0021-7557/09/85-06/495
Jornal de Pediatria

Prevalence of serotypes and antimicrobial resistance of invasive strains of pneumococcus in children: analysis of 9 years

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Abstract

Objective: To determine the prevalence of serotypes and antimicrobial susceptibility of strains of pneumococcus in children and to evaluate the implications for vaccine formulation.

Methods: Strains of pneumococcus obtained from children admitted with invasive diseases were isolated at Hospital de Clínicas of Universidade Federal de Uberlândia, Uberlândia, Brazil, and sent to Instituto Adolfo Lutz, São Paulo, Brazil, for further identification, serotyping, and determination of antimicrobial susceptibility.

Results: From April 1999 to December 2008, 142 strains of pneumococcus, obtained from children under 5 years of age, were analyzed. Seventy-five (52.8%) patients were male, and the age ranged from 1 to 60 months (mean age = 19±15.4 months; median = 15 months). The most common diagnoses were pneumonia [92 cases (64.8%)] and meningitis [33 cases (23.2%)]. The strains were mostly isolated from blood [61 samples (43%)], pleural fluid [52 samples (36.6%)], and cerebrospinal fluid [28 samples (19.7%)]. The most common serotypes were 14, 5, 6B, 1, 6A, 18C, 19A, 3, 9V, 19F, 23F, 9N, and 10A. There were 14 [9.9%] penicillin-resistant strains, which was detected only in the following serotypes: 14, 6B, 19F, 19A, and 23F, being predominant from 2004 to 2008 (p = 0.000). There was reduced susceptibility to co-trimoxazole (79.5%), erythromycin and clindamycin (11.3% each), and ceftriaxone (5.6%).

Conclusions: Penicillin resistance was detected in 9.9% of the strains, being predominant from 2004 to 2008. Twenty different pneumococcal serotypes were identified, and 71.9% of the serotypes were represented in the 7-valent conjugate vaccine (PN CRM7) currently available.

J Pediatr (Rio J). 2009;85(6):495-502: Streptococcus pneumoniae, serotyping, pneumococcal vaccines, drug resistance.

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This study was conducted at Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil, and at Instituto Adolfo Lutz (IAL), São Paulo, SP, Brazil.

No conflicts of interest declared concerning the publication of this article.

Suggested citation: Mantese OC, de Paula A, Almeida VV, de Aguiar PA, Wolkers PC, Alvares JR, et al. Prevalence of serotypes and antimicrobial resistance of invasive strains of pneumococcus in children: analysis of 9 years. J Pediatr (Rio J). 2009;85(6):495-502.

Manuscript submitted May 14 2009, accepted for publication Sep 30 2009.

doi:10.2223/JPED.1950

Introduction

Streptococcus pneumoniae is one of the most frequent cause of pneumonia, acute otitis media (AOM), meningitis, and sinusitis in children.[1,2] It is responsible for high rates of morbidity and mortality in children younger than 5 years old and adults older than 65 years old, mainly in developing countries.[2-4]

The clinical evolution of the pneumococcal infection is influenced by several aspects, and the appropriateness of the treatment chosen is among these factors. The treatment of pneumococcal infections is based on initial antibiotic therapy, which is usually empirical in terms of etiology and *in vitro* susceptibility to antimicrobial agents. The antibiotic therapy can be adequately adjusted after the performance of bacterial culture isolation and antibiogram test. Penicillin is the drug of choice for many pneumococcal diseases;[1,2] however, with the increasing description of penicillin-resistant strains since the 1980s, some alternative regimens have been suggested.[5-7] Based on the positive response to the treatment with β-lactam antibiotics (penicillin or ampicillin) of patients with invasive pneumococcal diseases (except for meningitis), even when caused by strains with minimum inhibitory concentration (MIC) up to 2.0 µg/mL, a redefinition of the susceptibility categories has been recommended.[5]

The prevention of invasive diseases is mainly based on active immunization.[1-3] According to the capsular polysaccharide antigen, 91 different serotypes[8,9] belonging to 46 pneumococcal serogroups[10] have been described so far. A 7-valent vaccine containing the capsular polysaccharide of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, conjugated to a carrier protein, was licensed in the USA in 2000.[3,10-12] The immunogenicity of the polysaccharide is increased due to the covalent binding to the protein molecule, especially in children younger than 2 years old.[3,10,11] In addition, this vaccine protects against nasopharyngeal colonization for those serotypes represented in it.[3,10,11]

With the purpose of monitoring the pattern of resistance to antimicrobial agents and the profile of pneumococcal serotypes in Latin America, the Pan American Health Organization (PAHO) created the Regional System for Vaccines (SIREVA) in 1993 with the participation of six countries, including Brazil. Since 2004, this program has been broadened to include other bacteria (*Haemophilus influenzae* and *Neisseria meningitidis* in addition to pneumococcus) and other countries (currently 20 countries take part in this project), being called SIREVA II.[13-15] It is a laboratory surveillance program that analyzes (counts and describes) laboratory characteristics such as serotype and *in vitro* resistance pattern of the strains collected. Since April 1999, Uberlândia, Brazil, has been participating in the national network of the SIREVA Project by sending pneumococcal strains isolated at the Clinical Analysis Lab of Hospital de Clínicas of Universidade Federal de Uberlândia (HC-UFU), in Uberlândia, to Instituto Adolfo Lutz (IAL), in São Paulo, Brazil.

As the resistance pattern of antibiotics and the prevalence of pneumococcal serotypes vary according to different populations from different geographic regions and, probably, throughout time as well, it is important to define the profile of the most prevalent serotypes in a given community and to establish the resistance rates to support the choice of the best vaccination coverage and the best initial empirical treatment.

The objective of the present study is to determine the prevalence of serotypes and antimicrobial susceptibility of invasive strains of pneumococcus in clinical specimens of children up to 5 years old admitted with invasive disease at HC-UFU and to evaluate the implications for antipneumococcal vaccine formulation.

Patients and methods

The HC-UFU is a teaching public hospital fully financed by the Brazilian public Unified Health System. This hospital is a 510-bed facility and, since it is a regional center of excellence, most beds are assigned to patients with severe diseases.

This is a prospective, case series, laboratory surveillance study, whose laboratory data on serotyping and *in vitro* susceptibility to antimicrobial agents of pneumococcal invasive strains were collected from patients hospitalized at HC-UFU. The index case is a pneumococcal strain isolated at the Clinical Analysis Lab from clinical specimens (blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, abscess fluid) of an inpatient. Aseptically collected specimens were appropriately processed and seeded in blood culture flasks (blood samples) or chocolate agar and blood agar plates (other types of samples) within the shortest time possible after collection and immediately after being delivered at the laboratory. Only one pneumococcal isolate per hospitalized patient was considered in the present study. Pneumococcal strains were isolated and identified at HC-UFU according to internationally described methods and sent to the Bacteriology Department of IAL, São Paulo, for specimen confirmation, serotyping and determination of *in vitro* susceptibility to antimicrobial agents.[16] Pneumococcal strains were freeze-dried in skim milk at 20% and adequately catalogued.

Antimicrobial susceptibility was assessed using the disc diffusion technique for oxacillin (1 µg), tetracycline, ofloxacin, chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim (co-trimoxazole), vancomycin, and clindamycin in Mueller-Hinton agar plates supplemented with sheep blood at 5% according to the standardized technique.[17,18] Oxacillin-resistant strains (inhibition halo ≤ 19 mm) were submitted to MIC determination for penicillin using broth

microdilution and classified, according to the criteria of the 2008 Clinical and Laboratory Standards Institute (CLSI),[19] as susceptible (S) when MIC ≤ 0.06 µg/mL) and resistant (R) when MIC ≥ 0.12 µg/mL for strains collected from a patient with meningitis; for those strains collected from patients without meningitis, the breakpoints considered were S ≤ 2 µg/mL, intermediate resistant (IR) = 4 µg/mL, and full resistant (FR) ≥ 8 µg/mL. Oxacillin-resistant strains were submitted to ceftriaxone MIC determination and considered S when MIC was ≤ 0.5 µg/mL, IR with MIC = 1 µg/mL, and FR with MIC ≥ 2 µg/mL for strains from patients with meningitis; for those strains collected from patients without meningitis, the breakpoints were MIC ≤ 1 µg/mL for S, MIC = 2 µg/mL for IR, and MIC ≥ 4 µg/mL for FR.[20] Oxacillin-susceptible strains (inhibition zone > 19 mm) were considered penicillin-susceptible and were not submitted to MIC determination according to the 2007 CLSI standard.[21] The breakpoints recently defined for penicillin[19] and for ceftriaxone[20] were used in the results obtained throughout this study and were stored in a database.

Serotyping was performed using the Neufeld-Quellung reaction according to the technique described elsewhere,[22] using polyclonal antisera.[8]

Data were collected from April 1999 to December 2008, and the results underwent statistical analysis. Proportions of the clinical diagnoses and antimicrobial susceptibility pattern in the different periods were compared using the chi-square test performed with the Monte Carlo simulation method with 10,000 resampling. Whenever necessary, samples underwent normality and homogeneity tests. Significance level of the null hypothesis was set at 5% ($p < 0.05$).

This study was approved by the Research Ethics Committee of UFU.

Results

During a period of 9 years and 8 months, from April 1999 to December 2008, 330 samples were sent to the IAL lab. Of these, 152 were collected from children up to 5 years old; and 142 samples were analyzed after exclusion of 10 non-viable strains. Seventy-five (52.8%) samples were collected from male patients, and the children's age ranged from 1 to 60 months (mean age = 19±15.4 months; median = 15 months). The number of isolates according to age group was 101 in children up to 24 months old and 41 among patients between 25 and 60 months old. The number of samples analyzed during each year from 1999 to 2008 was 22, 21, 14, 16, 24, 9, 11, 9, 10, and 6, respectively. Clinical diagnosis was pneumonia in 92 patients (64.8%), meningitis in 33 (23.2%), occult bacteremia in 12 (8.5%), sepsis in three (2.1%), soft tissue abscess in one (0.7%), and cellulitis in another one (0.7%). There was no significant difference between the proportions of diagnosis of meningitis and pneumonia throughout time. Strains were collected from blood in 61 samples (43%), pleural fluid in 52 (36.6%), cerebrospinal fluid in 28 (19.7%), and abscess fluid in one (0.7%).

A total of 20 different serotypes were identified, and the most common ones are shown in Table 1 (according to different age groups), in Table 2 (according to different periods of isolation), and in Table 3 (according to clinical diagnosis). There was no predominance of a serotype

Table 1 - Distribution of pneumococcal serotypes according to the age groups of children admitted with invasive diseases from 1999 to 2008

Serotypes	≤ 24 months	%	25-60 months	%	Total	%
14	49	48.5	15	36.5	64	45.1
5	6	6.0	4	9.8	10	7.0
6B	8	8.0	2	4.9	10	7.0
1	3	2.9	4	9.8	7	4.9
6A	5	5.0	2	4.9	7	4.9
18C	3	2.9	4	9.8	7	4.9
19A	3	2.9	3	7.3	6	4.3
3	5	5.0	0	0.0	5	3.6
9V	4	4.0	1	2.4	5	3.6
19F	3	2.9	1	2.4	4	2.8
23F	3	2.9	1	2.4	4	2.8
9N	0	0.0	2	4.9	2	1.4
10A	2	2.0	0	0.0	2	1.4
Other*	7	7.0	2	4.9	9	6.3
Total	101	100.0	41	100.0	142	100.0

* Other: ≤ 24 months = serotypes 4, 7F, 8, 18A, 18B, 20 and non-typeable (one from each); 25-60 months = 15B and serogroup G (one from each).

Table 2 - Distribution of pneumococcal serotypes collected from children admitted with invasive disease according to period of isolation from 1999 to 2003 and from 2004 to 2008

Serotypes	1999-2003	%	2004-2008	%	Total	%
14	41	42.2	23	51.1	64	45.1
5	10	10.3	0	0.0	10	7.0
6B	5	5.2	5	11.1	10	7.0
1	7	7.2	0	0.0	7	4.9
6A	5	5.2	2	4.5	7	4.9
18C	6	6.1	1	2.2	7	4.9
19A	5	5.2	1	2.2	6	4.2
3	3	3.1	2	4.5	5	3.5
9V	2	2.1	3	6.7	5	3.5
19F	3	3.1	1	2.2	4	2.9
23F	3	3.1	1	2.2	4	2.9
4	1	1	0	0.0	1	0.7
7F	1	1	0	0.0	1	0.7
Other	5*	5.2	6†	13.3	11‡	7.8
Total	101	100.0	41	100.0	142	100.0

* Other: Serotypes 8, 10A and serogroup G (one from each), serotype 9N (two).
† Other: Serotypes 10A, 15B, 18B, 20 and non-typeable (one from each).
‡ Other: 8, 15B, 18A, 18B, 20, G and non-typeable (one from each); 9N and 10A (two from each).

regarding age group, time period, or clinical diagnosis when the frequency was different from zero in the related column.

There were 51 (35.9%) oxacillin-resistant strains. According to the 2008 CLSI standard,[18] 14 (9.9%) strains were also penicillin-resistant (1.4% were classified as IR and 8.5% were FR), which was detected only in the following serotypes: 14 (10 strains), 6B, 19F, 19A, and 23F (one strain from each serotype). There was predominance of resistance in cases of meningitis [12 isolates (36.4%)] compared with the other diagnoses (two isolates (1.8%)] ($p = 0.000$) and in the period from 2004 to 2008 compared

Table 3 - Distribution of pneumococcal serotypes according to clinical diagnosis of children admitted with invasive disease in the period from 1999 to 2008

Serotypes	Meningitis	%	Pneumonia	%
14	15	45.5	46	50.0
18C	3	9.1	2	2.2
6A	3	9.1	3	3.3
6B	3	9.1	6	6.5
19F	2	6.1	0	0.0
1	1	3.0	6	6.5
10A	1	3.0	1	1.1
18B	1	3.0	0	0.0
23F	1	3.0	3	3.3
3	1	3.0	4	4.3
5	1	3.0	8	8.7
7F	1	3.0	0	0.0
19A	0	0.0	4	4.3
9V	0	0.0	4	4.3
9N	0	0.0	2	2.2
20	0	0.0	1	1.1
4	0	0.0	1	1.1
Total	33	100.0	92	100.0

Table 4 - Distribution of pneumococcal strains collected from children with invasive disease according to penicillin susceptibility and period of isolation from 1999 to 2003 and from 2004 to 2008

	Penicillin susceptibility		
Isolation period (year)	**Susceptible***	**Resistant (%)†**	**Total**
1999-2003	94	3 (3.1)	97
2004-2008	34	11 (24.4)	45
Total	128	14 (9.9)	142

Chi-square = 15,769; p = 0.000.
* Susceptibility suggested using the oxacillin disk test.
† According to the 2008 CLSI standard.

with the period from 1999 to 2003 (p = 0.000) (Table 4). Reduced susceptibility to co-trimoxazole was detected in 113 strains (79.5%); of these, 7% were IR and 72.5% were FR. Resistance to erythromycin and clindamycin was simultaneously found in 16 strains (11.3% each). Resistance to ceftriaxone was detected in eight of the 14 penicillin-resistant strains, resulting in a rate of 5.6% (8/142); and, of these, 4.9% were IR and 0.7% were FR. There was not resistance to chloramphenicol, ofloxacin, rifampicin, or vancomycin.

Discussion

Despite the small size of our sample, it is possible to detect similarities between our findings and the results from national[13-15,23] and international[10,12-15] studies. In general, we confirmed the most frequent serotypes found in developing countries (14, 6A/6B, 5,1, 19F/19A, 9N/9V, and 23F), highlighting the high prevalence of serotypes 1 and 5, which are commonly found in Latin America.[3,10,11,23]

In a meta-analysis involving approximately 60,000 pneumococcal invasive strains collected from children younger than 6 years old on the six continents from 1980 to 2007, the most common serotypes isolated in Latin American countries (18,788 strains assessed) were 14, 6B, 5, 1, 23F, 6A, 18C, 19F, 19A, 9V, 7F, 3, and 4.[12] The coverage rate of the 7-valent vaccine for this population was 57.9% (including serotype 6A). The SIREVA Project collected 6.548 pneumococcal invasive strains from different regions of Brazil during 8 years (from 2000 to 2005,[13] in 2006[14] and in 2007[15]); of these strains, 5,742 were serotyped and 2,575 were collected from children younger than 6 years old. The most frequently found serotypes were 14, 6B, 18C, 19F, 23F, 1, 6A, 5, 19A, 9V, 3, 7F, and 4, which resulted in a coverage rate of 72.1% of the 7-valent vaccine (including serotype 6A). In other international studies, the 7-valent vaccine has shown a coverage rate between 80 and 90% in the USA, Canada and Australia,[10,11] and rates of 70 and 75% have been found in Europe and Africa,[10,11] while in Asia, the rate was 50%.[10]

The 7-valent conjugate vaccine covers the antigen of serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, contained in the product and, probably, including also serotype 6A, for cross-protection.[12] According to data shown in Tables 1 and 2, the vaccination coverage in our population is 71.9% (when serotype 6A was included) excluding, among others, serotypes 1, 3 and 5, commonly isolated; however, serotypes 6B, 14, 19F, and 23F would be covered, and penicillin-resistant strains are among these serotypes (vaccination coverage of 92.7%). The inclusion of serotypes 1, 5, and 7F in the 10-valent vaccine[12] would increase the coverage for our population to 84.5% (with serotype 6A), and the inclusion of serotypes 1, 5, 3, 7F, 6A, and 19A in the 13-valent vaccine, which is in its final phase of development,[12] would increase the coverage to 92.2%.

In spite of the fact that it represents some technical difficulties, the objective is to include the largest possible number of the most prevalent serotypes. The products that are going through the licensing process include 10 and 13 different serotypes.[12] The immunogenicity of the conjugate vaccine depends, among other aspects, on the chemical and antigenic characteristic of the carrier protein.[3] The 7-valent vaccine (Prevenar®, Wyeth Pharmaceuticals, Maidenhead, United Kingdom) contains the capsular oligosaccharide of seven different serotypes individually conjugated to the non-toxic protein CRM_{197}, produced by the mutant of *Corynebacterium diphtheriae*.[3] It was licensed in the USA in 2000 and is currently present in more than 70 countries

being used in children older than 2 months. It is indicated for the prevention of invasive pneumococcal disease (IPD) and AOM.[12] It has an effectiveness rate of 97.4 and 57%, respectively, for the prevention of IPD and AOM caused by serotypes represented in the vaccine.[3] The 13-valent vaccine, produced by the same pharmaceutical laboratory, contains the carrier protein CRM_{197} and its immunogenicity is similar to that of the 7-valent vaccine.[3]

The 10-valent vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) includes three different carriers: protein D (which binds individually the capsular polysaccharide of serotypes 1, 4, 5, 6B, 7F, 9V, 14, and 23F), tetanus toxoid (which binds serotype 18C), and diphtheria toxoid (which binds serotype 19F).[3,12] Due to protein D inclusion, a surface protein originally produced by non-typeable *Haemophilus influenzae*, protection against the infection with this bacterium is expected.[3]

Probably because of the small size of our sample, the present study did not show significant predominance of the frequency of serotypes throughout time when the representation was different from zero during each one of the periods (Table 2). However, range of frequency in waves of invasive disease caused by serotype 1 has already been found before,[10] and the trend of reduction throughout time in the frequency of serotypes 1 and 5 can be recognized in Brazil.[13-15] The reasons for such trend have not be provided yet; and, in our population, both serotypes remain among the most common serotypes.

The same is true regarding the comparison of the proportions according to age group (Table 1). Despite its higher frequency in adults, being associated with severe forms of pneumonia,[3,10,23] serotype 3 is an important cause of invasive disease in newborns and young infants, which would justify its inclusion in a vaccine for protection of children younger than 2 years old.[10] In the present study, serotype 3 plays an important role among the most common serotypes in children younger than 2 years old.

Even though some serotypes are associated with a specific disease, there is no exclusiveness, and different serotypes may cause different diseases[3] (Table 3). Since these variables are not completely dependent, the correlation of the serotype profile with the type of disease can be influenced by other factors such as isolation period and age.[3] The predominance of serotype 19A (in children up to 5 years old) and serotype 9V (in children older than 5 years old and adults) in pneumonia, in comparison with meningitis, could be detected in a Brazilian study involving the period from 1977 to 2000.[23] In spite of the low frequency in Brazil[4,13-15,23] and in Latin American countries,[12] both serotypes are associated with penicillin-resistance,[3,10,24] and serotype 19A currently represents the main emergent serotype in the USA after the 7-valent conjugate vaccine was approved for universal use.[3,10]

New breakpoints of *in vitro* penicillin susceptibility have been recently suggested[19] and, when they were used in the present study, we found a resistance rate of 9.9% (14/142 strains), with 1.4% (two strains) being classified as IR and 8.5% (12 strains) as FR, a percentage very lower than the rates of 15% (at all ages) and 29.6% (in children up to 2 years) found in this same population in 2003[25] using the traditional criteria.[21] Significant increases in the penicillin resistance rates of pneumococcal invasive strains have been described in Brazil, with rates going from 10.2% in 1993 to 27.9% in 2004,[24] according to the traditional criteria.[21] It is possible to compare these values with those from another national study recently published[15] that used the new criteria of breakpoints for penicillin.[19] The authors analyzed 315 pneumococcal invasive strains collected from children up to 5 years old during 2007 and found a penicillin resistance rate, according to the current criteria,[19] of 39.4%, with 5.4% of IR and 34% of FR.[15] Such difference can be partially explained by the fact that, in the Brazilian study, 69.5% of the strains (219/315) were collected from patients with meningitis,[15] while in the present study only 23.2% (33/142) had the same source. It is important to highlight that for invasive strains collected from patients with meningitis, the breakpoints for penicillin remained the same ($S \leq 0.06$ μg/mL and $R \geq 0.12$ μg/mL).[19] Therefore, when the criteria suggested by the 2008 CLSI[19] are adopted, the impact on the reduction of the penicillin resistance rates will decrease as the number of strains from patients with meningitis increases in the sample analyzed. This fact can also explain, at least in part, the predominance of penicillin resistance in cases of meningitis compared with pneumonia in the present study.

Penicillin resistance was restricted to serotypes 14, 6B, 19A, 19F, and 23F, usually associated with drug resistance.[3,10,24] These are the so-called pediatric serotypes that are responsible for a large number of episodes of infection in children. Probably, the intrinsic characteristics of age-dependent immunogenicity and the extensive exposure to antimicrobial agents contribute, respectively, to the predominance among the younger children and to the development of penicillin resistance.[2,10,24] In the present study, it was possible to confirm the increase in the proportion of penicillin-resistant strains during the period from 2004 to 2008 (24.4%) compared with the period from 1999 to 2003 (3.1%), according to data shown in Table 4. The increasing trend of penicillin resistance in Brazil throughout time has already been demonstrated in a national study[24] and it is associated with the emergency and dissemination of two international clones called Spain 9V-3 and Tennessee 14-18. These clones, which express the capsule of serotype 14 and are characterized by drug resistance, became predominant in the South and Southeast regions of the country after 1998. Among the 14 penicillin-resistant isolates detected in the present study, 10 (71.4%) are from serotype 14 and,

therefore, it is possible to assume that, at least in part, the increase in the proportion of resistant strains is caused by the dissemination of these clones in this community.

The screening method for detection of penicillin resistance using oxacillin disks (1 µg) has been used for decades and its sensitivity rate (above 99%) is higher than its specificity rate (80%).[26] In the analysis shown in the present study, 72.5% (37 out of 51) of the strains classified as possibly resistant (using oxacillin disk) were actually sensitive to penicillin (according to MIC determination), which reinforces the need of reviewing the specificity rate of the method regarding the adoption of the new criteria proposed by the 2008 CLSI[19] and the importance of MIC determination for safe classification of the penicillin resistance of pneumococcal strains.[19]

The fact that eight ceftriaxone resistant pneumococcal strains were found (six IR and two FR) results in a resistance rate of 5.6%; thus it was lower than those found in Brazilian population surveillance studies,[14,15] when using the same breakpoints for ceftriaxone.[20] Among the 601 invasive strains collected from children up to 5 years old in 2006[14] and 2007,[15] there was a ceftriaxone resistance rate of 20.6% (124/601 strains), with 16% of IR and 4.7 of FR. Similarly to penicillin, such difference may be explained, at least partially, by the larger proportion of cases of meningitis in the Brazilian study[14,15] (69.5 vs. 23.2%). In the USA, the Active Bacterial Core Surveillance (ABCs) Report detected, among 3,514 invasive strains collected from patients belonging to different age groups during 2007, a cefotaxime resistance rate of 6.9% (IR = 5.5% and FR = 1.4%).[4]

Regarding the susceptibility to other antimicrobial agents, it possible to conclude that the high resistance rate found for co-trimoxazole (79.5%) is in agreement with the rates reported in other Brazilian studies (54.5,[27] 61.2,[13-15] 65,[24] and 65.7%[28] and may compromise the indication of this chemotherapy drug for the treatment of pneumococcal infections. Erythromycin (11.3%) and clindamycin (11.3%) resistance rates remained relatively low if compared with those rates reported in other Brazilian studies: erythromycin (3.8,[27] 5.5,[13-15] 5.7,[28] and 6.2%[24]) and clindamycin (2.9[28] and 3.1%[27]). The fact that all 16 erythromycin-resistant strains were also resistant to clindamycin suggests the manifestation of the MLS_B phenotype, characterized by the resistance to macrolides, lincosamides, and streptogramin B.[29,30] There was not *in vitro* resistance to chloramphenicol, ofloxacin, rifampicin, or vancomycin.

The initial treatment for most pneumococcal infections remains empirical in terms of etiology and susceptibility to drugs. Since antibiotic choice is influenced by the laboratory classification (as susceptible or resistant, according to the MIC), the adoption of new breakpoints must increase the rates of strains reported as being S and consolidate the importance of the use of penicillin for the treatment of nonmeningeal pneumococcal disease.

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