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BRIEF REPORT

Rapid Changes in Nasopharyngeal Antibiotic Resistance Gene Profiles After Short Courses of Antibiotics in a Pilot Study of Ambulatory Young Children

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We quantified antibiotic resistance genes before and after short antibiotic courses in nasopharyngeal specimens from ambulatory children. Carriage of certain bacteria and resistance genes was common before antibiotics. After antibiotics, we observed substantial reductions in pneumococcal and *Staphylococcus aureus* carriage and rapid expansion in the abundance of certain resistance genes.

Keywords. acute respiratory illness; antibiotic resistance; antibiotics; children; *Pneumococcus*; *Staphylococcus*.

Antibiotics are commonly prescribed for acute respiratory illnesses (ARIs) in children [1]. While often necessary, antibiotics are associated with several adverse effects, which may increase health care utilization and costs [2] and promotion of antibiotic resistance [3, 4]. Use of common antibiotics reduces nasopharyngeal (NP) colonization with specific pathogens, including pneumococcus, *Moraxella catarrhalis*, and *Haemophilus influenzae*, and leads to selection of antibiotic-resistant strains after treatment [5]. However, the overall impact of antibiotic use in the nasopharynx, including colonizing commensal bacteria, has not been clearly characterized.

We conducted a prospective study to examine changes in the abundance of common antibiotic resistance genes following short courses of antibiotics for ARI in young children. These assessments were independent of detection of specific colonizing bacteria. We hypothesized that antibiotic use leads to expansions in certain antibiotic-resistant genes in the nasopharynx, beyond what has been previously described in pathogen-specific studies.

METHODS

From August 2019 through March 2020, we enrolled a convenience sample of children aged >2 months and <5 years who were seen at a Vanderbilt outpatient clinic and prescribed an antibiotic for ARI (Table 1). Children were excluded if they had a tracheostomy, received home health nursing, resided outside of Davidson County, or had a caregiver who worked/studied in the medical field or was not English-speaking.

Patient Consent

The study was approved by the Vanderbilt University Institutional Review Board (IRB#190885). Caregivers of all participants provided written informed consent to participate before any study activities were conducted.

After written informed consent, a pre-antibiotic NP swab specimen was obtained, and a follow-up home visit was scheduled 6–8 days after enrollment, when a second, postantibiotic NP specimen was collected. Specimens were placed in skim milk, tryptone, glucose, and glycerol (STGG) medium, transported in a cooler, and stored at –80°C within 2 hours.

NP specimens were tested by quantitative polymerase chain reaction (qPCR) for several resistance genes commonly present in *Staphylococcus aureus* (*mecA*, *ermA*, *ermB*, and *ermC*) and *Streptococcus pneumoniae* (*mef*, *ermB*, and *pbp2b*) (Supplementary Table 1) [6–12]. See the Supplementary Materials for a detailed description of the methods. Briefly, to compare between baseline (pre-antibiotic) and follow-up (postantibiotic) time points, cycle threshold (CT) values were normalized using human RNase P for the total amount of human DNA in the specimen and reported as relative quantity (RQ), calculated from delta-delta CT (ddCT) values and representing the fold change of gene expression relative to control. qPCR was performed in triplicate, and mean RQ was reported. Specimens were also plated on trypticase soy agar (TSA) with 5% sheep blood agar to assess for growth of *Staphylococcus aureus* and *Streptococcus pneumoniae* [13, 14].

RESULTS

Twenty-five children met selection criteria, and 13 agreed to enroll in the study. One participant was lost to follow-up; thus 12 pairs of NP specimens were collected. Almost half of children were <12 months of age, 54% were female, and 85% were Black

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Table 1. Characteristics of Study Cohort, Nashville, Tennessee

Characteristics	No. of Participants (%) (n = 13)
Age	
2–12 mo	6 (46)
12–24 mo	5 (38)
24–60 mo	2 (15)
Gender	
Male	6 (46)
Female	7 (54)
Race	
Black	11 (85)
White	1 (8)
Hispanic	1 (8)
Diagnosis	
Acute otitis media	10 (77)
Preseptal cellulitis	2 (15)
Streptococcal pharyngitis	1 (8)
Antibiotic	
Amoxicillin	12 (92)
Clindamycin	2[a] (15)

[a]One participant received both amoxicillin and clindamycin.

(Table 1). Most children were diagnosed with acute otitis media, and 12/13 were prescribed amoxicillin (Supplementary Table 2).

Streptococcus pneumoniae

At baseline, pneumococcus was detected in 6 children by culture and *lytA* PCR (Figure 1A); all 13 children had detection of 2 or more pneumococcal antibiotic resistance genes. At follow-up, pneumococcus was detected in only 1 child. Among 12 participants with paired specimens, 11 had detection of 2 or more pneumococcal resistance genes at follow-up. In 6 of 7 children with *pbp2b* carriage at baseline, the gene was undetectable at follow-up (Figure 1B). All 12 children had *mef* detection at both baseline and follow-up; 8 demonstrated an increase in RQ at follow-up (Figure 1C). Among 10 with *ermB* detection at baseline, 9 had the gene detectable at follow-up, and 7 demonstrated an increase in RQ at follow-up. One child had new detection of *ermB* at follow-up (Figure 1D).

Staphylococcus aureus

Staphylococcus aureus was detected in 2/13 children at baseline and 0/12 children at follow-up. Nine of 13 had detection of at least 1, and 6/13 had 2 or more *S. aureus*–associated genes (*mecA*, *ermA*, *ermB*, and *ermC*) at baseline. At follow-up, 7/12 children had at least 1 *S. aureus* resistance gene detected, while 6/12 had 2 or more. Only 1 child had *ermA* detected at baseline, which was not detected at follow-up (Figure 1E). Among 6 children with *mecA* detection at baseline, levels increased in 1, decreased in 2, and were undetectable in 3 at follow-up. Three patients had new detection of *mecA* at follow-up (Figure 1F). Four children had *ermB* detection at baseline; 2 exhibited increases and 2 exhibited decreases in RQ at follow-up (Figure 1G). Two children had new detection of *ermB* at follow-up. Among 4 children with *ermC* at baseline, all demonstrated an increase in RQ, and 2 children had new detection of *ermC* at follow-up (Figure 1H).

DISCUSSION

Detection of antibiotic resistance genes was common in our cohort of young children, even before starting antibiotics. After 7 days of antibiotic treatment, pneumococcal and *S. aureus* nasopharyngeal colonization declined, with only 1 child remaining

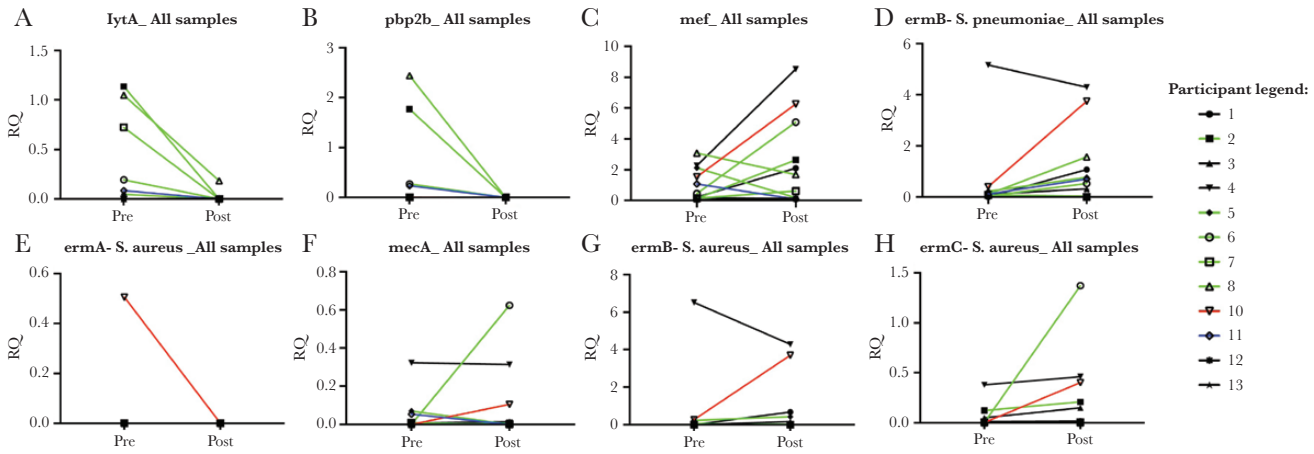

Figure 1. Measure of relative quantity (RQ) of antibiotic resistance genes, calculated from delta-delta cycle threshold via quantitative polymerase chain reaction analysis: (A) *lytA*, (B) *Streptococcus pneumoniae pbp2b*, (C) *Streptococcus pneumoniae mef*, (D) *Streptococcus pneumoniae ermB*, (E) *Staphylococcus aureus ermA*, (F) *Staphylococcus aureus mecA*, (G) *Staphylococcus aureus ermB*, and (H) *Staphylococcus aureus ermC*. The x-axes represent the pre- and postantibiotic visits. The y-axes represent the RQ of the antibiotic-resistant genes. Each participant is represented by a different shape, listed in the participant legend. The green lines reflect participants in whom *Streptococcus pneumoniae* was isolated by culture, the red lines reflect participants in whom *Staphylococcus aureus* was isolated by culture, the blue lines reflect participants in whom both *Streptococcus pneumoniae* and *Staphylococcus aureus* were isolated by culture, and the black lines reflect participants in whom neither *Streptococcus pneumoniae* nor *Staphylococcus aureus* was isolated by culture.

colonized with pneumococcus, and none with *S. aureus*. Despite these declines, the frequency and abundance of certain genes that encode for macrolide or macrolide-lincosamide-streptogramin (MLS) patterns of resistance increased in many children, suggesting that antibiotic treatment influences the composition of the nasopharyngeal resistome after only a few days. Given that nasopharyngeal colonization with pneumococcus and *S. aureus* was eradicated in nearly all children after 7 days of antibiotics, persistent detection of resistance genes suggests that other colonizing commensal bacteria may be an important reservoir of those genes, with the potential for horizontal transfer to more pathogenic co-colonizing organisms. A previous study demonstrated that commensal streptococci, nearly ubiquitous constituents of the human nasopharyngeal microbiome, frequently serve as reservoirs of beta-lactam resistance genes, which can subsequently be transferred to *S. pneumoniae* [15]. While *S. pneumoniae* and other pathogens may carry these resistance genes, with important clinical implications, our findings may suggest that treatment regimens targeting these infections may not be sufficient to contain the reservoir of these genes, and in fact may be associated with expansion of these genes, in an individual.

Disappearance of the pneumococcal penicillin resistance gene *pbp2b* in postantibiotic specimens, coinciding with near eradication of pneumococcal carriage, suggests that *pbp2b* may be more specific to pneumococci than other genes tested, which frequently persisted or expanded even when pneumococcal carriage was reduced. Rapid emergence or increased abundance of *S. aureus*–associated *ermA*, *ermB*, and *ermC*, associated with MLS resistance patterns, and *mecA*, associated with methicillin resistance (MRSA), after antibiotic exposure may have important implications for empiric treatment of suspected *S. aureus* infections.

Because we were interested in the early changes in antibiotic resistance genes associated with antibiotic use, we selected the 7-day time point for follow-up, suspecting that many children would continue to receive antibiotics at this time, given that guidelines support up to a 10-day duration of antibiotics for several common ARI diagnoses including otitis media, streptococcal pharyngitis, and pneumonia. Given that a single follow-up time point at 7 days after initiation of treatment was used, we are unable to ascertain the time at which any changes began or the duration of these changes with continued antibiotic exposure or after antibiotics were discontinued.

The limitations of our study include limited generalizability with small sample size given early study termination due to the coronavirus disease 2019 pandemic and enrollment restricted to English-speaking families. We enrolled a convenience sample of participants, who may not precisely reflect the sociodemographic composition of the overall clinic patient population. We selected a limited pool of resistance genes for this pilot study; several clinically relevant or emerging resistance genes associated with *S. pneumoniae* or *S. aureus* were not assessed. Without a control group, it is difficult to understand whether observed changes differ from the dynamic colonization patterns that may exist in individuals with ARI, even in the absence of antibiotic exposure. We did not adjust for confounders that may have influenced the nasopharyngeal resistome, such as antibiotic adherence. We isolated only *Streptococcus pneumoniae* and *Staphylococcus aureus*, with more detections of the former, an expected finding given the young age of the participants. While we detected resistance genes independent of bacteria, we could not determine whether those genes were functional or just remnants from recent colonizations. Demonstration of increases in RQ does not support the latter possibility. A major strength of the study is the calculation of RQ using ddCT values, normalizing against human and total microbial DNA presence, allowing calculation of true gene abundance without bias by variability in sample collection.

Our study is one of few to measure abundance of nasopharyngeal antibiotic resistance genes in young children before and after antibiotics, irrespective of bacterial presence. Given that children are often primary drivers of nasopharyngeal bacterial transmission, our findings may also elucidate an indirect impact of antibiotic use among close contacts of antibiotic recipients. These findings may have important implications for understanding nasopharyngeal bacterial carriage in children, underscore important potential consequences of short-term antibiotic use, and further support the need for enhanced antibiotic stewardship in the outpatient setting.

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