

## Analysis of invasive pneumonia-causing strains of *Streptococcus pneumoniae*: serotypes and antimicrobial susceptibility

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### Abstract

**Objectives:** To identify the most common pneumococcal serotypes in children hospitalized with invasive pneumonia, correlate isolated serotypes with those included in conjugate vaccines, and ascertain the sensitivity of the isolated pneumococcal strains to penicillin and other antibiotics.

**Methods:** From January 2003 to October 2008, a retrospective study of hospitalized children with a diagnosis of *Streptococcus pneumoniae* pneumonia was conducted at the university hospital of Universidade de São Paulo. Criteria for inclusion were: age greater than 29 days and less than 15 years, radiological and clinical diagnosis of pneumonia, and isolation of *Streptococcus pneumoniae* in blood cultures and/or pleural effusion.

**Results:** The study included 107 children. The most common serotypes were 14 (36.5%), 1 (16%), 5 (14.6%), 6B (6.3%) and 3 (4.2%). The proportion of identified serotypes contained in the heptavalent, 10-valent and 13-valent conjugate vaccines was 53.1, 86.5, and 96.9%, respectively. Pneumococcal strains were sensitive to penicillin (minimum inhibitory concentration, MIC  $\leq$  2  $\mu$ g/mL) in 100 cases (93.5%) and displayed intermediate resistance (MIC = 4  $\mu$ g/mL) in 7 cases (6.5%). No strains were penicillin-resistant (MIC  $\geq$  8  $\mu$ g/mL) according to the Clinical and Laboratory Standards Institute 2008 standards. Tested isolates were highly sensitive to vancomycin, rifampicin, ceftriaxone, clindamycin, erythromycin, and chloramphenicol.

**Conclusions:** Our results confirm a significant potential impact of conjugate vaccines, mainly 10-valent and 13-valent, on invasive pneumonia. Furthermore, susceptibility testing results show that penicillin is still the treatment of choice for invasive pneumonia in our setting.

*J Pediatr (Rio J)*. 2011;87(1):70-75: *Streptococcus pneumoniae*, pneumonia, serotype, antimicrobial resistance, pneumococcal conjugate vaccine.

### Introduction

The estimated incidence of community-acquired pneumonia among children under five years of age in developing countries is approximately 151.8 million new cases per year, 11-20 million of which require hospitalization.<sup>1</sup>

In developing countries, pneumonia occurs more often and is more severe than in developed nations, and carries higher incidence and mortality rates; pneumonia accounts for one-fifth of under-five deaths in the developing world.<sup>2</sup>

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According to a 2008 article published in the Bulletin of the World Health Organization,<sup>3</sup> Brazil is among the top 15 countries in incidence of pneumonia – 0.11 episodes per child-year among under-fives, which translates to 1.8 million cases per year.

In this age range, *Streptococcus pneumoniae* is the main causative agent of bacterial pneumonia, except in the neonatal period.

Vaccination is one of the most important ways of preventing pneumococcal infection. The heptavalent conjugate vaccine (PCV7), which contains seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) has been licensed for use in approximately 90 countries since 2000. Its composition is based on the serotypes most commonly implicated in infection in the United States, Canada, and some European countries.<sup>4</sup> The frequency with which the various serotypes of *S. pneumoniae* occur and the extent of their role in causing invasive disease varies with time and place. Therefore, the proportion of disease-causing serotypes present in PCV7 is high in the United States, Canada and Australia (80-90%), followed by Europe and Africa (70-75%), Latin America (roughly 65%) and Asia (roughly 50%).<sup>4</sup> This proportion is low in Latin America due to the high prevalence of serotypes 1 and 5 in the region.<sup>5-7</sup> Thus arises a need for new vaccines that contain additional serotypes responsible for invasive pneumococcal disease in various countries and regions worldwide. At the present time, two pneumococcal conjugate vaccines are available: the 10-valent vaccine, which provides additional coverage for serotypes 1, 5, and 7F, and was introduced to the Brazilian National Immunizations Program in 2010; and the 13-valent vaccine, which extends coverage to serotypes 1, 3, 5, 6 A, 7F and 19a.<sup>8</sup>

Regarding penicillin susceptibility of *S. pneumoniae*, minimum inhibitory concentration (MIC) breakpoints for penicillin were first established in the late 1970s, in response to the need for ensuring successful treatment of pneumococcal meningitis. Over the past three decades, increasing MICs to penicillin have emerged in pneumococci, and the percentage of penicillin-intermediate and penicillin-resistant strains has risen.<sup>9,10</sup> In a study conducted by the Regional Vaccine System Project (Projeto de Sistema Regional de Vacinas, SIREVA) between 2000 and 2005, of a total of 8,993 pneumococcal isolates (36.4% of which obtained from patients with pneumonia), 37.8% were penicillin-resistant.<sup>11</sup> In light of this growing resistance, penicillins are increasingly being rejected for both empirical and susceptibility-guided treatment, in favor of more expensive, broad-spectrum antibiotics. However, retrospective and prospective studies in adult and pediatric populations showed that the outcomes of pneumococcal pneumonias caused by purportedly resistant strains but treated with penicillin antibiotics did not differ from those obtained with other antimicrobial agents, suggesting that

meningitis breakpoints did not apply to pneumonia.<sup>12-14</sup> Later multicenter studies confirmed these results.<sup>15,16</sup>

On the basis of these findings and of a review and reassessment of microbiology, pharmacokinetics and pharmacodynamics data, in January 2008, the Clinical and Laboratory Standards Institute (CLSI) published new breakpoints for pneumococcal susceptibility to penicillin.<sup>17</sup> In non-meningeal disease, isolates are currently considered penicillin-sensitive with MIC  $\leq 2$   $\mu\text{g/mL}$ , penicillin-intermediate with MIC = 4  $\mu\text{g/mL}$ , and penicillin-resistant with MIC  $\geq 8$   $\mu\text{g/mL}$ . Meningitis isolates are considered sensitive with MIC  $\leq 0.06$   $\mu\text{g/mL}$  and resistant if MIC  $\geq 0.12$   $\mu\text{g/mL}$ .

As the epidemiology of pneumococcal disease varies with time and place, periodic reassessment with monitoring of prevalent serotypes and patterns of resistance is required for better therapeutic guidance and definition of control strategies. With that in mind, the main objectives of this study were: to identify the *S. pneumoniae* serotypes most frequently isolated from children hospitalized for invasive pneumonia; compare these serotypes with those included in conjugate vaccines; and analyze their susceptibility to the antimicrobial agents most often used in pediatric practice.

## Methods

This was a descriptive, retrospective study of pneumococcal pneumonia in pediatric inpatients of the Hospital Universitário da Universidade de São Paulo (HU-USP). HU-USP is a secondary-level teaching hospital that mostly cares for the population of the Butantã health district, on the west side of the city of São Paulo, Brazil. Children are usually admitted to the hospital from the pediatric emergency department; most are previously healthy and have no underlying conditions. Criteria for inclusion were: age greater than 29 days and less than 15 years, clinical and radiological diagnosis of pneumonia, and isolation of *Streptococcus pneumoniae* in blood cultures and/or pleural effusion between January 2003 and October 2008. Cases of hospital-acquired pneumonia – defined as clinical manifestations of pneumonia arising > 72 hours after admission in patients not undergoing invasive procedures – were excluded. As the study was conducted at a teaching hospital, complete blood counts, blood cultures, and, whenever possible, pleural effusion cultures were performed in all patients diagnosed with pneumonia.

*S. pneumoniae* strains were isolated from blood cultures using the BacT/ALERT® detection system (Biomérieux, France), whereas pleural fluid specimens were cultured on chocolate agar and sheep blood agar (Biomérieux, Rio de Janeiro, Brazil). Pure cultures were taken from blood agar. Organisms were identified at the HU-USP microbiology lab,

using the methods described by Spellerberg & Brandt<sup>18</sup> and an automated system (Vitek® 120, Biomérieux, France).

Susceptibility testing was performed using the disk diffusion method (Oxoid, UK), according to 2008 CLSI standards.<sup>18</sup> Penicillin MICs were determined by the Etest® gradient strip method (ABbiomerieux, Sweden) when resistance was demonstrated on the oxacillin screen test. All susceptibility tests used 2008 CLSI breakpoints for non-meningeal infections.<sup>17</sup>

Isolates were considered multidrug-resistant when drug susceptibility testing showed resistance to three or more of the following antimicrobial agents: penicillin, erythromycin, trimethoprim-sulfamethoxazole (TMP-SMX), tetracycline, chloramphenicol, clindamycin, rifampicin, or levofloxacin.

Serotyping was carried out at the Instituto Adolfo Lutz laboratory, using the Neufeld quellung reaction with polyclonal antisera manufactured by the Danish Statens Serum Institut (Copenhagen, Denmark).

Basic descriptive statistics were used to analyze data. Continuous variables were expressed as means, standard deviations, and medians and categorical variables were expressed as proportions or frequency tables. The Student *t* test was used for comparison of parametric variables. The level of significance was set at  $p = 0.05$ .

The present study was approved by the HU-USP Research Ethics Committee (CEP-HU/USP:653/06-SISNEP CAAE: 0009.0.198.000-06).

## Results

During the study period, the mean annual admission rate to the HU-USP pediatric ward was 1,535 patients. Of these, 720 per year (47%) were diagnosed with pneumonia. *S. pneumoniae* was isolated from blood or pleural fluid cultures in approximately 2.5% of children hospitalized for pneumonia.

The final study sample comprised 107 children with a clinical and radiological diagnosis of pneumonia and growth of *S. pneumoniae* in blood and/or pleural fluid cultures.

In 75 cases (70%), pneumococci were isolated in blood cultures; in 16 (15%), from pleural fluid; and in 16 others (15%), from both.

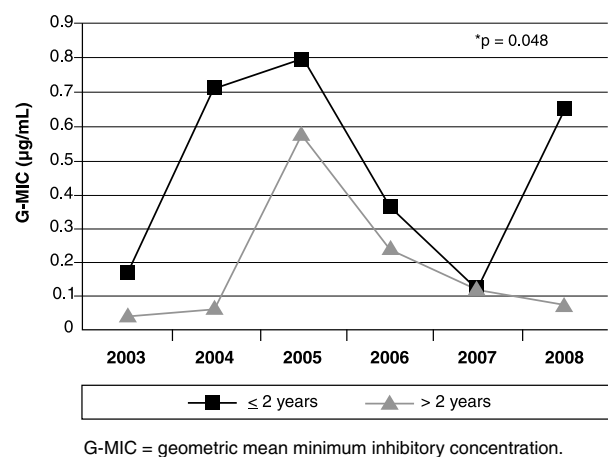
Median age was 23 months; 51.4% of patients were aged  $\leq 24$  months and 82.2% were  $\leq 5$  years of age.

Associated conditions were present in 48 cases (44.9%): wheezing in 37 (77.1%), chickenpox in 8 (16.7%), recurring pneumonia of undetermined etiology in 4 (8.3%), prematurity and/or bronchopulmonary dysplasia in 3 (6.3%), and heart disease in 3 (6.3%).

According to 2008 CLSI breakpoints, 100 isolates (93.5%) were penicillin-sensitive (MIC  $\leq 2$   $\mu\text{g/mL}$ ) and

seven (6.5%) were penicillin-intermediate (MIC = 4  $\mu\text{g/mL}$ ). No isolates were penicillin-resistant.

The geometric mean MIC (G-MIC) for penicillin followed an upward trend between 2003 and 2005, followed by a sharp decline in 2006 and 2007. The G-MICs for penicillin of pneumococcal strains isolated from children  $\leq 2$  years of age were higher than those of isolates from children  $> 2$  years of age. Comparison of MIC values showed a population-wide mean MIC of  $0.28 \pm 0.22$  for penicillin, with a significant difference ( $p < 0.05$ , Student *t* test) between MICs of isolates from children 2 years old or younger (mean MIC,  $0.47 \pm 0.29$ ) and those of isolates obtained from older patients (mean MIC,  $0.19 \pm 0.20$ ) (Figure 1).



**Figure 1** – Geometric mean MICs ( $\mu\text{g/mL}$ ) for penicillin over time and correlation with age range ( $\leq 2$  years vs.  $> 2$  years)

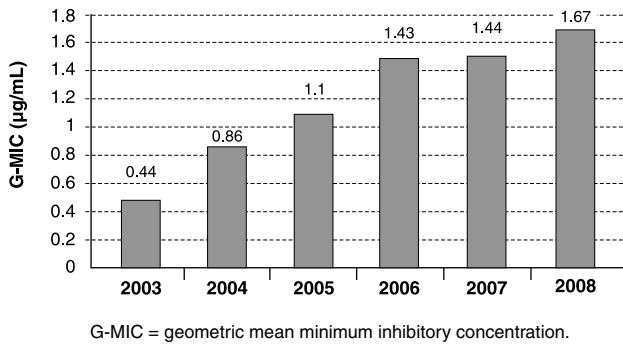
A total of 96 isolates from 96 cases of pneumonia were serotyped (89.7%). Eleven isolates lost viability before typing. The most prevalent serotypes were 14 (36.5%), 1 (16.7%), 5 (14.6%), 6B (6.3%) and 3 (4.2%). Other serotypes identified were 4, 6A and 19A (three isolates each); 7F, 18C, 23F and 19F (two isolates each); and 23B, 9V, 12F, 19F and 23F (one isolate each).

Median patient age for each of the most prevalent serotypes was 21 months (serotype 14), 70 months (serotype 1), 38.5 months (serotype 5), and 14 months (serotypes 6B and 3). Serotypes 1 and 5 were thus predominant in the  $> 24$  months age range, and serotypes 14, 6B and 3 in patients aged  $\leq 24$  months.

In all, 52 isolated strains belonged to the serotypes most commonly associated with penicillin resistance (6B, 9V, 14, 19F, 23F, 6A and 19A). Of these, seven (13.5%) had MICs of 4  $\mu\text{g/mL}$  for penicillin. The first strain with a MIC of 4  $\mu\text{g/mL}$  was isolated in 2004. Of the seven strains with MICs of 4  $\mu\text{g/mL}$ , five were serotype 14, one serotype 23F and one serotype 6B.

The G-MICs for penicillin of the most prevalent serotypes were as follows: serotype 14, 1 µg/mL; serotype 6B, 0.13 µg/mL; serotype 5, 0.06 µg/mL; serotype 4, 0.05 µg/mL; serotype 1, 0.03 µg/mL; and serotype 3, 0.02 µg/mL. The only statistically significant difference was for serotype 14.

The G-MIC of penicillin for serotype 14 increased progressively over the study period (Figure 2).



**Figure 2 -** Geometric mean minimum inhibitory concentration of penicillin (µg/mL) for serotype 14 over the study period

Susceptibility of the *S. pneumoniae* strains isolated in our sample to non-penicillin antimicrobial agents is shown in Table 1.

All tested strains were sensitive to rifampicin and vancomycin. Several other agents showed high susceptibility rates, including chloramphenicol (99%), clindamycin (97.2%), ceftriaxone (94.6%), and levofloxacin (97.6%). Erythromycin susceptibility was 97.2%. Of the three erythromycin-resistant strains isolated, two were cross-resistant to clindamycin as well.

Five pneumococcal strains were multidrug-resistant; in four, the MIC of penicillin was 4 µg/mL. Of these strains, two were serotype 14, one serotype 6B, and one serotype 23F. The remaining isolate was not serotyped.

Only one patient (age 5) had received the heptavalent pneumococcal conjugate vaccine. Serotype 12F was isolated from this patient's cultures.

**Discussion**

Worldwide, the *S. pneumoniae* serotypes most commonly associates with antimicrobial resistance are 6B, 9V, 19F, 14, 23F, 6A and 19A. The heptavalent and 10-valent conjugate vaccines each contain five of these serotypes (6B, 9V, 19F, 14 and 23F), whereas the 13-valent vaccine contains all. In the present study, the percentage of resistance-associated serotypes contained in the heptavalent and 10-valent vaccines was 94.2%. Only the 13-valent vaccine contains serotypes 19A and 6A; however, several studies have shown that vaccine serotype 6B provides partial cross-protection against serotype 6A.<sup>8</sup>

In Brazil, 63 serotypes of *S. pneumoniae* were identified in 4,858 cases of invasive pneumococcal disease reported between 1993 and 1999. Eleven capsular serotypes accounted for 86.2% of pneumonias. The serotypes most strongly associated with pneumonia were 14 (33.1%), 1 (12%), 5 (11.2%), 6A/6B (10.5%), 23F (4.4%) and 19A (3.7%).<sup>5</sup> In a study by the SIREVA II project (2000-2005) of 528 pneumococcal strains isolated from pneumonia cases in Brazil, the most common serotypes were 14 (48.5%), 1 (13.4%), 6B (7.6%), 19A (4.4%) and 5 (4.2%).<sup>11</sup> In a study conducted in Salvador, Bahia, the serotypes isolated from 70 invasive strains of *S. pneumoniae* (77% isolated from children and adolescents with pneumonia) were 14 (22.9%), 5 and 6A (10% of cases each), 6B and 19F (8.6%

**Table 1 -** Antimicrobial susceptibility of isolated pneumococcal strains, according to 2008 Clinical and Laboratory Standards Institute (CLSI) breakpoints

Agent	No. isolates		Sensitive isolates		%	
	≤ 2 years	> 2 years	≤ 2 years	> 2 years	≤ 2 years	> 2 years
Penicillin	55	52	50	50	90.9	96.2
Vancomycin	27	27	27	27	100	100
Rifampicin	54	51	54	51	100	100
Ceftriaxone	46	34	44	32	95.7	94.1
Clindamycin	55	51	52	51	94.5	100
Cloramphenicol	54	49	54	48	100	98
Levofloxacin	43	39	42	38	97.7	97.4
Erythromycin	55	51	52	49	94.5	96
TMP-SMX	54	51	14	16	25.9	31.4
Ciprofloxacin	12	9	6	6	50	33.3

TMP-SMX = trimethoprim-sulfamethoxazole.

each), 9V, 18C and 23F (5.7% each).<sup>19</sup> A similar distribution was found in the present study.

Of the serotypes isolated in our study, 53.1% are present in the heptavalent conjugate vaccine. This lower percentage versus that found in other countries is mostly due to the high prevalence of serotypes 1 and 5, which account for 16.7 and 14.6% of cases respectively. In Brazil, according to the SIREVA II project (2000-2005), 70% of pneumonia-causing serotypes are contained in the heptavalent vaccine, 82.4%, in the 10-valent vaccine and 92.9% in the 13-valent vaccine.<sup>11</sup>

Serotypes 14, 3 and 6B were dominant in patients under the age of 2 years, whereas serotypes 1 and 5 were more prevalent in older children. According to these findings, PCV7 would have provided 77.1% coverage in children aged 2 and under in our sample and only 29.4% coverage in children over the age of 2.

The 10-valent vaccine, which contains serotypes 1, 5, and 7F in addition to those provided in the heptavalent vaccine (and would thus provide coverage for 86.5% of serotypes isolated in the present study), was introduced to the Brazilian National Immunization Program in 2010.<sup>8</sup> After its introduction, the potential for protection of children aged  $\leq 2$  years and  $> 2$  years would be 79.2 and 93.8% respectively. The other available pneumococcal conjugate vaccine – the 13-valent vaccine, which contains serotypes 1, 5, 7F, 3, 6A and 19A in addition to those contained in PCV7 – would provide coverage for 96.9% of pneumococcal serotypes isolated in our sample.

Interestingly, the most prevalent serotypes of *S. pneumoniae* have remained the same across the most diverse regions of Brazil for over a decade.

Penicillin susceptibility data using 2008 CLSI breakpoints for non-meningeal pneumococcal disease<sup>17</sup> show that 95.3% of pneumonia-causing pneumococci isolated in our sample were penicillin-sensitive, with no strains displaying full-blown resistance. An assessment of 7,903 pneumococcal strains isolated in 10 U.S. states between 2006 and 2007, using the same breakpoints, found similar susceptibility rates.<sup>17</sup> In the SIREVA II (2000-2005) study,<sup>11</sup> 95.1% of respiratory disease isolates were penicillin-sensitive; in the later SIREVA II (2008) project,<sup>20</sup> 85.1% of non-meningeal disease-causing isolates from under-fives and 93.1% of those from older children were susceptible.

A study conducted in Uberlândia, state of Minas Gerais, Brazil, between 1999 and 2008 reinforces our findings. Penicillin resistance rates in 100 *S. pneumoniae* strains isolated from children aged 12 or younger with a diagnosis of pneumonia declined from 33 to 1% upon adoption of new CLSI breakpoints.<sup>21</sup> This wealth of data shows that penicillin antibiotics are still an excellent therapeutic option for treatment of pneumococcal pneumonia.

In the present study, seven *S. pneumoniae* strains were penicillin-intermediate (MIC = 4  $\mu\text{g}/\text{mL}$ ). Five of these were

serotype 14 (71.4%), one was serotype 23F and one was serotype 6B.

According to a study conducted by Brandileone et al. between 1993 and 2004, the penicillin G-MICs of invasive pneumococcal disease isolates have been on an upward trend.<sup>22</sup> A similar trend was found in the present study between 2003 and 2005, followed by a steep decline in 2007. This was likely due to the lower prevalence of serotype 14 isolates recorded in 2007, as serotype 14 has the highest G-MIC values for penicillin.

Increasing G-MICs for penicillin of serotype 14 strains were also reported by other Brazilian authors between 1993 and 2004.<sup>22</sup> Furthermore, the prevalence of serotype 14 has also increased in under-fives: from 25.1% in the 1993-1999 period to 32.1% between 2000 and 2004. This increase in G-MIC values has been associated with the emergence and rapid dissemination of two overseas clones of serotype 14 *S. pneumoniae*, Spain9V-3 and Tennessee.<sup>14-18</sup>

The main reasons for abandoning penicillin in the treatment of pneumococcal pneumonia are the progressive increase of penicillin resistance in *S. pneumoniae* and the absence of a consensus on optimal dosage.<sup>23</sup> Pharmacokinetics and pharmacodynamics data suggest that, in the treatment of pneumonia, beta-lactam antibiotics are effective against pneumococci with MICs of up to 4  $\mu\text{g}/\text{mL}$ . In children, therapeutic levels have been achieved at doses ranging from 100,000 to 300,000 IU/kg/day in 4 to 6 divided doses.<sup>24,25</sup>

For treatment of penicillin-intermediate strains (MIC = 4  $\mu\text{g}/\text{mL}$ ), which are still rare, adequate therapeutic levels can be achieved by doubling the daily dose of penicillin (200,000 IU/kg/day), even in the presence of complicated pleural effusions.<sup>26</sup> In the present study, G-MICs for penicillin were higher in isolates obtained from patients under the age of 2 years, probably due to greater antibiotic use and greater susceptibility to infection in this age range.

All pneumococcal strains isolated in the present study were highly sensitive to all antimicrobials except TMP-SMX, mirroring the findings of the SIREVA II project,<sup>20</sup> which found a resistance rate of 77.5% in isolates from patients aged  $\leq 14$  years.

Of the three erythromycin-resistant strains isolated, two were cross-resistant to clindamycin, suggesting expression of the MLS<sub>B</sub> phenotype. During the SIREVA II project study period,<sup>20</sup> the erythromycin resistance rate in Brazil (isolates from patients aged  $\leq 14$  years) was 8.9%.

Despite the low positivity rate of cultures obtained from patients with pneumonia, the present study provides a reminder of the importance of collecting samples to elucidate the etiology of cases whenever possible and thus guide choice of antimicrobial therapy. The main limiting factor of our investigation is that it was conducted in a single hospital, the catchment area of which covers only a single

region of the city of São Paulo, Brazil. We would like to stress the importance of conducting similar studies in the various regions of Brazil to monitor *S. pneumoniae* serotypes and their penicillin susceptibility, bearing in mind that the 10-valent pneumococcal conjugate vaccine has just been introduced as part of the Brazilian National Immunization Program and that postvaccine surveillance plays an essential role in continued epidemiological characterization of *S. pneumoniae* in the country and monitoring of antimicrobial resistance.

Data from the present study, as did prior Brazilian studies,<sup>5,11,19</sup> show that the 10-valent pneumococcal conjugate vaccine, particularly due to its inclusion of serotypes 1 and 5, contains a greater proportion of invasive pneumonia-causing pneumococcal strains than the heptavalent vaccine. Likewise, when it becomes available, the 13-valent vaccine will contain a greater proportion of disease causing serotypes than its 10-valent counterpart.

According to current susceptibility criteria and the sensitivity findings reported herein, penicillin should still be considered the first-line drug of choice for treatment of invasive pneumococcal pneumonia.

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