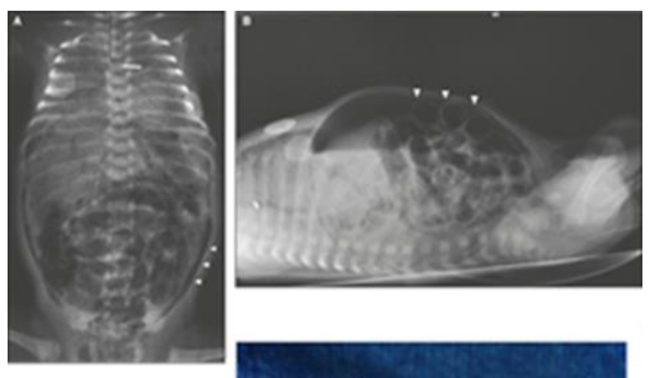

Introduction

Necrotizing enterocolitis (NEC) is one of the most serious diseases of the gastrointestinal tract of premature babies.
NEC - ischemic necrosis of intestinal mucosa.
Incidence 3-10% Lethality 20-30% especially ↑ for surgical NEC.
Pathogenesis: multifactorial.
Microbiome: changes since birth ↑ risk of NEC.
The results of microbiome and metabolome research could make a significant contribution to early diagnosis of NEC and initiate personalised treatment and improve outcomes.

Methodology

Aim:

To explore the microbiome and metabolome of a newborn with necrotizing enterocolitis, to determine new, minimally invasive diagnostic and prognostic biomarkers of NEC that can be used in clinical practice..

Materials and Methods:

A single center prospective cohort study.

From 2023-2024 stool and urine samples were collected.

Inclusion criteria: Premature infant (<7th day of life, <32GN, <1500 g birth weight).

Biomarkers:

- urine- L-D- lactate dehydrogenase, Serum Amyloid A, PGE-MUM, IFABP,
- feces- L-D- lactate dehydrogenase, Glycogen debranching enzyme.

Microbiome analysis: Shotgun metagenomic sequencing,

QIAseq FX DNA Library Kit, MetaPhlAn2i

Biomarker analysis: ELISA, Microsoft Excel 365, IBM SPSS Statistics 29.0

Results

- This study evaluated ***7 NEC preterm infants***, five female, three male infants, mean (SD) gestational age (GA) (weeks) 27 (24-30), mean (SD) birth weight (BW)(grams) 964 (673-1233). Common presenting symptoms across all cases in NEC included feeding intolerance, abdominal distension and bile-stained vomiting. Four of the infants were fed donor breastmilk, one received formula and two breastmilk.
- Biomarkers:
  - feces: LDH and Glycogen Debranching Enzyme (GDE) were undetectable
  - urine: Prostaglandin E2 (PGE2) and Lactate Dehydrogenase (LDH) were significantly elevated in NEC patients during the early days of diagnosis ($p<0.05$), while no significant differences were observed for I-FABP and Serum Amyloid A (SAA). (Figure 1.,2.)
  - Microbiome:The fecal microbiome composition analysis revealed significant dysbiosis, with Firmicutes dominating in four patients, Actinobacteria in one patient, Proteobacteria in one patient, and Ascomycota in one patient. Pathogenic species such as *Enterococcus faecalis*, *Staphylococcus haemolyticus*, and *Klebsiella* were predominant. There is a slight decrease in alpha diversity metrics after the NEC diagnosis, including reductions in species richness (Chao1 index 21.00 vs 17.00) and diversity (Shannon 3.56 vs 3.43 and Simpson indices 0.90 vs 0.89) (Figure 3,4, 5 and 6).

Conclusion

- Microbiome analysis for NEC showed diverse relative proportion of bacteria and should be continued with larger number of samples to obtain and create credible taxa paterns.
- NEC appears linked to gut microbiome dysbiosis, with an abundance of pathogenic species and reduced diversity.
- Feeding type influenced the microbiome, suggesting the importance of further research into microbiome-targeted interventions.
- This study identified potential PGE2 and LDH biomarkers for early NEC diagnosis and management, warranting validation in larger studies.

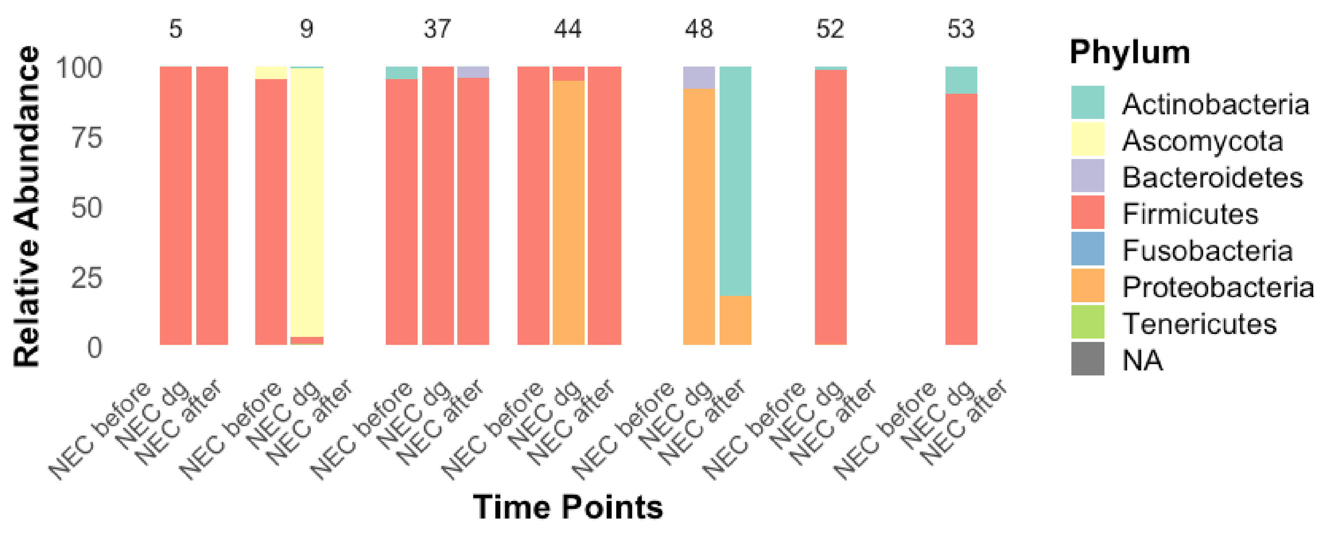

Fig.3. **Stacked bar plot of NEC group abundances at phylum level.**

	NEC, Mdn ng/ml (IQR)	Control, Mdn ng/ml (IQR)	p-value
1	0.51 (0.40-0.84)	0.34 (0.17-0.52)	***0.045 ****
2	0.51 (0.40-0.75)		***0.036 #***

Fig. 1. LDH # p value from One Sample Wilcoxon Signed Rank Test, * p value from Mann Whitney U test

	NEC, Mdn pg/ml (IQR)	Control, Mdn pg/ml (IQR)	p-value #
Day 1	0.45 (0.00-9.82)	23.43 (7.25-44.25)	0.003*
Day 2	5.25 (0.00-13.37)		0.028
Day 3	13.75 (4.58-19.56)		0.028
Day 4	7.14 (1.58-17.72)		0.043
Day 5	13.57 (3.83-20.00)		0.043
Day 6	1.13 (0.00-12.36)		0.066
Day 7	18.14 (18.14-18.14)		0.317
Day 8	7.81 (2.43-7.81)		0.180
Day 9	15.41 (15.41-15.41)		0.317

Fig. 1. PGE2 # p value from One Sample Wilcoxon Signed Rank Test, except * p value from Mann Whitney U test, Median values are presented with IQR (25%; 75%)

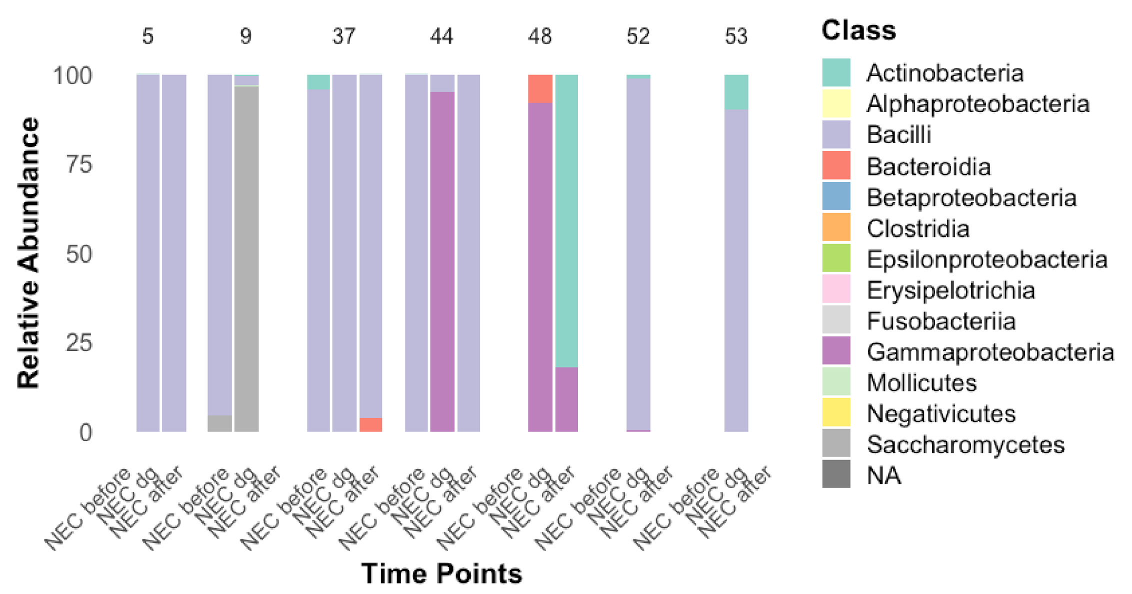

Fig.4. **Stacked bar plot of NEC group abundances at class level.**

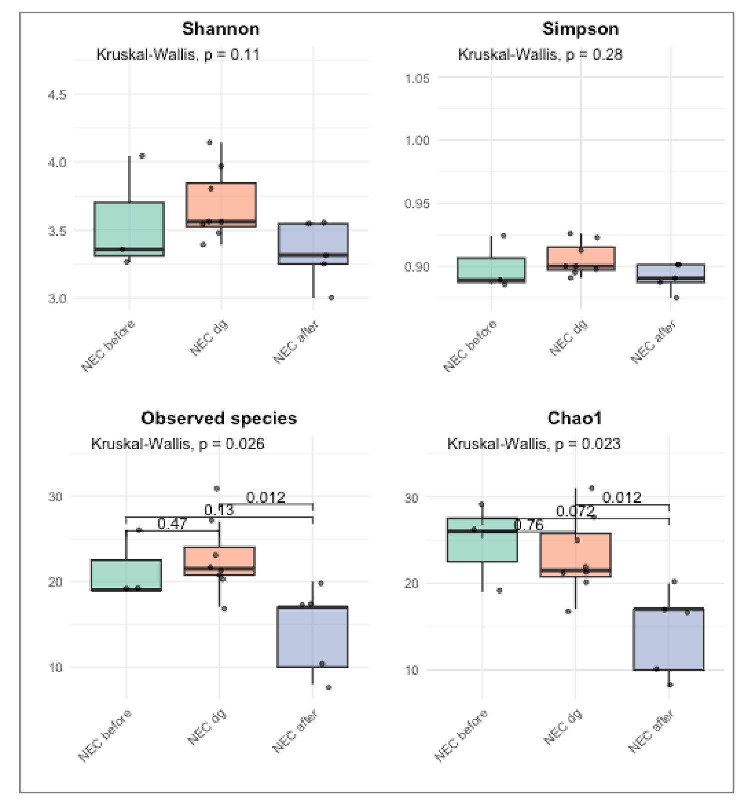

Fig.6. **Alpha-diversity metrics comparison of NEC patient gut microbiome at different time points.** A median of 21.00 species were observed at the NEC diagnosis state for each sample. As shown in figure, there is a slight decrease in alpha diversity metrics after the NEC diagnosis, including reductions in species richness (Chao1 index 21.00 [20.00 - 25.00] vs 17.00 [10.25 - 19.25]) and diversity (Shannon 3.56 [3.48 - 3.80] vs 3.43 [3.08 - 3.55] and Simpson indices 0.90 [0.90 - 0.91] vs 0.89 [0.88 - 0.90]). Before NEC diagnosis the alpha diversity metrics showed a larger dispersion (Chao1 index 17.00 [10.25 - 19.25], indicating greater variability in this smaller group (n = 3).

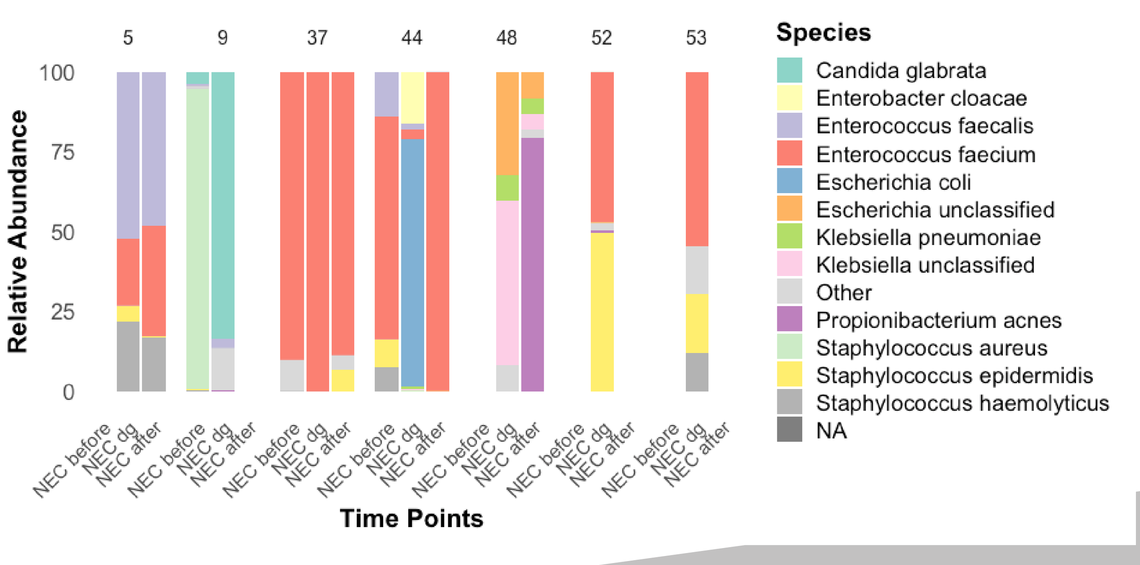

Fig.5. **Stacked bar plot of NEC group abundances at species level.**

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