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MAJOR ARTICLE

Rising Pneumococcal Antibiotic Resistance in the Post–13-Valent Pneumococcal Conjugate Vaccine Era in Pediatric Isolates From a Primary Care Setting

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Background. Antibiotic-resistant *Streptococcus pneumoniae* strains may cause infections that fail to respond to antimicrobial therapy. Results reported from hospitalized patients with invasive, bacteremic infections may not be the same as those observed in a primary care setting where young children receive care for noninvasive infections. Young children experience the highest burden of pneumococcal disease. The aim of this study was to determine the antibiotic susceptibility of *S. pneumoniae* strains isolated from children in a primary care setting in the post–13-valent pneumococcal conjugate vaccine (PCV13) era.

Methods. This was a prospective collection of 1201 isolates of *S. pneumoniae* from 2006 through 2016 in a primary care setting. Antibiotic susceptibility testing to 16 different antibiotics of 10 classes was performed. Participants were children aged 6–36 months. Nasopharyngeal swabs were obtained from patients during acute otitis media (AOM) visits and routine healthy visits. Middle ear fluid was obtained by tympanocentesis.

Results. After introduction of PCV13, antibiotic susceptibility of pneumococci, especially to penicillin, initially improved largely due to disappearance of serotype 19A, included in PCV13. However, beginning in 2013, antibiotic susceptibility among pneumococcal strains began decreasing due to new serotypes not included in PCV13. In addition to reduced susceptibility to penicillin, the most recent isolates show reduced susceptibility to third-generation cephalosporins, fluoroquinolones, and carbapenems, antibiotics commonly used to treat life-threatening, invasive pneumococcal diseases.

Conclusions. In recent years, pneumococcal nasopharyngeal and AOM isolates from children exhibit reduced susceptibility to penicillin, third-generation cephalosporin, fluoroquinolone, and carbapenem antibiotics. The new strains have a different profile of resistance compared to the pre-PCV13 era.

Keywords. *Streptococcus pneumoniae*; antibiotic susceptibility; pneumococcal conjugate vaccine; penicillin; acute otitis media.

Streptococcus pneumoniae (pneumococci) infections, including invasive (meningitis, bacteremic pneumonia, and sepsis) and noninvasive (acute otitis media [AOM], nonbacteremic pneumonia, sinusitis, and conjunctivitis) diseases, are major causes of morbidity and mortality worldwide [1, 2]. The emergence of penicillin-resistant pneumococcal strains, and multidrug-resistant strains, may result in antibiotic treatment failure. Since the 1980s, antibiotic-resistant pneumococcal strains have markedly increased globally and penicillin-resistant pneumococci are particularly common in developing countries [3].

In the United States (US), based on previous National Committee for Clinical Laboratory Standards criteria (identical to the current Clinical and Laboratory Standards Institute [CLSI] "oral penicillin" criteria), pneumococcal resistance to penicillin (minimum inhibitory concentration [MIC] ≥ 2 μg/mL) was approximately 5% before 1989, and increased to approximately 20% before introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in 2000 [4, 5]. In the timeframe of 2000 to 2010, penicillin-nonsusceptible isolates, encompassing intermediate (MIC = 0.12–1 μg/mL) and resistant isolates, increased to account for 30%–50% of all strains in both nasopharyngeal (NP) and invasive isolates, largely driven by the then–nonvaccine serotype 19A [6–8]. In the first years following introduction of the 13-valent conjugate vaccine (PCV13), a near elimination of serotype 19A NP colonization and infections resulted in a significant decrease in penicillin resistance among pneumococcal strains [9–11]. The decrease of resistance after introduction of PCV7 was also described for macrolides and fluoroquinolones [12].

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A similar phenomenon is occurring following introduction of PCV13 as occurred after introduction of PCV7. New strains expressing capsular serotypes not included in PCV13 are emerging to cause disease [9, 13], and strains that acquire antibiotic resistance are increasing in frequency due to their survival of the fittest advantage. Thus, Darwinian principles are at play in events occurring after introduction of two generations of PCVs.

Information on the susceptibility pattern of pneumococci gives insight to evolving selection advantages specific strains have acquired, and any shift in antibiotic resistance should be identified as it may impact successful treatment. Therefore, the US Centers for Disease Control and Prevention (CDC) monitors pneumococcal resistance patterns through its Active Bacterial Core surveillance (ABCs) network [14]. That network collects data from blood and cerebrospinal fluid isolates from hospitalized patients of all ages with invasive, systemic infections. In 2007, we reported emergence of a serotype 19A strain colonizing the nasopharynx and causing AOM infections in a pediatric primary care setting that was resistant to all US Food and Drug Administration–approved antibiotics for children [15]. At that time, during the PCV7 era, other than the unique serotype 19A strain, pneumococcal antibiotic resistance patterns and serotype distribution results from the nasopharynx and middle ear fluid (MEF) during AOM of children aged 6–36 months from primary care pediatric practices in Rochester, New York, were similar to those reported by the CDC [14, 16, 17].

The focus of reports from the CDC is invasive pneumococcal disease (IPD). However, the greatest disease burden and the dominant cost savings generated from PCVs reside in AOM infection prevention [18, 19]. The CDC reports resistance to penicillin based on cutoffs for anticipated efficacy for treatment of IPD using parenteral antibiotics (resistant for nonmeningitis infections, ≥8 μg/mL; for meningitis, ≥0.12 μg/mL), whereas in primary care settings, antibiotics are predominantly used to treat pneumococcal AOM, sinusitis, and nonbacteremic pneumonia by the oral route [20, 21]. For oral antibiotics, appropriate MICs used to define susceptibility to penicillin and other antibiotics are different from those used to define susceptibility to parenteral antibiotics [22, 23].

In this study, we sought to identify new strains of pneumococci expressing serotypes not included in PCV13 that are emergent in a primary care setting and to characterize antibiotic susceptibility of the isolates. A total of 1201 pneumococcal strains isolated from the nasopharynx during health and from the nasopharynx and MEF during AOM infections in children were studied.

METHODS

Demographics and Sample Collection

In this prospective cohort study, clinical samples were prospectively collected from children attending primary care pediatric practices in Rochester, New York. The children were predominantly from a middle-class, suburban sociodemographic population. Scheduled periodic NP samples were taken from children at age 6, 9, 12, 15, 18, 24, and 30–36 months and at the onset of any episode of AOM diagnosed by validated otoscopists. MEF samples were collected by tympanocentesis during AOM and, for NP samples, swabs were passed along the nasal floor to the posterior pharynx until resistance was met in the area of the adenoid. Children were not treated with antibiotics prior to the culture done unless it was a treatment failure episode, and only 1.5% of all AOM episodes in this cohort where pneumococci was isolated were treatment failure AOM cases and treated with antibiotics prior to culture. The study population and overall study design have been previously described [6, 9, 24, 25].

Children received PCV7 from 2006 until April 2010 and PCV13 after April 2010, according to the recommended US schedule. All subjects were up to date on vaccines except for minor delays and followed the 3 + 1 vaccine schedule in both the PCV7 and PCV13 eras. The investigational review boards of the University of Rochester (2006–2009) and Rochester General Hospital (April 2009–present) approved the study. Written informed consent was obtained from parents.

Pneumococcal Identification and Serotyping

Nasopharyngeal and MEF samples were inoculated onto blood agar plates. Pneumococci were identified by standard culture methods as previously described based on α-hemolysis and optochin sensitivity using Taxo P-discs [6], and serotypes were determined by Quellung reaction [9].

Antibiotic Susceptibility

The antibiotic susceptibility of pneumococci was determined with the VITEK-2 AST-GP68 or GP74 susceptibility cards (bioMérieux) in the clinical laboratories of Rochester General Hospital. On this card, benzylpenicillin, amoxicillin, ceftriaxone, cefotaxime, meropenem, ertapenem, ofloxacin, levofloxacin, moxifloxacin, erythromycin, telithromycin, vancomycin, linezolid, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) were tested by automated modified broth microdilution. The numerical values for each antibiotic were expressed in micrograms per milliliter and pneumococci were classified as susceptible, intermediate, or resistant based on 2017 CLSI breakpoints, with oral cutoffs used for penicillin (Supplementary Table 1) [22].

Nonsusceptible isolates were defined as isolates meeting either intermediate or resistant cutoff MIC values. As the AST-GP68 and GP74 cards differ in their upper quantitation limit for penicillin (with the former ending at ≥2 μg/mL and the latter at ≥8 μg/mL) for analyses, all GP74 card MIC reads of 4 or ≥8 μg/mL were "rounded down" to ≥2 μg/mL. Some isolates failed the testing requirements of VITEK-2 instrument conditions and those readouts were excluded in the analysis, except

Table 1. Description of the Study Cohort

Characteristic	No. (%)		
Total No. of children	448		
Sex			
Male	248 (55)		
Female	200 (45)		
Race/ethnicity			
Non-Hispanic white	351 (78)		
African American	27 (6)		
Hispanic/Latino	9 (2)		
Asian	8 (2)		
Mixed race	53 (12)		
	Total, No.	PCV7 Time Period, No. (%)	PCV13 Time Period, No. (%)
Total visits between 6 and 36 mo of age	1072 visits	336 (31)	736 (69)
Healthy visits	813	230 (28)	583 (72)
AOM visits	259	106 (41)	153 (59)
% of children with repeated AOM visits	…	26 (31)	38 (33)
Total pneumococci isolates	1201	398 (31)	803 (69)
Healthy visit, NP isolates	813	230 (28)	583 (72)
AOM visit, NP isolates	259	106 (41)	153 (59)
AOM visit, MEF isolates	129	62 (48)	67 (52)

Abbreviations: AOM, acute otitis media; MEF, middle ear fluid; NP, nasopharyngeal; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

erythromycin. Failed readings for erythromycin (approximately one-third of isolates) were tested by the Kirby-Bauer method.

Statistical Analysis

Modeling used log-transformed MIC data, given the skewed distribution. To estimate the general time trend of MIC, the moving average method was applied. The method does not make any distributional assumption about the data. Due to the repeated-measure characteristics of the data, a spline mixed-effect model on the smoothed trend was imposed; using a mixed-effect model addresses repeated measures and correlated data [26]. Models with best Akaike information criteria were selected. The *P* value for each data set using the mixed-effect model method indicates the statistical significance of the change in relationship of MIC and time. Moving average smoothing and fitting of spline mixed effect models were performed in R using packages "smooth" and "lme4."

RESULTS

From 2006 through 2016, 1201 pneumococci isolates were characterized from 1072 different visits of 448 children. The number of AOM episodes per child for the PCV7 era (2006 through June 2010) and PCV13 era (September 2010 through 2016) did not differ (0.82 vs 0.84 per child, respectively). The number of AOM episodes with culture-confirmed *S. pneumoniae* cases between the PCV7 and PCV13 eras was previously reported by our group as not different [10]. Demographic and visits information are shown in Table 1. 68% of the pneumococci were isolated from the nasopharynx during healthy visits, 22% from the nasopharynx during AOM, and 10% from MEF. The frequency of pneumococci from the nasopharynx during healthy visits, and at onset of AOM, remained relatively constant year to year at a mean of 33% (range, 29%–38%) and 55% (range, 41%–64%) of samples, respectively. The overall serotype distributions for NP carriage and AOM strains, including proportions that were PCV13 vaccine serotypes, have been published previously [9, 10]. Table 2 shows antibiotic nonsusceptibility of all the pneumococcal isolates and predominant serotypes in this cohort.

S. pneumoniae Antibiotic Susceptibility

The antibiotic MICs of pneumococcal isolates for 6 β-lactams and 2 quinolones collected from 2006 through 2016 are shown in Figure 1. There is a discernible time trend for changes in MIC that can be classified as U-shaped. The time range 2011–2013 (the immediate PCV13 era) plays a distinct role where lower MIC values were observed (mostly susceptible isolates to all the antibiotics), likely due to PCV13 vaccine effect. The time frame 2014–2016 plays a distinct role where higher MICs were noted. Similar analysis for 8 other antibiotics is shown in Supplementary Figure 1.

Antibiotic Susceptibility in NP Carriage and AOM Infection-causing Strains

A detailed analysis of *S. pneumoniae* antibiotic susceptibility to 4 β-lactams during healthy visits and AOM infection visits is shown in Figure 2. Statistical evaluation was limited to the time span 2013–2016, as pneumococcal antibiotic susceptibility during the years 2006–2013 have been described in our prior publications, where we reported significant increases in antibiotic susceptibility after PCV13 vaccine introduction [6, 9, 10, 24]. During healthy visits, the median MIC

Table 2. Percentage of Nonsusceptible Isolates of *Streptococcus pneumoniae* in This Cohort Including All Serotypes and Predominant Serotypes

		Vaccine Serotypes		Nonvaccine Serotypes									
Isolates	All Serotypes	19A	6A	11A	11C	15A	15B/C	21	22	23A	23B	35B	35F
No. of isolates	1201	158	32	65	32	35	163	94	32	51	113	159	22
% serotypes out of total serotypes tested	…	13.2	2.7	5.4	2.7	2.9	13.6	7.8	2.7	4.3	9.4	13.2	1.8
% isolated from AOM (nasopharynx and MEF)	33.6	50.0	46.7	33.8	34.4	20.0	37.4	31.9	12.5	25.5	26.5	23.9	31.8
Antibiotics	% Nonsusceptibilities (Including Intermediate and Resistant Isolates)												
Pencillin	37.1	88.6	75.0	4.6	6.2	45.7	9.8	2.1	…	50.9	17.7	84.3	50.0
Amoxicillin	2.7	1.9	…	…	…	…	…	…	…	…	0.9	11.9	15.6
Cefotaxime	2.6	43.0	28.1	…	…	5.7	…	…	…	…	…	32.7	37.5
Ceftriaxone	2.6	42.4	28.1	…	…	…	…	…	…	…	…	7.5	12.5
Meropenem	16.9	44.9	21.9	1.5	…	…	…	…	…	…	0.9	57.2	43.8
Ertapenem	1.2	5.7	…	…	…	…	…	…	…	…	…	1.3	…
Vancomycin	0.5	…	…	…	…	…	…	…	…	…	0.9	0.6	…
Erythromycin	32.9	57.6	78.0	21.5	25.0	40.0	37.4	2.9	15.6	21.6	28.6	55.9	37.5
Telithromycin	…	…	…	…	…	…	…	…	…	…	…	…	…
Tetracycline	11.5	51.3	21.9	1.5	3.1	42.8	6.7	…	…	3.9	1.8	0.6	…
Levofloxacin	…	…	…	…	…	…	…	…	…	…	…	…	…
Moxifloxacin	…	…	…	…	…	…	…	…	…	…	…	…	…
Ofloxacin	0.2	1.3	…	…	…	…	…	…	…	…	…	0.6	…
TMP-SMX	20.2	59.5	78.1	20.0	9.4	17.1	8.6	1.1	…	5.9	12.4	8.2	15.6
Chloramphenicol	0.4	…	18.8	…	…	…	…	…	…	…	…	…	…
Linezolid	…	…	…	…	…	…	…	…	…	…	…	…	…

Abbreviations: AOM, acute otitis media; MEF, middle ear fluid; TMP-SMX, trimethoprim-sulfamethoxazole.

(MIC_{50}) of pneumococci isolated was low (≤0.06 μg/mL) for all 4 β-lactam drugs tested and did not significantly change over the study years. In contrast, among the NP and MEF isolates during AOM, the MIC_{50} to penicillin, amoxicillin, ceftriaxone, and meropenem from 2013 through 2016 rose significantly (Figure 2). In the NP isolates collected at healthy visits, at onset of AOM and from MEF, the 90% MIC (MIC_{90}) to all β-lactam antibiotics increased significantly from 2013 through 2016 (Figure 2). Of note, the overall trend for MIC relating to macrolides decreased after introduction of PCV13 due to elimination of serotypes 19A and 6B (Supplementary Figure 2). Emergent strains of serogroups 11, 15, and 35 were frequently nonsusceptible to macrolides (Table 2) and increasing in prevalence since 2013 (Supplementary Figure 2).

Pneumococcal isolates from the nasopharynx of children during times of health may not reflect isolates in the nasopharynx at onset of AOM infections or the isolates that cause infection [23]. Pneumococci isolated from the MEF identify virulent strains since they have caused disease. Table 3 shows the clear tendency among some of the antibiotics for lower antibiotic susceptibility among the AOM isolates collected in 2013–2016.

Serotype-specific Antibiotic Susceptibility Analysis Correlation With Antibiotics

There are 2 natural explanations for changes over time in antibiotic susceptibility of pneumococci. The first is that the prevalence of specific serotypes may change over time, particularly when these have higher or lower antibiotic resistance than the population mean. The second is a change in antibiotic susceptibility within a specific serotype. Both explanations were identified in our population. Three serotypes were identified that can largely (but not exclusively) explain changes in susceptibility trends observed in our cohort. Serotypes 35B and 35F increased in β-lactam resistance during the time frame 2013–2016 (Figure 3) and in prevalence (Supplementary Figure 2). Serotype 11A strains had higher MICs to quinolones (Figure 3) and increased in prevalence during 2013–2016 (Supplementary Figure 2). The increase in isolation of 35B, 35F, and 11A strains occurred at healthy visits (Supplementary Figure 2*A*) and at onset of AOM (Supplementary Figure 2*B*). The rise in frequency of isolation of serotypes 35B, 35F, and 11A resembles that observed during the PCV7 era for serotype 19A (Supplementary Figure 2). Capsular switching among strains due to vaccine pressure can happen where prior resistant strains can escape and persist in the population [27]. However, based on our molecular analysis (multilocus sequence typing) of strains in our study, this is not the case here (data not shown). Antibiotic selection by clinicians may change over time as well and can impact antibiotic susceptibility, but this was not the case in our study cohort.

Supplementary Table 3 provides data for the drug resistance profile of the most recent *S. pneumoniae* strains, including enumeration of multidrug-resistant isolates.

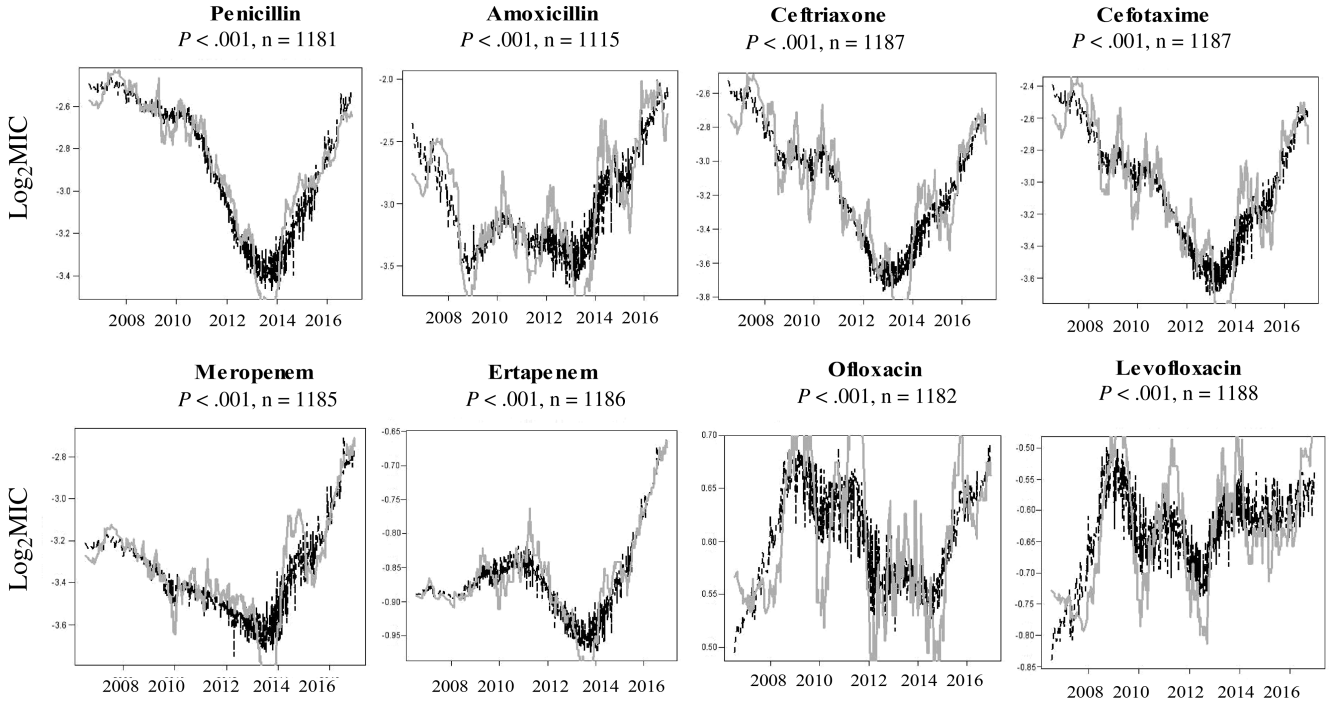

Figure 1. *Streptococcus pneumoniae* antibiotic minimum inhibitory concentration (MIC) trends over time curve based on measured MIC values for each β-lactam antibiotic and fluoroquinolone for all the isolates recovered from 2006 to 2016, with all serotypes pooled. A spline mixed effect model was used (see details in the Statistical Analysis section). First, a moving average smoothing was fit on the data. After that, a spline mixed-effect model (black lines) was superimposed on the smooth curve (gray lines). Data are displayed and analyzed with breakpoints for splines set 2 years apart. Similar results occurred when splines were set 1 year apart. The *P* value for each antibiotic indicates the statistical significance of the change in relationship of MIC and time. The reported *P* value is the significance level of the model analyzing overall change over time using package lmerTest in R. This package performs the Satterthwaite method and Kenward-Roger method.

DISCUSSION

In this article, we provide evidence of changing antibiotic susceptibility of pneumococci isolated in the post-PCV13 era from young children in a pediatric primary care setting. The study focused on young children because they experience the highest burden of pneumococcal disease and frequently receive antibiotics that may result in selection of resistant strains. Rapid and steady erosion of antibiotic susceptibility of pneumococci was observed beginning in 2013 until the end of the study at end of 2016. Pneumococci expressing capsular serotypes not included in PCV13, specifically 11A, 35B, and 35F, accounted for much of the change. The new strains have a different profile of antibiotic susceptibility compared to isolates in the pre-PCV13 era. Of note, a third generation of PCVs is in advanced development—PCV15 and PCV20. Both vaccines include serotypes 22F and 33F. PCV20 includes serotype 11A and 15B but neither of the third-generation PCVs includes serotypes 35B or 35F.

Antibiotic resistance among pneumococci is largely driven by pediatric use [28]. Typically, strains that are antibiotic resistant spread to older children and adults, including the elderly [29], although our results suggest that strains may have spread from adults to children, as well, as fluoroquinolones are rarely used in the US to treat infections in young children. The emergence of strains with antibiotic resistance occurs as a consequence of selection pressure created by antibiotic exposures [30]. PCVs nearly effectively eliminate NP carriage of strains expressing the serotypes included in the vaccines [10]. However, the ecological niche vacated by the eliminated strains becomes occupied by replacement strains [9]. Replacement strains that are antibiotic resistant come to dominate in the nasopharynx under selection pressure of antibiotic use [31]. Not all strains are equally virulent [32] but strains that possess virulence and antibiotic resistance genes increase over time and come to dominate to cause systemic and mucosal infections [33]. As an example, Davies et al [34] has shown similar increase in levofloxacin-resistant pneumococci in the nonvaccine serotypes under PCV7 pressure when replacement of vaccine serotypes happened.

For penicillin, 3 sets of MIC cutoff levels are used to define a "resistant" strain, depending on whether the infection is meningitis, or nonmeningitis and treated with intravenous penicillin, or oral penicillin, such as AOM, sinusitis, and nonbacteremic pneumonia. We report here MIC_{50} and MIC_{90} levels for oral penicillin and amoxicillin administration for pediatric NP colonization and AOM. The frequency of strains resistant to penicillin and amoxicillin decreased with the introduction of PCV13 but rebounded to levels similar to those before PCV13 introduction

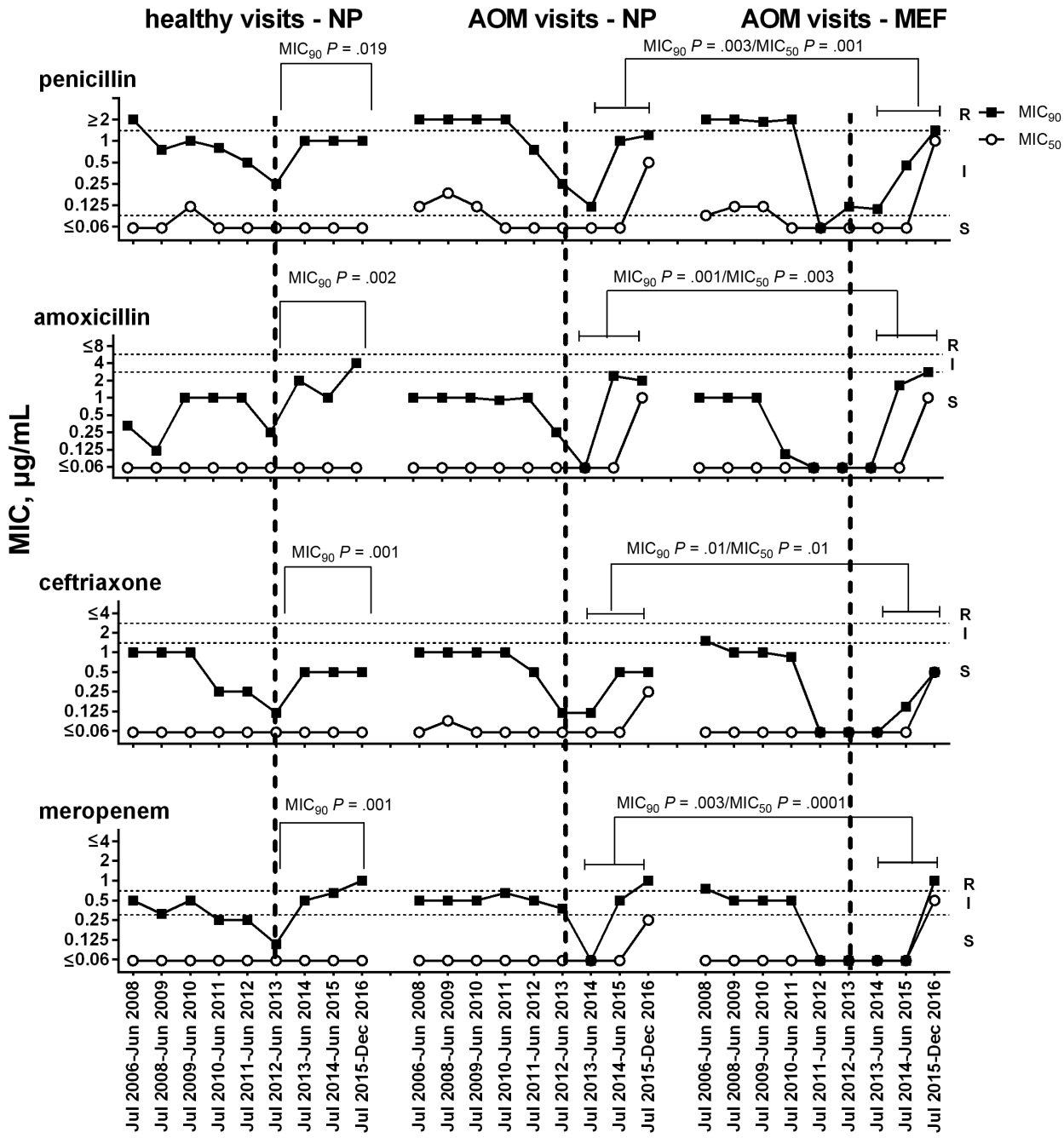

Figure 2. The β-lactam antibiotic minimum inhibitory concentrations (MICs) of pneumococcal isolates to show sensitive (S), intermediate (I), and resistant (R) strains. Isolates were classified by visit type and specimen source, as well as time period. Median and 90th percentile (MIC_{50} and MIC_{90}) measurements are shown. In healthy visits, the range of number of isolates included in the analysis was 49–137 for each year. The range was 23–66 isolates during acute otitis media (AOM) visits. Analysis was limited to the time span from 2013–2014 to 2015–2016 because pneumococcal resistance during the years 2006–2007 to 2012–2013 have been previously described in multiple prior publications [7, 10, 11, 25]. Analysis of AOM visits were plotted separately, but for statistical analysis, isolates collected from the nasopharynx (NP) were combined with isolates from middle ear fluid (MEF) due to the low number of isolates from MEF. The vertical dashed line represents the time point when data were included in statistical analysis (*P* values were calculated using linear regression comparing change in MIC_{90} with time of healthy visit isolates and MIC_{50}/MIC_{90} for AOM isolates). Segmented linear regression modeling method was used for this analysis (R package segmented). Outcome variables were MIC_{50} and MIC_{90} and the covariate was "time" of the measurement. MIC breakpoints are according to current Clinical and Laboratory Standards Institute guidelines. Nonmeningitic breakpoints are used for cefotaxime and ceftriaxone, in addition to oral penicillin breakpoints being used for penicillin.

by 2015–2016. Since the penicillins are the treatment of choice for pneumococcal AOM, sinusitis and nonbacteremic pneumonia in adults and children, the resistance rates observed in 2015–2016 are worrisome. If we analyze the frequency of fully resistant strains based on definitions used for nonmeningitis IPD infections treated with parenteral antibiotics, the fully resistant rates (MIC ≥ 8 µg/mL) would be lower and resemble those reported by the US CDC from the ABCs network for IPD [14, 35].

Table 3. Comparison of Minimum Inhibitory Concentration Between Acute Otitis Media and Healthy Visit *Streptococcus pneumoniae* Isolates

	Comparison of MIC Between AOM and Healthy Visit Isolates						
	AOM Isolates		Healthy Visit Isolates				
Antibiotic	No.	Mean Log MIC	No.	Mean Log MIC	Percentage Change in Mean MIC Between AOM and Healthy Isolates	*P* Value	Effective Sample Size
Penicillin	156	−3.10	319	−3.23	8.5	.0002	175.1
Amoxicillin	155	−3.01	322	−3.16	9.6	.0026	179.9
Ceftriaxone	156	−3.42	324	−3.50	5.4	.0032	180.9
Cefotaxime	156	−3.30	324	−3.41	7.0	.0013	178.4
Meropenem	155	−3.39	324	−3.48	5.9	.0111	176.5
Ertapenem	156	−0.88	325	−0.89	0.9	.3060	174.5
Ofloxacin	156	0.59	322	0.59	0.0	.7588	136.3
Levofloxacin	156	−0.59	326	−0.59	0.1	.4130	150.0
Moxifloxacin	156	−2.00	327	−2.00	0.0	1.0000	NA
Erythromycin	111	−1.11	277	−1.14	2.0	.8643	82.5
Telithromycin	146	−1.95	325	−1.95	0.1	.6380	113.7
Vancomycin	156	0.01	326	0.00	0.6	.2354	121.6
Linezolid	156	−2.00	324	−2.00	0.0	1.0000	NA
Chloramphenicol	154	1.02	319	1.02	0.1	.1717	66.4
TMP-SMX	156	3.63	326	3.62	0.9	.0064	157.5
Tetracycline	156	0.14	323	0.10	2.6	.0173	72.1

No. = effective sample size for year 2016–2017. *P* value based on permutation test comparing mean MIC of middle ear isolates vs nasopharyngeal isolates. Comparisons were made between isolates from the period 2013–2016 where we observed a change in antibiotic susceptibility as shown in Figure 1. Due to the nature of correlated data (repeated measurement), the effective sample size is smaller compared to the entire sample size. We used the R package "longpower" to calculate the effective sample size for the mixed-effect models used in the analysis of the data in this table.
Abbreviations: AOM, acute otitis media; MIC, minimum inhibitory concentration; NA, not applicable; TMP-SMX, trimethoprim-sulfamethoxazole.

Amoxicillin is the treatment of choice for empiric therapy of AOM and sinusitis in adults and children [36, 37]. Levels of amoxicillin achievable in the middle ear have been measured and are variable [38]. The variability is due to differences in absorption of amoxicillin (from 20% to 90% of administered dose absorbed) [39] and penetration into the infected middle ear or sinus space. Measurement of the MIC_{50} and MIC_{90} of pneumococci provide an opportunity for understanding the likelihood of treatment failure and the likelihood that antibiotic exposures may lead to selection of resistant strains in the nasopharynx.

A trend for increasing concentrations of antibiotic required to treat pneumococci portends to a risk of treatment failures in the future. Therefore, our observation of rising MICs to third-generation cephalosporins, fluoroquinolones, and carbapenems is significant for future treatment of IPD. Most often sepsis, meningitis, and lobar pneumonia are treated empirically until culture results become available. Third-generation cephalosporins, fluoroquinolones, and carbapenems are commonly used antibiotics for empiric treatment of these potentially life-threatening infections. Moreover, in the absence of definitive bacterial culture results, these classes are relied upon as bactericidal for a broad array of bacteria, including pneumococci.

As was observed by our group following introduction of PCV7, pneumococci expressing different capsular serotypes are emerging in the post-PCV13 era [10, 11]. However, emergences of strains expressing different capsular types vary in different parts of the world [21, 40]. The ABCs network has reported increased isolation of non-PCV13 vaccine serotypes causing IPD in the US [41]. In 2014–2016, the network also reported that the strains expressing capsular serogroup 35 emerged and increased in prevalence; the strains were β-lactam antibiotic nonsusceptible.

There were limitations to this study. The pneumococci evaluated were obtained from young children seen in a primary care setting. The pediatric practices had a patient population consistent with the demographic of suburban communities in the US. In prior collaborations, results regarding NP colonization and AOM infections caused by pneumococci in Rochester have been similar to those observed in other urban, suburban, and rural communities [39]. However, pneumococcal antibiotic susceptibility patterns are dynamic and may show geographic and/or demographic variation in other settings. Our results suggest the need for ongoing studies. We did not study antibiotic consumption or assess antibiotic treatment failure in this cohort. Some of our patients may have sought care for AOM at other facilities, so not all AOM episodes may have been captured, which is an additional limitation of the study.

We conclude that beginning in 2013, a decrease in antibiotic susceptibility of pneumococci isolated from children in the primary care setting occurred due to non-PCV13 vaccine serotypes. Recent isolates have decreasing susceptibility to penicillins, third-generation cephalosporins, fluoroquinolones, and carbapenems.

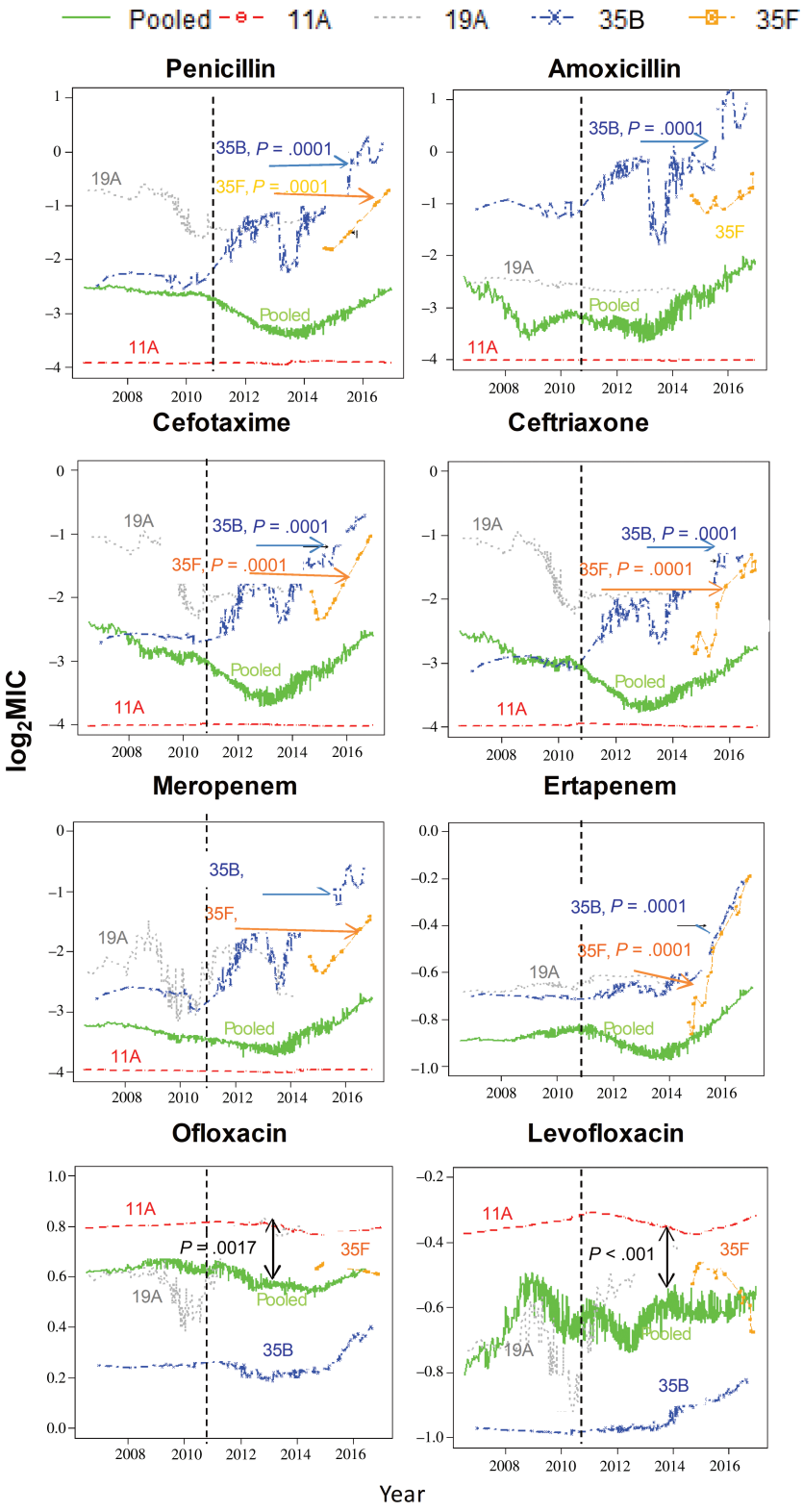

Figure 3. Serotype-specific time trend of minimum inhibitory concentration (MIC) values for antibiotics based on the measured MIC values. Serotypes 11A, 35B, and 35F are represented on each plot along with serotype 19A for comparison of change observed after introduction of 13-valent pneumococcal conjugate vaccine (PCV13). The serotype-pooled antibiotic susceptibility is superimposed for reference. Each curve is represented by a spline mixed-effect model estimate superimposed on a moving average smoother (see main text in methods for details). The analysis used spline mixed-effects model (package lmer in R). The splines' breakpoints were 2 years apart. All of the *P* value comparisons of the data of Figure 3 are included in Supplementary Table 3. *P* values are shown for the significance for serotypes 35B and 35F changes in MIC over time. For serotype 11, average MICs for ofloxacin and levofloxacin are significantly higher compared to the serotype-pooled susceptibility for all years studied. The vertical dashed line represents the time point when PCV13 vaccine was introduced.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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

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