Airway Microbiome and Development of Bronchopulmonary Dysplasia in Preterm Infants: A Systematic Review

Mohan Pammi, MD, PhD, MRCPCH[1], Charitharth Vivek Lal, MD[2], Brandie D. Wagner, PhD[3], Peter M. Mourani, MD[4], Pablo Lohmann, MD[1], Ruth Ann Luna, PhD[5], Amy Sisson, MS, MLS[6], Binoy Shivanna, MD, DM, PhD[1], Emily B. Hollister, PhD[5], Steven H. Abman, MD[3,4], James Versalovic, MD, PhD[5], Gary J. Connett, MBChB, DCH, FRCPCH, MD[7], Vineet Bhandari, MD, DM[8], and Namasivayam Ambalavanan, MD[2]

Objectives To summarize evidence regarding microbial dysbiosis of the airway associated with bronchopulmonary dysplasia (BPD) and to explore heterogeneity among studies.

Study design We included studies that evaluated the airway microbiome in preterm infants who developed BPD using culture-independent molecular techniques and reported alpha- and beta-diversity metrics and microbial profiles.

Results The 6 included studies had substantial clinical and methodological heterogeneity. Most studies reported the presence of an airway microbiome early after birth and an evolution in the first weeks of life with increasing bacterial loads. The early airway microbiome was dominated by *Staphylococcus* and *Ureaplasma* spp. Two studies reported differences in alpha- and beta- diversity indices in preterm infants with BPD compared with those who did not develop BPD. Increased microbial community turnover, changes in the relative abundance of Proteobacteria and Firmicutes, and decreased Lactobacilli were reported with BPD progression. Most included infants were born by cesarean delivery, and a majority were exposed to postnatal antibiotics. No data regarding feeding human milk or correlations with the development of gut microbiota (gut-lung axis) were available.

Conclusions Microbial dysbiosis may be associated with BPD progression and severity, and further study of microbiome optimization in preterm infants at risk for BPD is warranted. *(J Pediatr 2019;204:126-33).*

Bronchopulmonary dysplasia (BPD) is a form of chronic lung disease of preterm infants that is associated with considerable short-term and longer-term morbidity and risk of mortality.[1,2] The incidence of BPD has remained relatively constant despite improvements in perinatal care, newer ventilatory strategies, and increasing survival of extremely preterm neonates. The incidence of BPD varies inversely with gestational age (GA) and ranges from 48% to 68% in neonates born at <28 weeks of gestation.[3]

BPD is characterized by lung injury, inflammation, alveolar simplification, dysregulation of microvascular development, and pulmonary hypertension.[1,2,4] There has been a surge in interest in the role of the airway and lung microbiome in BPD as technology to evaluate the microbiome has advanced and become more readily available.[5,6] The airway is colonized with microbiota, with reports of as many as 10-100 bacterial cells per 1000 human cells.[7] The amniotic fluid, placenta, and vagina each has its own microbiome and may possibly seed the airways of the newborn either before or during birth.[8,9] The lung microbiome observed at birth evolves over the first weeks and months of postnatal life. In preterm infants, factors that can affect development of the lung microbiome include exposure to prenatal and postnatal antibiotics, use of respiratory support devices, sepsis, feeding and nutrition, concurrent development of the intestinal microbiome, and the surrounding environmental microbiome.[10]

Chorioamnionitis, transplacental infection, or abnormal colonization can create an inflammatory process that initiates and exacerbates the development of BPD.[11] The use of antibiotics early in life has been associated with an increased incidence of BPD.[12,13] An apparent link exists between the development of the gut and lung microbiome (gut-lung axis).[14] Microbial dysbiosis in the gut has been implicated in the development of the inflammatory disease necrotizing enterocolitis, and similarly, microbial dysbiosis in the respiratory tract may lead to lung

BPD	Bronchopulmonary dysplasia
ELBW	Extremely low birth weight
ETA	Endotracheal aspirate
GA	Gestational age
PCR	Polymerase chain reaction
PMA	Postmenstrual age

From the [1]Section of Neonatology, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX; [2]Division of Neonatology, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL; [3]Section of Pulmonary Medicine; [4]Pediatric Heart Lung Center, Section of Critical Care Medicine, Department of Pediatrics, School of Medicine, University of Colorado, Aurora, CO; [5]Texas Children's Microbiome Center, Texas Children's Hospital and Department of Pathology, Baylor College of Medicine; [6]Texas Medical Center Library, Houston, TX; [7]Department of Pediatrics, Southampton University Hospitals NHS Trust, Southampton, United Kingdom; and [8]Department of Pediatrics, Drexel University College of Medicine, Philadelphia, PA

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inflammation and BPD. In addition, the presence of normal commensal bacteria in the gut and lungs is necessary for the normal development of the immune system and immune homeostasis,[15] and elimination or disruption of normal commensal microbiota may lead to abnormal inflammatory responses, which may play a role in the pathogenesis of BPD.

We systematically reviewed studies that investigated the airway microbiome in preterm infants who developed BPD compared with controls and performed a qualitative synthesis of the data from the included studies. Our primary objective was to describe and summarize information on the neonatal airway microbiome that has been associated with BPD in preterm infants, as defined in the National Institutes of Health's consensus statement.[2] A secondary objective was to explore heterogeneity among studies that have investigated the airway microbiome to explain inconsistency in the reported results.

Methods

We performed our systematic review in accordance with the PRISMA guidelines[16] and the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) consensus statement.[17] We included prospective or retrospective, case-control, and cohort studies that evaluated the neonatal airway microbiome in preterm infants who later developed BPD compared with infants without BPD, using culture-independent molecular techniques and reporting at least one alpha- or beta-diversity metric or a microbial profile. Our search strategy and the databases searched are outlined in the **Appendix** (available at www.jpeds.com).

Data Collection and Statistical Analyses

All titles and abstracts identified by our search strategy were screened for relevance by a single author, and those deemed relevant were retrieved in full and evaluated for inclusion eligibility by 2 authors independently. All results were compared, and disagreements were resolved by mutual discussion. Relevant data were extracted from included studies and additional information to clarify the study design, and data was sought from the authors via e-mail in at least 3 attempts. The data extracted by the authors were examined for any discrepancies with input from a third author, and any conflicts were resolved by mutual discussion. The methodological quality of each study was assessed using relevant items in the checklist for observational studies proposed by Viswanathan et al.[18]

Results

We identified 98 reports using our search methods. After removing duplicate reports, we screened the resulting 77 reports for inclusion; of these, 70 reports that did not include our study population (preterm infants), evaluation (microbiome techniques), or outcome (BPD) of interest were excluded (PRISMA flow diagram, **Figure**; available at www.jpeds.com). Seven full text articles were reviewed for eligibility, of which 6 studies met our inclusion criteria (**Table I**). One study[25] was excluded because of use of culture methods for microbial evaluation. The methodological quality of the 6 included studies was assessed using the item checklist, and responses of "yes," "no," or "unclear" are reported (**Table II**; available at www.jpeds.com). The inclusion and exclusion criteria were similar in the comparison groups. Molecular methods of microbiome analysis were reported for all patients in all studies. All studies had appropriate comparison groups and reported all of our prespecified outcomes, and results were believable given the study limitations. Only one of the 6 included studies addressed the issue of confounding by matching or stratification to avoid known confounders.

The 6 eligible studies that met our inclusion criteria[19-24] included 2 studies conducted in the United Kingdom[19,20] and 4 studies performed in the US.[21-24] We have included the study reported by Mourani et al in 2011[21] as a separate study even though the data were included in the study reported by Wagner et al,[24] because the samples were sequenced on Roche 454 platform in the former study and by the Illumina MiSeq in the latter. The results reported in the included studies are tabulated in **Table III**.

We found significant heterogeneity among the 6 included studies in both methodological and clinical characteristics. Variations in microbiome results occurred owing to variations in the populations evaluated (possible differences in race and ethnicity, antenatal antimicrobial exposure), as well as to variations in techniques, such as differences in endotracheal aspirate (ETA) collection, airway secretions, DNA kits, DNA extraction and sequencing methods, sequencing platforms, and primers used to target the hypervariable regions.[26] The substantial heterogeneity among the studies precluded a quantitative synthesis of sequence data, and thus we present our findings qualitatively.

DNA was extracted using different extraction kits (**Table I**), and sequencing platforms also varied. Lohmann et al and Mourani et al used the Roche 454 sequencing platform,[21,22] and in later studies, Lal et al and Wagner et al used the Illumina MiSeq platforms.[23,24] The 2 studies from the United Kingdom reported terminal restriction fragment length polymorphisms or denatured gel electrophoresis techniques with species-specific polymerase chain reaction (PCR) for *Mycoplasma* and *Ureaplasma* species,[19,20] whereas the studies from the US were based on amplification of the variable regions of the 16S rRNA gene.[21-24] Mourani et al and Wagner et al used the V1-V2 region of the 16S sRNA gene (same place and an extended study),[21,24] Lohmann et al used the V3-V5 hypervariable region,[22] and Lal et al used the V4 region for PCR amplification and sequencing.[23] Differences in microbial composition have been reported due to variations in the regions of the 16S RNA variable regions evaluated.[27]

Microbial profiles in microbiome studies have been shown to vary by geographical locations.[10] The dates of the studies included in our review ranged from 2006 to 2017, a span of 11 years. All studies were prospective cohort studies, but with varying sample sizes. There were differences among studies in the birth weight and GA of the preterm infants enrolled and the time points at which the ETA sampling was performed.

Table I. Characteristics of microbiome evaluation in the included studies

Authors	Year	Methods of microbiome evaluation	DNA extraction	16S rDNA target region and PCR primers	Platform/sequence metrics
Stressmann et al[19]	2000	PCR amplification and restriction endonuclease digestion and T-RFLP analysis; species-specific PCR amplification of multibanded gene from *Ureaplasma urealyticum*	DNA extraction was performed with a customized method.	Forward primer, 8f 700: 5′-AGA GTT TGA TCC TGG CTC AG-3′; reverse primer, 926r: 5′-CCG TCA ATT CCT TTR AGT TT-3′	Sequencing from PCR products of 5 patients was performed at Macrogen (Seoul, Republic of Korea).
Payne et al[20]	2010	DGGE profiling; species-specific PCR to detect the presence of *M hominis*, *U urealyticum*, and *U parvum*	DNA was extracted using a Qiagen All Prep DNA/RNA Mini Kit (Qiagen, Germantown, Maryland).	A 334-bp section of the 16S rRNA gene specific to *M hominis* was amplified with primers RNAH1f and RNAH2r. The multiple-banded antigen (MBA) gene of *U urealyticum* was amplified with primers UMS-125f and UMA226r. The 16S rRNA gene was amplified with primers 357f (conserved for domain Bacteria, *E coli* positions 341-357) and 907rM (universal conserved primer, *E coli* positions 907-926). The primers amplify a 586-bp section of the 16S rRNA gene of members of the domain Bacteria, including the highly variable V3-V5 region.	DGGE was performed using a Bio-Rad D-Code DGGE system. For sequencing analysis, *U urealyticum*, *M hominis*, and DGGE PCR products were sequenced with primers UMS-125f, RNAH1, and 907rM, respectively. Sequencing was performed using ABI BigDye Terminator version 3.1 sequencing dye (Applied Biosystems Foster City, California).
Mourani et al[21]	2011	Quantitative real-time PCR assays for total bacterial load; pyrosequencing for microbiome evaluation	DNA was prepared from each sample using the Qiagen EZ1 Advanced platform (Qiagen, Germantown, Maryland), and the bacterial load of each sample was estimated using a TaqMan quantitative PCR assay.	DNA sequencing was performed using barcoded primers (27F-338R) compatible with the Roche 454 pyrosequencer forward primer, 5′-GCCTTGCCAGCCCGCTCAGTC AGAGTTTGATCCTGGCTCAG -3′. The reverse primer was 5′-GCCTCCCTCGCGCCATCAGNNNNNNNNCA TGCTGCCTCCCGTAGGAGT -3′, which targets the V1-V2 hypervariable region of the 16S rRNA.	Sequencing was performed with a Roche Genome Sequencer FLX system (Roche, 454 life sciences, Branford, Connecticut) at the Consortium for Comparative Genomics sequencing facility at the University of Colorado Denver.
Lohmann et al[22]	2014	Conventional cultures; PCR amplification of the V3V5 regions and 454 sequencing	Bacterial DNA was extracted using the Mo Bio PowerSoil kit (Qiagen, Germantown, Maryland) with modifications used in the Human Microbiome Project.	V3-V5 regions of the bacterial 16S rRNA gene (V3V5-357F: 5′-CCTACGGGAGGCAGCAG-3′; V3V5-926R: 5′-CCGTCAATTCMTTTRAGT-3′)	Roche 454 (Roche, 454 life sciences, Branford, Connecticut) sequencing technology was used, yielding an average of 3000 reads per region with an average read length of >500 bases.
Lal et al[23]	2016	PCR amplification of the V4 region of the 16S rRNA gene; only samples with >1000 reads were included.	DNA was extracted using a DNA Isolation Kit (D6010; Zymo Research, Irvine, California).	The sequence data covered the 16S rRNA V4 region with a PCR product length of ~255 bases and 250 paired-end reads. Degenerate PCR primers specific for the V4 region were used (Eurofins mwg/operon; http://www.operon.com).	PCR products were sequenced using NextGen sequencing on the Illumina MiSeq platform (Illumina, San Diego, California).
Wagner et al[24]	2017	16S rDNA amplification and sequencing	DNA was extracted using the Qiagen EZ1 Advanced automated extraction platform with the bacterial card and tissue extraction kit.	Amplicons were generated using primers targeting approximately 300 bp of the V1/V2 variable region of the 16S rRNA gene.	Illumina (Illumina, San Diego, California) paired-end sequencing was performed on the MiSeq platform.

DGGE, denaturing gradient gel electrophoresis; *T-RFLP*, terminal restriction fragment length polymorphism.

Table III. Patient characteristics and results from included studies

Authors	Year	Setting and period	Study design	Characteristics of participants	Reported results
Stressmann et al[19]	2010	Place: Southampton, United Kingdom Study period: Unclear	Prospective cohort study of intubated preterm infants studied within the first week of life by T-RFLP	n = 8 infants, 14 ETA samples Chronological age: All samples were collected in the first week of life except 1 sample collected at 31 d. PMA: 24 5/7 to 31 3/7 wk	A diverse array of bacteria, including *S aureus*, *Enterobacter* spp., *M catarrhalis*, *P aeruginosa*, and *Streptococcus* spp. were found in the endotracheal secretions. Species-specific PCR was more sensitive for the detection of *U urealyticum* (positive in 3 of 8 patients) than T-RFLP (positive in only 1 patient). *U urealyticum*, when present, persisted in all the samples from the same patient. All patients had an oxygen requirement at age 28 d, but only 3 of 8 were diagnosed with BPD (by study definition) at 36 wk PMA.
Payne[20]	2010	Place: Two level III neonatal intensive care units in the United Kingdom, at Southampton and Portsmouth Study period: 12 mo, exact dates unclear	Prospective cohort study of preterm infants born weighing <1.3 kg who were ventilated for at least 24 h and met clinical and radiologic criteria for physician-diagnosed respiratory distress syndrome. ETA and nasogastric aspirates were assessed by DGGE for bacterial community profiling and by species-specific PCR for *Mycoplasma* and *Ureaplasma* spp.	n = 55 Chronological age: just after the first 24 h of life or sooner PMA: mean, 26.2 wk (range, 23-30 wk)	DGGE identified bacterial species in 59% of combined NGA and ETA samples. Coagulase-negative *Staphylococcus* spp. (*S haemolyticus* [the most common microbe in ETA] and *S epidermidis*) were the most common. *Fusobacterium nucleatum* was the most common organism in NGA specimens. *M hominis* was detected in 9%-13% of ETA samples and in 25%-30% of NGA samples. *Ureaplasma* spp. were detected in approximately 50% of all samples; *U parvum* was the most prevalent, followed by *U urealyticum*. Among the 48 infants surviving up to 36 wk PMA, ordinal logistic regression showed an OR of 4.80 (95% CI, 1.15-20.13) for BPD or death when *Ureaplasma* was present (vs absent), with BPD defined using the National Institutes of Health definition.[2]
Mourani et al[21]	2011	Place: University of Colorado, Denver. This cohort was a part of a larger observational cohort of 49 preterm infants. Period: July 2006-2010	Prospective observational study. Serial ETA samples were collected from preterm infants (GA ≤ 34 wk) and infants with a birth weight of 500-1250 g, who required mechanical ventilation for at least 21 d. Of the 10 preterm infants, 1 died, 1 had mild BPD, 3 had moderate BPD, and 5 had severe BPD. Infants without BPD were not enrolled.	n = 10 Chronological age: Samples collected within 72 h and at age 7, 14, and 21 d PMA: 24-27 wk	Only 2 of 10 samples collected at 72 h of life contained adequate bacterial DNA for successful sequence analysis, but bacterial loads increased after day 3 of life. Seven of the 72 organisms observed represented the dominant organism (>50% of total sequences) in 31 of 32 samples with positive sequences. A dominant organism represented >90% of the total sequences in 13 samples. *Staphylococcus* spp, *U parvum*, and *U urealyticum* were the most frequently identified dominant organisms, but *Pseudomonas* spp, *Enterococcus* spp, and *Escherichia* spp were also present. The diversity of the bacteria in ETA was much lower in neonates than in older children or adults and was not associated with age or BPD severity.
Lohmann et al[22]	2014	Place: Texas Children's Hospital, Houston; single center Study period: June-December 2012	Twenty-five infants, born at ≤32 wk GA and intubated in the first 24 h, were enrolled.	n = 25 Chronological age: ETA was obtained at intubation and at age 3, 7, and 28 d. PMA: 24-32 wk	At the time of intubation (first 24 h), neonates who subsequently developed BPD (n = 10) had lower bacterial diversity (observed species count and Shannon diversity index) compared with those who did not develop BPD. The overall mean number of observed species and Shannon diversity index across time differed significantly between infants with BPD and those without BPD. Microbial profiles at the phylum level showed that Firmicutes increased and Proteobacteria decreased over time in the infants who developed BPD compared with the relatively diverse and stable community in the non-BPD group. *Acinetobacter* was the predominant genus in both groups, but the relative abundance of *Acinetobacter* spp. decreased longitudinally in the BPD group, with increasing amounts of *Staphylococcus* and *Klebsiella* spp.

(*continued*)

Table III. Continued

Authors	Year	Setting and period	Study design	Characteristics of participants	Reported results
Lal[23]	2016	Place: Discovery cohort, University of Alabama at Birmingham Regional Neonatal Intensive Care Unit; validation cohort, Drexel University, Philadelphia Study period: October 2014 to March 2015	Prospective observational cohort study. All 10 full-term infants enrolled were intubated within 6 h of age due to either surgical indications (congenital heart disease, abdominal wall defect) or perinatal depression (with no signs of meconium aspiration syndrome). A validation cohort of 14 ELBW infants, 7 with BPD and 7 without BPD, from another center was also studied.	n = 23 ELBW infants (10 with BPD and 13 without BPD), and 10 full-term infants and an additional 18 patients with established BPD Chronological age: All 23 ELBW infants and full-term infants were sampled on day 1 of age (at birth or within 6 h of age). For infants with established BPD, samples were obtained at 36 wk PMA at the time of ETT change. Mean PMA: ELBW infants, 24.5 ± 0.2 wk; full-term infants, 38.3 ± 2.2 wk; infants with established BPD, 37.6 ± 1.5 wk	A diverse airway microbiome was observed in both preterm and term infants at birth. Full-term infants had a different and more diverse microbiome compared with older preterm infants with established BPD. Both ELBW and full-term infants had a predominance of Firmicutes and Proteobacteria on the first day of age, in addition to the presence of Actinobacteria, Bacteroidetes, Tenericutes, Fusobacterium, Cyanobacteria, and Verrucomicrobia. Compared with newborn full-term infants matched for PMA, the airway microbiome of infants after diagnosis of BPD was characterized by increased Proteobacteria and decreased Firmicutes and Fusobacteria. Gamma Proteobacteria were more abundant and alpha Proteobacteria were less abundant in infants with BPD compared with newborn ELBW and full-term infants. The most abundant Proteobacteria in infants with BPD were Enterobacteriaceae. *Lactobacillus* was less abundant in the early airway microbiome of infants who later developed BPD in both the discovery and validation cohorts. Differences in Shannon alpha-diversity and beta-diversity by UniFrac distances were seen between infants with BPD and full-term and ELBW infants. Five ELBW preterm infants who were followed from birth until the development of BPD exhibited a distinct temporal dysbiotic change with a decreased relative abundance of Firmicutes and an increased relative abundance of Proteobacteria.
Wagner[24]	2017	Place: Two centers, University of Colorado School of Medicine, Anschutz Campus and Indiana University School of Medicine Period: July 2006 to February 2013 The data include the 10 preterm infants reported by Mourani et al, 2011.[21]	Prospective observational, longitudinal study of preterm infants with GA ≤34 wk and birth weight 500-1250 g. BPD was determined based on modified National Institutes of Health criteria with oxygen reduction tests; 51 infants had mild BPD, 49 had moderate BPD, and 52 had severe BPD. Infants without BPD were excluded due to low numbers.	n = 152 Chronological age: Mechanically ventilated infants had ETA samples collected serially at enrollment and at age 7, 14, and 21 d. Mean PMA: mild BPD, 25.69 ± 1.3 wk; moderate BPD, 25.61 ± 1.63 wk; severe BPD, 25.4 ± 1.56 wk	The cross-sectional dataset included only samples collected between 5 and 9 d of age and consisted of 79 subjects: 23 with mild BPD, 27 with moderate BPD, and 29 with severe BPD. The median total bacterial load for day 7 ETA samples, as well as the Shannon alpha-diversity index and evenness, were not significantly different across the BPD groups. Most samples were dominated by *Staphylococcus* (68%) and *Ureaplasma* (18%) spp, and the relative abundance of *Staphylococcus* was not significantly different by BPD severity. There were very poor associations of microbial markers, alpha diversity, total bacterial load and the relative abundance of individual taxa with the severity of BPD. For longitudinal analysis, 233 samples were sequenced from 94 subjects (25 with mild BPD, 30 with moderate BPD, and 39 with severe BPD). Preterm infants who eventually developed severe BPD exhibited greater bacterial community turnover with age, acquired less *Staphylococcus* in the first days after birth, and had a higher initial relative abundance of *Ureaplasma*.

NGA, nasogastric aspirate.

Although most recent studies used the National Institutes of Health's definition of BPD, the older studies used other definitions.

Discussion

Our review identified 6 eligible studies, which were qualitatively synthesized without meta-analysis. We assessed the methodological quality and risk of bias in these 6 studies using the key components of study design in observational studies, which is superior to quality scoring.[17] The methodological quality of the included observational studies was adequate.[18]

Four of the 6 studies identified bacteria at birth or early in life in both term and preterm infants.[19,20,22,23] In contrast, Mourani et al reported low or undetectable bacterial sequences in ETA samples obtained in the first 72 hours of life in preterm infants born at <28 weeks GA,[21] and in the study of Wagner et al, the airway microbiome at birth could not be assessed, because many samples were acquired at several days of age.[24] Most of the preterm infants (>60%) enrolled in the studies were born by cesarean delivery with intact membranes.[22,23] There was an evolution of microbial colonization, with increases in bacterial DNA loads during the first weeks of life.[21,22,24] Older infants with established BPD had more diverse microbiomes compared with preterm infants at birth.[24]

Payne et al reported that bacterial sequences related to coagulase-negative *Staphylococcus* spp (*S haemolyticus* and *S epidermidis*) were the most common. *Mycoplasma hominis* was detected in 9%-13% and *Ureaplasma* spp were detected in approximately 50% of the ETA samples in that study.[20] Stressmann et al reported *Staphylococcus aureus*, *Enterobacter* spp, *Moraxella catarrhalis*, *Pseudomonas aeruginosa, Streptococcus* spp, and *Ureaplasma* spp in the ETA samples.[19] Lal et al reported that the airway microbiome from both extremely low birth weight (ELBW) and full-term infants at birth showed a predominance of Firmicutes and Proteobacteria on the first day of life, along with the presence of Actinobacteria, Bacteroidetes, Tenericutes (including *Ureaplasma* spp), Fusobacteria, Cyanobacteria, and Verrucomicrobia.[23]

The studies reported by Lohmann et al and Lal et al compared ELBW infants with BPD and those without BPD.[22,23] Lohmann et al reported decreased bacterial diversity as estimated by the number of observed species and the Shannon diversity index in infants who developed BPD compared with those who did not, based on the sample obtained at intubation (in the first 24 hours of life).[22] Lal et al reported differences in microbe diversity and abundance (alpha diversity and beta diversity) between ELBW infants and infants with established BPD and between full-term infants and infants with established BPD.[23]

Mourani et al and Wagner et al compared airway microbiome against grades of severity of BPD; in both studies, preterm infants without BPD were few and not analyzed.[21,24] In the cross-sectional dataset of samples obtained from days 5-9, in preterm infants, the bacterial load, the Shannon alpha-diversity index and evenness were not significantly different across BPD groups. Stressmann et al reported BPD in 3 of 8 infants at 36 weeks postmenstrual age (PMA), but could not compare infants with and without BPD owing to their small sample size.[19]

The majority of studies examined microbial composition at the phylum and genus levels in patients with BPD. Lohmann et al reported that microbial communities at the phylum level showed increases in the relative abundance of Firmicutes and decreases in the relative abundance of Proteobacteria over time in the BPD group, in contrast to the relatively diverse and stable community in the non-BPD group.[22] *Acinetobacter* was the predominant genus in both groups, but the relative abundance of *Acinetobacter* spp decreased longitudinally in the BPD group, with increasing amounts of *Staphylococcus* and *Klebsiella* spp. In contrast to the study by Lohmann et al, Lal et al reported that the airway microbiome of infants after diagnosis of BPD was characterized by increased Proteobacteria and decreased Firmicutes and Fusobacteria compared with newborn full-term infants matched for PMA.[23] Variations in clinical characteristics of the included patients, in methodology, or in environmental ecology between the units where the studies were conducted may explain this difference. Gammaproteobacteria were more abundant and Alphaproteobacteria were less abundant in infants with BPD compared with ELBW and term infants. The most abundant Proteobacteria in the infants with BPD were Enterobacteriaceae. Lactobacillus were statistically significantly less abundant in the early airway microbiome of infants who later developed BPD, but the overall abundance of Lactobacillus was low.[23] In the cohort of Wagner et al, *Staphylococcus* (68%) and *Ureaplasma* (18%) spp dominated the microbiome, but the relative abundance of these bacteria was not significantly associated with severity of BPD.[24] Payne et al reported that among the 48 infants surviving up to 36 weeks PMA, ordinal logistic regression showed an OR of 4.80 (95% CI, 1.15-20.13) for BPD or death where *Ureaplasma* was present in ETA specimens.[20]

Longitudinal changes in the airway microbiome were reported in 2 studies.[23,24] Wagner et al analyzed 233 samples from 94 preterm infants and found that preterm infants who eventually developed severe BPD exhibited greater bacterial community turnover with age, as assessed by Shannon beta-diversity and Morisita-Horn pairwise comparison measures.[24] In this study, infants with more severe BPD acquired less *Staphylococcus* in the first days after birth and had a greater initial relative abundance of *Ureaplasma.* Lal et al followed 5 preterm infants longitudinally and found that despite multiple courses of antibiotics, the infants showed distinct temporal dysbiotic changes, with a decrease in Firmicutes and increase in abundance of Proteobacteria over time.[23]

We also examined a number of potential clinical determinants of the airway microbiome. An association of antibiotics-induced dysbiosis with BPD has been reported.[13] All patients in the cohort of Mourani et al received antibiotics in the first 21 days of life either empirically or for confirmed infection (median duration, 12 days; range, 2-19 days).[21] In the cohort of Payne et al, 52 of 55 infants (94.5%) received antibiotics, including 50 with cefotaxime and 2 with benzyl penicillin and gentamicin.[20] Sixty percent of the cohort of Lohmann et al received empiric treatment with ampicillin and gentamicin for

48 hours, and there were no significant differences in the Shannon index in the initial specimen between infants who received empiric antibiotic treatment and those who did not.[22] Lal et al reported that most mothers of preterm infants received antimicrobial therapy before delivery, but there were no differences in the airway microbiome between the infants of mothers who received prenatal antibiotics and the infants of mothers who did not.[23] Lal et al reported decreased *Lactobacillus* spp in infants who were exposed to chorioamnionitis.[23] In the cohort of Lohmann et al, *Acinetobacter* was the predominant genus in the airways of all infants at birth, and no differences in bacterial diversity and cytokines (lipopolysaccharides and lipoteichoic acid) were detected in ETA samples from infants with exposure and those without exposure to chorioamnionitis.[22]

In the cohort of Lal et al, of the 23 ELBW infants in the discovery cohort, 15 (65%) were born by cesarean delivery and 8 (35%) were born by vaginal delivery, and there were no statistically significant differences in airway microbiota by mode of delivery.[23]

The long-term effects of an altered airway microbiome in infants with BPD is unknown. Exactly when the relative proportions of each microbial class achieve the "adult" composition is not clear. More research is needed to understand the long-term effects of perturbation of the respiratory microbiome in the neonatal period.

There were some limitations to the studies that we reviewed. All the samples from preterm infants in the included studies were obtained from intubated infants. How the airway microbiome develops in infants without an endotracheal tube, such as in preterm infants on continuous positive airway pressure or with a high-flow nasal cannula is unknown. ETA serves as a surrogate to assess the respiratory microbiome, but how it relates to the peripheral lung microbiome remains unclear. Compared with the stool or intestinal samples, ETA samples have much less bacteria and are considered low-biomass samples. Analysis of low-biomass samples may be improved in the near future with newer and more efficient techniques. None of the included studies evaluated the microbiome by metagenomics, which may provide greater resolution up to strain level and information including the metabolic function of these microbiota in the airways and how they relate to respiratory disease.

Despite our extensive search strategy, it is possible that we missed some potentially relevant studies. However, we contacted experts in the field and are confident that all eligible studies were included. Publication bias in microbiome studies was not evaluated, because the number of included studies was fewer than 10.

In conclusion, the airway microbiome is identifiable early after birth and evolves over time with increasing bacterial loads and diversity. Firmicutes and Proteobacteria are the predominant phyla and *Staphylococcus* and *Ureaplasma* are the predominant genera of the early airway microbiome. In patients with BPD, increased microbial community turnover, changes in the relative abundance of Proteobacteria and Firmicutes, and decreased Lactobacilli were reported with BPD progression. Airway microbiome differences by mode of delivery or exposure to antibiotics could not be evaluated, because most included infants were born via cesarean delivery and were exposed to postnatal antibiotics. No data on feeding human milk or correlations with the development of gut microbiota (gut-lung axis) were available. The metabolic functions of the lung microbiota and their role in the causation of lung injury sequence and inflammation remain to be defined. Metagenomic and metabolomic assessments coupled with assessment of inflammation of the lung and airway samples may delineate BPD causation and progression. ■

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Reprint requests: Mohan Pammi, MD, PhD, MRCPCH, Section of Neonatology, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, 6621 Fannin Street, West Tower 6-104, Houston, TX 77030. E-mail: mohanv@bcm.edu

References

1. Collins JJP, Tibboel D, de Kleer IM, Reiss IKM, Rottier RJ. The future of bronchopulmonary dysplasia: emerging pathophysiological concepts and potential new avenues of treatment. Front Med (Lausanne) 2017;4:61.
2. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723-9.
3. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 2010;126:443-56.
4. Jobe AH, Ikegami M. Prevention of bronchopulmonary dysplasia. Curr Opin Pediatr 2001;13:124-9.
5. Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL, Birren BW, et al. The Human Microbiome Project: a community resource for the healthy human microbiome. PLoS Biol 2012;10:e1001377.
6. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
7. Marsland BJ, Yadava K, Nicod LP. The airway microbiome and disease. Chest 2013;144:632-7.
8. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237ra65.
9. DiGiulio DB. Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med 2012;17:2-11.
10. Taft DH, Ambalavanan N, Schibler KR, Yu Z, Newburg DS, Deshmukh H, et al. Center variation in intestinal microbiota prior to late-onset sepsis in preterm infants. PLoS One 2015;10:e0130604.
11. Gantert M, Been JV, Gavilanes AW, Garnier Y, Zimmermann LJ, Kramer BW. Chorioamnionitis: a multiorgan disease of the fetus? J Perinatol 2010;30(Suppl):S21-30.
12. Novitsky A, Tuttle D, Locke RG, Saiman L, Mackley A, Paul DA. Prolonged early antibiotic use and bronchopulmonary dysplasia in very low birth weight infants. Am J Perinatol 2015;32:43-8.
13. Cantey JB, Huffman LW, Subramanian A, Marshall AS, Ballard AR, Lefevre C, et al. Antibiotic exposure and risk for death or bronchopulmonary dysplasia in very low birth weight infants. J Pediatr 2017;181:289-93.e1.
14. Marsland BJ, Trompette A, Gollwitzer ES. The gut-lung axis in respiratory disease. Ann Am Thorac Soc 2015;12(Suppl 2):S150-6.
15. Surana NK, Kasper DL. Deciphering the tête-à-tête between the microbiota and the immune system. J Clin Invest 2014;124:4197-203.
16. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339:b2535.

17. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008-12.
18. Viswanathan M, Berkman ND, Dryden DM, Hartling L. Assessing risk of bias and confounding in observational studies of interventions or exposures: further development of the RTI Item Bank. AHRQ Publication No. 13-EHC106-EF. Rockville (MD): Agency for Healthcare Research and Quality; 2013
19. Stressmann FA, Connett GJ, Goss K, Kollamparambil TG, Patel N, Payne MS, et al. The use of culture-independent tools to characterize bacteria in endo-tracheal aspirates from pre-term infants at risk of bronchopulmonary dysplasia. J Perinat Med 2010;38:333-7.
20. Payne MS, Goss KC, Connett GJ, Kollamparambil T, Legg JP, Thwaites R, et al. Molecular microbiological characterization of preterm neonates at risk of bronchopulmonary dysplasia. Pediatr Res 2010;67:412-8.
21. Mourani PM, Harris JK, Sontag MK, Robertson CE, Abman SH. Molecular identification of bacteria in tracheal aspirate fluid from mechanically ventilated preterm infants. PLoS One 2011;6:e25959.
22. Lohmann P, Luna RA, Hollister EB, Devaraj S, Mistretta TA, Welty SE, et al. The airway microbiome of intubated premature infants: characteristics and changes that predict the development of bronchopulmonary dysplasia. Pediatr Res 2014;76:294-301.
23. Lal CV, Travers C, Aghai ZH, Eipers P, Jilling T, Halloran B, et al. The airway microbiome at birth. Sci Rep 2016;6:31023.
24. Wagner BD, Sontag MK, Harris JK, Miller JI, Morrow L, Robertson CE, et al. Airway microbial community turnover differs by BPD severity in ventilated preterm infants. PLoS One 2017;12:e0170120.
25. Imamura T, Sato M, Go H, Ogasawara K, Kanai Y, Maeda H, et al. The microbiome of the lower respiratory tract in premature infants with and without severe bronchopulmonary dysplasia. Am J Perinatol 2017;34:80-7.
26. Hiergeist A, Reischl U, Gessner A. Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high intercenter variability. Int J Med Microbiol 2016;306:334-42.
27. Barb JJ, Oler AJ, Kim HS, Chalmers N, Wallen GR, Cashion A, et al. Development of an analysis pipeline characterizing multiple hypervariable regions of 16S rRNA using mock samples. PLoS One 2016;11:e0148047.

50 Years Ago in *The Journal of Pediatrics*

Radiologic Findings in the Lungs of Premature Infants

Thibeault DW, Grossman H, Hagstrom JWC, Auld PA. J Pediatr 1969;74:1-10

Thibeault et al published a comprehensive examination of the lungs in premature infants requiring oxygen therapy, including chest radiographs, clinical outcomes, and in 2 cases, lung biopsy data. They were intent on identifying the role of oxygen in the pathology seen in premature infant lungs. Infants were categorized into 3 groups based on definite, probable, or no evidence of Wilson-Mikity syndrome (WMS). WMS was first described as delayed onset of respiratory distress in premature infants who required oxygen with minimal ventilatory support, with a characteristic radiographic appearance of "bubbly" cystic pulmonary interstitial emphysema (PIE).[1] WMS has been largely lost to time, subsumed into bronchopulmonary dysplasia (BPD), as ventilator support for premature infants has become standard treatment since the late 1960s.

A review of the radiographs provided by the authors shows classic findings of BPD, including reticular opacities and cystic lucencies, and late-stage findings of PIE, characterized by scarring and hyperinflation. One group of infants had higher birth weights and were less premature but still required early oxygen therapy for lung disease and subsequently showed evidence of WMS. Two of these infants underwent lung biopsy, which revealed interstitial fibrosis with compensatory emphysema of other alveoli, consistent with BPD of today.

Since this report, there have been numerous therapeutic advances in care for the premature infant lung, including antenatal steroids, postnatal surfactant delivery, improvements in mechanical ventilation technology, and targeting of lower supplemental oxygen provision. However, although many of the "old" causes of BPD have been minimized, BPD remains a clinical challenge. The next frontier in research may lie in the historic approaches of Thibeault et al, further subdividing cases of BPD into different histopathological patterns of injury—along with imaging and genetic studies—which may provide new insight into how an immature pulmonary-vascular system can be better coaxed into maturity.[2]

Meredith Laguna, MD, MPH
Benjamin Laguna, MD
Michael D. Cabana, MD, MPH
Department of Pediatrics and Radiology
University of California, San Francisco
San Francisco, California

References

1. Wilson M, Mikity V. A new form of respiratory disease in preterm infants. Am J Dis Child 1960;99:489-99.
2. Day CL, Ryan RM. Bronchopulmonary dysplasia: new becomes old again! Pediatr Res 2017;81:210-3.

Appendix

Search Strategies for Major Databases

1. PubMed search strategy

(((((((lung*[Title/Abstract]) OR airway*[Title/Abstract]) OR "air-way*"[Title/Abstract]) OR trachea*[Title/Abstract])) AND (((((((microbiota*[Title/Abstract]) OR "micro-biota*"[Title/Abstract]) OR microbiome*[Title/Abstract]) OR "micro-biome*"[Title/Abstract]) OR microbe*[Title/Abstract])) AND (((bacteria*[Title/Abstract] OR biologic*[Title/Abstract] OR microbiologic*[Title/Abstract] OR "micro-biologic*"[Title/Abstract])) AND (environment*[Title/Abstract] OR coloniz*[Title/Abstract] OR colonis*[Title/Abstract] OR characteriz*[Title/Abstract] OR characteris*[Title/Abstract])))) AND (("bronchopulmonary dysplasia"[Title/Abstract] OR "broncho-pulmonary dysplasia"[Title/Abstract] OR "chronic lung disease"[Title/Abstract]))) AND ((infant*[Title/Abstract] OR newborn*[Title/Abstract] OR "new-born*"[Title/Abstract] OR neonat*[Title/Abstract] OR "neo-nat*"[Title/Abstract] OR baby*[Title/Abstract] OR babies*[Title/Abstract] OR premature*[Title/Abstract] OR "pre-mature*"[Title/Abstract] OR preemie*[Title/Abstract] OR preterm*[Title/Abstract] OR "pre-term*"[Title/Abstract]))

2. MEDLINE by OVID Search

	Search terms	Number of articles retrieved
1	exp Lung/	254 816
2	exp TRACHEA/	35 084
3	(lung* or airway* or "air-way*" or trachea*).ti,ab,kw.	718 951
4	1 or 2 or 3	811 839
5	exp Microbiota/	15 751
6	(microbiota* or "micro-biota" or microbiome* or "micro-biome*" or microbe*).ti,ab,kw.	65 004
7	((bacteria* or biologic* or microbiologic* or "micro-biologic*") adj2 (environment* or coloniz* or colonis* or characteriz* or characteris*)).ti,ab,kw.	34 705
8	5 or 6 or 7	102 812
9	exp Bronchopulmonary Dysplasia/	3995
10	exp PULMONARY DISEASE, CHRONIC OBSTRUCTIVE/	47 574
11	("bronchopulmonary dysplasia*" or "broncho-pulmonary dysplasia*").ti,ab,kw.	5759
12	("lung disease*" adj3 chronic*).ti,ab,kw.	12 468
13	("chronic disease*" adj3 lung*).ti,ab,kw.	125
14	9 or 10 or 11 or 12 or 13	63 789
15	exp INFANT/	1 058 047
16	exp PREMATURE BIRTH/	10 621
17	(infant* or newborn* or "new-born*" or neonat* or "neo-nat*" or baby* or babies* or premature* or "pre-mature*" or preemie* or preterm* or "pre-term*").ti,ab,kw.	748 308
18	15 or 16 or 17	1 393 396
19	**4 and 8 and 14 and 18**	**45**

3. Embase search

1	'lung'/exp	344 959
2	'trachea'/exp	43 984
3	'lung*':ti,ab,kw OR 'airway*':ti,ab,kw OR 'air-way*':ti,ab,kw OR 'trachea*':ti,ab,kw	1 053 351
4	#1 or #2 or #3	1 141 022
5	'microflora'/exp	87 150
6	'microbiota*':ti,ab,kw OR 'micro-biota*':ti,ab,kw OR 'microbiome*':ti,ab,kw OR 'micro-biome*':ti,ab,kw OR 'microbe*':ti,ab,kw	83 179
7	((('bacteria*' NEAR/2 'environment*'):ti,ab,kw) OR (('bacteria*' NEAR/2 'coloniz*'):ti,ab,kw) OR (('bacteria*' NEAR/2 'colonis*'):ti,ab,kw) OR (('bacteria*' NEAR/2 'characteriz*'):ti,ab,kw)) AND (('bacteria*' NEAR/2 'characteris*'):ti,ab,kw)	70
8	((('biologic*' NEAR/2 'environment*'):ti,ab,kw) OR (('biologic*' NEAR/2 'coloniz*'):ti,ab,kw) OR (('biologic*' NEAR/2 'colonis*'):ti,ab,kw) OR (('biologic*' NEAR/2 'characteriz*'):ti,ab,kw)) AND (('biologic*' NEAR/2 'characteris*'):ti,ab,kw)	184
9	((('microbiologic*' NEAR/2 'environment*'):ti,ab,kw) OR (('microbiologic*' NEAR/2 'coloniz*'):ti,ab,kw) OR (('microbiologic*' NEAR/2 'colonis*'):ti,ab,kw) OR (('microbiologic*' NEAR/2 'characteriz*'):ti,ab,kw)) AND (('microbiologic*' NEAR/2 'characteris*'):ti,ab,kw)	14
10	((('micro-biologic*' NEAR/2 'environment*'):ti,ab,kw) OR (('micro-biologic*' NEAR/2 'coloniz*'):ti,ab,kw) OR (('micro-biologic*' NEAR/2 'colonis*'):ti,ab,kw) OR (('micro-biologic*' NEAR/2 'characteriz*'):ti,ab,kw)) AND (('micro-biologic*' NEAR/2 'characteris*'):ti,ab,kw)	-
11	#5 OR #6 OR #7 OR #8 OR #9 OR #10	35 314
12	'lung dysplasia'/exp	9750
13	'chronic lung disease'/exp	11 251
14	'chronic obstructive lung disease'/exp	108 377
15	'bronchopulmonary dysplasia*':ti,ab,kw OR 'broncho-pulmonary dysplasia*':ti,ab,kw	8361
16	('lung disease*' NEAR/3 'chronic*'):ti,ab,kw	19 235
17	('chronic disease*' NEAR/3 'lung*'):ti,ab,kw	245
18	#12 OR #13 OR #14 OR #15 OR #16 OR #17	135 126
19	'infant'/exp	1 050 819
20	'prematurity'/exp	100 134
21	'infant*':ti,ab,kw OR 'newborn*':ti,ab,kw OR 'new-born*':ti,ab,kw OR 'neonat*':ti,ab,kw OR 'neo-nat*':ti,ab,kw OR 'baby*':ti,ab,kw OR 'babies*':ti,ab,kw OR 'premature*':ti,ab,kw OR 'pre-mature*':ti,ab,kw OR 'preemie*':ti,ab,kw OR 'preterm*':ti,ab,kw OR 'pre-term*':ti,ab,kw	1 009 760
22	#19 OR #20 OR #21	1 518 839
23	**#4 AND #11 AND #18 AND #22**	**49**

4. Cochrane library

1	lung*:ti,ab,kw or airway*:ti,ab,kw or "air-way*":ti,ab,kw or trachea*:ti,ab,kw	62 295
2	microbiota*:ti,ab,kw or "micro-biota*" or microbiome* or "micro-biome*" or microbe*	2205
3	bacteria* or biologic* or microbiologic* or "micro-biologic*":ti,ab,kw	56 850
4	environment* or coloniz* or colonis* or characteriz* or characteris*:ti,ab,kw	93 078
5	#3 and #4	8533
6	"bronchopulmonary dysplasia" or "broncho-pulmonary dysplasia" or "chronic lung disease":ti,ab,kw	
7	infant* or newborn* or "new-born*" or neonat* or "neo-nat*" or baby* or babies* or premature* or "pre-mature*" or preemie* or preterm* or "pre-term*":ti,ab,kw	64 906
8	**#1 and #2 and #5 and #6 and #7**	**0**

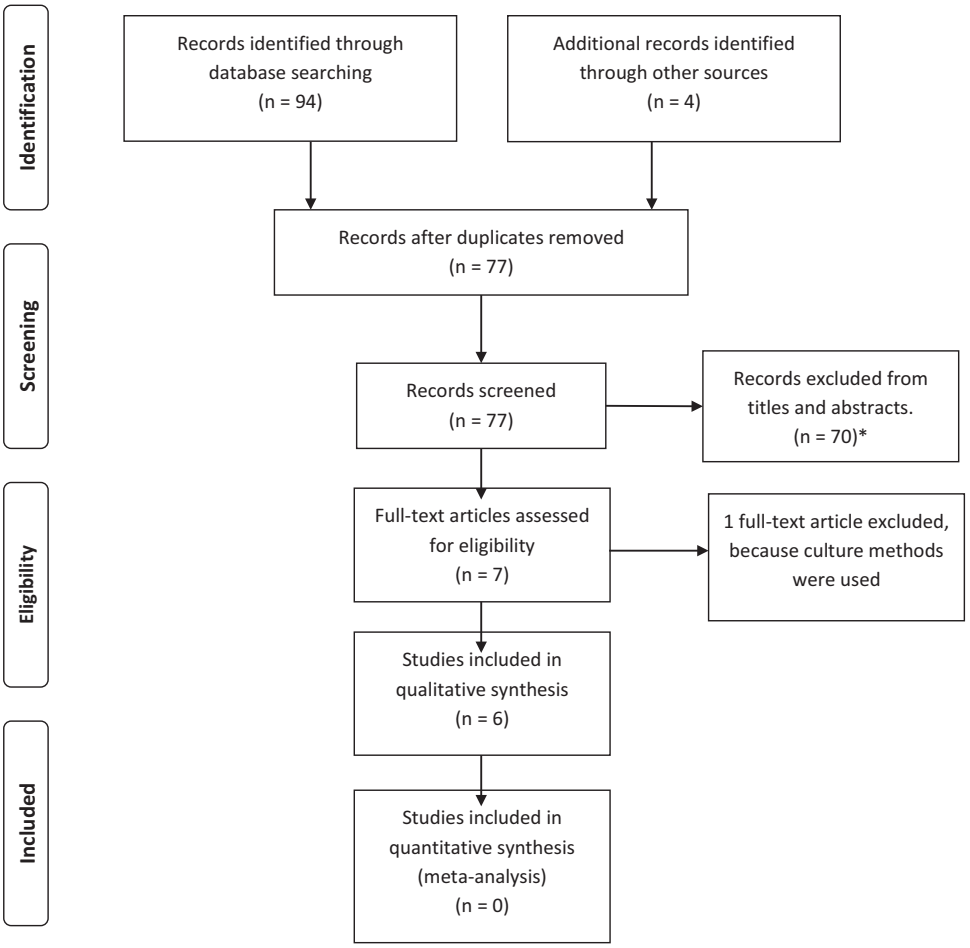

Figure. PRISMA flow diagram depicting the results of our literature search; the number of records identified, included, and excluded; and the reasons for exclusions. *Seventy excluded studies did not address the participants of interest (preterm neonates), method of evaluation (microbiome techniques), or outcome of interest (BPD).

Table II. Methodological assessment of the included studies

Assessment criteria	Payne et al, 2010[20]	Stressmann et al, 2010[19]	Mourani et al, 2011[21]	Lohmann et al, 2014[22]	Lal et al, 2016[23]	Wagner et al, 2017[24]
Do inclusion/exclusion criteria vary across comparison groups?	No	No	No	No	No	No
Is the selection of the comparison group inappropriate?	No	No	No	No	No	No
Were valid and reliable measures (outcomes) applied consistently across all study participants?	Yes	Yes	Yes	Yes	Yes	Yes
Are any important primary outcomes missing from the results?	No	No	No	No	No	No
Are results believable given the study limitations? (overall quality of the study)	Yes	Yes	Yes	Yes	Yes	Yes
To assess confounding						
Was these any attempt to balance allocation between groups (eg, stratification, matching)	No	No	No	No	No	No
Were important confounding variables (GA or birth weight) taken into account in the design/ and or analysis (by, eg, matching, stratification, multivariate analysis)?	No	No	No	No	Yes	No

Assessing methodological quality based on key components of study design is recommended over assigning quality scores in observational studies. The inclusion and exclusion criteria were similar in the comparison groups. Molecular methods of microbiome analysis were reported for all patients in all studies. All studies had appropriate comparison groups and reported all of our prespecified outcomes, and results were believable given the study limitations. Only 1 of the 6 included studies addressed the issue of confounding by matching or stratification to avoid known confounders.