Gut bacteria and necrotizing enterocolitis: cause or effect?

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Main Article

Necrotising enterocolitis (NEC) is multifactorial disease process which affects the gut, most typically the terminal ileum, and occurs almost exclusively in babies born significantly preterm (<32 weeks gestation). The main risk factors for NEC include gestational age, birth weight and feeding with infant formula, that are in turn associated with the immaturity of both the gut and the immune system. The gut bacteria have a significant role in developing barrier function, integrity, and mucosal and systemic immune function in the neonate. They also 'educate' the gut associated lymphoid tissue, allowing the infant's immune system to tolerate the presence of its microbiota [1]. As NEC does not occur in a sterile environment (e.g. *in utero* or germ free animal models), it may be that in some preterm infants this habituation by the immune system to the gut microbial community may be undermined initiating an exaggerated inflammation cascade that results in NEC [2].

Over the past four decades, researchers, driven by improvements in methodology, have trawled through microbiological datasets to determine the role of the preterm gut microbiota in NEC and in particular, whether the presence of specific bacteria are correlated with the

disease. There are conflicting reports in the literature, with some studies showing no association of the gut microbiota with incidence of NEC [3], whereas others studies identify particular taxa (i.e. *Klebsiella*; *Clostridium*) correlated with an increased risk of disease [4]. Many early molecular studies demonstrated differing abundances of bacteria in stool prior to NEC diagnosis, but were confounded by low and non-optimal sampling [5]. It is important to note that many studies of the gut microbiota present data where all NEC cases within an experimental cohort are pooled. Analyses such as these, while simple to understand, are misleading as any increase in a particular taxa are unlikely to be shared across all NEC infants. More recently, improved sampling coupled to advances in sequencing technology, have highlighted the diverse and individual nature of the gut microbiota both *within* infants on a single neonatal intensive care unit (NICU), but also *between* different NICUs [6].

The recent study by Raveh-Sadka *et al.* [7] is a powerful demonstration of how, using newly developed methodologies, it is possible to resolve sequence data from gut associated microbial communities into genomes. Using this approach they began to tease out the complexity of these microbial communities and their contribution, if any, to NEC. The study carried out metagenomic sequencing on 55 stool samples from 10 infants (6 NEC, 4 controls) during an NEC outbreak 2.5 times the average case in the NICU (Magee-Women's Hospital of the University of Pittsburgh Medical Centre). The study showed that there was no evidence for a common genomically distinct pathogenic agent causing NEC. An important unknown in any outbreak of NEC within in a NICU, is whether a single pathogenic strain is cross-colonising infants. While a review of NEC outbreaks failed to determine a consistent pattern of colonisation [8], it is plausible that identical strains could be found in infants diagnosed with NEC. However, Raveh-Sadka *et al.* showed that in their cohort each individual infant had a distinct bacterial profile down to strain level. They were also able to explore strains of individual taxa. In this case, *Enterococcus faecalis* present in several of the

babies. They were able to show each infant had its own pattern of specific strains which in turn threw up some fascinating questions, around the ecology of *E. faecalis.* For example, what was the size and diversity of the community from which these strains are derived? How might they disperse and colonise the host? What are the barriers to colonisation within the NICU? Are they able to competitively exclude other strains? Does dominance of a particular strain reflect strong selective pressures modulated by the host's genetic background and the microbial community resident in the gut? Given that *E. faecalis* has been implicated in beneficially modulating the immune response in neonates [9] these questions may have clinical as well as fundamental significance. The ability to resolve the community to bacterial strain level also demonstrated how different strains of the bacterium *Veillonella* responded to antibiotics with one strain being replaced by a second. This may help explain why some microbiome studies on preterm gut communities showed no significant changes to antibiotic administration at the level of family or genus [10]. It also highlights the limitations of sequence based studies on 16S rRNA gene diversity that cannot resolve the community to strain level.

Aside from bacterial colonisation in NEC, Raveh-Sadka *et al*. presented some intriguing and novel findings relating to the preterm gut microbiome. One aspect the authors explored was the potential capabilities for mobile elements such as plasmids and phages to move around the NICU, potentially contributing to NEC. Comparable to the results for strain level analysis, identical sequences were found within infants overtime and there were few cases where plasmids and phages were detected in different infants. However, there were no identical mobile elements that were shared between NEC infants.

With more questions than answers arising from a decade of molecular research into the role of the preterm gut microbiome in NEC, the study by Raveh-Sadka *et al*. clearly demonstrates the opportunities that rapidly evolving sequencing technologies offer to understand this

complex multi-factorial disease. In the short and medium term genome-resolved metagenomics will help better frame our research questions and identify potential interactions between microbial components of a community and its environment that are not otherwise visible. In the longer term unpicking the complexity of NEC will require greater collaboration and agreement on methodologies and the exploitation of bio-banks of stool, urine, serum, and resected tissue samples from preterm infants to help overcome issues around small disease cohorts. Moreover, researchers will need to exploit system based approaches including metabolomics and proteomics, of both microbial community and the host to more comprehensively characterise the mechanisms of NEC.

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