

Distribution of *Streptococcus pneumoniae* serotypes among global populations

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Revised Abstract

Background: *Streptococcus pneumoniae* remains a leading cause of disease in children and adults. Serotypes differ in invasiveness, virulence and antibiotic resistance; therefore, serotype surveillance is necessary to monitor the burden of pneumococcal disease, especially in the setting of pneumococcal vaccination programs. The serotypes and antibiotic susceptibilities of 2,173 *Streptococcus pneumoniae* isolates collected through the Tigecycline Evaluation Surveillance Trial, (TEST) 2004-2009, were evaluated. **Methods:** Serotypes were determined by PCR; results were confirmed by Quellung reactions. Minimum inhibitory concentrations were determined by broth microdilution. **Results:** Worldwide, serotypes 19A (28%), 19F (10%) and 14 (9%) were the most common in children under 4y. In adults over 16y, 19A (13%) 3, 6A and 7F (all 7%) were most common. Serotypes 3, 19A, and 6A accounted for more than 28% of all isolates, while 63% of isolates had serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. These data, although limited in numbers, add to the body of literature that suggests a substantial burden of pneumococcal disease can be attributed these serotypes. The association between pneumococcal serotypes and antibiotic resistance highlights the need for ongoing monitoring of the seroepidemiology of this important pathogen.

	<2y(n=170)	2-4y(n=146)	5-15y(n=144)	16-65y(n=1053)	>65y(n=660)
PCV13	74.7%	78.8%	72.2%	58.8%	61.1%
PCV7	30.6%	30.8%	31.3%	21.0%	21.7%

Discussion: This analysis of an existing isolate database found serotype 19A, a serotype associated with higher levels of antibiotic resistance, was the most common global serotype. The prevalence of serotypes contained only in PCV7 has been reduced to less than 23%. Serotypes 3, 19A, and 6A accounted for more than 28% of all isolates, while 63% of isolates had serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. These data, although limited in numbers, add to the body of literature that suggests a substantial burden of pneumococcal disease can be attributed these serotypes. The association between pneumococcal serotypes and antibiotic resistance highlights the need for ongoing monitoring of the seroepidemiology of this important pathogen.

Introduction

Streptococcus pneumoniae represents a leading cause of mortality and morbidity worldwide in children and adults, causing pneumonia, bacteremia and meningitis. The burden of disease is especially notable in the very young and those over 65 years of age [1]. *S. pneumoniae* has at least 92 known serotypes, although not all are associated with disease. Serotypes vary greatly in invasiveness and virulence, and age group distributions differ among populations [2]. Additionally, most antibiotic-resistant pneumococcal infections are attributed to five specific serotypes (6B, 9V, 14, 19F, and 23F) [3]. A number of multivalent vaccines have been developed, using a variety of serotypes. Data on serotype distribution of pneumococcal disease is needed to monitor and assess the pneumococcal burden of disease in the population, especially in the setting of pneumococcal vaccination immunization programs. The Tigecycline Evaluation Surveillance Trial (TEST) is an on-going global surveillance program begun in 2004. In this analysis, serotyping of *Streptococcus pneumoniae* isolates collected in 47 countries throughout the world through the TEST program was undertaken to document serotype distribution in children and adults of different age groups.

Materials & Methods

2,181 *S. pneumoniae* isolates from sterile body sites from the TEST program were included in this analysis. All isolates were collected from 2004-2009. Sterile body sites included body fluids, cardiovascular system, including blood, central nervous system, lymph, ovary, lung, respiratory sinus and bone. Based on the seven- reaction sequential multiplex PCR described previously [4], we devised a PCR using only four reactions to identify the pneumococcal serotype, followed by confirmation via the conventional Quellung reaction. This PCR-based approach is based on serotype-specific genes within the capsular polysaccharide synthesis genes (the *cps* locus) of SPN. While this scheme could not detect all serotypes without doing a traditional serotyping checkerboard, it served to streamline the project and reduce cost by reducing the number of Quellung reactions needed. Using primers targeted at different loci within the *cps* gene, multiplex PCR reactions were set up in a sequential fashion, including an internal *cps* control. When the PCR assigned isolates to a serotype subset, conventional serotyping was performed based on the subset type. All PCR serotype results were confirmed by Quellung reaction. Isolates that were negative by PCR were serotyped by Quellung reactions alone. Pneumococcal antisera were purchased from Statens Serum Institute (Copenhagen, Denmark).

Minimum inhibitory concentrations (MICs) were determined by the Clinical and Laboratory Standards Institute (CLSI) recommended broth microdilution testing method [5] using panels prepared at IHMA, Inc. (Schaumburg, IL). MIC interpretive criteria followed published CLSI guidelines [6].

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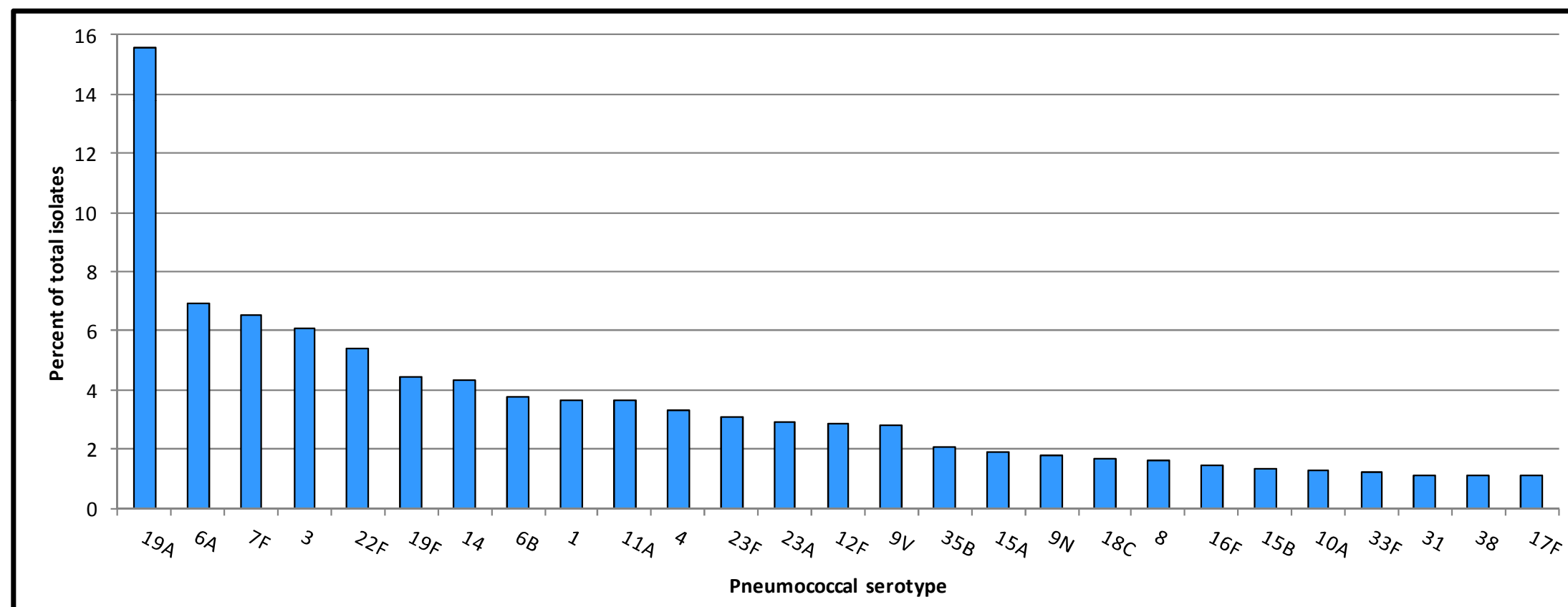
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Results

Results are shown in the following figures and tables.

Figure 1. Serotypes* of *S. pneumoniae* from sterile body sites from global^b isolates, 2004 – 2009 (all ages).



*Serotypes contributing less than 1% not shown; includes 23B, 5, 15C, 35F, 20, 13, 34, 7C, 18B, 33A, 12A, 24F, 21, 10F, 18A, 18F, 35A, 9L, 11D, 29, 19B, 25F, 28A, 28F, 33B, 7A, 7B, 9A and Factor 13.
^bGlobal regions include Latin America, Asia/Pacific, Africa, Middle East, Europe and North America.

Table 1. Age and serotype distribution of 144 *S. pneumoniae* from Latin America.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
14	19 (13.2)		4 (28.6)	3 (20.0)	9 (12.5)	3 (9.7)
1	15 (10.4)		1 (7.1)		11 (15.3)	3 (9.7)
6A	13 (9.0)	1 (8.3)		2 (13.3)	6 (8.3)	4 (12.9)
6B	11 (7.6)	2 (16.7)	2 (14.3)		5 (6.9)	2 (6.5)
7F	9 (6.3)	1 (8.3)		2 (13.3)	4 (5.6)	2 (6.5)
19A	8 (5.6)	2 (16.7)	1 (7.1)	1 (6.7)	3 (4.2)	1 (3.2)
3	7 (4.9)				4 (5.6)	3 (9.7)
19F	7 (4.9)	2 (16.7)	1 (7.1)		3 (4.2)	1 (3.2)
22F	6 (4.2)	1 (8.3)			2 (2.8)	3 (9.7)
9V	5 (3.5)		1 (7.1)	2 (13.3)	2 (2.8)	
5	4 (2.8)	1 (8.3)	2 (14.3)		1 (1.4)	
11A	4 (2.8)			2 (13.3)	1 (1.4)	1 (3.2)
18C	4 (2.8)		1 (7.1)		3 (4.2)	
23F	4 (2.8)			2 (13.3)		2 (6.5)
9N	4 (2.8)				3 (4.2)	1 (3.2)
4	3 (2.1)	1 (8.3)			2 (2.8)	
17F	3 (2.1)				2 (2.8)	1 (3.2)
23A	3 (2.1)				3 (4.2)	
Other	15 (10.4)	1 (8.3)	1 (7.1)	1 (6.7)	8 (11.1)	4 (12.9)
Total N	144	12 (8.3)	14 (9.7)	15 (10.4)	72 (50.0)	31 (21.5)

Latin American countries include: Argentina n=58, Brazil n=18, Chile n=17, Colombia n=14, Honduras n=1, Jamaica n=5, Mexico n=18, Panama n=4, Venezuela n=9.

Table 2. Age and serotype distribution of 42 *S. pneumoniae* from Africa.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
19F	6 (14.3)	3 (30.0)	2 (28.6)		1 (6.3)	
6B	6 (14.3)	1 (10.0)	2 (28.6)	2 (25.0)		1 (100)
4	4 (9.5)				4 (25.0)	
23F	4 (9.5)	2 (20.0)		1 (12.5)	1 (6.3)	
14	3 (7.1)	2 (20.0)			1 (6.3)	
19A	3 (7.1)	1 (10.0)			2 (12.5)	
1	2 (4.8)			2 (25.0)		
8	2 (4.8)				2 (12.5)	
15B	2 (4.8)		2 (28.6)			
16F	2 (4.8)	1 (10.0)		1 (12.5)		
6A	2 (4.8)				2	
9V	2 (4.8)			1 (12.5)	1 (6.3)	
3	1 (2.4)				1 (6.3)	
11A	1 (2.4)			1 (12.5)		
23A	1 (2.4)				1 (6.3)	
28F	1 (2.4)		1 (14.3)			
Total N	42	10 (23.8)	7 (16.7)	8 (19.0)	16 (38.1)	1 (2.4)

African countries include: South Africa n=38 and Mauritius n=4.

Table 3. Age and serotype distribution of 156 *S. pneumoniae* from Asia/Pacific.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
3	16 (10.8)	2 (9.1)		1 (11.1)	6 (9.0)	7 (13.5)
19A	16 (10.8)	2 (9.1)	1 (16.7)	1 (11.1)	4 (6.0)	8 (15.4)
14	13 (8.3)	4 (18.2)		1 (11.1)	6 (9.0)	2 (3.8)
19F	12 (7.7)	1 (4.5)	2 (33.3)		5 (7.5)	4 (7.7)
6A	10 (6.4)	2 (9.1)		2 (22.2)	3 (4.5)	3 (5.8)
6B	9 (5.8)	1 (4.5)	1 (16.7)		4 (6.0)	3 (5.8)
22F	8 (5.1)				6 (9.0)	2 (3.8)
23F	8 (5.1)	2 (33.3)			3 (4.5)	3 (5.8)
17F	7 (4.5)	2 (9.1)			4 (6.0)	1 (1.9)
4	6 (3.8)	1 (4.5)			2 (3.0)	3 (5.8)
18C	5 (3.2)				3 (4.5)	2 (3.8)
8	4 (2.6)				3 (4.5)	1 (1.9)
13	4 (2.6)	2 (9.1)			2 (3.0)	
7F	4 (2.6)	1 (4.5)			1 (1.5)	2 (3.8)
Other	30 (19.2)	4 (18.2)		4 (44.4)	11 (9.4)	9 (17.3)
Total N	156	22 (14.1)	6 (3.8)	9 (5.8)	67 (42.9)	52 (33.3)

Asia/Pacific countries include: Australia n=28, China n=3, Malaysia n=2, Philippines n=4, Singapore n=10, South Korea n=4, Taiwan n=1.

Table 4. Age and serotype distribution of 84 *S. pneumoniae* from the Middle East.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
19A	12 (14.3)	3 (27.3)	4 (28.6)		1 (3.6)	4 (20.0)
1	9 (10.7)		1 (7.1)	4 (36.4)	2 (7.1)	2 (10.0)
6A	7 (8.3)	2 (18.2)	1 (7.1)	1 (9.1)	2 (7.1)	1 (5.0)
5	5 (6.0)		3 (21.4)		2 (7.1)	
9V	5 (6.0)				3 (10.7)	2 (10.0)
4	4 (4.8)			1 (9.1)	3 (10.7)	
8	4 (4.8)				4 (14.3)	
14	4 (4.8)		1 (7.1)		1 (3.6)	2 (10.0)
11A	4 (4.8)			3 (27.3)		1 (5.0)
19F	4 (4.8)			1 (9.1)	1 (3.6)	2 (10.0)
6B	4 (4.8)	2 (18.2)	2 (14.3)			
7F	4 (4.8)	2 (18.2)			1 (3.6)	1 (5.0)
23F	3 (3.6)		1 (7.1)		1 (3.6)	1 (5.0)
10A	2 (2.4)				2 (7.1)	
12F	2 (2.4)				2 (7.1)	
Other	11 (13.1)	2 (18.2)	1 (7.1)	1 (9.1)	3 (3.6)	4 (20.0)
Total N	84	11 (13.1)	14 (16.7)	11 (13.1)	28 (33.3)	20 (23.8)

Middle Eastern countries include: Israel n=18, Oman n=1, Saudi Arabia n=1.

Table 5. Age and serotype distribution of 1279 *S. pneumoniae* from North America.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
19A	268 (21.0)	33 (38.4)	34 (44.2)	20 (25.6)	123 (18.4)	58 (15.6)
6A	94 (7.3)	5 (5.8)	6 (7.8)	4 (5.1)	43 (6.4)	36 (9.7)
7F	91 (7.1)	6 (7.0)	4 (5.2)	5 (6.4)	54 (8.1)	22 (5.9)
22F	82 (6.4)	8 (9.3)	2 (2.6)	4 (5.1)	41 (6.1)	27 (7.3)
3	67 (5.2)		2 (2.6)	2 (2.6)	35 (5.2)	28 (7.5)
23A	54 (4.2)	3 (3.5)	2 (2.6)	1 (1.3)	31 (4.6)	17 (4.6)
11A	52 (4.1)	2 (2.3)	2 (2.6)	2 (2.6)	28 (4.2)	18 (4.9)
12F	48 (3.8)	3 (3.5)	1 (1.3)	1 (1.3)	39 (5.8)	4 (1.1)
19F	48 (3.8)	5 (5.8)	7 (9.1)	7 (9.0)	24 (3.6)	5 (1.3)
35B	39 (3.0)	1 (1.2)	1 (1.3)		16 (2.4)	21 (5.7)
4	35 (2.7)	1 (1.2)		4 (5.1)	21 (3.1)	9 (2.4)
15A	34 (2.7)		2 (2.6)	2 (2.6)	13 (1.9)	17 (4.6)
6B	26 (2.0)	1 (1.2)	1 (1.3)	3 (3.8)	12 (1.8)	9 (2.4)
Other	341 (26.7)	18 (20.9)	13 (16.9)	23 (29.5)	187 (28.0)	100 (27.0)
Total N	1279	86 (6.7)	77 (6.0)	78 (6.1)	667 (52.2)	371 (29.0)

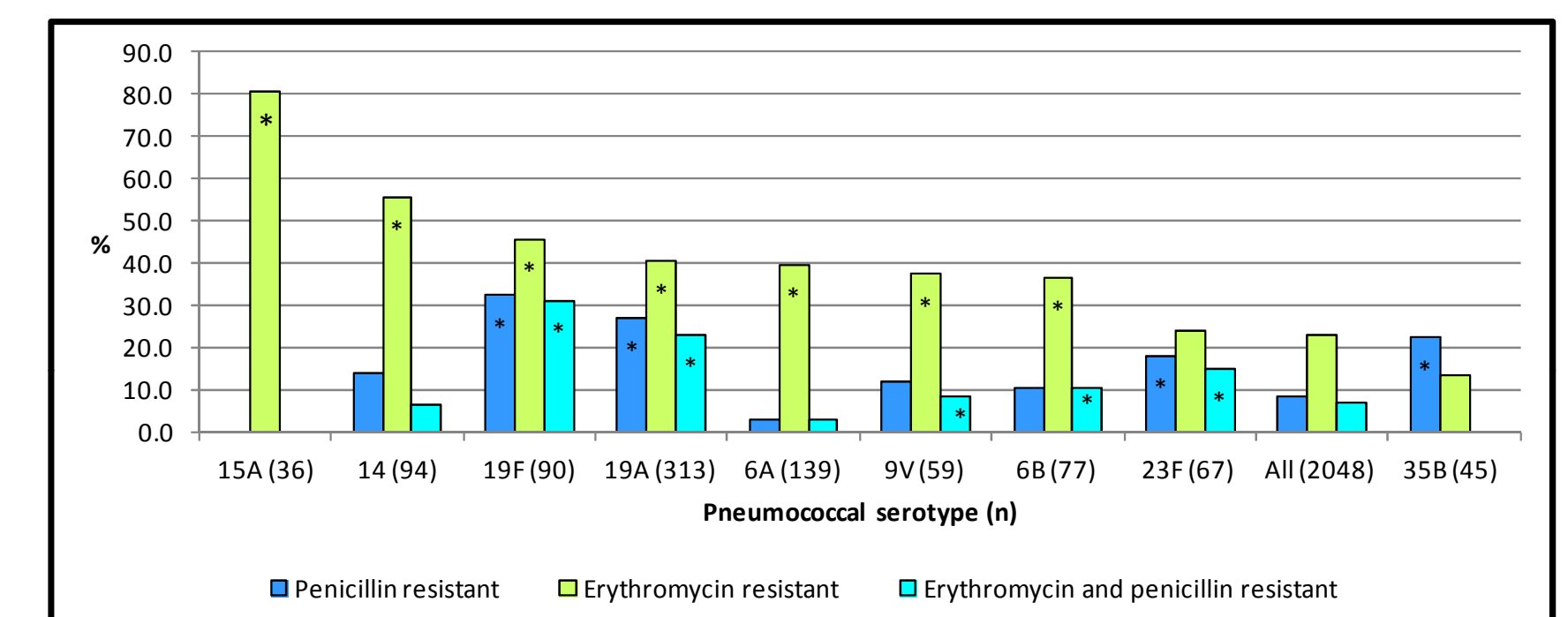
North American isolates include: Canada n=17, United States n=354.

Table 6. Age and serotype distribution of 468 *S. pneumoniae* from Europe*.

Serotype	Total N (%)	Age in years				
		<2	2-4	5-17	18-65	>65
3	40 (8.5)	3 (10.3)	1 (3.6)		19	17
14	36 (7.7)	9 (31.0)	6 (21.4)		5	16
1	35 (7.5)		2 (7.1)	5	20	8
7F	34 (7.3)			5	14	15
19A	31 (6.6)	3 (10.3)	3 (10.7)	3	8	14
6B	26 (5.6)	2 (6.9)		4	13	7
23F	24 (5.1)		3 (10.7)	5	9	7
6A	24 (5.1)	3 (10.3)	3 (10.7)	1	10	7
9V	22 (4.7)			1	12	9
22F	21 (4.5)	1 (3.4)	1 (3.6)	4	8	7
4	20 (4.3)	1 (3.4)		1	6	12
19F	19 (4.1)	5 (17.2)	2 (7.1)		2	10
11A	18 (3.8)				13	5
8	13 (3.8)				5	8
18C	12 (2.6)		1 (3.6)	2	5	4
9N	12 (2.6)		3 (10.7)		5	4
12F	11 (2.4)				5	6
Other	70 (15.0)	2 (6.9)	3 (10.7)	3 (8.8)	33 (17.2)	29 (15.7)
Total N	468	29 (6.2)	28 (6.0)	34 (7.3)	192 (41.0)	185 (39.4)

European countries include: Austria n=2, Belgium n=43, Croatia n=6, the Czech Republic n=9, Denmark n=27, Finland n= 10, France n=56, Germany n=56, Greece n=15, Hungary n=8, Ireland n=3, Italy n=95, Latvia n=8, Lithuania n=8, Poland n=6, Portugal n=4, Slovenia n=45, Spain n=79, Sweden n=14, Switzerland n=13, the Netherlands n=8, and the United Kingdom n=10.
*European data presented previously at ICAAC, Chicago, IL, 2011.

Figure 2. Resistance to erythromycin and penicillin of 2048 *S. pneumoniae* from a global population by serotype, all ages.



Conclusions

- This analysis of organisms collected through the TEST program demonstrates that for all age groups in all regions combined, serotype 19A (15.6%) was the most common serotype isolated during 2004-09 globally. Serotype distribution varied by age group, with 27.5% of isolates from children 4 years and under expressing 19A, with next most frequent serotypes 19F (9.5%) and 14 (8.5%) in this age group. Serotype 1 was found in 9.0% of isolates from children 5-15 years, while 7F was found in 7.6% of this age group. The most common serotypes in isolates from adults over 15 years of age were 19A (13.3%), 3 (7.1%), 6A (6.9%) and 7F (6.8%). Serotype distribution varies by region, with the North American region isolates contributing 59% of all isolates in this analysis.
- Antibiotic susceptibility testing showed some of the most common serotypes, notably 15A, 19A, 6A, 19F and 14, to be associated with increased resistance to erythromycin ($p < 0.05$, Fisher's exact test), with 19A and 19F more likely to be resistant to both erythromycin and penicillin ($p < 0.0001$, Fisher's exact test). Ten isolates of varying serotypes were levofloxacin resistant, with seven of the ten originating in North America (data not shown).
- In this study, serotypes 3, 19A and 6A accounted for more than 28% of all *S. pneumoniae* clinical isolates, with 63% of all isolates collected among serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F. The prevalence of serotypes contained only in PCV7 has been reduced to less than 23%.
- Although limited in numbers, these data suggest that a significant burden of pneumococcal disease worldwide can be attributed to these serotypes. The association of emerging serotypes with antibiotic resistance highlights the need for ongoing monitoring of the seroepidemiology of this important pathogen.