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MAJOR ARTICLE

Bacterial Colonization and Antibiotic Resistance in a Prospective Cohort of Newborn Infants During the First Year of Life

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Background. Infants are virtually sterile at birth and frequently use antibiotics; our objective was to (1) characterize the longitudinal colonization with bacterial pathogens and associated antibiotic resistance in a cohort of community-dwelling infants in Northeast Ohio and (2) describe longitudinal concurrent antibiotic and daycare exposures.

Methods. For 35 newborns, nasopharyngeal swabs were cultured for Streptococcus pneumoniae, anterior nasal for Staphylococcus aureus, and perirectal for extended-spectrum beta-lactamase (ESBL)-producing Gram-negative enteric bacteria, at 3-month intervals for 12 months. Infant and household antibiotics and daycare exposure were assessed longitudinally.

Results. Thirteen infants received perinatal or nursery antibiotics. By 3 months, at least 22 were colonized with Gram-negative bacteria; 2 with S pneumoniae (type 19A, resistant; 15C, susceptible), 5 with methicillin-susceptible S aureus. By 12 months, at least 22 of 35 infants received antibiotics, 20 had household members with antibiotics, and 12 attended daycare; 7 more had household members with daycare exposure. The ESBL-producing organisms were not identified. At least 10 infants were colonized at some time with an antibiotic-resistant organism, 3 more with pathogens displaying intermediate resistance. Pathogen colonization and resistance were intermittent and inconsistent.

Conclusions. In a community-based cohort followed from birth, early antibiotic and daycare exposures are common, especially considering perinatal maternal exposures. Colonization patterns of Gram-negative bacteria, S pneumoniae, S aureus, and resistant pneumococci are strikingly dynamic. Further research can identify key areas for potential interventions to maximize clinical antibiotic outcomes while minimizing future resistance.

Keywords. antibacterial agents; drug resistance; infant; *Staphylococcus*; Streptococcus pneumonia.

Young infants in the United States receive, on average, >1 antibiotic course per year [1], and children can be major community reservoirs of resistant organisms, especially transmittable under relatively crowded conditions, such as within families and child-care settings [2, 3]. Much of the US data on the temporal course of infant colonization come from before the pneumococcal conjugate vaccine era and before the rising prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing Gram-negative organisms [4–11]. Most interest in pediatric colonization with resistant organisms has focused on *Streptococcus pneumoniae*, *S aureus*, and *Enterobacteriacae* resistant to the expanded-spectrum β-lactam antibiotics (ESBL-producing organisms) [7–13].

Although almost sterile before birth, infants' nasopharyngeal and gastrointestinal tracts become progressively colonized with a diverse, complex, and interacting array of microorganisms [14]. This creates an opportunity to study colonization and resistance starting from a microbiological "tabula rasa." The objective of this study was to characterize the longitudinal colonization with bacterial pathogens and associated antibiotic resistance in a cohort of community-dwelling infants in Northeast Ohio, to describe antibiotic and daycare exposures, and to pilot procedures for a larger cohort study powered for hypothesis testing.

METHODS

In this prospective community-based observational study, we enrolled a cohort of 35 infants, and we longitudinally assessed bacterial colonization of the respiratory and gastrointestinal tracts every 3 months during their first year of life. A sample size of 35 was feasible with our available resources, which was adequate to study baseline parameters for these infants and to pilot necessary procedures.

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Eligibility and Enrollment

Research associates offered enrollment to a convenience sample of mothers of newborn infants at a large urban university-affiliated hospital in Cleveland, Ohio from June 1, 2011 to November 11, 2011. Inclusion criteria required the following: the infant was admitted to the well baby nursery; the mother was ≥18 years of age, had legal custody, and could read, speak, and understand the English language; and infants' and mothers' physicians had provided consent to approach the infant's mother. An infant was excluded from enrollment if he/she was transferred out of the well baby nursery to the neonatal intensive care unit.

Data Collection

Mothers were interviewed to obtain demographic data and pregnancy-related medical history, including infant gestational age in weeks, mode of delivery, maternal infections, and antibiotic exposure during pregnancy, delivery, or after delivery, and whether infants received antibiotics after delivery. Additional information collected included nutrition, age of all additional members of the infants' households and their current antibiotic use, household daycare attendance (defined as out-of-home care including >5 children attended >3 days per week and >4 hours per day) [15], and household member smoking status. Infants' birth weight and length were obtained from nursery records, and baseline cultures were obtained; these included nasopharyngeal cultures for *S pneumoniae*, anterior nasal cultures for *S aureus*, and perirectal cultures for Gram-negative enteric bacteria.

At follow-up visits (scheduled at 3, 6, 9, and 12 months of age), mothers were asked about their infants' and household's interim history since the previous study visit, including infant and household antibiotic use, breast versus formula feeding versus both, daycare attendance, hospital admissions, and household smoking. Each follow-up visit included repeat infant nasopharyngeal, anterior nasal, and rectal cultures.

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools. This study was approved by the University Hospitals of Cleveland Institutional Review Board, and parental written informed consent was obtained.

Cultures

Nasopharyngeal specimens were inoculated into Todd-Hewitt broth supplemented with yeast extract and serum for transport. On receipt in the laboratory, broths were incubated for 6 hours and then subcultured onto blood agar plates for pneumococcal isolation, susceptibility, and serotyping, using the Centers for Disease Control and Prevention *Streptococcus* laboratory protocol [16]. Anterior nasal swabs were directly inoculated onto mannitol salt agar, and *S aureus* isolates were tested for methicillin, erythromycin, and clindamycin susceptibility by disk diffusion. Rectal swab specimens were diluted 1:100 in sterile saline and inoculated onto 2 MacConkey agar plates, 1 containing cefotaxime at 2 μg/mL, and recovered colonies were identified and susceptibility testing performed by disk diffusion from cefotaxime-containing plates [16–18]. Susceptibility testing for *S pneumonia* and *S aureus* were performed by broth microdilution and interpreted according to methods and standards of the Clinical and Laboratory Standards Institute (pages and specific antibiotics tested listed in Table A1) [16]. Pneumococcal and staphylococcal isolates were categorized as either showing sensitivity, intermediate resistance, or resistance to tested antibiotics (Table A1).

Antibiotics

For follow-up study visits, the infant was considered antibiotic-exposed if he/she received antibiotics since the previous visit (or within the previous 3 months, if the previous visit was missed). Household member exposures were similarly defined.

Outcomes and Analysis

We report colonization with *S pneumoniae*, *S aureus*, *Escherichia coli*, antibiotic-resistant *S pneumoniae* and *S aureus*, and third-generation cephalosporin-resistant Gram-negative bacteria. Pneumococcal isolates were stratified as either serotypes included in the 13-valent pneumococcal conjugate vaccine or nonvaccine serotypes.

Pearson's correlation coefficient was calculated using Stata SE 13.1, StataCorp. With 35 infants, we did not have the power for hypothesis testing; accordingly, we planned a descriptive analysis.

RESULTS

Participants

In this study, 35 infants were enrolled and 18 were male (Table 1). Mean gestational age was 39.2 weeks (standard deviation = 1.2; range, 35.9–41.3 weeks). Thirteen mothers reported

Table 1. Infant Characteristics at Enrollment

Nursery Cohort N = 35		Standard Deviation	Range
Male, N	18		
Residence, N			
North Cleveland or East Cleveland	17		
Other	18		
Gestational age, in weeks, mean	39.2	1.2	35.9–41.3
Growth parameters, mean			
Weight, kg	3.3	0.56	2.3–4.3
Length, cm	49.6	2.4	45.0–53.7
Antibiotics, N			
Maternal			
Pregnancy	13		
Labor/delivery	12		
Postdelivery	1		
Infant			
Postdelivery	2		
During or postdelivery	13		

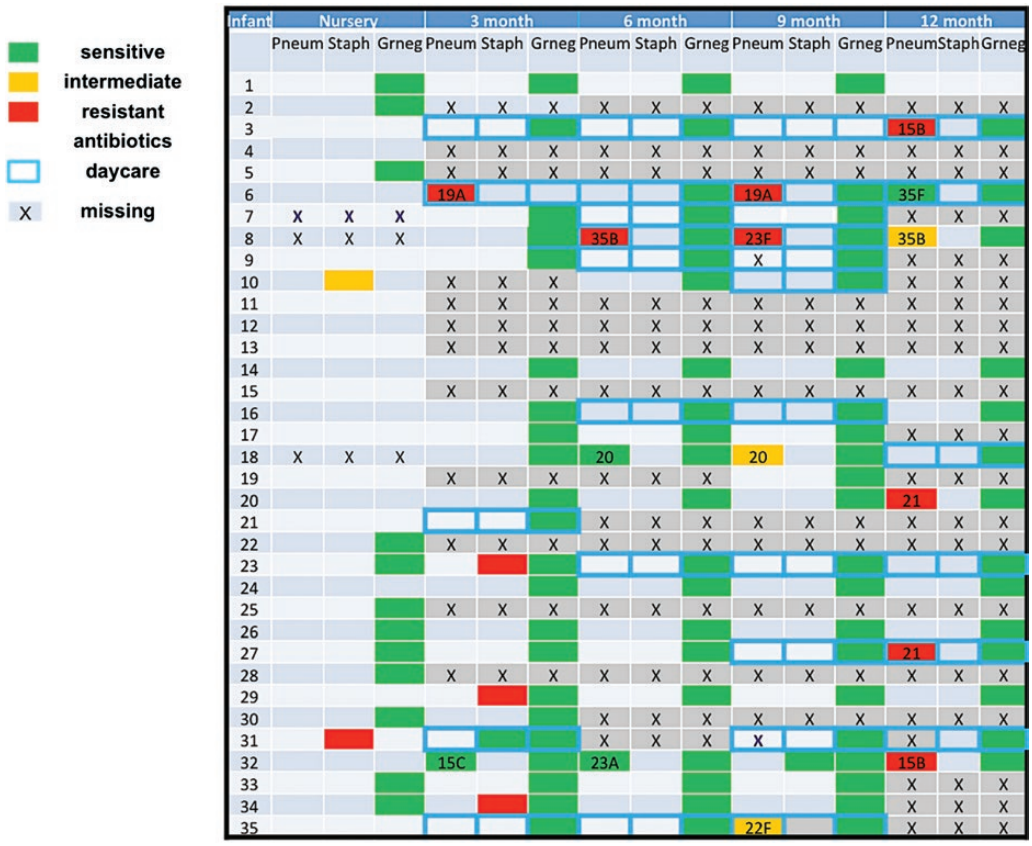

Figure 1. Longitudinal antibiotic and daycare exposures, colonization with *Streptococcus pneumonia*, *Staphylococcus aureus*, and Gram-negative enteric bacteria and associated antibiotic resistance for each infant over the first year of life. Each row represents longitudinal data for 1 infant for each of the study visits, every 3 months over the first year of life. X means the visit was missed. Cultures: Column under each visit (0, 3, 6, 9, and 12 months of age) indicates colonization with that organism: Pneum, *Strreptococcus pneumoniae*; Staph, *Staphylococcus aureus*; Grneg, Gram-negative enteric bacteria. Color indicates whether the organism was sensitive to all antibiotics tested (green), was resistant to at least 1 antibiotic (red), or showed intermediate resistance (yellow). Pneumococcal isolates were tested for penicillin, amoxicillin, ceftriaxone, azithromycin, clindamycin, and levofloxacin resistance. Staphylococcal isolates were tested for oxacilln, erythromycin, clindamycin, ciprofloxacin, and trimethoprim-sulfamethoxazole resistance. Serotype is indicated for pneumococcal organisms. Exposures: Outline indicates whether that infant had been exposed to antibiotics (dark blue) since the previous study visit, exposed to daycare (bright blue), or exposed to both.

receiving antibiotics during pregnancy: 12 during delivery and 1 after delivery.

Exposures

Delivery mode data were not collected until the midpoint of the study. Of the 13 infants for whom we have delivery mode, 5 were delivered by C-section and 8 were delivered vaginally.

Tables 1 and 2 and Figure 1 depict infant antibiotic exposure reported at each study visit. Two infants received antibiotics in the nursery after delivery; 13 were either exposed to antibiotics during labor or after delivery. By 12 months of age, at least 22 of the 35 enrolled infants were exposed to antibiotics at least once, at least 6 were exposed twice, and 2 were exposed to antibiotics 3 times.

Table 2 depicts infant and household antibiotics, daycare, and other exposures. By 12 months, at least 20 of the 35 enrolled infants had ever had another household member exposed to antibiotics. By 12 months, at least 14 of 35 infants were exposed to daycare and at least 19 had either personal or household member daycare. Infant exposure to antibiotics and infant daycare were correlated (r = 0.28, P = .002) (Figure 2). By 12 months of age, 7 infants had been exposed to household tobacco smoking.

At enrollment, 20 mothers intended to exclusively breastfeed their infants, 10 intended exclusive formula feeding, and 5 used both breastfeeding and formula. Table 2 presents the number of infants exclusively breastfeeding and the number receiving some breastfeeding at each of the study visits. By 3 months of age, most of the breastfeeding infants were also receiving some infant formula

Bacterial Colonization

Table 2 and Figure 1 summarize culture results among the infants presenting to each study visit. In Figure 1, each row represents longitudinal data for 1 infant, over each of the study visits—at birth and 3, 6, 9, and 12 months of age. We isolated pneumococcus from nasopharyngeal swabs, *Staphylococcus* from anterior nares swabs, and Gram-negative enteric organisms from perirectal swabs. Antibiotic resistance is shown for isolated species (see Figure 1): red

Table 2. Infant Characteristics Over the Study Period

N = 35	Nursery, N = 35	3 Months, N = 24	6 Months, N = 21	9 Months, N = 23	12 Months, N = 15
Infant antibiotics	13	2	8	5	2
Infant daycare	0	5	8	10	6
Infant exclusive breastfeeding	20	8	5	4	1
Infant any breastfeeding	25	14	11	8	2
Immunizations	N/A	24	20	9	8
Number of household members (mean)	3.4	3.4	3.9	3.1	2.9
Number of household members <2 years (mean)	0.2	0.1	.3	0	0
Number of household members <5 years (mean)	0.9	0.8	0.8	0.6	0.6
Household antibiotics, any	12	8	7	4	1
Household daycare, any	11	7	4	6	3
Household smoking, any	X	5	3	4	2
Infant colonization, any					
Pneumococcus, any	0	2	3	4	6
Resistant pneumococcus, any	0	1	1	4	5
Staphylococcus, any	2	4	0	1	0
Resistant *Staphylococcus*	2	3	0	0	0
Gram-negative enteric, any	12	22	21	22	14

Abbreviations: N/A, not applicable; X, not asked.

for resistant to at least 1 organism, yellow for intermediate resistance, and green for organisms sensitive to all antibiotics. Figure 1 also depicts infants' longitudinal antibiotic and daycare exposure, with dark and bright blue outlines, respectively. Figure 1 demonstrates that colonization was dynamic, with varying colonizing organisms, serotypes, and resistances during the 12 months of the study. By 12 months of age, 10 infants were colonized at some time with an antibiotic-resistant organism, 3 more with pathogens displaying intermediate resistance.

Gram-Negative Enterics

Twelve of the 35 infants had Gram-negative enteric organisms recovered in the nursery. By 3 months of age, 22 of 23 infants cultured harbored Gram-negative enteric organisms. Despite instances of antibiotic exposure, no ESBL-producing organisms were isolated.

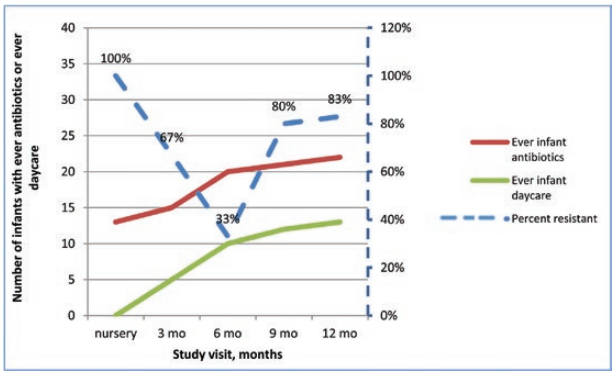

Figure 2. Infant cumulative antibiotic and daycare exposures and colonization with antibiotic-resistant *Streptococcus pneumonia* and *Staphylococcus aureus*.

Streptococcus Pneumoniae

We found a total of 15 pneumococcal isolates in 8 different infants; 3 isolates were 13-valent pneumococcal conjugate vaccine (PCV13) strains (19A, 23F). Two infants were colonized with pneumococci by 3 months of age, 1 was pansensitive, and 1, from an infant enrolled in daycare, showed intermediate resistance to ceftriaxone and resistance to all other tested antibiotics except levofloxacin; although these infants did not receive antibiotic treatment in the nursery, both were born to mothers who received antibiotics during labor or delivery. Of the 3 isolates at 6 months, 2 were pansensitive and 1 was resistant to penicillin and amoxicillin, with intermediate resistance to ceftriaxone. Of the two 9-month estimates, 1 was pansensitive and 1 was resistant to penicillin, amoxicillin, azithromycin, and clindamycin with intermediate resistance to ceftriaxone. Finally, at 12 months, 2 isolates were pansensitive, and 1 was resistant to penicillin and amoxicillin and 1 with intermediate resistance to ceftriaxone, Although 4 isolates were antibiotic susceptible, at some point during their first year of life, each of the 8 infants colonized with pneumococci harbored an organism expressing at least intermediate resistance to at least 1 antibiotic. Two infants with resistant pneumococcal isolates were exposed to antibiotics during the preceding 3 months, and all colonized infants were exposed to antibiotics at some time before that study visit.

We isolated PCV13 serotype 19A twice, at 3 months and at 9 months. We also isolated a pneumococcal 7-valent (PCV7) isolate, 23F, at 9 months. All vaccine-related isolates exhibited resistance to penicillin and at least intermediate resistance to ceftriaxone.

As depicted in Figure 1, serotypes and susceptibilities were highly variable within individual infants. For example, 1 infant was colonized with a resistant serotype 35B pneumococcus

at 6 months, a resistant serotype 23F at 9 months, and by 12 months again with serotype 35B but with intermediate resistance. Another infant had a resistant serotype 19A pneumococcus isolated at 3 and 9 months, and at 12 months the infant harbored a pansusceptible serotype 35F strain. Only 1 infant was colonized with the same serotype, serotype 20, for 2 consecutive visits, with a pansusceptible organism at 6 months and an organism with intermediate susceptibility to ceftriaxone at 9 months; neither this infant nor a household member were exposed to antibiotics during this time period, but a household member did attend daycare. A different infant was colonized with a penicillin-resistant serotype 23A at 3 months and at 9 months, but this organism was not isolated at 6 months. For the single infant with a susceptible pneumococcal isolates who had recurrence of the same serotype later in the year, the recurrent isolate exhibited resistance (serotype 20).

Staphylococcus Aureus

Seven *S aur*eus isolates were recovered from 6 infants. Two infants were colonized with *S aureus* in the nursery: 1 with intermediate resistance only to erythromycin and the other erythromycin resistant. At 3 months, 4 infants had *S aureus* isolates, 1 of whom was sensitive to all tested antibitics; the other 3 were erythromycin resistant. Only 1 infant possessed staphylococci isolated after 3 months of age: at 9 months, this infant harbored an isolate sensitive to all tested antibiotics. Of the 7 isolates, all were methicillin- and clindamycin-susceptible, and 5 exhibited at lease intermediate resistance to erythromycin. One infant with erythromycin-resistant *S aureus* isolated at birth was exposed to antibiotics through maternal antibiotic treatment during labor or delivery. No clindamycin-resistant organism was isolated from an infant with previous antibiotic or daycare exposure. All *S aureus* isolates were susceptible to trimethoprim sulfamethoxazole (Table 2, Figure 1). Again, colonization was dynamic, with persistent or recurrent colonization only apparent in 1 infant colonized with erythromycin-resistant *S aureus* in the nursery, who had a pansusceptible strain recovered at the 3-month visit; this infant attended daycare during this interim 3-month period but was exposed to no further antibiotics.

Figure 2 shows the number of infants at each study visit who had ever been exposed to antibiotics and who had ever attended daycare, alongside the percentage of colonizing *S pneumonia* and *S aureus* organisms isolated during that study period that showed at least intermediate resistance to at least 1 antibiotic. Although daycare and antibiotic exposure are correlated, antibacterial resistance looks more inconsistant.

Two infants were hospitalized during the study period; both received parenteral treatment with third-generation cephalosporin antibiotics during their hospital stay (Figure 1). Infant no. 6 was admitted with respiratory syncytial virus bronchiolitis between 3 and 6 months of age. For this infant, pneumococcus serotype 19A, resistant to at least 1 of the tested antibiotics, was isolated at 3 months and again at 9 months but not at the 6-month visit after the hospital admission. Infant no. 8 was admitted with an *E coli* urinary tract infection (pansensitive) between birth and the 3-month visit. No pneumococcus was isolated at 3 months; this infant was subsequently colonized with pneumococcus at each of the follow-up visits, at 6, 9, and 12 months, serotypes 35B (resistant), 25F (resistant), and 35B (intermediate), respectively. None of the enrolled infants experienced invasive pneumococcal or staphylococcal infections during their study period.

DISCUSSION

Although hospital-acquired colonization and infections have received recent attention [19, 20], there are scarce previous data describing how community-dwelling newborn infants become colonized with potentially pathogenic organisms, especially in the post-PCV13 era. Our data add some important contributions in this area.

Similar to previous studies, Gram-negative enteric colonization was already established in some infants in the nursery and in almost all tested infants by 3 months of age [21, 22]. Among infants in this cohort, despite their common antibiotic exposure at birth and during their first year of life, no ESBL-producing Gram-negative bacteria were isolated from these infants.

We found that colonization with pneumococci and staphylococci was intermittent and strikingly dynamic in our small cohort. Five of our 6 infants with *S aureus* were colonized by 3 months of age, none with MRSA; antibiotic-resistant *S aureus* was not isolated for 2 consecutive visits from any infant. Previous studies found that staphylococcal colonization was most prevalent early during the first year but tended to report higher prevalence of MRSA, up to 50%, and longer duration of carriage from 24 to 40 months, even with decolonization efforts [23, 24]. Groups believed to be at increased risk of infection with MRSA include neonates and children in general, urban underserved populations, and daycare attendees, all highly represented in our infant population.

In a pre-PCV7 cohort, most infants became colonized with their first strain of pneumococcus by 6 months of age [6]. In a post-PCV7 but pre-PCV13 cohort, 31% of children <24 months were colonized with *S pneumoniae* [25]. Post-PCV13 introduction, by 2011–2012, only 13% of Canadian children were colonized with *S pneumoniae* at 12–18 months [26]. Antibiotic susceptibility was not addressed in these previous studies. In our cohort, by 12 months at least 8 of 35 infants were colonized with pneumococci, and only 1 of 6 isolates was susceptible to all tested antibiotics. Only 2 vaccine-related organisms were isolated, and both of these were antibiotic resistant.

We found that although only 1 infant was colonized with the same pneumococcal serotype for 2 successive months, 2 additional infants eventually experienced recurrence of a similar isolate at subsequent study visits; 2 of 3 of these repeat appearances

exhibited different resistance profiles. It is of interest to note that in 30% of their longitudinal cohort, Gray et al [6] reported "reacquisition" of a previously carried type; it is not clear whether our infants reacquired these bacteria or whether they were actually present but in lower numbers and were not cultured [27].

Increasing evidence supports the importance of early antibiotic exposure on the developing infant intestinal microbial community [14]. Most previous studies considering bacterial colonization and related antibiotic resistance in the newborn infant consider only infant's direct antibiotic treatment after delivery [24, 28, 29]; however, maternal antibiotics administered during labor and delivery also expose the infant. For this reason, we report neonatal antibiotic exposure both ways, with or without considering maternal antibiotics received during labor or delivery. Only 2 of 35 infants were directly antibiotic-exposed after delivery during the neonatal period; however, 13 had antibiotic exposure if maternal antibiotics are also taken into account. This finding strongly suggests that maternal antibiotic should be considered when describing and studying newborn exposure. Many previous studies took a cross-sectional approach and did not consider previous episodes of antibiotic use and other individual- and household-level covariates [30–37]. This study suggests that each individual antibiotic exposure cannot be considered in isolation; this is supported by other work at the molecular level [38]. Considering breastfeeding as an ordinal, continuous, or time-varying, rather than dichotomous, covariate may better described the mixed nutrition sources experienced by many infants.

Our main limitation is small sample size and loss to follow up, although our retention is comparable with previous studies [39]. Our small sample size allowed us to focus attention on specific details and patterns of these infants' exposures and colonization. Athough others found good performance from perirectal relative to stool cultures, our yield was low [40]; we preferred stool specimens, but obtaining them was surprisingly challenging in the outpatient setting. This study served as a pilot to support a larger cohort study for which we are completing data collection, powered to assess the quantitative relationship between antibiotic use and resistant colonization during the first year of life; the role of other infant, household, and environmental exposures will also be explored. For a subset of these infants, we will use 16S ribosomal ribonucleic acid molecular techniques to examine the comprehensive gastrointestinal microbiome. Moreover, our technique isolated only the predominant bacterial organisms; culture-based methods do not detect the diversity of colonizing organisms. Finally, we were not able to validate parent history of infant and household antibiotic use.

CONCLUSIONS

In summary, in a community-based cohort followed from birth, early antibiotic exposures are common, especially considering perinatal maternal exposures. Colonization patterns of Gram-negative bacteria, *S pneumoniae*, *S aureus*, and resistant pneumococci and staphylococci were strikingly dynamic over time, even within the same infant. Future research, including a larger cohort study in progress, will shed further light on the process of early colonization, the effects of early antibiotic exposure on infants' future colonization with antibiotic reslstant bacteria, and the impact of daycare and household exposures. Ultimately, we must identify key areas for potential interventions to maximize antibiotics' clinical outcomes while minimizing future resistance [41–45].

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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APPENDIX

Table A1. Pneumococcus and *Staphylococcus* Antibacterial Resistance Testing

Organism	Antibacterial Drugs Tested	Reference Table and Pages Performance Standards for Antimicrobial Susceptibiilty Testing: Twenty-Fourth Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute; January 2014. M100-S24 [1]
Streptococcus pneumoniae		
	Penicillin	Table 2G, p. 88–92
	Amoxicillin	
	Ceftriaxone	
	Azithromycin	
	Clindamycin	
	Levofloxacin	
Staphyococcus aureus		
	Oxacillin	Table 2C, p. 68–75
	Erythromycin	
	Clindamycin	
	Ciprofloxacin	
	Sulfamethoxazole	
Enterobacteriaceae		
	Ceftazidime	Table 2A, p. 50–57