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 tetracycline was 22.0%.
Conclusions: Our population of pneumococci represents a transition era, soon after the introduction of 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.