MICROBIOME

Antibiotics and the infant microflora

Functional selection of novel antibiotic resistance genes and metagenomic sequencing reveal how antibiotic treatment and bacterial resistance genes interact to shape the fragile microbiome of premature infants.

Kristen Meyer and Kjersti Aagaard

When it comes to the gut microbiome, not all antibiotics necessarily impart a similar impact. In fact, we have known for several years that antibiotic resistance genes in our metagenome long preceded human use of antibiotics by thousands of years[1]. As premature or low-birth-weight neonates and infants often receive multiple courses of antibiotics, understanding how different antibiotic classes interact with antibiotic resistant (AR) genes to shape the gut microbiome of premature infants is imperative for designing optimal treatment regimens.

Writing in *Nature Microbiology*, Gibson *et al.*[2] provide an in-depth look at the dynamics of the premature gut microbiome and antibiotic resistome (the collection of AR genes harboured by the gut microbiome) in response to varying antibiotic exposures. The authors performed extensive analysis of longitudinally collected stool samples (401 in total) from a cohort of 84 hospitalized premature infants. While all premature neonates were exposed to antibiotics within the first week of life, 51 of the infants received one or more additional antibiotic courses. Stool samples from these infants were compared with those from the 33 infants who did not receive further antibiotics; this group was used to account for 'normal' variation in the developing preterm infant gut microbiome. To account for AR genes not currently present in annotated gene databases, the authors carried out a functional selection of AR genes from faecal metagenomic expression libraries prepared from a subset of 21 samples, allowing for the culture-unbiased identification of 794 AR genes, 79% of which had not been previously classified as conferring antibiotic resistance.

As reported previously[3], the authors found that the preterm gut microbiome has relatively low bacterial diversity compared with full-term infants at equivalent post-birth ages. Additionally, Gibson *et al.* report that AR genes are not evenly distributed among commensal species and potential pathogens. Rather, three potentially pathogenic species, *Klebsiella pneumonia*,

© PHOTODISC/GETTY IMAGES

Escherichia coli and *Enterobacter cloacae*, harbour a disproportionate number of AR genes, potentially providing resistance to multiple antibiotic classes. In contrast, putative beneficial commensals such as *Bifidobacterium* spp. carry relatively few AR genes. It is therefore tempting to speculate that, owing to the uneven distribution of AR genes, antibiotic treatments may kill putatively beneficial commensal bacteria, potentially allowing multidrug resistant (MR) pathogens to dominate the neonate's generally low-diversity microbiome.

Gibson *et al.* further compared how different classes of antibiotics impact the gut microbiome: they found that overall increased antibiotic exposure (regardless of class) is associated with reduced bacterial diversity, but some antibiotics affected species richness more than others. After accounting for several other factors, including post-menstrual age (gestational age plus day(s) of life), breastfeeding, individual variability and overall health status of the infant, they found that treatment with the antibiotics meropenem, cefotaxime, or ticarcillin/clavulanate was associated with a significant reduction in species richness and enrichment of specific

species and AR genes. For example, after treatment with ticarcillin-clavulanate (a carboxypenicillin/beta-lactamase inhibitor combination), there was a significant enrichment of *K. pneumonia* and AR genes encoding resistance to carboxypenicillins and aminopenicillin/beta-lactamase inhibitors. Interestingly, AR genes encoding resistance to other classes of antibiotics were also enriched, which may be explained by the presence of plasmid- and genome-encoded MDR gene clusters carried by the enriched species. In contrast, ampicillin had no effect, while vancomycin and gentamicin effects were variable.

This work provides a crucial glimpse into how antibiotic therapy may impact the premature gut microbiome. It illustrates the complexity of the impact, inclusive of the class of antibiotic given, the presence of specific bacterial species and the AR genes carried by these species. While one notable limitation of this study is the lack of association with clinical outcomes, other studies have reported that increased antibiotic exposure[4] and increased abundance of specific potential pathogens may be associated with increased risk for necrotizing enterocolitis in premature infants. These findings provide another step forward in providing optimal antibiotic regimens for premature infants, which may require screening for specific AR genes — as well as concurrent breastfeeding to potentially increase the abundance of commensal bacteria[5,6].

Although the results of this study are notable, there are several unanswered questions that are crucial for meaningful interpretation of these findings. First and foremost, as the gut microbiome varies substantially between infancy and adulthood[7], and even between premature and full-term infants[3], these findings cannot be generalized to other age groups. Therefore, the importance of antibiotic class in shaping the gut microbiome must be restricted, in its current interpretation, to low-birth-weight and premature infants. Second, it is unknown if the observed reduction in species richness early in life has

any significant impact on subsequent health outcomes. Finally, it is important to consider confounding factors intrinsically linked to the variables under study, such as the initial reason for administering additional antibiotic treatments, and associated maternal infectious exposures. For example, women who are known to harbour vaginal Group B *Streptococcus* (GBS) will receive intrapartum prophylaxis for prevention of neonatal GBS sepsis and, if mothers are suspected of having an intra-amniotic infection (chorioamnionitis), antibiotics will be continued among the neonates immediately following birth.

This study represents a necessary deep interrogation into the premature gut microbiome and resistome, and it highlights the complex interaction between AR gene carriage patterns and responses to antibiotic exposures. Caution should be taken to avoid over-interpreting the current findings, which should be considered along with the knowledge that antibiotics save lives and improve disease-free survival. Further studies determining both the short- and long-term clinical implications of antibiotic-associated depletion of bacterial richness are necessary to evaluate if, and how, careful antibiotic selection and breastfeeding may be used to restore bacterial diversity and bolster development of the premature gut microbiome. ❐

Kristen Meyer and Kjersti Aagaard are in the Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine, Baylor College of Medicine, Houston, Texas 77030, USA. K.A. is also in the Department of Molecular & Human Genetics, Division of Maternal Fetal Medicine, Baylor College of Medicine, Houston, Texas 77030, USA.
e-mail: aagaardt@bcm.edu

References
1. D'Costa, V. M. *et al. Nature* **477,** 457–461 (2011).
2. Gibson, M. K. *et al. Nature Microbiol.* **1,** 16024 (2016).
3. Arboleya, S. *et al. FEMS Microbiol. Ecol.* **79,** 763–772 (2012).
4. Alexander, V. N., Northrup, V. & Bizzaro, M. J. *J. Pediatr.* **159,** 392–397 (2011).
5. LaTuga, M. S., Stuebe, A. & Seed, P. C. *Semin. Reprod. Med.* **32,** 68–73 (2014).
6. Fallani, M. *et al. J. Pediatr. Gastroenterol. Nutr.* **51,** 77–84 (2010).
7. Yatsunenko, T. *et al. Nature* **486,** 222–227 (2012).