International Journal of Infectious Diseases 20 (2014) 47–51

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Susceptibility profiles and correlation with pneumococcal serotypes soon after implementation of the 10-valent pneumococcal conjugate vaccine in Brazil

Mariana Mott [a], Juliana Caierão [a,*], Gabriela Rosa da Cunha [a], Leandro Reus Rodrigues Perez [b], Roberto Matusiak [c], Katia Ruschel Pilger de Oliveira [d], Pedro Alves d'Azevedo [a], Cícero Dias [a,b]

[a] Universidade Federal de Ciências da Saúde de Porto Alegre, Sarmento Leite, 245, Porto Alegre, Rio Grande do Sul, Brazil
[b] Hospital Mãe de Deus, Porto Alegre, Rio Grande do Sul, Brazil
[c] Grupo Hospitalar Conceição, Porto Alegre, Rio Grande do Sul, Brazil
[d] Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 19 November 2013
Accepted 20 November 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Streptococcus pneumoniae
Resistance
Serotypes
Vaccines

SUMMARY

Objectives: To evaluate the susceptibility patterns among *Streptococcus pneumoniae* recovered during the years 2010–2012 and to correlate these with serotypes.
Methods: Pneumococci from invasive sites were serotyped by sequential multiplex PCR and/or Quellung reaction. Etest strips were used to determine the minimal inhibitory concentrations, and the Clinical and Laboratory Standards Institute (CLSI) guidelines were used for interpretation. Genetic determinants of macrolide resistance were assessed by PCR, and the occurrence of the D phenotype was analyzed following the recommendations of the CLSI.
Results: One hundred fifty-nine *S. pneumoniae* were studied; most were recovered from blood and were associated with serotypes 14, 3, 4, 23F, 20, 7F, 12F, 19A, and 19F. Pneumococcal conjugate vaccine PCV7, PCV10, and PCV13 and 23-valent polysaccharide vaccine serotypes represented 38.2%, 48.7%, 64.5%, and 85.5%, respectively. β-Lactam non-susceptibility (non-meningitis) was basically related to serotype 19A. For meningitis, it was observed in 21.4% (serotypes 14, 3, 9V, 23F, and 24F). Resistance to erythromycin occurred in 8.2% and *mef*A was the most common macrolide genetic determinant. One isolate was resistant to levofloxacin. Non-susceptibility to trimethoprim-sulfamethoxazole was 37.7% and to

PCV10. Non-susceptible patterns were found to be associated with classical PCV serotypes (especially serotype 14), which is still highly prevalent, and non-PCV10 ones (19A), which may disseminate, occupying the biological niche left by the vaccine serotypes.

1. Introduction

The microbiological diagnosis of pneumococcal diseases is frequently associated with challenging situations, such as the lack of sensitivity of culture-related methodologies and the use of antimicrobials prior to specimen collection.[1] Thus, the final diagnosis is commonly based on clinical and epidemiological characteristics of the disease, which results in empirical therapy.[2] However, antimicrobial resistance among *Streptococcus pneumoniae* has become a subject of concern, and as a result, empirical therapeutic choices may be compromised.[3]

Penicillin is the most important antibiotic against pneumococcal diseases. Strains with decreased susceptibility to penicillin were first reported in the 1960s, and since that period, resistance to this agent and other antimicrobials has been increasing constantly, to various degrees, from one region to another.[4–8] For the laboratory detection of penicillin and ceftriaxone resistance, the Clinical and Laboratory Standards Institute (CLSI) currently defines different breakpoints for meningitis and non-meningitis isolates.[9]

Studies have reported that some resistance patterns may be related to specific serotypes or clones, and the Pneumococcal Molecular Epidemiology Network (PMEN) has described the most

* Corresponding author.
E-mail address: julianaca@ufcspa.edu.br (J. Caierão).

1201-9712
http://dx.doi.org/10.1016/j.ijid.2013.11.009

relevant clones of antibiotic-resistant pneumococci.[10] The increase in penicillin non-susceptibility in the USA following the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), which was strongly associated with the dissemination of serotype 19A, is a good example of the clone–resistance pattern correlation.[11]

The introduction of different formulations of conjugate vaccines, as well as other variables, has contributed to changes in antimicrobial resistance worldwide.[8] In Brazil, a 10-valent vaccine (PCV10) has been introduced for children aged less than 2 years as part of the national program of immunization; however its effect on serotype distribution and antimicrobial resistance in this country are yet to be determined. As the diversity of serotypes and the increasing resistance to antibiotics are two essential elements that must be taken into account for the prevention and management of pneumococcal infections,[5,12] the objective of the present study was to evaluate the susceptibility patterns among *S. pneumoniae* recovered during the years 2010–2012 and to correlate these with serotypes.

2. Materials and methods

2.1. Bacterial isolates

A total of 159 non-duplicate *S. pneumoniae* isolates were included in the study. They were isolated from January 2010 to April 2012 in three general hospitals in Porto Alegre, Brazil. The study included isolates from patients with invasive pneumococcal diseases (IPD). The isolates were maintained at −80 °C and the species identification was done by routine tests: colony morphology, optochin susceptibility, and sodium deoxycholate lysis.[13]

2.2. Serotyping

Isolates were serotyped using a sequential multiplex PCR[14] targeting the 30 most common serotypes related to IPD in Latin America plus the capsular polysaccharide (*cps*) gene. For most isolates that presented amplification of only the *cps* gene, the Quellung reaction was performed using pool-, type-, and factor-specific antisera kindly provided by the US Centers for Disease Control and Prevention (CDC).

2.3. Susceptibility tests

Etest strips (AB Biodisk, Stockholm, Sweden) were used to determine the minimal inhibitory concentration (MIC), following the manufacturer's instructions. MICs for penicillin, ceftriaxone, vancomycin, meropenem, erythromycin, levofloxacin, tetracycline, and trimethoprim–sulfamethoxazole were evaluated. Interpretation of the results was done in accordance with the CLSI guidelines (2013),[9] taking into account the site of isolation to set the penicillin and ceftriaxone susceptibilities. Etest MICs were rounded up to a standard two-fold agar dilution scale. The reference strain *S. pneumoniae* ATCC 49619 was used for quality control.

2.4. Inducible resistance to clindamycin

To determine the erythromycin inducible resistance to clindamycin, isolates presenting resistance to erythromycin and susceptibility or intermediate resistance to clindamycin were submitted to D-zone test, following the 2013 CLSI recommendations.[9]

2.5. Genetic determinants of macrolide resistance

Isolates presenting a MIC ≥0.5 μg/ml for erythromycin were submitted to a duplex PCR reaction for the detection of the *erm*B and *mef*A genes, in accordance with Widdowson and Klugman.[15] Briefly, approximately 100 ng of DNA were used as template in a 20-μl reaction with 0.75 μM of each primer and 2 U of Taq DNA polymerase, at an annealing temperature of 56 °C. PCR products were visualized on a 2% TBE (Tris–borate–ethylenediaminetetraacetic acid (EDTA)) agarose gel, containing 0.5 μg/ml of ethidium bromide.

3. Results

We studied 159 *S. pneumoniae* isolates obtained from invasive sites, including blood (n = 124), cerebrospinal fluid (CSF) (n = 28), pleural fluid (n = 5), peritoneal fluid (n = 1), and joint fluid (n = 1). Patients ranged in age from 0 to 94 years, with an average of 47.9 years. Seventeen patients (10.7%) were aged ≤5 years and 39 (24.5%) were aged ≥65 years. Age was not available in the records for 13 patients and three others were identified as 'pediatric' without a defined age.

The distribution of serotypes found among isolates (in general and stratified by age) is shown in Table 1. Three isolates could not

Table 1
Serotype distribution of 159 pneumococcal isolates from invasive infections; Porto Alegre, Brazil, 2010–2012

Serotype	Vaccine formulation[a]	Number of isolates		
		Total	≤5 years old	≥6 years old
1	PCV10, PVC13, and PV23	3	-	3
3	PCV13 and PV23	13	2	11
4	PCV7, PCV10, PCV13, and PV23	15	-	15
5	PCV10, PVC13, and PV23	2	-	2
6A	PCV13	4	1	3
Serogroup 6	?	1	-	1
6B	PCV7, PCV10, PVC13, and PV23	5	1	4
6C	-	2	1	1
7C	-	1	-	1
7F	PCV10, PVC13, and PV23	11	-	11
8	PV23	5	1	4
9A	-	1	-	1
9N	PV23	1	-	1
9V	PCV7, PCV10, PVC13, and PV23	6	-	6
10A	PV23	1	-	1
11A	PV23	4	-	4
12F	PV23	11	-	11
14	PCV7, PCV10, PVC13, and PV23	16	6	10
15B	PV23	1	-	1
16F	-	2	-	2
17F	PV23	1	-	1
18A	-	1	-	1
18C	PCV7, PCV10, PVC13, and PV23	1	-	1
19A	PVC13, and PV23	7	1	6
19F	PCV7, PCV10, PVC13, and PV23	5	2	3
20	PV23	12	-	12
22F	PV23	1	-	1
23F	PCV7, PCV10, PVC13, and PV23	10	1	9
24F	-	4	-	4
28A	-	1	-	1
34	-	1	-	1
35F	-	2	-	2
38	-	2	1	1
Non-typeable	-	3	-	3
Not available for serotyping	-	3	-	3
Total		159	17	142

[a] PCV 7, 10, and 13 = pneumococcal conjugated vaccine with 7, 10, and 13 serotypes; PV23 = 23-valent polysaccharide vaccine.

Table 2
Prevalence of resistance and the MIC_{50} and MIC_{90} among 159 invasive pneumococcal isolates; Porto Alegre, Brazil, 2010–2012

	Susceptibility			MIC	
	R (%)	I (%)	S (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Penicillin (non-meningitis)	0	1 (0.6)	158 (99.4)	0.025	1
Ceftriaxone (non-meningitis)	0	2 (1.3)	157 (98.7)	0.016	0.5
Meropenem	1 (0.6)	6 (3.8)	152 (95.6)	0.006	0.25
Erythromycin	13 (8.2)	0	146 (91.8)	0.125	0.25
Tetracycline[b]	30 (19)	5 (3.2)	123 (77.8)	0.25	16
Levofloxacin	1 (0.63)	0	158 (99.4)	1	1
Trimethoprim–sulfamethoxazole	32 (20.1)	28 (17.6)	99 (62.3)	0.25	4

MIC, minimal inhibitory concentration; R, resistant; I, intermediate; S, susceptible.
[a] Statistically significant.
[b] One isolate was not available for tetracycline testing.

be recovered for serotyping, and one isolate belonging to serogroup 6 (according to the sequential multiplex PCR procedure) was not available for Quellung reaction. A total of 33 different serotypes were identified. Isolates belonging to serotypes 14 ($n = 16$), 4 ($n = 15$), 3 ($n = 13$), 20 ($n = 12$), 7F ($n = 11$), 12F ($n = 11$), 23F ($n = 10$), 19A ($n = 7$), 9V ($n = 6$), 6B ($n = 5$), and 19F ($n = 5$) were the most frequently found. Three isolates were defined as non-typeable by Quellung reaction. Serotypes included in the 7-, 10-, and 13-valent conjugate vaccines and the 23-valent polysaccharide vaccine represented 38.2% ($n = 58$), 48.7% ($n = 74$), 64.5% ($n = 98$), and 85.5% ($n = 130$), respectively, of the 152 isolates with a defined serotype. Among children aged ≤5 years ($n = 17$), serotype 14 was again the most common, but at this time more representative (35.5% vs. 7.0% in the population >5 years old). Vaccine coverage in this group was 58.8%, 58.8%, and 82.3% for the 7-, 10-, and 13-valent conjugate vaccines, respectively. In the even more restrictive group (under 2 years of age, $n = 12$), the vaccine coverage was 58.3%, 58.3%, and 83.3%. Taking into account people aged ≥65 years, these numbers decreased further: 30.8% (12/39), 33.3% (14/39), and 41.0% (18/40) for the 7-, 10-, and 13-valent conjugate vaccines, respectively. However, the polysaccharide vaccine (PPV23) had 71.8% coverage in this specific population.

Table 2 presents the prevalence of resistance and the MIC_{50} and MIC_{90} for the 159 pneumococcal isolates. One isolate was not available for tetracycline testing. Regardless of resistance rates, the most potent drugs against pneumococci were meropenem and erythromycin ($MIC_{90} = 0.25$ μg/ml). All isolates were susceptible to penicillin and ceftriaxone (non-meningitis breakpoints); however, intermediate resistance was found to penicillin (one isolate) and ceftriaxone (two isolates), all isolates serotyped as 19A. Considering the meningitis breakpoint (≥0.125 μg/ml), resistance to penicillin was observed in 6/28 (21.4%) isolates obtained from CSF, belonging to the following serotypes: 14 (two isolates), 3, 9V, 23F, and 24F (one isolate each). All CSF isolates were susceptible to ceftriaxone (meningitis breakpoint). Characteristics of pneumococci presenting resistance to penicillin (intermediate, non-meningitis), to ceftriaxone (intermediate or full, meningitis or non-meningitis), and to meropenem (intermediate or full resistance), the serotypes, and resistance to other antimicrobials are detailed in Table 3. Penicillin MICs higher than 0.06 μg/ml were more frequently associated with serotypes 14 (32.0%), 23F (12.0%), 9V (12.0%), 19A (8.0%), and 19F (6.0%), representing 70.0% of all cases.

Resistance (MIC ≥1 μg/ml) to erythromycin was observed in 8.2% (13/159) of the isolates. The *mef*A gene alone was amplified in 6/13 (46.1%) and the *erm*B gene alone in 4/13 (30.8%) pneumococci; both genes were amplified in 3/13 (23.0%). The presence of *erm*B (alone or with *mef*A) was associated with a MIC ≥256 μg/ml. Table 4 shows the distribution of genes involved in erythromycin resistance, the results of the D-test, and serotypes found among THESE pneumococci.

Isolates were fully susceptible to vancomycin (MIC_{50} and $MIC_{90} = 0.5$ μg/ml). Only one *S. pneumoniae*, isolated from a blood culture, was resistant to levofloxacin, with a MIC of 16 μg/ml; this isolate belonged to serotype 23F. Higher levels of non-susceptibility

Table 3
Characteristics of isolates presenting resistance to penicillin (intermediate, non-meningitis), to ceftriaxone (intermediate or full, meningitis or non-meningitis), and to meropenem (intermediate or full resistance)

Isolate number	Serotype	Specimen	Patient age (years)	MIC (μg/ml)					*erm*B gene	*mef*A gene
				PEN	CRO	MER	ERY	TET		
033.12	14	Blood	27	2	1	0.5	0.047	0.125	ND	ND
008.11	14	Blood	Pediatric	1	0.5	0.5	24	0.125	Neg	Pos
112.11	14	Pleural fluid	2	0.5	0.5	0.5	24	0.25	Neg	Pos
162.11	14	blood	4	1	0.5	0.5	16	<0.25	Neg	Pos
164.11	14	blood	4	1.5	0.5	0.5	8	1	Neg	Pos
025.12	19A	Blood	67	4	2	1	>256	6	Pos	Pos
167.11	19A	Blood	67	2.0	2	0.5	>256	8	Pos	Pos

MIC, minimal inhibitory concentration; PEN, penicillin; CRO, ceftriaxone; MER, meropenem; ERY, erythromycin; TET, tetracycline; ND, not done.

Table 4
Distribution of the genes involved among the 13 erythromycin-resistant isolates, serotypes found, minimal inhibitory concentrations, and results of the D-test

Genes involved	*n*	Serotypes (number in parenthesis)	MIC or MIC range (μg/ml)	D-test results
*mef*A	6	14 (5), 19A, non-typeable (1)	1.5–24	Neg
*erm*B	4	6B (1), 19F (1), 23F (1), 24F (1)	>256	Neg (2); Pos (2)
*mef*A + *erm*B	3	19A (2), 19F (1)	>256	Pos

MIC, minimal inhibitory concentration.

were observed for trimethoprim–sulfamethoxazole (37.7%: 20.1% intermediate and 17.6% resistant) and tetracycline (22.0%: 18.9% intermediate and 3.1% resistant).

4. Discussion

Pneumococcal disease is a global public health problem, especially in developing countries. In these regions, epidemiological surveillance focusing on the serotype distribution and resistance profiles of pneumococci is of great importance to reduce the burden of the disease and to improve therapy. A comprehensive understanding of antimicrobial resistance and the influence of serotypes on the susceptibility profiles should be obtained from investigations with pneumococci gathered from different parts of the world. In this context, the present study generated epidemiological data on the pneumococci circulating in the South of Brazil, taking into account the occurrence of antimicrobial resistance and its correlation with serotypes.

Many studies around the world have demonstrated an increasing trend in resistance of pneumococci to various classes of antimicrobials,[4–8,12] both in regions where a conjugated vaccine is available and efficiently provided to the population,[8] and where there is no active public vaccination program.[5] In the former context, the fact is possibly explained by the replacement of vaccine serotypes with others, which may present worrying resistance rates; serotype 19A is a classical example. In the second group, the reduced susceptibility may be especially related to the circulation of vaccine serotypes typically associated with antimicrobial resistance, such as 14, 19F, and 23F. Therefore, regardless of the existence or not of a vaccination program, resistance among pneumococci is becoming a matter of concern.

Our pneumococci represent post-vaccination isolates. However, considering that our study population consisted mostly of adults, we may expect the findings to represent a transition era, i.e., not enough time has elapsed to observe the effects of vaccination (herd immunity) on the adult population. Indeed, almost half (47.4%) of our pneumococcal isolates represented PCV10 serotypes, while the remaining isolates represented mixed non-PCV10 ones. According to Afonso et al.,[16] vaccine coverage in Porto Alegre is around 80%, which is lower than in other cities of Brazil. Altogether, these data support this being a transitional period.

Dos Santos et al.[17] first evaluated the distribution of serotypes among invasive disease before and after the implementation of PCV10 in Brazil. As expected, the incidence of PCV10 serotypes decreased in a comparison of the two periods, although the reduction was statistically significant only for the population aged <2 years. This reinforces the concept that a longer period of time needs to elapse from the implementation of PCV10 to observe its effect on the adult population (transition period). Among PCV10 serotypes, 14, 1, 5, and 7F were in the group of serotypes with a higher incidence during both periods observed by Dos Santos et al. Regarding non-PCV10 serotypes, 19A, 3, and 12F were predominant in this group. Although these data are from a different geographical region of Brazil, our serotype distribution results are, in general, consistent with those observed in that study. Our isolates present a wide distribution of serotypes, which include members of the different vaccine formulations, but 17.7% of them are not included in the most comprehensive of the vaccines, PPV23.

Among children (age ≤5 years), the proportion of isolates belonging to serotypes included in the vaccine (PCV10) was lower compared to values observed in other Brazilian studies (58% vs. 75.7–80%[17,18]). However, it is necessary to note that our data are derived from a relatively limited number of isolates, which compromises the interpretation of the results.

With regard to our general population, PCV10 coverage was even lower. This may be explained in part by the circulation of serotypes not related to PCV10, such as 12F, 7F, 20, and 19A. Attention should be drawn to the fact that serotypes such as 12F and 20, which were among the six most frequent, are not included in the available conjugate vaccines. This highlights the possibility of the emergence of these serotypes in the future.

In general, our resistance rates are lower than those observed in other countries with vaccination programs.[6,8,19] In groups where there is no active vaccination, or where vaccine coverage is not wide, resistance rates have also been higher than ours. In these cases, the most common serotypes recovered have been 14, 23F, 6B, and 19F, classical PCV7 ones.[4,5] In Latin America, knowledge of the circulating pneumococci is limited. Data from the SIREVA project, connected to the Pan American Health Organization, have shown reduced susceptibility to penicillin in 38% of isolates, with an increasing trend in some countries, including Brazil.[20] Data from Brazil are still scarce and fragmented. Some studies have shown low[21,22] or no[23] rates of non-susceptibility to penicillin, whereas others have indicated rates around 20%;[24–25] serotypes 14, 23F, 6B, and 19F have been most commonly associated with this resistance in brazilian studies.[24,25]

Full resistance (MIC >4.0 μg/ml) to penicillin was not found among the isolates included in this study. However, pneumococci showing intermediate or full resistance to β-lactams were observed, and this was associated with serotypes 14 and 19A. The characteristics of patients are distinct among those with serotype 14 and 19A. Patients with infection due to resistant serotype 14 isolates were younger (four children and one aged 27 years), whereas patients with 19A infections were older (>65 years). Also, β-lactam-resistant isolates of serotype 14 were resistant to erythromycin due to the presence of the *mef*A gene, while those belonging to serotype 19A harbored the *erm*B gene and had higher erythromycin MICs (>256 μg/ml). Both of these serotypes contain clones associated with resistance that are internationally distributed, such as sequence types ST156 (serotype 14) and ST320 (serotype 19A). While serotype 14 has historically (pre-vaccination period) been associated with penicillin resistance, with ST156 distributed worldwide including in Brazil,[25] many studies in the post-vaccination era have pointed to the important relationship between 19A and penicillin non-susceptibility.[8,18,26]

As noted above, among patients infected with non-susceptible serotype 14 pneumococci, four out of five were children, one of them without a defined age ('pediatric'). Two of them were 4 years old at the time of specimen collection (2011), which suggests that these patients did not receive the 2010 PCV10 immunization. The remaining patients were 2 years old (2011). These data may explain the occurrence of resistant serotype 14 among our pediatric population. However, as the number of children included in the study was low, statistical power is lacking to support information considering serotype distribution and antimicrobial susceptibility characteristics in this specific age group.

Another important therapeutic choice for pneumococcal infections is the macrolides. Resistance to erythromycin varies, in general from 25% to near 50%.[4,5,8] However, rates as high as 92% have been reported in some regions.[6] As shown by Mendonça-Souza et al.[27] in a large prospective study of isolates from three major cities in Brazil, pneumococci belonging to serotype 19A have been circulating in our region since 1996, recovered particularly from non-invasive sites and presenting resistance to erythromycin, tetracycline, and penicillin (previous 2008 CLSI breakpoints). Vaccination may provide conditions for the emergence of non-PCV10 serotypes, such as 19A. Further studies involving genotyping and clonality are necessary to better determine the influence of such specific clones on antibiotic resistance in Brazil. It is also important to highlight the occurrence of isolates exhibiting both *erm*B and *mef*A genes among serotypes 19A and 19F.

In a recent study in Brazil,[23] no quinolone resistance and only one isolate with intermediate resistance was observed among invasive and non-invasive pneumococci. Similar results were found in the current study (one fully resistant isolate) and by another group of authors in Latin America.[28] Although our data are also in agreement with those of other Brazilian studies with regard to tetracycline resistance (between 20% and 30%), some authors have shown higher trimethoprim–sulfamethoxazole resistance rates (up to 60%) in other regions of the country.[24,25]

Our study has some limitations. As we did not have a pre-vaccination population, we were unable to evaluate the impact of vaccination on resistance rates in our region. We also emphasize that serotyping the strains before starting a vaccination campaign is highly recommended for future experience. Also, as mentioned, our population included a small number of patients less than 5 years of age. Thus, we were not able to evaluate the initial effects of the vaccination appropriately, once children became the target for first-stage processing.

Nevertheless, our data provide important information on the susceptibility profile of pneumococci soon after the introduction of the vaccination. To our knowledge, this is among the first works to provide information on the pneumococcal serotype distribution following implementation of vaccination in Brazil. Our data show the occurrence of non-susceptibility to penicillin and other β-lactams among serotypes 14 and 19A. It is expected that the finding of serotype 14 in invasive disease will be massively reduced as the effect of PCV10 is increasingly observed. On the other hand, because serotype 19A is not included in the current vaccine formulation, non-susceptible isolates may increase in our region. In this context, epidemiological studies should be done systematically to evaluate the long-term effects of vaccination on serotype distribution and how changes in the distribution of serotypes in the post-vaccination era will impact resistance rates in the pneumococcal population.

Acknowledgements

We are grateful for the financial support received from the Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (11/2058-5, FAPERGS/CAPES; 473607/2008-5, CNPq; and 10/0026.1, FAPERGS). We also thank the Streptococcus Laboratory (especially Dr Lesley McGee) of the US Centers for Disease Control and Prevention for technical support in the Quellung serotyping. We also thank Andreia Duprat, Janira Prichula, and Rebeca Inhoque for technical assistance.

Conflict of interest: No conflict of interest to declare.

References

1. Werno AM, Murdoch DR. Laboratory diagnosis of invasive pneumococcal disease. *Clin Infect Dis* 2008;**46**:926–32.
2. Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. *Clin Infect Dis* 2011;**52**:S296–304.
3. Jones RN, Jacobs MR, Sader HS. Evolving trends in *Streptococcus pneumoniae* resistance: implications for therapy of community-acquired bacterial pneumonia. *Int J Antimicrob Agents* 2010;**36**:197–204.
4. Charfi F, Smaoui H, Kechrid A. Non-susceptibility trends and serotype coverage by conjugate pneumococcal vaccines in a Tunisian pediatric population: a 10-year study. *Vaccine* 2012;**30**:G18–24.
5. Tali-Maamar H, Laliam R, Bentchouala C, Touati D, Sababou K, Azrou S, et al. Reprint of: Serotyping and antibiotic susceptibility of Streptococcus pneumoniae strains isolated in Algeria from 2001 to 2010. *Vaccine* 2012;**30**:G25–31.
6. Tsai HY, Lauderdale TL, Wang JT, Chen YS, Liu JW, Huang JH, et al. Updated antibiotic resistance and clinical spectrum of infections caused by *Streptococcus pneumoniae* in Taiwan: emphasis on risk factors for penicillin non-susceptibilities. *J Microbiol Immunol Infect* 2012;**46**:345–51. http://dx.doi.org/10.1016/j.jmii.2012.07.012.
7. Munier AL, de Lastours V, Varon E, Donay JL, Porcher R, Molina JM. Invasive pneumococcal disease in HIV-infected adults in France from 2000 to 2011: antimicrobial susceptibility and implication of serotypes for vaccination. *Infection* 2013;**41**:663–8. http://dx.doi.org/10.1007/s15010-013-0419-x.
8. Jones RN, Sader HS, Mendes RE, Flamm RK. Update on antimicrobial susceptibility trends among Streptococcus pneumoniae in the United States: report of ceftaroline activity from the SENTRY Antimicrobial Surveillance Program (1998–2011). *Diagn Microbiol Infect Dis* 2013;**75**:107–9.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 23rd Informational supplement. M100-S23. Wayne, PA: CLSI; 2013.
10. McGee L, McDougal L, Zhou J, Spratt BG, Tenover FC, George R, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the Pneumococcal Molecular Epidemiology Network. *J Clin Microbiol* 2001;**39**:2565–71.
11. Moore M, Gertz RE, Woodbury RL, Barkocy-Gallagher GA, Schaffner W, Lexau C, et al. Population snapshot of emergent Streptococcus pneumoniae serotype 19A in the United States, 2005. *J Infect Dis* 2008;**197**:1016–27.
12. Isaacman DJ, McIntosh ED, Reintert RR. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int J Infect Dis* 2010;**14**:e197–209.
13. Spellerberg B, Brandt C. Streptococcus. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, editors. *Manual of clinical microbiology*. 10th ed., Washington, DC: American Society for Microbiology; 2011 p. 331–49.
14. Dias CA, Teixeira LM, Carvalho MG, Beall B. Sequential multiplex PCR for determining capsular serotypes of pneumococci recovered from Brazilian children. *J Med Microbiol* 2007;**56**:1185–8.
15. Widdowson CA, Klugman KP. Emergence of the M phenotype of erythromycin-resistant pneumococci in South Africa. *Emerg Infect Dis* 1998;**4**:277–81.
16. Afonso ET, Minamisava R, Bierrenbach AL, Escalante JJ, Alencar AP, Domingues CM, et al. Effect of 10-valent pneumococcal vaccine on pneumonia among children, Brazil. *Emerg Infect Dis* 2013;**19**:589–97.
17. Dos Santos SR, Passadore LF, Takagi EH, Fujii CM, Yoshioka CR, Gilio AE, et al. Serotype distribution of *Streptococcus pneumoniae* isolated from patients with invasive pneumococcal disease in Brazil before and after ten-pneumococcal conjugate vaccine implementation. *Vaccine* 2013;**31**:6150–4. http://dx.doi.org/10.1016/j.vaccine.2013.05.042.
18. Andrade AL, Oliveira R, Vieira MA, Minamisava R, Pessoa Jr V, Brandileone MC, et al. Population-based surveillance for invasive pneumococcal disease and pneumonia in infants and young children in Goiânia, Brazil. *Vaccine* 2012;**30**:1901–9.
19. Oftadeh S, Gidding HF, Gilbert GL. Laboratory surveillance of invasive pneumococcal disease in New South Wales, Australia, before and after introduction of 7-valent conjugate vaccine: reduced disease, but not antibiotic resistance rates. *Epidemiol Infect* 2012;**25**:1–10.
20. Castañeda E, Agudelo CI, Regueira M, Corso A, Brandileone MC, Brandão AP, et al. Laboratory-based surveillance of *Streptococcus pneumoniae* invasive disease in children in 10 Latin American countries: a SIREVA II project, 2000–2005. *Pediatr Infect Dis J* 2009;**28**:e265–70.
21. Bedran MB, Camargos PA, Leocádio Filho G, Bedran RM, Najar HC. Susceptibility of *Streptococcus pneumoniae* to penicillin in the state of Minas Gerais, Brazil, from 1997–2004. *Braz J Infect Dis* 2005;**9**:390–7.
22. Mouro A, Kiffer C, Koga PC, Monteiro AM, Camargo EC, Pignatari AC. Spatial exploration of *Streptococcus pneumoniae* clonal clustering in São Paulo, Brazil. *Braz J Infect Dis* 2011;**15**:462–6.
23. Rossi F, Franco MR, Rodrigues HM, Andreazzi D. *Streptococcus pneumoniae*: susceptibility to penicillin and moxifloxacin. *J Bras Pneumol* 2012;**38**:66–71.
24. Menezes AP, Campos LC, dos Santos MS, Azevedo J, Dos Santos RC, Carvalho Mda G, et al. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* prior to introduction of the 10-valent pneumococcal conjugate vaccine in Brazil, 2000–2007. *Vaccine* 2011;**29**:1139–44.
25. Barroso DE, Godoy D, Castañeiras TM, Tulenko MM, Rebelo MC, Harrison LH. β-Lactam resistance, serotype distribution, and genotypes of meningitis-causing *Streptococcus pneumoniae*, Rio de Janeiro, Brazil. *Pediatr Infect Dis J* 2012;**31**:30–6.
26. Richter SS, Heilmann KP, Dohrn CL, Riahi F, Beekmann SE, Doern GV. Changing epidemiology of antimicrobial-resistant *Streptococcus pneumoniae* in the United States, 2004–2005. *Clin Infect Dis* 2009;**48**:e23–33.
27. Mendonça-Souza CR, Carvalho MD, Barros RR, Dias CA, Sampaio JL, Castro AC. Occurrence and characteristics of erythromycin-resistant *Streptococcus pneumoniae* strains isolated in three major Brazilian states. *Microb Drug Resist* 2004;**10**:313–20.
28. Mendes C, Marin ME, Quiñones F, Sifuentes-Osornio J, Cuilty Siller C, Castanheira M, et al. Antibacterial resistance of community-acquired respiratory tract pathogens recovered from patients in Latin America: results from the PROTEKT surveillance study (1999–2000). *Braz J Infect Dis* 2003;**7**:44–61.