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Decreased Ceftriaxone Susceptibility in Emerging (35B and 6C) and Persisting (19A) *Streptococcus pneumoniae* Serotypes in the United States, 2011-2012: Ceftaroline Remains Active *In Vitro* among β-Lactam Agents

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Totals of 8.7% (103/1,190) and 21.0% (249/1,190) of the *Streptococcus pneumoniae* isolates recovered from specimens collected in the United States during the 2011-2012 AWARE (Assessing Worldwide Antimicrobial Resistance Evaluation) Surveillance Program were ceftriaxone nonsusceptible according to the CLSI (≤1 μg/ml for susceptible) and EUCAST (≤0.5 μg/ml for susceptible) criteria, respectively. Decreased susceptibility to ceftriaxone (MIC, 1 μg/ml) was frequently observed among serotypes 19A (51.4%; 128/249) and 35B (29.7%; 74/249), which were most often observed in the East South Central and South Atlantic U.S. Census regions. Ceftaroline ($MIC_{50/90}$, 0.12/0.25 μg/ml) remained active (≥96.8% susceptible) when tested against these less susceptible isolates.

Streptococcus pneumoniae is an important pathogen responsible for community-acquired bacterial pneumonia (CABP), bacteremia, meningitis, and otitis media and continues to be a major cause of morbidity and mortality worldwide (1). In 2000, the seven-valent pneumococcal conjugate vaccine (PCV7) was introduced in the U.S. childhood vaccine schedule, followed by the PCV13 in 2010 (2). Conjugate vaccines were shown to be immunogenic and prevented pneumococcal disease in children and even in immunocompromised patients (3). However, it has been known that vaccine use modifies the epidemiology of pneumococcal disease and colonization, and investigations have documented increases in the rates of carriage and infections caused by non-PCV7 and later non-PCV13 serotypes (1, 4–6).

Recently, the serotype distribution of *S. pneumoniae* isolates recovered in the United States (2011-2012) was investigated (7). Serotypes 19A, 3, and 35B were found to be the most prevalent serotypes among *S. pneumoniae* recovered from sampled patients. In addition, serotypes 19A and 35B comprised the majority (81.1%) of *S. pneumoniae* isolates with elevated ceftriaxone MIC values (≥1 μg/ml). In this study, we investigated the serotypes and geographic distributions of *S. pneumoniae* isolates that demonstrated elevated MIC results (≥1 μg/ml) for ceftriaxone, a commonly used agent for invasive pneumococcal disease (IPD). Furthermore, the activities of ceftaroline and other comparator agents were quantified when tested against these less-susceptible pneumococcal isolates.

A total of 1,187 *S. pneumoniae* clinical isolates received from July 2011 through June 2012 as part of the AWARE (Assessing Worldwide Antimicrobial Resistance Evaluation) program, a component of the SENTRY Antimicrobial Surveillance Program, were included in this investigation. These isolates were recovered from hospitalized patients in 63 medical centers located in the nine U.S. Census regions. Isolates were recovered from blood or lower respiratory tract cultures (7). Bacterial identification was performed by the participating microbiology laboratory and confirmed by the central monitoring laboratory (JMI Laboratories, North Liberty, IA, USA). Bacterial identification was confirmed by colony morphology, biochemical algorithms, and the Vitek2 system, as needed. When the bacterial identification was questionable using phenotypic methods or an untypeable serotyping result was obtained by the applied methodology, isolates were subjected to PCR assay for further identification (8).

Isolates were tested for susceptibility by broth microdilution methods, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (9). MIC results for several anti-Gram-positive agents were obtained using panels manufactured by Thermo Fisher Scientific (Cleveland, OH, USA). Validation of the MIC values was performed by concurrent testing of the quality control (QC) strain *S. pneumoniae* ATCC 49619 (10). In addition, the inoculum density was monitored by colony counts to ensure an adequate number of cells for each testing event. MIC interpretations were based on the CLSI M100-S24 (10) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (11).

Isolates were subjected to PCR assays for amplification of the *cpsB* gene as previously described by Leung et al. (12). Amplicons were sequenced on both strands, and the nucleotide sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via PubMed (see http://www.ncbi.nlm.nih.gov/blast/). Due to sequence homology among certain serotypes, those showing a nucleotide sequence similarity of >99% were grouped (e.g., 9V/9A, 7F/7A, 11A/11D, 15A/15F, 22F/22A, and 15B/15C). All isolates determined to be serogroup 6 by sequencing analysis were subjected to multiplex PCR assays for confirmation and discrimination between 6A/6B and 6C/6D (13).

Overall, ceftriaxone had MIC_{50} and MIC_{90} results of ≤0.06 and 1 μg/ml, respectively (Table 1). Totals of 8.7 and 21.0% of all *S.*

Received 4 April 2014 **Accepted** 17 May 2014
Published ahead of print 27 May 2014
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doi:10.1128/AAC.02976-14

TABLE 1 MIC distribution and antimicrobial activity of ceftriaxone when tested against specific serogroups/types of *S. pneumoniae*[a]

Serogroup/type (no. tested)	MIC (μg/ml)		No. (cumulative %) of isolates inhibited at MIC (μg/ml) of:								
	50%	90%	≤0.06	0.12	0.25	0.5	1	2	4	8	>8
All (1,190)	≤0.06	1	688 (58.0)	118 (67.9)	67 (73.5)	65 (79.0)	146 (91.3)	88 (98.7)	6 (92.4)	8 (99.9)	1 (100.0)
3 (96)	≤0.06	0.25	84 (87.5)	2 (89.6)	9 (99.0)	0 (99.0)	1 (100.0)				
4 (5)	≤0.06		5 (100.0)								
6A/6B (20)	0.25	2	5 (25.0)	2 (35.0)	3 (50.0)	6 (80.0)	1 (85.0)	3 (100.0)			
6C/6D (69)	0.25	1	22 (31.9)	3 (36.2)	10 (50.7)	27 (89.9)	6 (98.6)	0 (98.6)	0 (98.6)	1 (100.0)	
7C/7B/40 (18)	≤0.06	≤0.06	17 (94.4)	1 (100.0)							
7F/7A (29)	≤0.06	≤0.06	29 (100.0)								
8 (15)	≤0.06	0.25	12 (80.0)	0 (80.0)	2 (93.3)	1 (100.0)					
9N/9L (34)	≤0.06	≤0.06	33 (97.1)	1 (100.0)							
9V/9A (5)	1		0 (0.0)	0 (0.0)	0 (0.0)	2 (40.0)	1 (60.0)	2 (100.0)			
10A (22)	≤0.06	0.25	19 (86.4)	0 (86.4)	1 (90.9)	0 (90.9)	2 (100.0)				
11A/11D (71)	≤0.06	≤0.06	66 (93.0)	3 (97.2)	1 (98.6)	0 (98.6)	0 (98.6)	1 (100.0)			
12F/12A/44/46 (13)	≤0.06	0.12	11 (84.6)	2 (100.0)							
13 (9)	≤0.06		8 (88.9)	1 (100.0)							
14 (2)	≤0.06		1 (50.0)	0 (50.0)	0 (50.0)	0 (50.0)	1 (100.0)				
15A/15F (50)	0.12	0.5	13 (26.0)	20 (66.0)	10 (86.0)	4 (94.0)	3 (100.0)				
15B/15C (66)	≤0.06	0.25	41 (62.1)	14 (83.3)	11 (100.0)						
16F (22)	≤0.06	0.12	19 (86.4)	1 (90.9)	0 (90.9)	0 (90.9)	1 (95.5)	1 (100.0)			
17F (20)	≤0.06	0.12	16 (80.0)	2 (90.0)	1 (95.0)	0 (95.0)	0 (95.0)	1 (100.0)			
18 (18A/18B/18C/18F) (1)	≤0.06		1 (100.0)								
20 (11)	≤0.06	≤0.06	11 (100.0)								
21 (12)	≤0.06	0.5	8 (66.7)	0 (66.7)	1 (75.0)	3 (100.0)					
22F/22A (68)	≤0.06	≤0.06	64 (94.1)	1 (95.6)	0 (95.6)	1 (97.1)	1 (98.5)	0 (98.5)	1 (100.0)		
23A (75)	0.12	0.25	19 (25.3)	41 (80.0)	9 (92.0)	3 (96.0)	2 (98.7)	1 (100.0)			
23B (53)	≤0.06	0.12	33 (62.3)	16 (92.5)	1 (94.3)	1 (96.2)	1 (98.1)	1 (100.0)			
23F (4)	0.12		1 (25.0)	1 (50.0)	0 (50.0)	0 (50.0)	1 (75.0)	1 (100.0)			
24 (24A/24B/24F) (2)	≤0.06		1 (50.0)	0 (50.0)	1 (100.0)						
28F/28A (1)	≤0.06		1 (100.0)								
31 (30)	≤0.06	≤0.06	30 (100.0)								
33F/33A/37 (20)	≤0.06	≤0.06	19 (95.0)	0 (95.0)	0 (95.0)	0 (95.0)	1 (100.0)				
34 (16)	≤0.06	0.5	14 (87.5)	0 (87.5)	0 (87.5)	2 (100.0)					
35F/47F (13)	≤0.06	≤0.06	13 (100.0)								
38/25F/25A (12)	≤0.06	≤0.06	12 (100.0)								
48 (1)	≤0.06		1 (100.0)								
Nontypeable (11)	0.5	1	4 (36.4)	0 (36.4)	1 (45.5)	2 (63.6)	4 (100.0)				
35B (92)	1	1	7 (7.6)	0 (7.6)	0 (7.6)	11 (19.6)	70 (95.7)	4 (100.0)			
19A (164)	1	2	22 (13.4)	6 (17.1)	6 (20.7)	2 (22.0)	47 (50.6)	70 (93.3)	4 (95.7)	6 (99.4)	1 (100.0)
19F (35)	≤0.06	2	26 (74.3)	1 (77.1)	0 (77.1)	0 (77.1)	3 (85.7)	3 (94.3)	1 (97.1)	1 (100.0)	

[a] The CLSI and EUCAST breakpoints for ceftriaxone susceptibility are ≤1 μg/ml and ≤0.5 μg/ml, respectively.

pneumoniae isolates were categorized as nonsusceptible to ceftriaxone according to CLSI (≤1 μg/ml for susceptible, nonmeningitis cases) and EUCAST (≤0.5 μg/ml for susceptible) breakpoint criteria, respectively. *S. pneumoniae* isolates exhibiting ceftriaxone MIC values of ≥1 μg/ml were predominantly (84.3%; 210/249) serotypes 19A, 19F, and 35B, while remaining isolates were associated with 15 other serotypes and 4 nontypeable strains. Serogroup 19 represented 83.5% (86/103) and 54.6% (136/249) of isolates that were nonsusceptible to ceftriaxone according to CLSI and EUCAST criteria, respectively, and these isolates exhibited markedly decreased susceptibility to several agents (0.0 to 36.8% susceptible), except for ceftaroline (≥94.1% susceptible), levofloxacin (99.3% susceptible), linezolid (100.0% susceptible), and vancomycin (100.0% susceptible) (Table 2).

The vast majority of the 35B isolates (95.7%; 88/92) were susceptible to ceftriaxone when applying the CLSI breakpoint (≤1 μg/ml for susceptible) (Table 1). In stark contrast, only 19.6% of the 35B isolates were considered susceptible when the EUCAST criteria were used (≤0.5 μg/ml for susceptible), since this serotype (76.1%; 70/92) showed a ceftriaxone modal MIC result at 1 μg/ml, as previously described (14). The 35B isolates with elevated ceftriaxone MIC values of ≥1 μg/ml had low susceptibility rates to other agents as well, and similar to the results for serogroup 19, ceftaroline, levofloxacin, linezolid, and vancomycin remained active (≥98.6% susceptible), in addition to clindamycin (Table 2). Moreover, penicillin was active against 35B isolates only when a higher CLSI breakpoint (i.e., ≤2 μg/ml for susceptible) was applied. Of note, isolates from serogroup 6 exhibited a bimodal MIC distribution for ceftriaxone, with modal MIC values of ≤0.06 and 0.5 μg/ml (Table 1). In addition, ceftriaxone inhibited 89.9% (62/69) of the emerging 6C/6D serotype isolates at ≤0.5 μg/ml (EUCAST breakpoint).

Among the parenteral cephalosporins, ceftaroline ($MIC_{50/90}$, 0.12/0.25 μg/ml) remained active when tested against these isolates with decreased susceptibility to ceftriaxone (i.e., MIC, ≥1 μg/ml), with overall MIC results 8-fold lower than those for ceftri-

TABLE 2 Activity of ceftaroline and comparator antimicrobial agents when tested against the most common serotypes of *S. pneumoniae* isolates displaying elevated (≥1 μg/ml) ceftriaxone MIC results

Serogroup/type population (no. tested) and antimicrobial agent	MIC (μg/ml)			% susceptible/% resistant[a]	
	Range	50%	90%	CLSI	EUCAST
19 (136)[b]					
Ceftaroline	0.06 to 0.5	0.12	0.25	100.0	94.1/5.9
Ceftriaxone	1 to >8	2	2	36.8/9.6	0.0/9.6
Penicillin	0.12 to 8	4	8	12.5/10.3[c]	NA
Penicillin	0.12 to 8	4	8	0.0/97.1[d]	0.0/87.5
Amoxicillin/clavulanate	≤1 to >8	8	>8	5.9/90.4	NA
Erythromycin	≤0.12 to >16	>16	>16	3.7/96.3	3.7/96.3
Clindamycin	≤0.25 to >2	>2	>2	19.1/80.1	19.9/80.1
Levofloxacin	0.5 to 4	1	1	99.3/0.0	99.3/0.7
Trimethoprim-sulfamethoxazole	≤0.5 to >4	>4	>4	0.7/99.3	0.7/99.3
35B (74)					
Ceftaroline	0.03 to 0.25	0.12	0.12	100.0	100.0/0.0
Ceftriaxone	1 to 2	1	1	94.6/0.0	0.0/0.0
Penicillin	1 to 4	2	2	98.6/0.0[c]	NA
Penicillin	1 to 4	2	2	0.0/86.3[d]	0.0/1.4
Amoxicillin/clavulanate	2 to 8	2	4	51.4/1.4	NA
Erythromycin	≤0.12 to >16	8	>16	34.2/65.8	34.2/65.8
Clindamycin	≤0.25	≤0.25	≤0.25	100.0/0.0	100.0/0.0
Levofloxacin	0.5 to >4	1	1	98.6/1.4	98.6/1.4
Trimethoprim-sulfamethoxazole	≤0.5 to >4	≤0.5	>4	82.4/12.2	86.5/12.2
All (249)					
Ceftaroline	≤0.015 to 0.5	0.12	0.25	100.0	96.8/3.2
Ceftriaxone	1 to >8	1	2	58.6/6.0	0.0/6.0
Penicillin	≤0.06 to 8	4	4	47.8/6.1[c]	NA
Penicillin	≤0.06 to 8	4	4	1.2/89.1[d]	1.2/52.2
Amoxicillin/clavulanate	≤1 to >8	8	8	30.5/51.8	NA
Erythromycin	≤0.12 to >16	>16	>16	15.3/84.7	15.3/84.7
Clindamycin	≤0.25 to >2	≤0.25	>2	50.4/48.8	51.2/48.8
Levofloxacin	0.5 to >4	1	1	98.8/0.8	98.8/1.2
Trimethoprim-sulfamethoxazole	≤0.5 to >4	>4	>4	29.3/67.1	31.3/67.1
Linezolid	0.25 to 2	0.5	1	100.0	100.0/0.0
Vancomycin	≤0.12 to 0.5	0.25	0.5	100.0	100.0/0.0

[a] Criteria as published by the CLSI (10) and EUCAST (11). NA, not available.
[b] The serogroup was composed of 8 and 128 isolates of 19F and 19A, respectively.
[c] Criteria as published by the CLSI (10) for "penicillin parenteral (nonmeningitis)."
[d] Criteria as published by the CLSI (10) for "penicillin (oral penicillin V)."

axone ($MIC_{50/90}$, 0.5 μg/ml) (Table 2). Ceftaroline also demonstrated a MIC_{50} result of 0.12 μg/ml when tested against isolates of serogroup 19 or those of the 35B serotype. *S. pneumoniae* isolates with elevated ceftriaxone MIC results (≥1 μg/ml) were observed in all U.S. Census regions, but most commonly in the South Atlantic (37.9% of *S. pneumoniae* isolates within this region) and East South Central (27.8%) regions. Susceptibility rates among other regions varied from 11.9% (West North Central) to 24.9% (East North Central) (Fig. 1). Occurrences of 19A isolates with elevated ceftriaxone MIC results (≥1 μg/ml) varied from 55.6% in the West North Central to 92.3% in the East South Central and South Atlantic regions (Fig. 1), and 35B strains with decreased ceftriaxone susceptibility were also more prevalent in the East South Central and South Atlantic regions (91.7% to 100.0%).

The prevalence of serotype 19A increased in the United States during the post-PCV7 era (2004-2009) (4, 5, 15, 16), but recent investigations have reported that this serotype has decreased since the introduction of PCV13 (6, 17) or possibly prior to its introduction (18). Although serotype 19A has become less prevalent, studies indicated that the nonsusceptibility rates for 19A have increased even further (7, 18), likely due to antimicrobial pressure and consequent expansion of multidrug-resistant (MDR) populations within 19A isolates (i.e., clonal complex 320) (18). PCV7 successfully decreased the prevalence of targeted serotypes and also decreased the nonsusceptibility rates of these serotypes (19). Based on earlier study results (6, 17) and past experience with PCV7, the use of PCV13 should impact pneumococcal disease in a similar fashion and will likely reduce the prevalence of targeted serotypes and nonsusceptibility rates, especially for serotype 19A. However, given the fact that non-PCV13 serotypes seem to be emerging (6, 7, 17), and certain serotypes (i.e., 35B and 6C) exhibit decreased susceptibility to several agents (including ceftriaxone), it appears likely that decreased antimicrobial susceptibility will continue to be a clinical problem (20).

A previous study demonstrated geographic variations in the *S. pneumoniae* nonsusceptibility rates within the United States, and similar to our findings, higher nonsusceptibility rates were observed in the Southeast (Tennessee and Georgia) (20). The Infec-

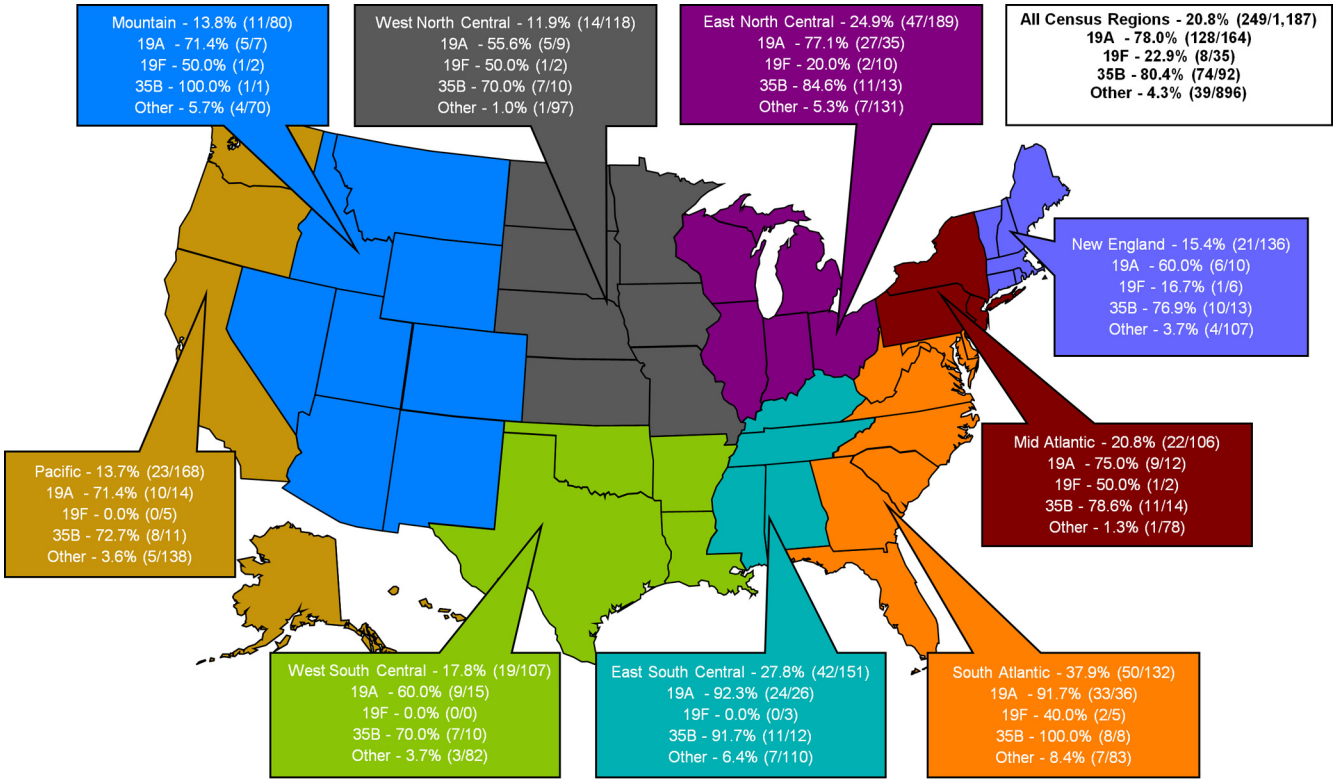

FIG 1 Distribution of *S. pneumoniae* and specific serotypes displaying elevated (≥1 μg/ml) ceftriaxone MIC results within the U.S. Census regions in 2011-2012.

tious Diseases Society of America (IDSA) guidelines for the treatment of community-acquired pneumonia in infants and children (2011) recommend empirical treatment with third-generation parenteral cephalosporins (ceftriaxone or cefotaxime) for hospitalized infants and children who are not fully immunized, in regions where the local epidemiology of IPD strains documents high-level penicillin resistance, or for infants and children with life-threatening infection, including those with empyema (21). Additional surveillance studies over time will be required to better understand the pneumococcal epidemiology after introduction of PCV13, but these and other early data suggest the emergence of non-PCV13 serotypes and decreased susceptibility to the recommended parenteral cephalosporins (21). Herein, ceftriaxone covered 79.0 and 91.3% of isolates using current EUCAST and CLSI criteria, respectively (Table 1); therefore, it would seem prudent that revision of the IPD empirical treatment guidelines should be considered, especially for the South Atlantic and East South Central regions.

ACKNOWLEDGMENTS

We thank JMI Laboratories (North Liberty, IA, USA) staff members S. Benning, M. Castanheira, A. Costello, S. Costello, L. Deshpande, and M. Gerst and other staff members for technical support and manuscript assistance.

D.B. and I.A.C. are employees of Cerexa, Inc. (Oakland, CA), a wholly owned subsidiary of Forest Laboratories Inc. (New York, NY), the sponsor of this study.

JMI Laboratories, Inc., received research and educational grants in 2011-2013 from the American Proficiency Institute (API), Anacor, Astellas, AstraZeneca, Bayer, Cempra, Cerexa/Forest, Contrafect, Cubist, Daiichi, Dipexium, Enanta, Furiex, GlaxoSmithKline, Johnson & Johnson (Ortho McNeil), LegoChem Biosciences Inc., Meiji Seika Kaisha, Merck, Nabriva, Novartis, Pfizer, Rempex, Rib-X Pharmaceuticals, Seachaid, Shionogi, The Medicines Company, Theravance, Thermo Fisher, and some other corporations. Some JMI employees are advisors/consultants for Astellas, Cubist, Pfizer, Cempra, Cerexa/Forest, J&J, and Theravance.

REFERENCES

1. **Rosen JB, Thomas AR, Lexau CA, Reingold A, Hadler JL, Harrison LH, Bennett NM, Schaffner W, Farley MM, Beall BW, Moore MR, CDC Emerging Infections Program Network.** 2011. Geographic variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. Clin. Infect. Dis. **53:**137–143. http://dx.doi.org/10.1093/cid/cir326.
2. **Nuorti JP, Whitney CG, Centers for Disease Control and Prevention.** 2010. Prevention of pneumococcal disease among infants and children—use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine—recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. **59:**1–18.
3. **Luján M, Burgos J, Gallego M, Falco V, Bermudo G, Planes A, Fontanals D, Peghin M, Monso E, Rello J.** 2013. Effects of immunocompromise and comorbidities on pneumococcal serotypes causing invasive respiratory infection in adults: implications for vaccine strategies. Clin. Infect. Dis. **57:**1722–1730. http://dx.doi.org/10.1093/cid/cit640.
4. **Jacobs MR, Good CE, Bajaksouzian S, Windau AR.** 2008. Emergence of *Streptococcus pneumoniae* serotypes 19A, 6C, and 22F and serogroup 15 in Cleveland, Ohio, in relation to introduction of the protein-conjugated pneumococcal vaccine. Clin. Infect. Dis. **47:**1388–1395. http://dx.doi.org/10.1086/592972.
5. **Ampofo K, Pavia AT, Chris S, Hersh AL, Bender JM, Blaschke AJ, Weng HY, Korgenski KE, Daly J, Mason EO, Byington CL.** 2012. The changing epidemiology of invasive pneumococcal disease at a tertiary children's hospital through the 7-valent pneumococcal conjugate vaccine era: a case for continuous surveillance. Pediatr. Infect. Dis. J. **31:**228–234. http://dx.doi.org/10.1097/INF.0b013e31823dcc72.
6. **Richter SS, Heilmann KP, Dohrn CL, Riahi F, Diekema DJ, Doern GV.** 2013. Pneumococcal serotypes before and after introduction of conjugate

vaccines, United States, 1999-2011. Emerg. Infect. Dis. **19:**1074–1083. http://dx.doi.org/10.3201/eid1907.121830.

7. **Mendes RE, Costello AJ, Jacobs MR, Biek D, Critchley IA, Jones RN.** 23 May 2014. Serotype distribution and antimicrobial susceptibility of USA *Streptococcus pneumoniae* isolates collected prior to and post introduction of 13-valent pneumococcal conjugate vaccine. Diagn. Microbiol. Infect. Dis. http://dx.doi.org/10.1016/j.diagmicrobio.2014.05.020.
8. **Prèree MF, Fayet OA.** 2011. A specific polymerase chain reaction test for the identification of *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. **70:**45–53. http://dx.doi.org/10.1016/j.diagmicrobio.2011.02.005.
9. **Clinical and Laboratory Standards Institute.** 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard–9th edition. Approved standard M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
10. **Clinical and Laboratory Standards Institute.** 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
11. **European Committee on Antimicrobial Susceptibility Testing.** 2014. Breakpoint tables for interpretation of MICs and zone diameters, version 4.0. http://www.eucast.org/clinical_breakpoints/.
12. **Leung MH, Bryson K, Freystatter K, Pichon B, Edwards G, Charalambous BM, Gillespie SH.** 2012. Sequetyping: serotyping *Streptococcus pneumoniae* by a single PCR sequencing strategy. J. Clin. Microbiol. **50:**2419–2427. http://dx.doi.org/10.1128/JCM.06384-11.
13. **Pai R, Gertz RE, Beall B.** 2006. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. J. Clin. Microbiol. **44:**124–131. http://dx.doi.org/10.1128/JCM.44.1.124-131.2006.
14. **Beall B, McEllistrem MC, Gertz RE, Jr, Boxrud DJ, Besser JM, Harrison LH, Jorgensen JH, Whitney CG, Active Bacterial Core Surveillance/Emerging Infections Program Network.** 2002. Emergence of a novel penicillin-nonsusceptible, invasive serotype 35B clone of *Streptococcus pneumoniae* within the United States. J. Infect. Dis. **186:**118–122. http://dx.doi.org/10.1086/341072.
15. **Tan TQ.** 2012. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. Clin. Microbiol. Rev. **25:**409–419. http://dx.doi.org/10.1128/CMR.00018-12.
16. **Sharma D, Baughman W, Holst A, Thomas S, Jackson D, da Gloria Carvalho M, Beall B, Satola S, Jerris R, Jain S, Farley MM, Nuorti JP.** 2013. Pneumococcal carriage and invasive disease in children before introduction of the 13-valent conjugate vaccine: comparison with the era before 7-valent conjugate vaccine. Pediatr. Infect. Dis. J. **32:**e45–e53.
17. **Kaplan SL, Barson WJ, Lin PL, Romero JR, Bradley JS, Tan TQ, Hoffman JA, Givner LB, Mason EO, Jr.** 2013. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. Pediatr. Infect. Dis. J. **32:**203–207. http://dx.doi.org/10.1097/INF.0b013e318275614b.
18. **Hulten KG, Kaplan SL, Lamberth LB, Barson WJ, Romero JR, Lin PL, Bradley JS, Givner LB, Tan TQ, Hoffman JA, Mason EO.** 2013. Changes in *Streptococcus pneumoniae* serotype 19A invasive infections in children from 1993 to 2011. J. Clin. Microbiol. **51:**1294–1297. http://dx.doi.org/10.1128/JCM.00058-13.
19. **Hampton LM, Farley MM, Schaffner W, Thomas A, Reingold A, Harrison LH, Lynfield R, Bennett NM, Petit S, Gershman K, Baumbach J, Beall B, Jorgensen J, Glennen A, Zell ER, Moore M.** 2012. Prevention of antibiotic-nonsusceptible *Streptococcus pneumoniae* with conjugate vaccines. J. Infect. Dis. **205:**401–411. http://dx.doi.org/10.1093/infdis/jir755.
20. **Link-Gelles R, Thomas A, Lynfield R, Petit S, Schaffner W, Harrison L, Farley MM, Aragon D, Nicols M, Kirley PD, Zansky S, Jorgensen J, Juni BA, Jackson D, Moore MR, Lipsitch M.** 2013. Geographic and temporal trends in antimicrobial nonsusceptibility in *Streptococcus pneumoniae* in the post-vaccine era in the United States. J. Infect. Dis. **208:**1266–1273. http://dx.doi.org/10.1093/infdis/jit315.
21. **Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harrison C, Kaplan SL, Mace SE, McCracken GH, Jr, Moore MR, St Peter SD, Stockwell JA, Swanson JT.** 2011. Executive summary: the management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin. Infect. Dis. **53:**e25–e76. http://dx.doi.org/10.1093/cid/cir531.